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Editorial introductions

Current Opinion in Rheumatology was launched in 1989. It is one of a successful series of review journals whose unique format is designed to provide a systematic and critical assessment of the literature as presented in the many primary journals. The field of rheumatology is divided into 15 sections that are reviewed once a year. Each section is assigned a Section Editor, a leading authority in the area, who identifies the most important topics at that time. Here we are pleased to introduce two of the Journal’s Section Editors for this issue.

Section Editors

George C Tsokos, MD

Dr. Tsokos obtained his Medical Degree at the University of Athens, Greece, and finished his training in Internal Medicine at the Veterans Administration Hospital and Georgetown University in Washington, D.C. After a fellowship in Immunology and Rheumatology at the National Institutes of Health, Bethesda, MD, he joined the staff of the Uniformed Services University in Bethesda where he is a Professor of Medicine and Cell/Molecular Biology, Director of the Immunology/Rheumatology Division and Vice Chair of for Research Programs in the Department of Medicine. He also serves as Chief of the Department of Cellular Injury at the Walter Reed Army Institute of Research.

Dr. Tsokos studies immune cell signaling and gene transcription aberrations and mechanisms of tissue injury in SLE. His recent contributions indicate that SLE immune cells display unique biochemical and molecular aberrations that are responsible for increased response to antigen/autoantigen on one hand and for failure to produce sufficient amounts of interleukin-2 on the other. He is involved in the editorial activities of several journals including Section Editor of the Journal of Immunology and Associate Editor-in-Chief of Clinical Immunology. Dr. Tsokos is President-elect of the Clinical Immunology Society and serves on the Board of Directors of the Research Foundation of the American College of Rheumatology.

Robert A Colbert, MD, PhD

Dr. Colbert obtained his MD and PhD degrees through the Medical Scientist Training Program at the University of Rochester School of Medicine, and following a residency in pediatrics, trained in Pediatric Rheumatology at the Duke University/University of North Carolina program. He joined the Division of Rheumatology at Cincinnati Children’s Hospital Medical Center and the University of Cincinnati College of Medicine, where he became a tenured Associate Professor of Pediatrics in 2001 and Professor in 2005. He is an Associate Director of the Division of Rheumatology, and also serves as Associate Director of the medical school’s NIH-funded Physician Scientist (MD/PhD) Training Program, and directs the Pediatric Rheumatology Training Grant (NIH T32).

Dr. Colbert’s research focuses on the immunobiology of HLA-B27 and its role in the pathogenesis of spondyloarthritis. Recent efforts have led to the discovery that HLA-B27 is a very unusual allele in that it misfolds, a property that can cause activation of a stress response pathway known as the unfolded protein response. This abnormal response may contribute to the well-recognized ability of this allele to confer susceptibility to spondyloarthritis. He is also interested in juvenile onset spondyloarthritides, particularly how to improve diagnosis at the earliest stages of disease, and defining novel methods for predicting outcome. He is a member of the Medical and Scientific Advisory Board of the Spondylitis Association of America.

Dr. Colbert has served on many American College of Rheumatology (ACR) committees including a Blue Ribbon panel to address the future of Pediatric Rheumatology, and more recently the Executive Committee of
the Pediatrics Section. He is co-chairing the organizing committee for the next North American Pediatric Rheumatology Meeting formerly known as the ‘Park City’ meeting. Dr Colbert has served on numerous national and international grant review panels, and is currently a member of the Arthritis, Connective Tissue and Skin Sciences (ACTS) study section at NIH. He reviews papers for several journals, and is an Advisory Editor for Arthritis & Rheumatism. He received the James R. Klinenberg Science Award from the Arthritis Foundation in 1999, was an ACR/Immunex Visiting Professor in 2002, and this year was chosen as an ACR representative for the ACR/EULAR International Academic Exchange Program.
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In this issue a panel experts has tried to capture the major advances that surfaced during the past year. Due to space limitations, certain choices had to be made but we hope that the state-of-the-art short reviews presented here will encourage readers to seek all that has happened recently.

Immune cells from patients who have systemic autoimmune diseases respond to excessively available autoantigen. Immune cells from patients with systemic lupus erythematosus (SLE) have, for still unclear reasons, lost their tolerant status. Conversely, it appears that autoantigen is produced in these patients in increased rates. Addressed in the review by Graham and Utz (pp. 513–517) are the mechanisms that are responsible for the production and availability of autoantigen and how and why certain autoantigens represent prime targets for the autoimmune response. Several antigens appear to undergo post-translational modification during apoptosis, whereas increased granzyme B activity during apoptosis has been shown to be responsible for the production of distinct autoantigens. Viruses have long been implicated in the induction of autoimmunity through the so-called ‘molecular mimicry’ process. Herein the role of viral proteases in the generation of autoantigens is discussed. Interferons appear to elicit the production of autoantigens whereas increased rate of alternative spicing of antigens appears to contribute to the number of available target autoantigens. It is still difficult to understand, however, why only certain autoantigens are selected to serve as a target of the autoimmune response, or conversely, why certain autoantigens elicit an autoimmune response. Studies like those reported by McClain et al. [1] shed light on the order of appearance of the autoimmune response and inferentially point out the antigens that initiate the autoimmune response.

We have learned much in recent years about the contribution of genes in the expression of autoimmunity in both humans and mice. Bagavant and Fu (pp. 523–528) discuss recent evidence supporting the role of susceptibility genes for end-organ damage and autoreactive T cells in determining disease outcome. The authors note the complex interactions between innate and adaptive immunity resulting in end-organ damage.

Also in this issue, Croker and Kimberly (pp. 529–537) update us on specific genetic variants to the SLE phenotype from populations of different backgrounds. The authors also discuss evidence supporting the notion of susceptibility alleles common to multiple autoimmune conditions such as rheumatoid arthritis, psoriasis, and autoimmune thyroid disease. Although a single common foundation to autoimmunity seems unlikely, some shared building blocks seem probable. Also discussed are the epistatic interactions between susceptibility alleles that contribute to disease development and the factors that may contribute to phenotype severity. Approaches being developed to determine the

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Abbreviation

SLE systemic lupus erythematosus

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That complement is involved in the pathogenesis of systemic autoimmunity has been documented. Complement is important for the selection of the immune repertoire and its absence has been shown to favor the propagation of an autoimmune repertoire. Simultaneously, complement is important in the execution of tissue pathology [7]. Karp (pp. 538–542) reviews the most recent developments in the role of complement in the expression of autoimmune pathology. The antiphospholipid syndrome, a major feature of SLE, is characterized by prothrombotic events that depend on complement activation. Heparin was shown to be of therapeutic value in a mouse model of antiphospholipid syndrome because of its ability to inhibit complement activation rather than because of its anticoagulant activity. Karp also reviews a study demonstrating that anti-C1q autoantibodies are pathogenic only when C1q complexes are deposited in the kidneys. Both studies imply that autoantibodies become pathogenic when another factor facilitates their deposition to tissues. Similar conclusions were reached in other studies showing that autoantibodies deposit only in tissues that have been conditioned by ischemia [8,9]. These studies are a start in explaining why patients with SLE who have sufficient quantities of circulating autoantibodies do not necessarily have active disease and why following exposure to a stressor these patients present with disease flare.

One of the limitations in the planning and execution of therapeutic trials in SLE is the lack of proper disease biomarkers to accurately diagnose and predict disease flares and remissions [10]. Liu et al. (pp. 543–549) discuss in a logical manner the concepts that should govern the development of SLE biomarkers. They also introduce a new biomarker, the presence of C4d on the surface membrane of erythrocytes, that may serve as an accurate diagnostic test and possible predictor of disease flares and remissions.

Sfikakis et al. in this issue (pp. 550–557) discuss the available rationale for the planning and execution of B-cell-depleting treatment in SLE. The accumulated evidence clearly suggests that rituximab (anti-CD20 antibody) monotherapy or rituximab used in conjunction with a classic immunosuppressive agent can be of therapeutic value in patients with severe disease. It appears that the short-term data are not shadowed by severe or morbid side effects. We have learned also that B-cell depletion does not affect the levels of protective antibody in the short term, although it appears to decrease the levels of autoantibody. The data for interpretation are limited, but they suggest that the requirements for the production of protective antibody and autoantibody are different. Although some of the clinical effect may be associated with the decrease in the autoantibody level, it appears that B-cell depletion has a profound effect in the generation and preservation of activated T cells and probably in the presentation of autoregion. It is obvious that proper, controlled clinical trials are needed to determine accurately the clinical efficacy of treatment with B-cell-depleting biologics.

Finally, Hansen et al. (pp. 558–565) discuss recent advances in the immunopathogenesis of Sjögren’s syndrome and their implications in the treatment of the disease. It has become clear that B-cell hyperreactivity and enhanced levels of B cell activating factor of the tumor necrosis factor family/B lymphocyte stimulator (BAFF/BlyS) play a central role in the expression of the disease. Studies have suggested the use of flow cytometry to detect B-cell subsets, the measurement of soluble forms of cell surface molecules, cytokines, and ligands (BAFF/BlyS) of receptors as diagnostic tools. Some of these may be considered for development as biomarkers. The information reviewed points to a central role for B cells in the expression of the disease and suggests the consideration of B-cell-depleting therapies for clinical trials in patients with Sjögren’s syndrome.

References

Sources of autoantigens in systemic lupus erythematosus
Kareem L. Graham and Paul J. Utz

Purpose of review
A hallmark of systemic lupus erythematosus is the production of autoantibodies that recognize nuclear antigens. However, the underlying events and mechanisms that lead to the selection of these molecules for the autoimmune response remain poorly understood. In this review, we will examine some of the proposed explanations for sources of systemic lupus erythematosus-specific autoantigens. We will focus on events related to apoptosis, viral infection, cytokine production, innate immune system components, and alternative splicing of pre-mRNA transcripts.

Recent findings
Definitive proof of a viral etiology for lupus remains elusive. However, recent observations have added to increasing evidence that viruses contribute to the bypass of tolerance in systemic lupus erythematosus. Also, events associated with apoptosis — most notably proteolytic autoantigen cleavage by caspases and granzyme B — have been implicated in the initiation of autoimmune responses for over a decade. Results obtained from animal models and human systems suggest complex functions for pro-apoptotic pathways in the regulation of immune responses. Inducible antigen expression and alternatively spliced transcripts may represent additional ways of generating autoantigenic material. Finally, toll-like receptor family members may play critical roles in the induction of antibody responses to nucleic acids in systemic lupus erythematosus.

Summary
Several factors may contribute to the generation of systemic lupus erythematosus-specific autoantigens. Determining the underlying causes of autoantibody production may provide important insight into the etiology and pathogenesis of this disease.

Keywords
autoantibodies, autoantigen, autoimmunity, systemic lupus erythematosus

Abbreviations
ANA antinuclear antibodies
CTL cytotoxic T lymphocyte
EBNA-1 Epstein—Barr virus nuclear antigen-1
EBV Epstein—Barr virus
IFN-α interferon-alpha
DNA-PKcs DNA-dependent protein kinase, catalytic subunit
PARP poly (ADP-ribose) polymerase
SLE systemic lupus erythematosus
TLR toll-like receptor

Introduction
Systemic lupus erythematosus (SLE) is a chronic autoimmune disease of unknown etiology. Like most autoimmune diseases, the development of SLE is believed to be influenced by a combination of genetic, environmental, and hormonal factors [1].

SLE is characterized by the presence of antinuclear autoantibodies (ANAs), which are primarily directed against molecules that have roles in important cellular processes. A key issue in lupus is how these largely intracellular antigens become targets of the autoimmune response. The heterogeneous nature of SLE suggests that numerous factors may be involved in generating the autoantigens that are associated with this disease. Several findings point to a role for the pro-apoptotic protease granzyme B in breaking self-tolerance in SLE. It has been shown that granzyme B cleaves autoantigens that are targeted across the spectrum of human systemic autoimmune disease, producing unique autoantigenic fragments that are not seen during caspase-mediated or other forms of cell death. These observations implicate granzyme B activity as a major contributor to the generation of novel, immunogenic epitopes. However, a number of SLE-associated autoantigens are inefficiently cleaved by granzyme B, indicating a possible role for other mechanisms. Although a viral etiology has been proposed for SLE and many other autoimmune diseases, formal proof of a viral origin for any autoimmune disorder remains difficult to establish. In this article, we will examine some of the prevailing hypotheses on the origins of autoantigens in SLE. We will focus on the role of proteolytic autoantigen cleavage by granzyme B, as well as two proposed mechanisms of virus-induced autoimmunity. We will also discuss the implications of inducible autoantigen expression, alternative splicing of autoantigen mRNA transcripts, and activation of toll-like receptor (TLR) family members (Table 1).

Apoptosis and autoimmunity
Apoptosis, or programmed cell death, is critical for immune system homeostasis and embryonic development,
and its morphologic and biochemical properties have been well-defined [2]. The link between apoptosis and autoimmunity has been widely studied, and several observations support a role for programmed cell death in inciting or propagating autoimmune disease. Ultraviolet irradiation – a common method of inducing apoptosis – results in the redistribution of lupus-associated autoantigens to the cell surface of cultured keratinocytes, where these antigens can then be recognized by human lupus sera, and presumably also by T and B cells [3,4]. In addition, immunization with apoptotic cells induces autoantibody production and glomerular IgG deposition in normal mice [5]. Numerous mechanisms have been proposed to explain how programmed cell death may contribute to the breakdown of immune tolerance. Excessive apoptosis in SLE patients may liberate DNA, histones, and other intracellular antigens that drive the autoimmune response [6]. Defects in the clearance of apoptotic debris may promote the release of antigens that are normally sequestered. These sequestered antigens may, in turn, trigger autoimmune responses [7]. The reduced phagocytic ability of macrophages derived from diseased lupus mice further supports a role for apoptotic cell removal in the progression of disease [8].

Notably, many lupus-associated autoantigens are posttranslationally modified during apoptosis. Examples include the La antigen which is dephosphorylated [9], and vimentin, which undergoes citrullination [10]. Thus, posttranslational modifications that occur during programmed cell death may allow these antigens to subvert normal mechanisms of peripheral tolerance, contributing to the immunogenicity of certain self-proteins in SLE (reviewed in [11]).

### Proteolytic autoantigen cleavage by granzyme B

Proteolytic cleavage is an important component of a myriad of processes, including antigen processing and presentation [12] and regulation of cell signaling [13]. Caspases – cysteine proteases that cleave substrates immediately after aspartic acid residues – are the key effector molecules of apoptosis [14], and a small subset of lupus-associated autoantigens is cleaved by caspases during apoptosis. Examples include poly (ADP-ribose) polymerase (PARP) and the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs), two autoantigens that play critical roles in DNA repair. It has been proposed that cleavage of these and other proteins may serve to abolish essential homeostatic activities and insulate faithful execution of the apoptotic program [15]. However, proteolytic autoantigen cleavage may also serve as a general mechanism for the initiation of autoimmunity.

Engagement of the appropriate T lymphocyte or natural killer cell receptor triggers the release of cytotoxic granules. These granules contain perforin, a pore-forming protein, and serine proteases known as granzymes. Several granzymes have been identified, with granzymes A and B being the most abundant in mice and humans [16]. Granzyme B has been implicated in rapid induction of apoptosis, being the most abundant in mice and humans [16]. Granzyme B cleaves directly after aspartic acid residues (similar to caspases), and is capable of activating caspase-dependent and caspase-independent pathways of cell death [16].

Casciola-Rosen et al. analyzed in detail the interaction of several autoantigens with granzyme B [20,21]. Although most of these autoantigens are also cleaved by one or more caspases, but incubation with granzyme B generates unique protein fragments that are not seen during caspase-mediated forms of cell death [21]. Importantly, ‘non-autoantigens’ do not appear to be substrates for granzyme B. Also, the tissue where an autoantigen is expressed may influence its susceptibility to proteolysis [22].

In support of a role for granzyme B in triggering autoimmune responses, it has been reported that human anti-centromere protein B autoantibodies selectively bind granzyme B-generated autoantigen fragments [23]. In addition, studies performed by Blanco et al. suggest that CD8+ cytotoxic T lymphocyte (CTL) effector status correlates with SLE disease activity. CD8+ CTLs isolated from SLE patients generated unique granzyme B-dependent autoantigen
fragments upon incubation with K562 human erythroleukemia cells [24]. These results suggest a pathogenic role for granzyme B in SLE. However, granzyme B is not required for the initiation of autoantibody responses after immunization with the mineral oil pristane. In the pristane-induced model of SLE, granzyme B-deficient mice produce autoantibodies to the U1-70 kDa antigen, a well-characterized unique substrate for granzyme B. Pristane-primed mice also produce antibodies to the nuclear factor 90 antigen, a novel substrate for granzyme B [25]. Differences between mouse and human enzymes and autoantigens may reconcile these seemingly disparate findings. Also, the pristane-induced pathway of lupus may differ from human disease, as the mechanism whereby pristane triggers lupus-like autoimmune remains unknown. It is also unclear whether this mineral oil induces activation of the perforin/granzyme pathway. However, immunization with squalene, a precursor of cholesterol, has been shown to induce lupus autoantibodies in mice. These observations suggest that the ability to stimulate autoimmunity is shared by chemically diverse hydrocarbon oils, and indicate that the pristane model is of some physiologic relevance [26]. In addition, the observation that CD4+ regulatory T-cells can use the perforin pathway to kill autologous target cells suggests a regulatory role for cytotoxic granule components in humans [27**]. Together, the data indicate that perforin and granzyme B have complex roles in regulating immune responses.

**Molecular mimicry in systemic lupus erythematosus**

Many diseases that are characterized by autoimmune phenomena may actually be infectious in nature. In support of this view, viral infections are associated with a variety of autoimmune conditions, including multiple sclerosis and type I diabetes [28]. However, the mechanisms responsible for virus-induced autoimmunity remain poorly understood.

Molecular mimicry – defined as cross-reactivity between microbial and self-determinants recognized by the adaptive immune system – is perhaps the most popular explanation for the clinical association between microbial infection and autoimmune disease [28]. Epstein–Barr (EBV) virus infection has long been linked with SLE [29]; however, the significance of this association has not been entirely clear. Previously, James and Harley et al. have noted similarity between a region of the EBV nuclear antigen-1 (EBNA-1) and an epitope of the Sm-BB autoantigen. Immunization of rabbits with the Sm-BB-derived PPPGM RPP and PPPGIRGP octapeptides, which resemble the PPPGRRP epitope of EBNA-1, induced autoantibodies to other regions of the Sm-BB protein, as well as epitope spreading to other splicosomal components [30]. Recently, a potential role for EBV-induced molecular mimicry in the initiation of SLE has been reexamined. In an elegant study, McClain et al. analyzed serum samples collected from lupus patients prior to their diagnosis with clinical disease. These authors determined that antibodies directed against the initial epitope of the human Ro-60-kDa (Ro-60) autoantigen directly cross-react with a region of EBNA-1. Interestingly, the initial Ro-60 epitope shares no primary sequence homology with the EBNA-1 linear epitope. Rabbits immunized with the first epitope of Ro-60 or the cross-reactive EBNA-1 epitope developed autoantibodies directed against multiple epitopes of Ro and splicosomal autoantigens, and eventually developed clinical symptoms of lupus [31**].

Taken together, these observations provide strong support for the hypothesis that anti-Ro-60 and anti-Sm-BB’ autoantibodies in human lupus arise through molecular mimicry. However, this hypothesis would only account for autoantibodies seen in a subset of SLE patients [32*]. There may be other cross-reactive regions in EBNA-1 or different EBV proteins that are involved in triggering SLE. Furthermore, EBV is extremely prevalent, with more than 90% of the world’s population presumed to serve as carriers [33]. Therefore, other factors – genetic, hormonal, environmental, or stochastic – must play a role in EBV-induced autoimmune responses. Other viruses may also promote SLE.

**Viral proteases**

Other mechanisms for virus-induced autoimmunity have been proposed, including presentation of virus/self-protein complexes to autoreactive lymphocytes [34] and bystander activation [35]. In addition, the phenomenon of autoantigen cleavage by viral proteases may represent another relevant mechanism for virally induced autoimmunity.

An important event in the life cycle of many viruses is the interaction of virus-encoded proteases with host cell proteins. This can result in site-specific cleavage of molecules that have pivotal roles in host cell metabolism, thereby promoting viral replication and viral release from infected cells. In addition to inhibiting host cell transcription and translation, proteolytic cleavage of host proteins by viral proteases may have another consequence: the generation of novel self-epitopes that can trigger autoimmune responses. Importantly, such a scenario would not exclude bystander activation, molecular mimicry, or other potentially valid mechanisms.

To date, a limited number of autoantigens have been identified as substrates for viral proteases. These include histone H3, a substrate for a foot-and-mouth disease virus protease [36], as well as the La antigen, which is cleaved by the poliovirus 3C protease [37]. Recently, we have shown that DNA-PKcs is cleaved by a picornavirus 2A protease [38], indicating that viral proteases may contribute to the generation of novel autoantigenic epitopes. More comprehensive screens may identify additional substrates for viral proteases. However, the immunogenicity of these protease-generated cleavage fragments must be formally
determined. As a corollary to this, a number of important substrates for viral proteases – such as poly (A) binding protein and TATA-binding protein – have not been identified as autoantigens in human disease. Therefore, the precise contribution of autoantigen cleavage by viral proteases to the bypass of self-tolerance remains to be established. The current data suggest that virus-induced autoimmune responses are likely to result from interplay between several factors. These may include molecular mimicry, novel epitope generation by viral proteases, and the proinflammatory conditions that are associated with viral infection. Similarly, granzymes are expressed by cells that have important roles in controlling viruses and tumors. Autoimmunity arising as a result of granzyme B activity may stem from a legitimate immune response to a microbe, malignancy, or some other insult.

**Inducible autoantigens and alternative splicing**

Microarray analysis of blood mononuclear cells derived from SLE patients has identified an interferon biosignature. This biosignature is characterized by the presence of elevated transcripts for interferon-inducible genes [39,40]. In particular, there is considerable literature supporting a major pathogenic role for type I interferons such as interferon-alpha (IFNα) (reviewed in [41•]). Notably, IFNα has been shown to induce dendritic cell differentiation [42], and plasmacytoid dendritic cell (pDC) production of IFNα has been implicated in the activation of plasma cells [43].

Observations from several other groups have also focused attention on IFNα as a central regulator of SLE. However, the role of type I interferons in murine lupus models is somewhat controversial. NZB mice lacking the α-chain of the IFNα/β receptor develop less severe clinical disease than their wild-type counterparts [44]. Conversely, deficiency in this same subunit exacerbates disease in the MRL/lpr model of lupus nephritis [45]. It has been shown that some of the autoantigens targeted by autoantibodies in a transgenic murine lupus model are IFNα-inducible [46]. These results suggest that some self-antigens may be selected for autoimmune responses when their expression is induced by specific cytokines or conditions in the periphery.

Recent studies have also implicated a role for transcript splicing in the initiation of SLE and other autoimmune diseases [47••]. Ng et al. noted that autoantigen transcripts undergo alternative splicing at greater frequency than transcripts of non-autoantigens. These alternatively spliced isoforms may encode novel (‘untolerized’) epitopes that are not expressed in central lymphoid organs. The events that regulate expression of these untolerized isoforms remain to be determined. It is also unclear whether the proteins encoded by alternatively spliced transcripts are preferentially recognized by autoimmune sera or autoreactive T lymphocytes. However, these observations suggest yet another intriguing potential source of autoantigens in SLE.

**Immunogenicity of nucleic acids**

Members of the mammalian TLR gene family are involved in the recognition of various microbial components and products. Most TLRs are located on the plasma membrane; however, activation of TLR9 takes place intracellularly [48]. TLR9 is engaged by hypomethylated CpG motifs, commonly found in bacterial DNA. However, co-engagement of the B cell receptor (BCR) and TLR9 by mammalian chromatin-containing immune complexes may play a pivotal role in the activation of autoreactive B cells [49]. Recently, chromatin (not bound by antibody) has been implicated as an endogenous ligand for TLR9. In this model, chromatin (released from apoptotic cells, for example) sequentially engages the BCR and TLR9. This leads to activation of chromatin-reactive B cells and eventual formation of chromatin-containing immune complexes. These immune complexes may further activate B cells, dendritic cells, and pDCs, thus providing a link between the innate and adaptive immune systems in the production of anti-DNA antibodies [50]. Mechanisms that may be involved in rendering mammalian chromatin immunogenic have been described [51]. Future studies may implicate other TLRs (e.g., TLRs 3, 7, and 8) in the development of autoantibodies to RNA and RNA-associated complexes.

**Conclusion**

Studies aimed at uncovering mechanisms involved in SLE pathogenesis are complicated by the incredibly diverse nature of the disease. It appears that no single gene is necessary or sufficient for complete disease expression, and the role of specific environmental contributions is difficult to pinpoint. There is a tremendous need to clarify the relative importance of the factors involved in the initiation of SLE. Doing so may facilitate the development of rational preventive and therapeutic approaches in the clinical setting.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest

Sources of autoantigens in systemic lupus erythematosus

Graham and Utz


33 This is a comprehensive review of the autoantibodies associated with SLE, along with their clinical associations, correlations with disease activity, and other relevant information.


42 The authors present their perspective on the role of cytokines in regulating the balance between protective immunity and tolerance.


48 Ng B, Yang F, Huston DP, et al. Increased noncanonical splicing of autoantigen transcripts provides the structural basis for expression of unmodified epitopes. J Allergy Clin Immunol 2004; 114:1463—1470. This study applied an approach using bioinformatics to identify autoantigens with alternatively spliced isoforms. Importantly, these alternatively spliced isoform-specific regions occur within candidate contact sites for antigen presenting molecules and autoantibodies.


Immune cells and cytokines in systemic lupus erythematosus: an update
Vasileios C. Kyttarisa,b,c, Yuang-Taung Juanga,b and George C. Tsokosa,b

Purpose of review
Systemic lupus erythematosus is characterized by overactive B cells that differentiate into autoantibody-forming cells, aberrant T cell function that provides help to B cells, and the production of pro-inflammatory cytokines. This article reviews recent studies unraveling the complex interplay between cytokines and lymphocytes in systemic lupus erythematosus.

Recent findings
In systemic lupus erythematosus, T cells are characterized by heightened calcium responses early after activation of their surface receptor. Alterations of the T cell receptor/CD3 complex, namely the substitution of the FcγR IIIa for the T cell receptor ζ chain, and increased mitochondrial potentials can account for this ‘overexcitable’ phenotype. At the same time, this heightened calcium signal leads to a block of the transcription of the IL-2 gene, a pivotal cytokine for the immune response. The end result is increased spontaneous apoptosis and decreased activation-induced cell death of T cells in systemic lupus erythematosus that in turn leads to enhanced help to B cells and potentially decreased regulatory function. The B cells, on the other hand, are shown to be directly activated by immune complexes by way of Toll-like receptors independently of T cells. Finally, recent studies have tried to elucidate the role of cytokines such as interferon-α in systemic lupus erythematosus and, following the paradigm of rheumatoid arthritis, to establish targets for treatment.

Summary
The increased apoptosis and aberrant T cell activation coupled with nonspecific activation of B cells lead to the production of auto-antigen: auto-antibody complexes that are the hallmark of systemic lupus erythematosus. Future treatments aiming at correcting the intracellular and intercellular signaling abnormalities may prove effective in restoring immune tolerance in systemic lupus erythematosus.

Keywords
B cells, interferon, interleukin-10, systemic lupus erythematosus, T cells

Introduction
Although the exact events leading to the break of tolerance in systemic lupus erythematosus (SLE) are unknown, innovative studies have helped us understand the immunologic aberrancies in SLE. SLE is characterized by exaggerated B cell responses that lead to the production of an array of autoantibodies, mainly against nuclear material. These responses are initiated, propagated, or both by activated immune cells (T cells, dendritic cells) and soluble mediators (pro-inflammatory cytokines, chemokines). Herein, we review recent key studies analyzing the intracellular and intercellular immune system signaling in SLE.

T cells
T cells in SLE exhibit increased spontaneous apoptosis and at the same time impaired activation and activation-induced cell death (AICD) [1,2]. Intracellular events following the engagement of the T cell receptor (TCR)/CD3 complex with the antigen-major histocompatibility complex lead to this distinct phenotype. In particular, SLE T cells exhibit exaggerated initial calcium influx, aberrant tyrosine phosphorylation, and heightened mitochondrial potentials following the activation of the TCR/CD3 complex [3,4].

The exaggerated calcium response upon engagement of the T cell receptor (TCR) is thought to be due to the substitution of the FcγR IIIa for the ζ chain in the TCR/CD3 complex [5]. Decreased transcription of the TCRζ chain gene is in part responsible for the decreased amount of ζ chain; this mechanism, though, cannot fully account for this decrease [6]. Another hypothesis tested by Chowdhury et al. [7*] is that the stability of TCR ζ chain mRNA in SLE T cells is decreased. Indeed, the investigators found that T cells from SLE patients predominantly express an alternatively spliced ζ chain mRNA that lacks nucleotides 672 to 1233 within exon VIII at the 3’ untranslated terminal region (3’-UTR). This 3’-UTR AS mRNA is translated into the 16kDa ζ chain molecule at a decreased
rate, as shown in both live COS7 cells and in-vitro experiments. In a complimentary study by Tsuzaka et al. [8], MA5.8 cells that are deficient in ζ chain protein were transfected with either the wild-type or a 3' UTR alternatively spliced ζ chain mRNA. The cells transfected with the 3' UTR alternatively spliced ζ chain mRNA showed lower expression of TCR/CD3 complex than those transfected with the wild-type ζ chain mRNA. When anti-CD3 antibody was used to activate these cells, interleukin-2 production was lower in the cells transfected with the 3' UTR alternatively spliced ζ chain mRNA than in the ones transfected with the wild-type ζ chain mRNA. The findings of these two studies suggest that mutations within the 3' UTR region of the ζ chain mRNA have significant effect on the stability of the molecule and its subsequent translation.

Despite the exaggerated calcium responses, the SLE T cells do not produce sufficient amounts of interleukin-2 upon stimulation [9]. The mechanism behind the decrease in the production of interleukin-2, a crucial cytokine for the stimulation, growth, and eventual AICD of T cells, was explored in a study by Juang et al. [10*] (Fig. 1a). In this study, serum from patients with SLE but not from control individuals was shown to induce the binding of the transcription repressor CREM at the -180 site of the IL-2 promoter in T cells from normal individuals. This serologic effect resides within the IgG fraction of the serum and is due to anti-TCR/CD3 auto-antibodies. The effect of these auto-antibodies on normal T cells is mediated by Ca2+/calmodulin-dependent kinase IV (CaMKIV). Blocking CaMKIV abrogates the effect of SLE serum on normal T cells, proving the pivotal role of this kinase in T cell function in SLE.

A different paradigm was observed in lupus-prone Fas-intact MRL (MRL/+/Fas+/+) mice [11*]. Naïve CD4+ T cells from these mice show a lower threshold of excitation when stimulated with anti-CD3 and anti-CD28 antibodies, and a hyperexcitable phenotype with high calcium influx, similar to T cells from humans with SLE. The interleukin-2 production, though, is high in this lupus model in contrast to the depressed interleukin-2 production in human SLE. The differences between the human disease and this mouse model could be attributed to the expression of transcriptional repressors such as CREM in the human SLE T cell.

T cells from patients with SLE exhibit, for unknown reasons, a characteristically persistent mitochondrial hyperpolarization upon TCR–antigen interaction, a key event for the T cell activation and AICD [3]. Nitric oxide, a small signaling molecule that can influence mitochondrial hyperpolarization, is the focus of a study by Nagy et al. [12*]. In this study, T cells from patients with SLE were shown to have higher mitochondrial mass and number than control individuals as well as higher concentrations of intracytoplasmic and intramitochondrial calcium. Nitric oxide production by monocytes from the same patients correlated with the mitochondrial mass. In addition, enhancement in mitochondrial biogenesis in normal T cells with nitric oxide induced a signaling profile similar to that of SLE (i.e. heightened calcium concentration early upon activation of the cell through TCR). This study provides another important mechanism for the heightened calcium mediated signaling in SLE.

A signaling abnormality that leads to increased spontaneous apoptosis of a unique subset of T cells, the natural killer T cells, was the focus of a report by Tao et al. [13*]. The investigators report that natural killer T cells have decreased amounts of the adhesion molecule CD226 and also decreased amounts of the cell survival molecule survivin that make these cells particularly prone to CD95-mediated apoptosis; these findings are more pronounced in patients with active SLE and can help explain
the observed decrease in the number of natural killer T cells in these patients. Given that natural killer T cells can play a role as regulatory T cells, the authors hypothesized that as their numbers decline as a result of increased apoptosis, autoreactive T cells are left unregulated, leading to disease exacerbation [14].

In SLE, T cells are also characterized by hypomethylation of DNA in the context of CpG dinucleotides, which is thought to contribute to their overexcitable phenotype by upregulating pro-inflammatory cytokines and adhesion molecules [15]. In a study that examined the methylation status of the perforin gene promoter, CD4+ cells but not CD8+ cells from patients with SLE were shown to have hypomethylated perforin promoter and higher perforin expression [16*]. Both hypomethylation of the promoter and protein expression were more pronounced in patients with active SLE. Perforin expression on CD4+ cells was shown in the same study to mediate the CD4-induced monocytes/macrophage killing; this in turn can lead to decreased clearance of the apoptotic material by the reticuloendothelial system that is a hallmark of SLE. The same group has also evaluated the effect of hypomethylation on the T cell–B cell interaction [17*]. Normal T cells treated with a methyltransferase inhibitor upregulated the surface molecule CD70. These T cells stimulated the production of immunoglobulin G (IgG) when cocultured with B cells, and this effect was attenuated when anti-CD70 antibody was added. These findings help explain the mechanisms of medication-induced lupus and provide significant insight into the pathophysiology of SLE.

B cells
Although the T cell–B cell interaction is important for the production of high-affinity auto-antibodies such as the IgG anti-dsDNA antibody, there is accumulating evidence that B cells can be activated directly by immune complexes [14,18].

Marginal zone B cells possess Toll-like receptor-9 that recognizes hypomethylated CpG motifs on microbial DNA and by doing so induces the production of IgM antibodies. This T cell–independent antibody production is essential for the immune response against microorganisms in the first days of an infection. He et al. [19*] reported that the CpG DNA–Toll-like receptor-9 interaction in co-operation with interleukin-10 initiated class switching in naïve B cells; subsequent activation of the B cell by way of B cell receptor cross-linking or BAFF (a cytokine produced by dendritic cells in response to CpG DNA inducible cytokines, such as IFN-α) leads to production of T cell–independent IgG antibodies. This novel mechanism of T cell–independent IgG antibody production may be of particular importance in the production of high-affinity pathogenic auto-antibodies in SLE, a disease characterized by high levels of both interleukin-10 and BAFF [20,21].

Cytokines
In recent years, treatments aiming specifically at blocking pro-inflammatory cytokines such as tumor necrosis factor (TNF) have resulted in significant clinical improvement in patients with autoimmune diseases such as Crohn’s colitis and rheumatoid arthritis [22,23]. Although the cytokine milieu is clearly altered in SLE, a ‘signature’ cytokine that can be specifically targeted in SLE remains elusive.

Interferons
Interest in interferons has been renewed recently after the finding that interferon-inducible genes were upregulated in peripheral blood mononuclear cells (PBMC) from patients with SLE [24]. This ‘interferon signature’ in gene expression in SLE was primarily seen in active lupus patients who also tended to have more severe disease manifestations such as brain and kidney involvement. Interestingly, direct measurement of interferon in the serum of these patients did not show any difference between patients with SLE and control individuals.

Searching for the specific interferon that is responsible for the up-regulation of the interferon inducible genes in SLE, investigators reported that interferon-α but not interferon-γ is the instigating factor for the ‘interferon signature’ in SLE [25*]. The authors found that the levels of mRNA transcribed from genes that are inducible by interferon-α were significantly higher in SLE PBMC than in control PBMC. By contrast, the levels of mRNA from interferon-γ inducible genes did not differ between SLE and control PBMC. The authors furthermore showed that plasma from patients with SLE up-regulates interferon-α inducible genes in normal PBMC.

Building on previous studies, Lovgren et al. [26*] showed that material from apoptotic or necrotic U937 cells, when combined with IgG from patients with SLE, induces the production of interferon-α from purified plasmacytoid dendritic cells. A similar effect was seen when necrotic but not apoptotic PBMC were used in lieu of U937 cells. The IgG from SLE patients was essential for this effect, suggesting that the increase in interferon-α production is caused by the presence of immune complexes containing apoptotic or necrotic material and auto-antibodies.

These results are complemented by a study of the effect of purified non-protein–containing immune complexes from patients with SLE (Fig. 1b) [27••]. The investigators showed that DNA containing immune complexes can activate plasmacytoid dendritic cells (PDC) to produce interferon-α and interleukin-8. The effect of immune complexes on PDC was mediated though cooperation of Toll-like receptor-9 with the FcγRIIa (CD32). The activated PDC were able to produce many other cytokines and chemokines that can activate immature dendritic
cells, monocytes, and T cells but not B cells. These findings suggest a link between the apoptosis caused by environmental factors (such as infection or ultraviolet light), the decreased clearance of apoptotic material in SLE, and the stimulation of plasmacytoid dendritic cells to produce interferon-α.

**Tumor necrosis factor-α**

This pleiotropic pro-inflammatory cytokine has been shown to contribute significantly to the pathogenesis of various autoimmune diseases. Its role in SLE, though, is unclear given that TNF-α levels are similar in SLE patients and control individuals. Low overall production of TNF-α may be contributing to the decrease in AICD that is characteristic of SLE, whereas local production of TNF-α due to environmental factors (infection, ultraviolet light) may be contributing to triggering of disease exacerbations [1]. In particular, ultraviolet light-B, a known cause of disease exacerbation, is shown to trigger TNF-α production in the skin. A study that examined the effect of TNF-α on the skin showed that TNF-α up-regulates the expression of the 52kd Ro/SSA protein and mRNA in keratinocytes, an effect mediated via the TNF receptor I [28*]. 52kd Ro/SSA protein is expressed on apoptotic bodies, and anti-Ro antibodies have been associated with cutaneous lupus. Taking these findings together, one can hypothesize that TNF-α plays a role in the pathogenesis of cutaneous lupus in patients with anti-Ro antibodies.

**Interleukin-10**

Interleukin 10, known to be increased in the sera of SLE patients, is thought to be an anti-inflammatory cytokine. In a study by Sharif et al. [29*], its role in conditions of interferon-α priming of cells was explored. The investigators showed that interleukin-10 has a different effect on human macrophages depending on whether the cells had been primed with interferon-α. Interleukin-10 suppresses the production of TNF-α by macrophages in response to lipopolysaccharide. When these macrophages were incubated (primed) with low-dose interferon-α, interleukin-10 had the opposite effect and induced higher production of TNF-α. Moreover, interleukin-10 under these conditions induced STAT-1, a key mediator in the interferon-γ signaling pathway. This differential pro-inflammatory effect of interleukin-10 in the context of high interferon-α is relevant in SLE, in which both cytokines are elevated, and provides a rationale for targeting interleukin-10 (with or without interferon-α) for the treatment of the disease.

**Conclusion**

Systemic lupus erythematosus is characterized by an overactive B cell compartment of the immune system. B cells are shown to be activated without specific cognate help by the T cells. By contrast, T cells show increased spontaneous apoptosis and can therefore provide nuclear material for the formation of immune complexes. The dendritic cells can influence this T-B interaction by producing interferon-α, an important cytokine in SLE. Although the initiating factor for the immunologic dysfunction in SLE is not known, several factors that perpetuate the autoimmune response have been described and can help guide treatment in the future.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

* • of special interest
** • of outstanding interest

10. Juang YT, Wang Y, Solomou EE, et al. Systemic lupus erythematosus serum IgG increases CREM binding to the IL-2 promoter and suppresses IL-2 production through CalM2K6. J Clin Invest 2005; 115:996—1005. Serum from patients with SLE causes an increase in binding of the transcription repressor CREM to the IL-2 gene promoter via CalM2K6, leading to a decrease of IL-2 production. IgG with anti-TCR specificity is shown to be responsible for this effect.
11. Zielenki CE, Jacob SN, Bouzahzah F, et al. Naive CD4+ T cells from lupus-prone Fas-intact MRL mice display TCR-mediated hyperproliferation due to intrinsic threshold defects in activation. J Immunol 2005; 174:5100—5109. T cells from Fas-intact MRL mice show an ‘overexcitable’ phenotype when stimulated by way of their surface receptor. In contrast to human lupus, T cells from these mice have increased IL-2 production.
12. Nagy G, Barca M, Gonchoroff N, et al. Nitric oxide-dependent mitochondrial biogenesis generates Ca2+ signaling profile of lupus T cells. J Immunol 2004; 173:3676—3683. T cells from patients with SLE are shown to have higher mitochondrial mass and numbers than controls, a phenomenon attributed to NO production by monocytes. NO causes normal T cells to exhibit an ‘overexcitable’ phenotype similar to that of lupus T cells.


The IFN-α—dependent gene expression in PBMC from SLE patients is explored in this paper.


Apoptotic nuclear material and lupus IgG induce production of IFN-α by plasmacytoid dendritic cells.


This paper shows that DNA-containing immune complexes bind to FcRγ on the surface of dendritic cells, get internalized in Toll-like receptor-9-containing lysosomes and activate dendritic cells to produce pro-inflammatory cytokines.


The authors show that TNF-α up-regulates Ro auto-antigen in keratinocytes, and propose a role of TNF locally in areas of inflammation in SLE.


IL-10 is shown to act as a pro-inflammatory cytokine in cells that have been primed with IFN-α.
New insights from murine lupus: disassociation of autoimmunity and end organ damage and the role of T cells
Harini Bagavanta,b and Shu Man Fu,a,b,c

Purpose of review
This review summarizes current literature on genetic regulation of different phenotypes in systemic lupus erythematosus in context of end-organ disease. Recent findings conflicting with the current paradigm that loss of tolerance to chromatin is the critical step for end-organ injury are discussed.

Recent findings
Systemic lupus erythematosus is a prototype immune complex disease with circulating autoantibodies to chromatin, histone proteins, Sm/La, and other nuclear and cytoplasmic proteins. Extensive studies have been carried out on the regulation of B-cell and autoantibody production in lupus mice. However, the hypothesis that autoantibodies are primary mediators of organ damage fails to explain the heterogenous presentation in patients. Studies in murine models of systemic lupus erythematosus clearly dissociate genetic control of autoantibody responses to classic lupus antigens and kidney disease. There is increasing evidence to support the role of autoreactive T cells and genetic control of end organ susceptibility. These studies suggest complex interactions between innate and adaptive immunity resulting in end-organ damage. This review focuses on autoimmune responses and renal involvement in spontaneous systemic lupus erythematosus using murine models of lupus nephritis.

Summary
Studies in murine models demonstrate complex genetic interactions regulating spontaneous systemic lupus erythematosus. Although detection of serum autoantibodies is considered a hallmark for clinical diagnosis of systemic lupus erythematosus, recent evidence shows that autoantibodies to classic lupus antigens are neither required nor sufficient for end-organ damage. Thus, murine models provide new insights into the pathogenesis of systemic lupus erythematosus.

Keywords
acute and chronic lupus glomerulonephritis, lupus susceptibility genes, NZM2328 mice, renal failure, systemic lupus erythematosus

Introduction
Systemic lupus erythematosus (SLE) is a complex autoimmune disease characterized by circulating autoantibodies to nuclear and cytoplasmic antigens. It affects multiple systems including skin, kidney, brain, joints, heart, lungs, and other organs. Renal involvement is associated with significant mortality and morbidity and is the primary focus of this review.

Murine models of SLE have been studied over 3 decades. Mouse strains like MRL/lpr, BXSB, (NZWxNZB)F1, NZM2410, NZM2328, and (SWRxNZB) F1 are some of the examples of spontaneous murine SLE. Spontaneous SLE in murine models is characterized by serum autoantibodies to chromatin, double-stranded DNA (dsDNA), histone proteins, and other antigens. The mice also develop histopathological changes of glomerulonephritis with glomerular immune complex deposits, glomerular mesangial expansion, glomerulosclerosis, fibrosis, and fatal renal failure. In addition to these strains, genetic mapping studies have allowed the generation of numerous congenic mice that dissect the regulation of distinct disease phenotypes in SLE.

One of the earliest autoantigens in SLE identified using sera from patients was DNA [1,2]. Indeed, DNA and anti-DNA antibody complexes were also demonstrated in sera of patients with SLE. Immunoglobulins eluted from diseased kidneys were first shown to recognize DNA, leading to the concept that dsDNA antibodies are a hallmark of SLE and nephritis. These developments have led to the hypothesis that the initial loss of tolerance to DNA and chromatin was followed by deposition of immune complexes in tissues, and the subsequent inflammatory response was the pathologic basis of organ injury.

Recent literature discussed here continues to focus on antibodies to DNA as an important marker of SLE. These
Intrinsic immune factors influence systemic lupus erythematosus

In mice, the appearance of class-switched immunoglobulin G(IgG) autoantibodies to DNA, histone, and small nuclear ribonucleoproteins (snRNPs) is associated with onset of disease. This autoimmune response may be initiated and sustained by intrinsic factors like lowered threshold for activation of antigen-specific T cells [3*,4,5], hyper-responsive and/or increased frequency of autoreactive B cells [6*,7*], increased intracellular antigen availability as a result of apoptosis [8], or decreased apoptosis of autoreactive T cells [9*]. C57BL/6 mice lacking the inhibitory Fcγ receptor, Fcγ receptor IIB (FcγRIIB), on their B cells develop lupus-like disease [10]. In addition, injection of bone marrow cells transfected with FcγRIIB can prevent disease in spontaneously lupus mice [11*]. Other single gene deletions (CD28, CD40L, interferon-γ) can prevent disease in a murine model of SLE induced by heavy metal mercury [12*,13]. Spontaneous lupus in MRL/lpr mice is influenced by interferon-γ or interleukin-4 deficiency [14], in addition, NZM2410 mice deficient in cytoplasmic proteins STAT6 and STAT4 that regulate T helper 1 and T helper 2 cytokine production respectively develop different SLE phenotypes [15]. Deletion of type I interferon receptor 1 accelerated disease in MRL/lpr mice, while deletion of both type I and II interferon receptors prevented disease, demonstrating a diverse role of interferons in lupus [16*]. No single gene mutation or aberrant gene expression has been identified as a causative factor in spontaneous SLE in mice or humans. These models are useful, however, in identification of immunologically relevant factors that prevent or promote disease.

Systemic lupus erythematosus is a complex disease controlled by multiple genes and influenced by complex epistatic interaction between different gene segments. Mapping of SLE susceptibility genes has been carried out in several inbred mouse strains and is reviewed elsewhere [17**]. This review focuses on the recent work in New Zealand Mixed (NZM) mouse strains and the insights into pathogenesis of lupus glomerulonephritis gained from these studies.

Autoantibodies to double-stranded DNA and chromatin, acute glomerulonephritis, and chronic glomerulonephritis are distinct phenotypic traits

New Zealand Mixed mice were derived from intercrosses of New Zealand White (NZW) and New Zealand Black (NZB) strains [18]. The large numbers of different NZM strains have varying genetic contributions from the parental NZB and NZW genes and are associated with widely differing incidence of lupus-like disease. Two NZM strains, NZM2410 and NZM2328, have been most extensively studied. Both mouse strains spontaneously develop autoantibodies to dsDNA and chromatin in males and females. Fatal renal disease has a significant female bias in NZM2328, similar to human SLE. In addition, renal disease in female NZM2328 mice occurs later in life in comparison with NZM2410. Backcross analysis (C57L/J x NZM2328) F1 X NZM2328 was carried out to map the SLE susceptibility genes [19]. Cohorts were studied at 12 months of age, and genetic analyses using micro-satellite markers showed distinct genetic intervals associated with the serum anti-nuclear, anti-DNA, or anti-DNA/histone autoantibodies (Adaz1 between D4Mit175 to D4Mit187 on chromosome 4; putative Adaz2 D1Mit15 to D1Mit155 on chromosome 1), acute proliferative glomerulonephritis (Agnz1 on chromosome 1 – D1Mit37 to D1 Mit17; Agnz2 D17Mit130; H-2-Th6 on chromosome 17), and chronic glomerulonephritis and severe proteinuria (Cgnz1 D1Mit15-D1Mit37).

The mapping studies were confirmed by generating two congenic mouse strains in which the segments containing Agnz1 and Cgnz1 on chromosome 1 and Adaz1 on chromosome 4 from NZM2328 mice were replaced with the corresponding chromosomal segments from lupus-resistant C57L/J mice [20**]. The congenic strains were designated NZM2328.C57Lc1 and NZM2328.C57Lc4. As expected, NZM2328.C57Lc1 failed to develop acute or chronic glomerulonephritis, and NZM2328.C57Lc4 had significantly reduced incidence of anti-nuclear, anti-dsDNA, or anti-DNA/histone autoantibodies. Surprisingly, NZM2328.C57Lc1 also failed to develop autoantibodies, suggesting a locus Adaz2 on chromosome 1 affecting autoantibody responses. Alternatively, the chromosome region 1 segment has epistatic interactions with the chromosome 4 segment that influences autoantibody responses. A dramatic finding in the congenic study was that NZM2328.C57Lc4 mice developed fatal glomerulonephritis comparable to the parent NZM2328 mice despite the absence of lupus autoantibodies. This genetic finding dissociates lupus autoantibody production from lupus glomerulonephritis and questions the current paradigm that autoantibodies to dsDNA and related autoantigens are the primary pathogenic mediators of disease. NZM2328.C57Lc1 contains susceptibility genes for the three phenotypes, dsDNA antibodies, acute glomerulonephritis, and chronic glomerulonephritis; therefore, the
generation of intra-chromosomal recombinant congenic lines will identify the loci for these three distinct phenotypes.

Mapping of systemic lupus erythematosus susceptibility genes in NZM2410 mice

Extensive genetic analyses have also been done in NZM2410 mice using a different approach. A backcross analysis of (C57BL/6 x NZM2410) F1 x NZM2410 was used to identify lupus susceptibility genes [21]. Three different loci named Sle1, Sle2, and Sle3 were identified on chromosomes 1, 4, and 7, respectively. Each of these loci was significantly associated with susceptibility to glomerulonephritis. Susceptibility to autoantibody production did not map significantly to any genetic region. Congenic mouse strains were generated in C57BL/6 mice containing Sle1, Sle2, or Sle3 genetic segments from NZM2410 (C57BL/6.Sle1, C57BL/6.Sle2, and C57BL/6.Sle3, respectively [22,23]). None of these congenic lines developed glomerulonephritis. Double congenic strains with either Sle1 and Sle2 or Sle1 and Sle3 and triple congenic strain with Sle1, Sle2, and Sle3 developed glomerulonephritis, suggesting interaction between the different susceptibility genes [24]. Although the single congenic lines (C57BL/6.Sle1, C57BL/6.Sle2, and C57BL/6.Sle3) failed to develop glomerulonephritis despite the original mapping studies, each of these lines has demonstrated some abnormality of immune function [25–28]. For example, Sle1 had the strongest association with glomerulonephritis, and C57BL/6.Sle1 mice spontaneously develop anti-dsDNA and anti-nuclear antibodies. C57BL/6.Sle2 congenic mice have a lowered threshold for B-cell activation with increased polyclonal IgM, and C57BL/6.Sle3 mice show CD4+ T-cell expansion with resistance to apoptosis after anti-CD3 stimulation. The C57BL/6.Sle1, C57BL/6.Sle2, and C57BL/6.Sle3 mice suggest that loss of tolerance to chromatin along with a hyper-responsive immune system are the primary requirements for SLE, while the NZM2328.C57Lc4 congenic mice clearly demonstrate that classic lupus autoantibodies are not required for glomerulonephritis. Thus, the different approaches and choices of resistant strains in studies of lupus susceptibility genes in NZM strains have yielded different results.

Intra-chromosomal recombinant strains within the C57BL/6.Sle1 region Sle1a, Sleb, Slec, and a putative Sle1d have been used to identify candidate genes that may contribute to lupus susceptibility [29]. The location of Sle1d remains to be defined. C57BL/6.Sle1 mice have no significant glomerulonephritis; therefore, it will be difficult to identify Sle1d using the approach of Morel et al. [29]. The signaling lymphocyte activation molecules (SLAM/CD2) cluster of genes is present on the Sle1b segment chromosome 1. Within this region is Ly108, an isoform of which is constitutively up-regulated on lymphocytes from C57BL/6.Sle1b mice. Thus, the Ly108 is a candidate gene for hyper-responsiveness in NZM2410 mice and, thereby, lupus susceptibility [30**].

Recently published studies using different lupus-susceptible and resistant strains like BALB/c, SWR, and NZW in the backcross analyses reveal genetic segments from the resistant strains that contribute to lupus susceptibility [31,32,33**,34,35]. In addition, results from generation of double congenic mice carrying different lupus susceptibility genes like Sle1 and lpr [36], FcyRIIB deficiency with yaa or lpr [37], and type I and type II interferon receptor deficiency with lpr [16*] affect SLE. These data show the complex genetics of SLE and epitope interactions that influence disease phenotypes.

Pathogenic autoantibody responses in systemic lupus erythematosus

There is an increasing body of literature that revisits the role of anti-DNA antibodies in SLE. Earlier studies with passive transfer of anti-DNA antibody-producing B-cell hybridomas into naïve recipient mice showed that cross-reactivity of the antibodies with glomerular antigens was an important criterion determining pathogenic potential. Further, proteinuria and glomerulonephritis were greater in the anti-DNA antibodies depositing in the peripheral capillary loops compared with the mesangial regions. Thus, not all DNA antibodies are pathogenic. These studies have been discussed in a recent review [38**]. A large panel of monoclonal antibodies from NZM2410 mice also showed that glomerular binding was associated with pathogenicity [39]. In another study, monoclonal antibodies generated from CD19+ cells from nephritic MRL/lpr kidneys showed increased reactivity to glomerular antigens as well as DNA [40]. A quantitative analysis of immunoglobulins eluted from nephritic kidneys in patients with SLE showed that less than 10% of the total IgG reacts with a panel of ‘lupus’ antigens like DNA, histone, the collageng-like region of C1q complement, Sm proteins, chromatin, and other antigens [41]. Thus, antigens recognized by most potentially pathogenic autoantibodies in lupus nephritis remain unknown.

The NZM2328.C57Lc4 congenic mice provide the most direct evidence that immune responses to antigens other than dsDNA are pathogenic in SLE [20**]. These mice lack circulating anti-nuclear, anti-DNA, or anti-DNA/histone antibodies in serum. They have renal immune complex deposits and severe glomerulonephritis, however. Immunoglobulins eluted from nephritic NZM2328.C57Lc4 kidneys react with kidney and liver proteins by Western blots but not with dsDNA or histone proteins. These data confirm the original genetic study showing that autoantibody responses and glomerulonephritis are distinct phenotypes.

Dissociation of the autoantibody responses from renal disease is also seen in NZM2410 mice deficient in STAT6 or STAT4. The STAT6-deficient mice develop high anti-DNA antibody titers and some mesangial hypercellularity but fail to progress to glomerulosclerosis. In contrast,
STAT4-deficient NZM2410 mice have low autoantibody titers and develop severe glomerulonephritis [15].

**Antibody diversification in systemic lupus erythematosus**

Analysis of patient sera shows an ordered appearance of autoantibodies recognizing different lupus antigens [42]. Over time, the specificity of the antibody response spreads to other proteins within a complex (intermolecular diversification) or different epitopes on the same protein (intramolecular diversification) [43]. The particle hypothesis proposed to explain the mechanism of autoantibody diversification suggests that an autoreactive T cell with a single specificity can help autoantibody production by B cells recognizing other regions within the same protein or other proteins complexed with the T-cell antigen. This paradigm fails to explain autoantibody reactivities to proteins not complexed to each other, often seen in sera from patients with SLE.

We have established a model of autoantibody diversification using two different antigen systems, the Ro/La antigens and the snRNP proteins [44-47]. Immunization of A/J mice with recombinant mouse Ro60 protein or a peptide of Ro60 can induce antibody to other parts of the Ro60 molecule (intramolecular spread) and to La, SmD, and U1-RNP proteins. The antibody reactivity to all the proteins was completely absorbed by the immunizing antigen, however. Thus, cross-reactivity between non-homologous lupus antigens is an important mechanism of antibody spreading. As described, cross-reactivity to kidney antigens, not DNA, is the important pathogenic specificity in SLE.

In another model of autoantibody diversification, immunization with SmD protein resulted in antibodies specific to A-RNP, which did not cross-react with SmD. Significantly, the specific antibody to A-RNP protein was preceded by a T-cell response to A-RNP protein. In this model, diversification of the T-cell response is required for B cells with diverse autoantibody specificity. A similar sequence of events may occur in spontaneous lupus mice, in which loss of tolerance to an unrelated antigen may finally spread to kidney reactive immune response and lupus glomerulonephritis.

**Evidence for a pathogenic T-cell response to kidney antigens**

Several examples cited show a clear dissociation between anti-dsDNA, anti-nuclear, anti-DNA/histone antibodies and lupus glomerulonephritis. In addition, it is now apparent that a kidney-specific B-cell and T-cell response is required for the development of lupus glomerulonephritis in mouse models. Past and recent studies show that deposition of immune complexes in the kidney is not sufficient for fatal renal failure. Indeed, animal models of immune complex glomerulonephritis induced by antibodies to glomerular basement membrane develop transient, though severe, proteinuria that rarely progresses to the lupus-like pathology of chronic glomerulonephritis. In our study of (SWRxBALB)F1 mice, neonatal thymectomy protected mice from fatal glomerulonephritis despite accelerated serum autoantibody responses and renal immune complex deposits [48]. Thus, renal immune complexes and autoantibody per se, even in genetically susceptible mice, are not sufficient to induce fatal glomerulonephritis.

The significance of nephritogenic T cells in renal disease was demonstrated by Chan et al. [49] using a novel approach. They generated MRL/lpr mice deficient in serum immunoglobulin but expressing membrane bound anti-(4-hydroxy-3-nitrophenyl) acetyl transgenic antibody. These mice developed glomerulosclerosis and interstitial nephritis, showing that renal immune complexes were not required for chronic renal disease.

T-cell infiltration in kidneys is seen with increasing severity of glomerulonephritis in mouse models [50]. In NZB/W F1 lupus mice, blockade of co-stimulatory T-cell function using murine CTLA4-immunoglobulin blocked rapid progression of established nephritis [51]. This protection was associated with reduced T-cell and B-cell infiltration in the kidneys and not with any changes in renal immune complex deposits. In nephritic NZM2328 mice, increased frequencies of activated CD69+ T cells are seen in kidney draining lymph nodes compared with axillary or inguinal lymph nodes [52]. Additional evidence comes from kidney biopsies of patients with nephritis, in which differences between Vβ usages of T-cell receptors in the kidney compared with peripheral blood suggest a preferential oligoclonal expansion of T cells infiltrating the kidney [53]. Deletion of kidney reactive T cells by intra-thymic injection of syngeneic kidney cells, not splenocytes, protected MRL/lpr mice from fatal glomerulonephritis [54]. All these data support the hypothesis that kidney reactive T cells play a direct role in pathogenesis of glomerulonephritis.

**Role for end organ in lupus glomerulonephritis**

Although an autoimmune response is the primary mediator of renal injury in SLE, the intrinsic susceptibility or resistance of the end organ and its response to immune injury play an important role in the progression of disease. The contribution of the end-organ factors to disease is unclear. In a model of immune complex disease induced by active immunization of rabbit immunoglobulins followed by injection of rabbit anti-glomerular basement membrane antibody, 12 inbred mouse strains tested showed differential susceptibility to glomerulonephritis and proteinuria [55,56]. The severity of glomerulonephritis varied despite comparable immune responses to rabbit immunoglobulin and glomerular immune complex deposition. In another model, the FcyRIIB-deficient mice develop fatal glomerulonephritis on the C57BL/6 but not on the BALB/c genetic
background [10]. In NZM2328 mice, neonatal thymectomy induces severe acute proliferative glomerulonephritis in both male and female mice. Only females develop chronic glomerulonephritis and renal failure, however; males do not [57]. Thus, in addition to genetic differences between strains, sex-related factors might also contribute to end-organ resistance.

Conclusion

Murine models of SLE have been critical in understanding the pathogenesis of SLE, a complex, multigenic autoimmune disorder. Genetic studies in spontaneous lupus mice like the NZM2328 and its congenic strains have offered new insights into the mechanisms of renal disease and have revealed the necessity to re-examine the old paradigms of systemic autoimmunity in SLE based on anti-DNA antibody responses. The literature presented supports a direct role for nephritogenic T cells in the induction and progression of lupus glomerulonephritis. The contribution of the end organ to the final outcome needs further examination. These issues are readily addressed by further genetic analysis involving NZM2328 and its congenics.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
- of special interest
- of outstanding interest


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Genetics of susceptibility and severity in systemic lupus erythematosus
Jennifer A. Croker and Robert P. Kimberly

Purpose of review
The genetic basis of systemic lupus erythematosus, a complex genetic trait, may provide important insights into autoimmune disease. Innovation in both practical and theoretical approaches will assist in accelerating the pace of discovery and our understanding of pathogenesis.

Recent findings
Significant progress has been made in the last year with respect to the refinement of genetic intervals to promising candidate genes involved in systemic lupus erythematosus pathogenesis and specific phenotype susceptibility. This review highlights these discoveries and suggests platforms that may affect the future of analysis of this complex disease.

Summary
Understanding the genetic basis for systemic lupus erythematosus disease and sub-phenotype susceptibility will have a substantial effect on the therapeutic interventions used to care for patients.

Keywords
admixture, susceptibility, systemic lupus erythematosus genetics

Introduction
Systemic lupus erythematosus (SLE) is a prototypic autoimmune disease that affects primarily women, especially those of reproductive age. Women of African American or Hispanic American ethnicity appear to have a higher risk of lupus [1], and an evident familial tendency for disease expression suggests a genetic basis for disease [2]. Identical twins show a concordance rate of 24–58% compared with less than 10% in fraternal twins. Furthermore, SLE familial risk estimates indicate a λs ~ 20 [3]. Taken together, these data suggest that lupus is a complex genetic trait with a threshold effect for expression. The variance in disease expression, however, suggests that certain environmental or epidemiologic factors also affect clinical manifestation [4].

Genetics of systemic lupus erythematosus susceptibility
Genome-wide linkage studies have been very successful in identifying genetic loci with association to the SLE phenotype in multiplex families. These regions typically encompass at least several megabases, however, and the multiplicity of regions proposed has created a challenge in prioritization. Fine mapping within these regions has moved from microsatellites to single nucleotide polymorphisms (SNPs), and such efforts have identified several candidate genes. Nonetheless, the task remains formidable, and the relative lack of power of linkage analysis to detect relatively small genetic effects has led to resurgent interest in family-based and case-control–based association studies. Although association has been used within regions of linkage or for candidate genes, the development of high-density SNP genotyping technologies has opened the prospect of genome-wide association studies (see review [5]). With candidate genes, the use of SNPs of known biologic function has been fruitful, especially with large sample sets providing sufficient power to detect modest effects. Both linkage and association approaches have been used to define a large set of genes and genomic regions involved in SLE susceptibility [6,7] (Table 1).

Major histocompatibility complex
The major histocompatibility complex human leukocyte antigen region includes >200 genes on human chromosome six involved in self/non-self recognition, antigen presentation, and immune regulation. Consistent associations have been observed between DRB11501 (DR2) and DRB10301 (DR3) alleles in white patients with SLE [46,47]. Dense microsatellite mapping in affected sib-pairs and in simplex families with SLE has revealed significant transmission
Table 1. Systemic lupus erythematosus candidate genes and associated polymorphisms

<table>
<thead>
<tr>
<th>Gene</th>
<th>Polymorphism</th>
<th>Susceptibility allele</th>
<th>Confirmation</th>
<th>Association parameters</th>
<th>Tested ethnicity</th>
<th>References</th>
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<tr>
<td>CRP</td>
<td>+1846G/A</td>
<td>+1846A</td>
<td>No</td>
<td>F-B</td>
<td>ECa</td>
<td>[8]</td>
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<td></td>
<td></td>
<td></td>
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<td></td>
<td></td>
<td></td>
<td>(EC, AC, IA, A, M)b</td>
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<td>+49G</td>
<td>No</td>
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<td>AA, C, S, J*, Metaa</td>
<td>[9,10,11,12,13]</td>
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<tr>
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<tr>
<td>FCRL3</td>
<td>−169 T/C</td>
<td>−169T</td>
<td>No</td>
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<td>C*</td>
<td>[29]</td>
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<td>MBL</td>
<td>G54D</td>
<td>D54</td>
<td>Yes</td>
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<td>J*</td>
<td>[30]</td>
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<tr>
<td>MCP-1</td>
<td>−2518 A/G</td>
<td>−2518G</td>
<td>Yes</td>
<td>C-C</td>
<td>C*, AA*, A*, M*, S*</td>
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<td>MHC</td>
<td>DRB1</td>
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<td>Yes</td>
<td>F-B</td>
<td>C*</td>
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<td>PD-1.3 A/G</td>
<td>PD-1.3A</td>
<td>Yes</td>
<td>C-C, F-B</td>
<td>C*, H*, AA, SW*, S</td>
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<td>PTPN22</td>
<td>R620W</td>
<td>W620</td>
<td>Yes</td>
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<td>C*, S*</td>
<td>[43*,44*]</td>
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<td>Meta, C-C, F-B</td>
<td>SW*, F, Metaa</td>
<td>[45]</td>
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<tr>
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<td>S684</td>
<td>No</td>
<td>Meta, C-C, F-B</td>
<td>SW*, F, Metaa</td>
<td>[45]</td>
</tr>
</tbody>
</table>

*aThose ethnicities, cohorts, or meta analyses in which a significant association was detected. bInsufficient data to test ethnicities independently—represented ethnicities are in parantheses. F-B, family-based; EC, European Caucasian; AC, Afro-Caribbean; IA, Indo-Asian; A, Asian; M, mixed ethnicity; Meta, meta-analysis; C-C, case-controlled; AA, African American; C, Caucasian; S, Spanish; J, Japanese; H, Hispanic; K, Korean; G, German; T, Thai; GR, Greek; CH, Chinese; NA, Native American; SW, Swedish.

distortion and enrichment of the human leukocyte antigen class II DRB11501 (DR2) haplotype in white individuals. No significance has been seen among common haplotypes in non-white individuals, although power has been limited [39].

Major histocompatibility complex class III includes genes encoding several complement proteins that have been implicated in SLE susceptibility for some time [48]. Patients with rare, complete complement C4 (A and B) deficiencies are at increased risk for SLE, glomerulonephritis, or lupus-like symptoms [49,50]. The C4 locus is complex with significant gene duplication and variation in copy number, referred to as copy number polymorphisms (CNPs). The copy number of C4 genes (gene dosage) in a diploid human genome ranges from two to seven in a diverse population of patients and healthy family members [51]. Ethnic differences in the C4 copy number have also been observed. Likewise, C4 protein serum levels in the population range from 80–1000 μg/ml [52]. Like other gene variants, the differential C4 copy number is related to SLE manifestation. Some data suggest that even in the absence of homozygous null alleles, C4A and C4B may participate in disease susceptibility and severity, respectively [51]. These observations suggest that CNPs and gene dosage effects may play an important role in SLE and other complex genetic diseases.

**Classic immunoglobulin receptors (Fcγ receptors)**

Among the nine published genome-wide linkage scans, a linkage effect in the region encompassing 1q23-24 has been identified in five studies [1,53–55,56]. This region includes the eight genes of the classical immunoglobulin receptor cluster that encode the three highly homologous, but distinct, families of classical IgG Fc receptors (FcRs; FcγRI [CD64], FcγRII [CD32], and FcγRIII [CD16]). These receptors are membrane-bound glycoproteins that interact with the constant (Fc) region of an antibody to elicit different immune responses, including phagocytosis, degranulation, endocytosis, immune complex clearance, antibody-dependent cell cytotoxicity, and transcriptional regulation of cytokine and chemokine expression [57].

Both linkage and association of the ligand binding site alleles of FCGR2A (FcγRIla) and FCGR3A (FcγRIla) with the SLE phenotype have been confirmed. Of the two co-dominantly expressed FcγRIla alleles, the arginine-131 (R131) allele, which binds human IgG2 poorly, is associated with SLE [14—19]. Similarly, of the two co-dominantly expressed FcγRIla alleles, the phenylalanine-176 (F176) allele, which binds human IgG1 and human IgG3 much less efficiently than the valine-176 allele, is enriched in patients with SLE, and may constitute a risk factor for renal disease [15,20—23]. Despite a modest contribution to genetic risk, with odds ratios of 1.5–2.0, association of both genes with SLE has been demonstrated with model-dependent and model-independent approaches [23]. Not surprisingly, not all case-control association studies have been positive. These studies have varied in study population size and corresponding statistical power, in the self-declared ethnicity and ancestral background of study participants, and in genotyping technologies applied to these highly homologous gene families. Meta-analyses of case-control association
studies in several ethnic populations support a role for these genes as disease risk genes [58–60].

The NA polymorphism of FCGR3B (FcγRIIIb), expressed predominantly on neutrophils, was originally proposed as a potential candidate gene for lupus by Goldstein and colleagues [61]. This polymorphism exists as two alleles that affect signal intensity and IgG affinity [62–65], and original work by Hatta et al. [66] suggested a population-based association with SLE. Subsequent work from this group and others, however, suggests that the apparent association of FcγRIIIb with SLE is a result of strong linkage disequilibrium with FCGR2B [17].

FCGR2B, which encodes the immunoreceptor tyrosine-based inhibition motif (ITIM)-containing inhibitory FcγRIIb protein, has a non-synonymous coding SNP in exon six that changes the isoleucine (I) to threonine (T) at amino acid 232 in the transmembrane domain, which alters the inhibitory potential of the receptor [17,26]. The T232 allele is associated with SLE, and perhaps lupus nephritis, in Chinese, Japanese, and Thai patients, but not in North American Caucasian patients, African American patients, or Northern European patients [24,25,26–28]. This difference may emphasize the roles of genetic heterogeneity and different ancestral backgrounds in defining disease risk alleles. Alternately, this disparity may be a result of linkage disequilibrium with recently identified regulatory SNPs (rSNPs) in the FCGR2B promoter that have been associated with the SLE phenotype. The FCGR2B 2B.4 promoter haplotype (-386C-120A) has increased binding affinities for GATA binding protein 4 and YinYang1 transcription factors, resulting in elevated FCGR2B expression levels and increased cellular response as measured by Ca²⁺-flux and B-cell viability [67•]. This particular haplotype is enriched in Caucasian patients with SLE compared with case-controls and provides an additional mechanistic role for FCGR2B alleles in autoimmune pathogenesis [29•].

**Immunoglobulin receptor homologues**

In addition to the classical FcγRs, in-silico homology searches of the genome have identified a newly recognized cluster of related immunoglobulin superfamily genes within the 1q21-23 locus. Teged FcR homologue (FcRH)/immunoglobulin superfamily related translocation associated/anti-IgM activating sequence (BXMAS)/immunoglobulin-superfamily-FcR-GP42 (IFGP)/Fc receptorlike genes, some members of this family are expressed primarily in mature B cells, including naive, memory, and to a lesser extent, germinal B cells and plasma cells [68–77]. These genes are polymorphic and show both non-conservative variation in coding region (e.g., the P660L substitution in FcRH3 [71]) and marked differences in allele frequencies according to ethnicity. In Japanese patients, an association of an FcRH3 promoter polymorphism with rheumatoid arthritis and SLE has been identified using linkage disequilibrium mapping of the region [30•]. Supporting evidence of an SLE association with the FcRH gene cluster in 1q21 has been reported (Gibson et al., unpublished data, January 2005).

**Protein kinases, phosphatases, and cell-signaling molecules**

Murine models suggest that alterations in kinase and/or phosphatase activity can lead to an autoimmune phenotype. The role of interferon signaling in lupus pathogenesis has been established in disease-prone mouse strains. In humans, several observations implicate a role of interferon signaling in SLE manifestation, including elevated interferon serum levels in patients with lupus and development of SLE in patients using interferon-α as a therapeutic agent for viral hepatitis or carcinoid tumors [78]. As a result of recent interest in the type I interferon pathway as a contributor to SLE pathogenesis, members of the janus kinase/signal transducers and activators of transcription pathway, including the tyrosine kinase 2 (TYK2) protein, have been explored as candidate genes. Using both family-based and case-control approaches, a joint linkage/association study of Swedish, Finnish, and Icelandic patients with SLE has identified strong associations of SLE susceptibility and polymorphisms in TYK2 and IFN regulatory factor 5 (IRF5) genes. Three rSNPs were identified in flanking or intronic regions of IRF5 that appear to be associated with SLE in Swedish and Finnish ethnicities. Two polymorphisms within TYK2 coding regions, A13430C (V362F) and T19107C (I684S), were associated with the disease in Swedish patients [45]. These variants may affect not only interferon-α signaling but also other cytokine-regulated signaling pathways. Recognition of the potential for epistasis with other cytokine polymorphisms may provide insight into some of the other proposed genetic associations with SLE, including interleukin-10 [79,80].

Protein tyrosine phosphatase N22 (PTPN22), which encodes the lymphoid protein tyrosine phosphatase originally characterized in T-cell receptor signaling in memory/effector T lymphocytes, has a non-synonymous coding SNP leading to an arginine to tryptophan substitution (R620W) that interrupts the interaction site for the negative regulatory kinase, Csk [81,82]. This polymorphism is associated with human rheumatoid arthritis (odds ratio = 1.65; 95% confidence interval, 1.23–2.20) and type I diabetes (odds ratio = 1.83; 95% confidence interval, 1.284–2.596), and case-control studies indicate an increased risk of SLE attributed to the W620 allele in North American white individuals (odds ratio = 1.37; 95% confidence interval, 1.07–1.75) and in Spanish Caucasian individuals (odds ratio = 1.55; 95% confidence interval, 1.05–2.29) [43•,44•,82,83]. Interestingly, the genomic locus for PTPN22, 1p13.3-1p13.1, does not fall into any previously identified linkage region.
Mice lacking the cell surface receptor progranil cell death 1 (PDCD1 or PD-1) develop spontaneous lupus-like disease phenotypes, including arthritis and glomerulonephritis [84]. The human homologue, which functions to down-regulate lymphocyte proliferation and cytokine secretion, has been mapped to the 2q37.3 locus, previously associated with SLE susceptibility [85]. A polymorphism (PD-1.3A) in PDCD1 has been associated with SLE susceptibility in Mexican and European populations (but not in African American, Swedish, or Finnish patients) [40,41,45]. Located within an intrinsic enhancer, this polymorphism alters the binding site of the runt-related transcription factor 1 (RUNX1) or acute myeloid leukemia 1 (AML1) which may affect transcriptional regulation of PDCD1, suggesting a mechanism for the development of SLE [40].

Another cell surface receptor, cytotoxic lymphocyte antigen 4 (CTLA-4), regulates T-cell activation, particularly under inflammatory conditions. Data regarding an association of CTLA-4 with SLE have been inconsistent. Independent studies of African American, North American Caucasian, Iberian Caucasian, and Japanese patients have suggested no statistically significant association of CTLA-4 polymorphisms with SLE [9,10,11,12]. A meta-analysis of eight previous studies, however, including those previously mentioned, revealed an increased susceptibility for SLE conferred by the +49 G allele, and decreased SLE risk by the +49 A allele [11,12]. This allele, in combination with a 3’ untranslated region microsatellite polymorphism (CT60), was also associated with SLE in Spanish patients with odds ratios of 1.71 (95% confidence interval, 1.18–2.49) [13]. A high degree of linkage disequilibrium between CTLA-4 polymorphisms may contribute to the varied observations [86]. The association data parallel the findings of CTLA-4 polymorphisms in type I diabetes and suggest that CTLA-4 may function as a modest SLE susceptibility loci or possibly a disease modifier [13,87].

Cytokines and chemokines
Monocyte chemoattractant protein 1 (MCP-1) has been related to glomerulonephritis in animal models [88]. In humans, the G allele of the rSNP at −2518 (A/G) is related to elevated MCP-1 protein levels, and using a candidate gene approach to study the association between SLE susceptibility, Tucci et al. [37] found the G allele to be enriched in patients with SLE (odds ratio = 4.1; 95% confidence interval, 1.44–14.83), particularly those with lupus nephritis (odds ratio = 4.5; 95% confidence interval, 1.322–15.71) [89]. Interestingly, in a survey of twice as many Spanish patients and matched controls, no association of MCP-1 with SLE was observed. An MCP-1 association was observed for the development of cutaneous vasculitis (odds ratio = 2.2; 95% confidence interval, 1.18–4.25), but not lupus nephritis, in these patients, however [38]. These studies suggest that the presence of the −2518G polymorphism may contribute to the development of SLE and/or may be a risk factor for the development of SLE sub-phenotypes in certain patient populations. Replication studies in substantially powered populations will help differentiate the role of MCP-1 in SLE susceptibility and severity in varying ethnicities.

Opsonins of the innate immune system
As a result of the role of C-reactive protein (CRP) in the handling and clearance of apoptotic material, its pathophysiology makes CRP an attractive candidate gene for study. On the basis of this rationale and its location in a region of linkage on 1q23.2, several groups have studied CRP polymorphisms in relation to both susceptibility and severity of SLE [8,90,91,92,93]. The coding region of CRP contains several synonymous but no significant non-synonymous SNPs. Analysis of several CRP polymorphisms in United Kingdom simplex SLE families revealed an association between a SNP adjacent to the 3’ untranslated region of CRP (+1846 G/A) and the development of SLE [8]. The basis for this relation is currently unclear, but the recent demonstration of rSNPs falling within E-box motifs in the CRP promoter, which regulate CRP protein levels, suggests that linkage disequilibrium between 3’ untranslated region markers and promoter haplotypes might be usefully explored [92]. Promoter haplotypes may also influence the intensity of the immune response and affect disease severity and sub-phenotypes.

Mannose (or mannan) binding lectin (MBL) is an opsonin of the innate immune system that facilitates activation and fixation of complement. Several different alleles of MBL affect MBL protein production, and studies have uncovered an association of MBL polymorphisms and SLE in various ethnicities [31–34,94]. Specifically, a glycine to aspartate change at codon 54 (G54D) has been shown to be a risk factor for SLE. Meta-analysis of eight previous studies suggests MBL polymorphisms confer a 1.6 times increased risk for SLE (odds ratio = 1.6; 95% confidence interval, 0.99–2.5) [95].

Systemic lupus erythematosus sub-phenotypes
Given both genetic and clinical heterogeneity of SLE, there has been considerable interest in genetic effects that might be more closely related with a specific clinical manifestation than with the global phenotype of SLE.

Several studies have established precedent for allelic contributions to renal involvement in patients with SLE. The R131 allele of FcγRIIa and the F158 allele of FcγRIIa have previously been associated with the development of lupus nephritis in this cohort [59,96–98]. A similar study of MCP1 polymorphisms suggests that particular rSNPs not only are related to SLE susceptibility but also may contribute to the development of lupus nephritis or cutaneous
vasculitis in patients with SLE [37,99]. The same MCP1 polymorphism has been associated, in case-control studies, with the development of arthritis by Chinese patients with SLE [100]. A study refining the statistical significance of MBL polymorphisms and SLE susceptibility revealed an association of the MBL codon 54 (G54D) allele and predisposition to renal involvement [34]. The same conclusion was drawn from a study of PDCD1 polymorphisms in Caucasian patients with SLE. In this study, a significant association was observed between an intronic rsNP affecting a ZEB transcription factor binding site and lupus nephropathy [101]. These data suggest that several genes may contribute to the manifestation for various SLE sub-phenotypes.

**Systemic lupus erythematosus disease severity and outcome**

Longitudinal study of patients with SLE has demonstrated disease progression over time, and the possibility that some genetic variants might contribute to the tempo and/or type of disease progression is of significant interest. For example, PROFILE, a large, multi-institutional effort studying a multiethnic, longitudinal cohort of patients with SLE, has investigated renal involvement and damage and has revealed a marked difference between Hispanics from Puerto Rico or Mexico, including higher disease activity, more damage accrual, increased frequency of anti-double stranded DNA antibodies, and more major organ involvement in the group of Hispanics from Mexico [102]. Interestingly, the FcγRIIIa F176 allele (particularly when homozygous) was highly correlated to renal involvement and progression (Alarcon et al., unpublished data, October 2004).

Given the association between cardiovascular arterial events and elevated levels of CRP, the relation between CRP alleles and CRP protein levels, and the significantly elevated risk for myocardial infarction and atherosclerosis compared with the general public or age-matched controls, polymorphic alleles of CRP have been explored as susceptibility alleles for cardiovascular events in patients with SLE [90,103–105]. Indeed, a GT expansion (GT<sup>20</sup>) in the first CRP intron is enriched in Hispanic and African American (not Caucasian) patients with SLE who have vascular arterial events. This suggests that CRP alleles may be a risk marker for cardiovascular complications in individuals diagnosed with SLE [93].

**Anticipated areas for progress**

On the foundations of linkage analyses and candidate gene associations, several innovations in methodological platforms may assist in accelerating the pace of discovery and our understanding of SLE pathogenesis.

**Gene expression microarrays**

The ability to assess quantitative gene expression patterns for large numbers of genes is now available through microarray analyses of messenger RNA levels. Several SLE studies have observed a significant change in interferon pathway gene expression, among other effects [106–108]. As with any technique, microarray analysis presents several challenges, including the examination, clustering, and prioritization of the large number of profile differences. These differences may reflect underlying genetic characteristics and provide another means to understand the genetic complexity of SLE (reviewed by [109]). They may also be secondary consequences of persistent inflammation, however, and reflect processes common to many autoimmune diseases that affect cell cycle progression, differentiation, migration, and apoptosis [110].

**High-throughput single nucleotide polymorphism chips**

Microarray technology has recently expanded to high-throughput, genome-wide SNP genotyping platforms. John et al. [111••] have compared linkage analysis using an 11,245 SNP chip compared with a microsatellite-based whole-genome screen in multicase families with rheumatoid arthritis. The genome-wide SNP approach identified several known rheumatoid arthritis-related loci within significantly smaller linkage intervals. In this case, the interval of linkage was 13 cM less than that identified by microsatellite scan, likely as a result of the increased information content across the genome in the chip analysis, which may facilitate downstream fine mapping. The increased information content may also contribute to the identification of additional loci, previously unidentified by the microsatellite scan. Efforts to minimize the false discovery rate in each method were implemented, including reducing genotyping error; however, it is possible that genotyping and map errors, information content limitations, and the presence of linkage disequilibrium may reveal false positives. In this study, additional evidence has been observed to support the verity of novel loci.

**Table 2. Susceptibility genes associated with autoimmune conditions in addition to Systemic lupus erythematosus**

<table>
<thead>
<tr>
<th>Gene</th>
<th>Autoimmune disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>C4</td>
<td>Multiple sclerosis</td>
<td>[120]</td>
</tr>
<tr>
<td></td>
<td>Type I diabetes</td>
<td>[120]</td>
</tr>
<tr>
<td>CTLA-4</td>
<td>Rheumatoid arthritis</td>
<td>[121–123]</td>
</tr>
<tr>
<td></td>
<td>Graves’ disease</td>
<td>[124–126]</td>
</tr>
<tr>
<td></td>
<td>Autoimmune thyroid disease</td>
<td>[124–126]</td>
</tr>
<tr>
<td></td>
<td>Type I diabetes</td>
<td>[126]</td>
</tr>
<tr>
<td>FCGR3A</td>
<td>Rheumatoid arthritis</td>
<td>[127–129]</td>
</tr>
<tr>
<td>FCRL3</td>
<td>Rheumatoid arthritis</td>
<td>[30]</td>
</tr>
<tr>
<td>MBL</td>
<td>Rheumatoid arthritis</td>
<td>[36,130]</td>
</tr>
<tr>
<td></td>
<td>Celiac disease</td>
<td>[131]</td>
</tr>
<tr>
<td></td>
<td>Adult dermatomyositis</td>
<td>[132]</td>
</tr>
<tr>
<td></td>
<td>Sjögren’s syndrome</td>
<td>[36]</td>
</tr>
<tr>
<td>MCP-1</td>
<td>Giant cell arthritis</td>
<td>[133]</td>
</tr>
<tr>
<td>PDCD1</td>
<td>Rheumatoid arthritis</td>
<td>[134–136]</td>
</tr>
<tr>
<td></td>
<td>Type I diabetes</td>
<td>[137]</td>
</tr>
<tr>
<td>PTPN22</td>
<td>Rheumatoid arthritis</td>
<td>[44*,83,118*,138]</td>
</tr>
<tr>
<td></td>
<td>Graves’ disease</td>
<td>[139,140]</td>
</tr>
<tr>
<td></td>
<td>Juvenile idiopathic arthritis</td>
<td>[138]</td>
</tr>
<tr>
<td></td>
<td>Type I diabetes</td>
<td>[82,118*,139,141]</td>
</tr>
<tr>
<td></td>
<td>Celiac disease</td>
<td>[138]</td>
</tr>
<tr>
<td></td>
<td>Hashimoto thyroiditis</td>
<td>[118*]</td>
</tr>
</tbody>
</table>
This study also analyzed linkage disequilibrium of an area of interest, suggesting that the SNPs could be used as a fine-mapping scaffold. Ultimately, this study serves as the proof of principle for the use of SNP chips in linkage analysis of complex, multigenic diseases to detect susceptibility loci. The high-density SNP chip was more precise, had lower genotyping error rates, and reduced the resource need and time for gene mapping. It is very likely that evaluation of SNPs via this high-throughput mechanism will be used for whole-genome association studies in case-control–designed SLE studies.

Admixture mapping

Admixture mapping, also known as mapping by admixture disequilibrium, relies on the availability of highly informative markers with differences in allele frequencies across major ethnic groups. The recent publication of a high-density map of more than 3000 validated SNPs having an approximate spacing of 1.2 cM and differing between European and African ethnicities has enabled studies of admixture [112,113]. This technique can be used to localize genes that underlie ethnic variation of disease (reviewed in [114]) and may provide a key to understanding the differential susceptibility to SLE of certain ethnicities at various loci.

Admixture mapping has been used to identify genetic loci affecting hypertension as one model of a complex, multigenic disorder [115**]. Scanning of 269 microsatellite markers in African American patients compared with Nigerian and European American controls revealed seven susceptibility loci on two chromosomes. In addition to confirming a few previously reported loci [116], this study identified several novel susceptibility loci. Although admixture mapping may have lower resolution than high-density whole-genome SNP-based association analyses, it is particularly useful when there is a prominent ethnic disparity of phenotype as seen in SLE. Development of high-resolution marker sets may also allow better whole-genome scans [113,117].

Conclusion

Differences in the contribution of specific genetic variants to the SLE phenotype from populations of different ancestral backgrounds are becoming increasingly apparent. While this observation underscores genetic heterogeneity in disease manifestation and emphasizes the opportunities in admixture mapping, it also cautions investigators to be suitably careful in the design of case-control studies. At the same time that genetic heterogeneity is becoming more clear empirically, evidence is gathering to support the notion of susceptibility alleles common to multiple autoimmune conditions such as rheumatoid arthritis, psoriasis, and autoimmune thyroid disease [118,119] (Table 2). A single common foundation to autoimmunity seems unlikely, but some shared building blocks seem probable. Among the challenges will be defining the epistatic interactions between susceptibility alleles to understand the basis for disease development and the factors that may contribute to phenotype severity. Among the confounders in analysis may be CNPs, as seen for the complement component C4. New technologies for high-density SNP genotyping, for splice variant detection, and for characterization of ancestry will provide powerful tools, however. Also, the goals of defining new pathways for intervention and of tailoring therapy to each patient on the basis of defined predispositions and our understanding of the relation among disease, genetics, environment, and lifestyle are both an exciting and an important pursuit.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest •• of outstanding interest

and functionally relevant promoter haplotype of FcγRIIIa and colleagues provide evidence of an association between a newly identified FcγRIIIa polymorphism and Spanish patients with systemic lupus erythematosus. Eur J Immunogenet 2002; 29:301—306.


This study confirms previously reported data [17,26] regarding the association of a non-synonymous and functionally relevant FcγRIIB SNP with SLE, independent of FcγRIIIa, as well as an association with the development of lupus nephritis, in Asian patients with SLE.


Su and colleagues provide evidence of an association between a newly identified and functionally relevant promoter haplotype of FcγRIIB and SLE.


This study provides the first published evidence of an association between SLE and one of a newly identified family of FcγRIII proteins (FcγRIII) using linkage disequilibrium mapping. Despite mapping to a similar linkage region (1q23) as classical Fc receptors, no linkage disequilibrium was observed between the variants in the Japanese cohort.


An analysis of this cohort of patients with provides the primary evidence for an association with an MCP allele, supported by very strong odds ratios.


In addition to defined associations with type 1 diabetes [80] and rheumatoid arthritis [81] Kyogoku et al. provide more evidence for a role of PTPN22 in autoimmune disease manifestation by demonstrating the first association with SLE.


This study confirms previous work establishing an association between a PTPN22 polymorphism and multiple autoimmune conditions, rheumatoid arthritis, and SLE.


This group revealed significant joint linkage/associations of two interferon signaling pathway genes, TYK2 and IRFS, with SLE in Swedish and Finish patients. These observations highlight the probable role of the interferon pathway in autoimmune disease etiopathogenesis and may be ideal candidates for epistasis analyses.


Although the association of complement with SLE susceptibility is not novel, this group has identified and evaluated the clinical effect of very rare complete C4 deficiencies. They have also characterized putative polymorphic differences that may contribute to epidemiological studies of SLE and other autoimmune diseases.


Salmon JE, Edberg JC, Kimberly RP. Fc gamma receptor III on human neutrophil
function by the glycosyl-phosphatidylinositol-anchored form of Fc gamma receptor. Immunogenetics 1998; 48:222—232.


Su et al. define the functional significance of two FcγRIIb promoter polymorphisms shown to associate with SLE pathogenesis [27], providing evidence of an underlying mechanism for disease susceptibility.


This study compared linkage analysis using a SNP chip compared with a microsatellite-based whole-genome screen in multicase families with rheumatoid arthritis. It serves as the proof of principle for the use of SNP chips in linkage analysis of complex, multicase diseases to detect susceptibility loci. The high information content also may be useful in linkage disequilibrium studies to refine genetic maps.


This group has created a very useful high-density map of more than 3000 validated single-nucleotide polymorphisms in multicase families with rheumatoid arthritis. This study contributes to a growing body of evidence suggesting that certain susceptibility alleles are associated with multiple autoimmune conditions.


Complement and systemic lupus erythematosus
David R. Karp

Purpose of review
It is well recognized that the complement system plays multiple roles in systemic lupus erythematosus. Activation of the classical pathway by immune complexes leads to the generation of inflammatory mediators, thus promoting tissue injury. Complement activation also plays an important role in the maintenance of tolerance to self-antigens. This review discusses recent insights in the role of complement in the pathogenesis of systemic lupus erythematosus.

Recent findings
The antiphospholipid syndrome is a major feature of systemic lupus erythematosus. New findings have clearly demonstrated that the prothrombotic effects seen in a mouse model of this syndrome depend on complement activation, whereas the protective effects of heparin are due to its anticomplementary effects rather than its anticoagulant action. Secondly, a potential mechanism explaining the association of anti-C1q autoantibodies with lupus glomerulonephritis has been elucidated in a mouse model system.

Summary
New findings have helped to reinforce the role of complement in the etiology and tissue damage of systemic lupus erythematosus. These findings point to more precise, mechanism-based therapies for autoimmune and inflammatory disease.

Keywords
antiphospholipid syndrome, C1q, complement, glomerulonephritis, major histocompatibility complex, systemic lupus erythematosus

Introduction
A role for complement in systemic lupus erythematosus (SLE) has been assumed for many years. Immune complexes formed by self-antigens and autoantibodies activate the classical pathway, generating inflammatory mediators and resulting in tissue damage. A more complicated picture has emerged over the past decade, however. First, it has become clear that genetic or acquired deficiency of the early complement components C1q or C4 (and to a lesser extent, C2) predisposes to the development of lupus [1–3]. This is supported by mouse models and illustrates a role for complement in the routine disposal of apoptotic cells containing autoantigens [4–6]. Second, a previously unknown dependence on complement activation has been shown in a mouse model of antiphospholipid syndrome (APS) [7]. Lastly, complement proteins themselves may become targets of autoantibodies, altering the regulation of inflammation [8]. This article reviews recent findings linking the complement system to SLE and autoimmunity.

Antiphospholipid syndrome
The APS is characterized clinically by venous and arterial thrombosis as well as pregnancy loss in association with a variety of autoantibodies directed against phospholipids and lipoproteins (aPLs). APS can occur as a primary autoimmune disorder or as a feature of SLE (so-called secondary APS). Overall, aPLs are present in approximately 40% of patients with SLE, and APS can similarly be seen in about one-third of SLE patients [9–11]. aPLs are present in 10%–50% of non-SLE patients with venous or arterial thromboembolism and are seen in up to 20% of women with recurrent pregnancy loss [12].

Numerous studies have shown that aPLs bind to and activate endothelial cells, monocytes, neutrophils, and platelets, causing the release of both inflammatory mediators as well as procoagulants. In the past 4 years, a substantial body of work has emerged demonstrating the essential role of the complement system in a mouse model of APS. In this model, pregnant mice are injected with polyclonal or monoclonal aPL antibodies, resulting in the death of up to 50% of the implanted embryos. Mice deficient in C3, or mice that have been treated with inhibitors of complement activation, are protected from fetal loss [13].

Studies to investigate the mechanism of complement-dependent aPL-induced fetal loss also demonstrated dependence on both C4 and factor B, suggesting that classical pathway activation and subsequent alternative
pathway amplification are both necessary. Other genetic models resistant to the effects of aPLs included C5-deficient mice, C5a receptor-deficient mice, mice treated with a peptide inhibitor of C5a-C5a receptor interaction, and mice depleted of neutrophils [14]. Interestingly, mice deficient in activating Fc receptors were still susceptible to fetal loss. Together, these data suggest a mechanism whereby complement activation generates C5a, which, in turn, activates neutrophils, resulting in a proinflammatory, prothrombotic state.

Additional evidence for this mechanism comes from recent experiments showing that tumor necrosis factor (TNF-α) levels in plasma rise nearly 10-fold in the first 2 hours following aPL injection [15•]. The increase in TNF-α was not seen in C5-deficient mice, demonstrating the absolute requirement for complement activation in the generation of inflammatory cytokines. Fetal resorption was not seen in mice that were either genetically deficient in TNF-α or mice that had been treated with pegylated soluble TNF receptor. Given the availability of several biologic therapies to block TNF-α therapeutically (infliximab, etanercept, and adalimumab), cytokine inhibition may be a useful treatment to prevent recurrent pregnancy loss in women with APS.

Two studies have addressed the role of complement inhibition as therapies for experimental APS. The traditional therapy for women who have had recurrent pregnancy loss due to APS is either unfractionated or low-molecular-weight heparin during most of the pregnancy. It has been assumed that this prevents the formation of placental microthrombi. Heparin has long been known to inhibit the activation of the complement system [16,17], however. Administration of either low-molecular-weight or unfractionated heparin to pregnant mice prevented the fetal loss caused by aPL antibodies [18••]. Fondaparinux is a pentasaccharide mimic of the antithrombin-binding region of heparin. Although it effectively inhibits factor Xa, it does not have anticomplementary activity. Likewise, the thrombin inhibitor hirudin is an anticoagulant without any effect on complement activation. Neither fondaparinux nor hirudin could prevent fetal resorption induced by aPL antibodies, demonstrating that it is the anticomplementary activity of heparin, not the anticoagulant effect of heparin, that is essential. In both mouse and human serum, heparin, but not fondaparinux or hirudin, was 100% effective in preventing complement activation as judged by the binding of C3b to trophoblast-like BeWo cells and the generation of C3a desArg. Heparin did not block the binding of aPL antibodies to placental tissue in vivo.

The authors conclude that the major therapeutic effect of heparin in this model, and likely in women treated to prevent pregnancy loss, is due to its ability to block activation of the complement pathway and not to its anticoagulant effect. Lastly, a novel monoclonal antibody to mouse factor B was tested in this mouse APS model [19••]. This antibody was developed by immunizing factor B-deficient mice with a recombinant protein consisting of the Fc portion of mouse IgG1 fused to the portion of factor B essential for hemolytic activity. This monoclonal antibody, designated 1379, was 100% effective in blocking zymosan-induced alternative pathway activation in vitro using serum from human, mouse, rat, baboon, rhesus monkey, cynomolgus monkey, pig, and horse as a complement source. Treatment with monoclonal antibody 1379 resulted in a decrease in the frequency of fetal resorption from 40.5% ± 8.7% to 20.3% ± 6% (P < 0.01). Deposition of C3 on decidual tissue and generation of C3a desArg in serum were similarly inhibited by the antibody. The authors conclude that blockade of the alternative pathway is an attractive potential therapy for APS, particularly for patients for whom anticoagulation is contraindicated. Specific alternative pathway blockade also circumvents potential problems with susceptibility to bacterial infection that could complicate strategies that block activation via the classic and mannann-binding lectin pathways.

**Role of anti-C1q in lupus nephritis**

The C1q subunit of C1 can interact with antibody in two distinct ways. In the classical activation pathway, the globular head of C1q binds specifically to the Fc portion of selected immunoglobulin. In addition, autoantibodies to C1q bind to the collagenlike tail region through their Fab domains. Such autoantibodies have been described in a number of autoimmune conditions, notably SLE and hypocomplementemic urticarial vasculitis [20,21]. A strong association has been seen between the presence of such antibodies and proliferative lupus nephritis [22–24,25•]. The significance of this finding is corroborated by the presence of similar anti-C1q autoantibodies in several mouse models of SLE characterized by the occurrence of glomerulonephritis [26,27•].

A potential mechanism for the association of anti-C1q with lupus nephritis was recently proposed. Trouw et al. [28••] developed a monoclonal antibody (JL-1) to the collagenlike tail of C1q that reacts with both human and mouse proteins. When JL-1 was administered to normal mice, there was deposition of C1q-anti-C1q complexes in the glomerulus but no evidence of nephritis. This effect was not seen in Rag2−/− mice, suggesting that C1q was acting as a ‘planted antigen’ binding to low levels of immunoglobulin present normally in the kidney. When mice were given subnephritogenic doses of rabbit anti-glomerular basement membrane (GBM) serum, JL-1, but not control monoclonal antibody, led to substantial C1q/anti-C1q glomerular deposition as well as albuminuria and histologically evident glomerulonephritis. The effect was clearly dependent classical complement pathway activation, as anti-GBM serum that did not fix C1q was
ineffective in this assay. In addition, mice genetically deficient in C1q, C4, C3, and all Fcγ receptors did not display renal dysfunction.

This leads to a more detailed mechanism for the development of lupus nephritis in the face of humoral autoimmunity. Following the deposition of immune complexes (e.g., DNA/anti-DNA) or antiglomerular antibodies, C1q is fixed. When anti-C1q antibodies are present, complement activation is amplified through a classical pathway-dependent manner, leading to generation of C3a/C5a and the membrane attack complex, recruitment of neutrophils and mononuclear cells, and resultant renal damage. Whether human polyclonal antibodies act in a manner identical to JL-1 is unknown, although they do recognize similar epitopes on C1q [20,23]. If the epitopes of pathogenic anti-C1q are sufficiently restricted, this suggests therapeutic options to block or remove such autoantibodies in lupus patients.

**C1q and dendritic cell function**

A second mechanism whereby anti-C1q autoantibodies can lead to SLE has been proposed [29]. Patients with C1q deficiency often have severe SLE [30], as do C1q knockout mice [31], although the exact phenotype depends on genetic background [32] and is not C3 dependent [33]. It is known that C1q targets apoptotic cells and effects clearance of apoptotic blebs containing auto-antigens [5,31]. Such inappropriate clearance may diminish self-tolerance. C1q mediates the binding of apoptotic Jurkat T cells to human immature dendritic cells [34**]. This uptake stimulated interleukin-6, interleukin-10, and TNF-α production by dendritic cells but did not cause production of interleukin-12p70. This suggests that the interaction of C1q opsonized apoptotic cells with dendritic cells leads to their safe removal without dendritic cell maturation and potential T-cell activation. In another system, C1q has recently been shown to actually suppress the lipopolysaccharide-induced production of interleukin-12p40 by bone marrow-derived dendritic cells [35**] in vitro whereas production of TNF-α was not affected. In vivo, the serum concentration of interleukin-12p40 in C1q-deficient mice given lipopolysaccharide was approximately twice that of wild type. Both interleukin-12p40 protein and message were decreased in dendritic cells treated with C1q. Whereas the levels of proteins related to lipopolysaccharide signaling (TLR4, MD-2, MyD88) were not affected by C1q, there was a decrease in nuclear factor-κB binding to the interleukin-12p40 promoter and a delay in extracellular signal-related kinase (ERK) phosphorylation.

Lastly, it has been shown that lupus-prone MRL/Mp mice that are genetically deficient in C1q have a marked increase in the number of splenic monocytes compared with C1q-sufficient animals [36**]. These mice also had larger numbers of activated T cells, plasma cells, and serum IgM concentration compared with the wild-type MRL/Mp mice. IgM antichromatin autoantibodies were significantly increased in the C1q−/− mice (1.9 vs 0.6 units/mL, P < 0.05). Together, these data suggest that C1q plays an important regulatory role in maintenance of self-tolerance and autoimmunity. Antibodies to C1q may alter levels of this protein and thus predispose individuals to the development of additional autoantibodies, as well as play a direct role in the pathogenesis of immune-mediated glomerulonephritis.

**C4 and systemic lupus erythematosus**

Numerous studies have demonstrated an association with complete or partial C4 deficiency and lupus. Between a third and a half of all lupus patients have a deficiency in the expression of C4A relative to C4B [37–39], although a high frequency of C4B deficiency is seen in some ethnic groups (e.g., Spanish, Mexican, and aboriginal Australians) [40,41]. The argument that C4 deficiency plays a role in the pathogenesis of lupus is supported by the finding that C4-null mice are more likely to develop autoantibodies and immune complex glomerulonephritis. About two dozen humans have been reported with homozygous deficiency of both C4A and C4B [42]. Nearly all these patients had SLE or immune complex glomerulonephritis. The molecular basis for complete C4 deficiency in seven patients from four independent families was recently described [43**]. Each of these patients had kidney disease, either mesangial or membranoproliferative glomerulonephritis. Five of the seven carried the diagnosis of SLE and one had Henoch-Schönlein purpura with skin and gastrointestinal involvement. Three patients were homozygous for HLA A30 B18 DR7 and the other four were homozygous for HLA A24 B38 DR13. The first group lacked C4A genes but had duplicated C4B genes with identical mutations in an intronic splice donor sequence. The second group had no C4B genes and had a two-basepair deletion in exon 13 of the C4A gene. Molecular testing to screen for these mutations in patients with autoimmune disease was developed.

C4A and C4B both form covalent bonds with activating substrates, but with different specificities [44]. C4A reacts preferentially with proteins such as immune complexes forming amide bonds. Functionally, this results in the prevention of immune complex precipitation. This activity is reduced in SLE patients, particularly those with low C4 levels. The genetics of C4 differs in various ethnic groups. Yu and Whitacre [45**] have studied more than 2000 individuals including lupus patients, their family members, and healthy controls. In whites, three-quarters of the C4 genes are the ‘long’ form, due to the insertion of a human retroviral sequence that downregulates C4 expression. In African Americans, more than 40% of the C4 genes are the short (high-expression) form. Individuals with four long C4 genes have 40% less C4 in their serum
than individuals heterozygous for long and short genes, which may account for the observed higher level of C4 in African Americans compared with whites. Thus, the role of C4 in lupus pathogenesis is complex. In some individuals, a relative deficiency promotes loss of tolerance to self and development of lupus (disease susceptibility), whereas in others a higher level of C4 promotes immune complex damage (disease severity).

Conclusion
The role of the complement system in the etiopathogenesis of SLE continues to evolve. Generation of anaphylatoxins and the membrane attack complex constitute prominent mediators of immune complex damage. The range of pathology caused by complement activation must be expanded now to include initiation of thrombosis in APS. If the findings from the mouse model can be extended to humans, it suggests that more directed therapy can be as effective, but with increased safety. Future studies that use antibodies to C1q and complement levels as biomarkers will be useful strategies to predict susceptibility to SLE, as well as propensity to develop glomerulonephritis.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest

A study that begins to unravel the mechanism whereby complement participates in the safe disposal of apoptotic cells. Dendritic cells are the key to tolerance vs an immune response and are shown in this paper to interact in a distinct manner with pattern recognition elements of the complement system.

Further demonstration that complement recognition of self-antigens can alter the physiology of dendritic cell responses.

Evidence that failure to bind complement to self-antigens influences the proliferation of immune cells without the development of autoimmunity unless the correct background is present.


An extensive review of the molecular genetics of human C4. Describes the basis for quantitative and qualitative variation that may underlie racial differences in lupus.
Biomarkers for systemic lupus erythematosus: a review and perspective
Chau-Ching Liu a,b, Susan Manzi a,b,c and Joseph M. Ahearn a,b

Purpose of review
Despite decades of extensive work in the understanding of the etiopathogenesis of systemic lupus erythematosus, few biomarkers have been validated and widely accepted for this disease. The lack of reliable, specific biomarkers not only hampers clinical management of systemic lupus erythematosus but also impedes development of new therapeutic agents. This paper reviews briefly the historical aspects of systemic lupus erythematosus biomarkers and summarizes recent studies on candidate biomarkers.

Recent findings
Recognizing the urgent need for lupus biomarkers, a Lupus Biomarker Working Group has recently been initiated to facilitate collaborative efforts aimed at identifying and validating biomarkers for systemic lupus erythematosus. Based on available data, several laboratory markers have shown promise as biomarkers for susceptibility, diagnosis, and disease activity. These include Fc receptor genes (disease susceptibility), complement C4d-bound erythrocytes (diagnosis or disease activity), CD27 high plasma cells (disease activity), ‘interferon signature’ (disease activity), and anti-C1q antibodies (disease activity and organ involvement).

Summary
There is a longstanding and recently rejuvenated enthusiasm for biomarkers that precisely and specifically reflect the pathophysiologic and clinical changes in systemic lupus erythematosus. Promising candidate biomarkers have been identified but must still be validated through rigorous, large-scale multicenter studies.

Keywords
biomarker, disease activity, systemic lupus erythematosus

Introduction
Systemic lupus erythematosus (SLE) is the most clinically and serologically diverse autoimmune disease. The spectrum of disease manifestations among patients with SLE is broad, ranging from subtle symptoms to life-threatening multiorgan failure. Because of its heterogeneous presentation and unpredictable course, clinical management of SLE remains one of the greatest challenges to physicians. Despite decades of advances in our understanding of SLE pathogenesis, few biomarkers for SLE have been validated and widely accepted [1**,2**]. The lack of reliable, specific biomarkers for SLE not only hampers precise assessment of disease activity and prompt identification of patients at risk for flares and organ damage, but also impedes accurate evaluation of responses to treatment [3]. Comprehending the urgent need for lupus biomarkers, the Food and Drug Administration and the National Institute of Arthritis and Musculoskeletal and Skin Diseases have recently convened several strategic meetings and established a Lupus Biomarker Working Group to facilitate identification and validation of such markers. This article reviews briefly the historical background for biomarkers and summarizes recent developments in the continuing search for biomarkers of SLE.

Biomarkers: definition and potential utility in systemic lupus erythematosus
A biomarker can be defined as a genetic, biologic, biochemical, or molecular event whose alterations correlate with disease pathogenesis or manifestations that can be evaluated qualitatively or quantitatively in laboratories [1**,4]. Several methodologic criteria are required for a laboratory measure to serve as a reliable biomarker for a disease, including the following: it must be biologically and pathophysiologically relevant; it must be simple for routine practice; and it must accurately and sensitively respond to changes in disease activity (see Illei et al. [1**,2**] for an extensive review).
Due to the heterogeneous and complex nature of SLE, it is unlikely that a single biomarker will demonstrate universal utility. Pertinent biomarkers have several potential uses in SLE [1••,2••]. For example, some may be used to facilitate accurate and early diagnosis of SLE; some may help identify individuals prone to develop SLE or patients at risk for severe disease and poor prognosis; some may be useful in determining disease severity or monitoring disease progression; some may indicate systemic or specific organ involvement; and some may be used to evaluate response to treatment.

Classification of potential lupus biomarkers
Although the etiology of SLE is still incompletely understood, numerous lines of evidence support that genetic, hormonal, and environmental factors are clearly involved [5,6]. Clinical manifestations of SLE are likely the consequence of an immune-inflammatory process, which evolves in different phases of the disease and is characterized by production of autoantibodies and activation of the complement system [6]. Based on these etiopathogenic features of SLE, we will categorize and discuss potential lupus biomarkers in different classes.

Biomarkers for susceptibility
Genetic factors clearly play a major role in predisposing an individual to development of SLE. The genetic susceptibility is clearly influenced by environmental factors, although these triggers remain poorly identified. A recent study showed that autoantibodies are present in patients with SLE many years before the development of clinical symptoms [7]. Moreover, the emergence of various autoantibodies appears to follow a preset course while patients are asymptomatic [7]. These observations suggest that the immune system of genetically predisposed individuals is inherently perturbed. Recent searches for ‘SLE genes’, primarily through candidate gene-association studies and genomewide linkage analyses, have clearly demonstrated that no single gene is responsible for causing SLE. Multiple genes are involved, perhaps in a hierarchical, interactive manner, to trigger disease onset [8•,9•]. Initial genetic studies focused on genes that are historically considered to be key components of immune responses, such as major histocompatibility complex genes. Specific alleles of major histocompatibility complex class II have been found to be associated with SLE in ethnic groups [10,11].

Currently, the loci that most warrant being referred to as ‘SLE genes’ are those encoding the components of the classical complement pathway [12]. Complete complement deficiency is a rare cause of SLE, however. Recent genetic studies of SLE have focused on correlating SLE (susceptibility, disease spectrum, and severity) with polymorphisms of hypothetical candidate genes that encode mannose-binding lectin [13], cytokines (e.g. interleukin-6, interleukin-10, tumor necrosis factor [TNF]-α, and osteopontin) [14–17], chemokines (e.g. monocyte chemotactrant protein-1) [18], cytokine receptors/antagonists (e.g. type II TNF-α receptor and interleukin-1 receptor antagonist) [19,20], Fc receptors (e.g. FcγRIIa, FcγRIIB, and FcγRIIA) [21–24], and other cell surface receptors (e.g. cytotoxic T-lymphocyte antigen-4 and programmed death protein-1) [25,26]. In some cases, reports have not been consistent among different laboratories, possibly reflecting heterogeneous genetic susceptibility influenced by ethnicity and environment. Recently, genomewide linkage analyses using families with multiple SLE cases and sibpairs have identified several SLE susceptibility loci and positional candidate genes, such as genes encoding C-reactive protein, pre-B-cell leukemia transcription factor, and polyadenosine-diphosphate-ribosyl transferase [27,28]. These studies have recently been summarized in several excellent reviews [8•,9•,29].

In conclusion, the field of SLE genetics holds great promise. It has not yet sufficiently matured to have provided practical and universal genetic biomarkers for SLE susceptibility, however.

Biomarkers for diagnosis
Once clinical symptoms have developed, prompt diagnosis and proper management of SLE remain great challenges to physicians. The current standard approach to the diagnosis of SLE relies on revised American College of Rheumatology criteria published in 1982 [30,31], but this approach is problematic in routine clinical practice. For example, development of four of 11 criteria for a definite SLE diagnosis may take years to decades in some patients. Such delay in diagnosis of SLE may deprive patients of timely treatment and predispose them to irreversible organ damage. Laboratory tests or biomarkers that facilitate early and accurate diagnosis of SLE are essential.

Traditionally, determination of autoantibodies such as antinuclear antibodies, antiretractable nuclear antigen antibodies (e.g. anti-Ro/SSA, anti-La/SSB, anti-snRNP, and anti-SM), and anti-double-stranded DNA (dsDNA) are used in diagnosing SLE. There are considerable drawbacks to use these immunologic markers, however (see Reveille [32•] for further discussion). In search of biomarkers with better specificity and sensitivity for SLE diagnosis, Manzi et al. [33••] recently investigated the possibility that abnormal levels of erythrocyte-bound complement activation product C4d (E-C4d) and complement receptor 1 (E-CR1) may serve this purpose. Using flow cytometric analysis, these investigators showed that patients with SLE had significantly higher E-C4d and lower E-CR1 levels than did patients with other autoimmune disease or healthy controls [33••]. The E-C4d/E-CR1 test was shown to be 81% sensitive and 91% specific for SLE vs healthy controls and 72% sensitive and 79% specific for SLE vs other diseases, with an overall negative predictive value of 92%. It was also estimated that 86% of
the patients with SLE had abnormal E-C4d/E-CR1 level at the time of the study visit, compared with 47% who had a positive anti-dsDNA test at the same visit \cite{33**}. These data suggest that simultaneous determination of E-C4d and E-CR1 by flow cytometry may have significant impact on the accuracy and timing of diagnosis of SLE and should be studied further as a lupus diagnostic biomarker.

**Biomarkers for disease activity**

Currently, disease activity in SLE is often assessed using composite disease activity indices, such as SLE Activity Index (SLEDAI), Systemic Lupus Activity Measure (SLAM), European Consensus Lupus Activity Measure (ECLAM), and British Isles Lupus Assessment Group (BILAG), which comprise a variety of clinical and laboratory parameters \cite{34,35}. These indices are exclusively used by academicians and lupus aficionados, however. They are too complex and cumbersome for use in routine clinical practice.

Autoantibody production and complement activation are considered two of the hallmark features of SLE, and laboratory measures of complement and autoantibodies are components of most disease indices. Numerous studies have been conducted to identify the associations of various autoantibodies (particularly anti-dsDNA) and complement proteins (including native molecules and activation products) with disease activity/severity in SLE. The results, however, are inconsistent \cite{36–41}. These inconsistent results may also confound the assessment of disease activity with the widely used disease indices. Consequently, the value of conventional tests measuring serum complement and autoantibodies as markers of SLE disease activity is being revisited. Several recent reviews have summarized the controversial issues concerning traditional markers and provided insightful perspective \cite{42,43}. This section focuses on several potential candidate biomarkers that have recently emerged.

Recent studies of murine lupus models and human lupus have suggested that B cells play a central role in the pathogenesis of SLE \cite{44,45}. A cardinal change reflecting B-cell abnormalities is alteration of peripheral B-cell homeostasis. In adult SLE patients, the frequency and absolute numbers of CD19^+CD20^+CD27^high plasma cells decreased significantly, whereas activation of type I interferon resulted in increased severity of renal disease and autoantibody production \cite{56}. These observations have led to a rejuvenated interest in the role of interferon in SLE (see Baechler et al. \cite{51*}, Crow and Kirou \cite{52*}, and Ronnblom and Alm \cite{58} for excellent reviews).

Several investigators have recently used gene-profiling methods to investigate interferon-α-induced genes as an alternative approach for assessing the role of interferon in SLE \cite{50,59–61}. To identify pathways that might be dysregulated in peripheral blood mononuclear cells of patients with SLE, Baechler et al. \cite{50} used microarray
techniques to study gene expression profiles in SLE patients and found a striking pattern of upregulated interferon-inducible genes (subsequently termed ‘interferon signature’) in a subset of SLE patients. They further observed that the interferon signature predicts more severe disease, such as cerebritis, nephritis, and hematologic involvement, in those patients. Similar findings have been reported by three other groups [59–61]. A recent study using quantitative real-time polymerase chain reaction analysis further confirmed that interferon-α is primarily responsible for inducing the observed interferon signature gene expression pattern by peripheral blood mononuclear cells from SLE patients [62]. Taken together, these data suggest that overexpression of interferon-inducible genes is a prominent and reproducible phenomenon in patients with SLE, especially those with active/severe disease. A longitudinal study is focusing on following changes in the interferon signature and other gene expression patterns in patients with SLE and determining whether such changes correlate or predict clinical outcomes such as disease flares, new organ involvement, and response to treatment [51*]. These upcoming results should yield insights on the candidacy of the interferon signature as a biomarker for SLE disease activity.

In recent years, a growing list of humoral factors, including cytokines (e.g. interleukin-6, interleukin-10, interleukin-16, and interleukin-18) [63–66], soluble cytokine receptors (e.g. soluble interleukin-2 receptor) [67], soluble adhesion molecules (e.g. soluble intercellular adhesion molecule and soluble vascular cell adhesion molecule) [68,69], acute-phase proteins (e.g. C-reactive protein and ferritin) [70], autoantibodies (e.g. antinucleosome and anti-C-reactive protein) [71], and soluble thrombomodulin [72,73], has been investigated in terms of their associations with the activity and severity of SLE (Table 1). Hopefully, this promising array of candidates will yield several validated SLE biomarkers.

### Table 1. Potential biomarkers for systemic lupus erythematosus

<table>
<thead>
<tr>
<th>Category</th>
<th>Marker</th>
</tr>
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<tbody>
<tr>
<td>Disease susceptibility</td>
<td>Complement deficiency</td>
</tr>
<tr>
<td></td>
<td>FcγRlla polymorphism</td>
</tr>
<tr>
<td></td>
<td>FcγRllb polymorphism</td>
</tr>
<tr>
<td></td>
<td>FcγRlla polymorphism</td>
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<tr>
<td></td>
<td>MBL Polymorphism</td>
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<td></td>
<td>MHC class II alleles</td>
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<td></td>
<td>Interleukin-10, Interleukin-6, TNE-α polymorphism</td>
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<tr>
<td></td>
<td>TNFR, Interleukin-1Ra polymorphism</td>
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<tr>
<td></td>
<td>PD-1 polymorphism</td>
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<td></td>
<td>CTLA-4 polymorphism</td>
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<tr>
<td></td>
<td>Interferon signature</td>
</tr>
<tr>
<td>Disease diagnosis</td>
<td>Anti-dsDNA</td>
</tr>
<tr>
<td></td>
<td>Serum complement levels: C3, C4</td>
</tr>
<tr>
<td>Disease activity</td>
<td>Anti-dsDNA</td>
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<tr>
<td></td>
<td>Serum complement and activation product levels</td>
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<tr>
<td></td>
<td>C3, C4, C3a, C5a, C3d, C4d, Bα, Bβ, sC5b-9</td>
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<td></td>
<td>Serum cytokine levels</td>
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<tr>
<td></td>
<td>interleukin-6, interleukin-10, interleukin-12, interleukin-13, 16,</td>
</tr>
<tr>
<td></td>
<td>interleukin-15, interleukin-16, interferon-α, interferon-γ, TNF-α</td>
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<tr>
<td></td>
<td>Soluble cytokine receptors</td>
</tr>
<tr>
<td></td>
<td>interleukin-2R, TNFR, interleukin-1Ra</td>
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<tr>
<td></td>
<td>Soluble cell surface molecules</td>
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<tr>
<td></td>
<td>CD27, CD154, BAFF (BlyS)</td>
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<tr>
<td></td>
<td>Endothelial activation markers</td>
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<tr>
<td></td>
<td>Soluble ICAM, sVCAM, thrombomodulin</td>
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<td></td>
<td>Acute phase proteins</td>
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<td></td>
<td>CRP, ferritin</td>
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<tr>
<td></td>
<td>Other autoantibodies</td>
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<tr>
<td></td>
<td>Antinucleosome, anti-C1q</td>
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<tr>
<td></td>
<td>Cellular markers</td>
</tr>
<tr>
<td></td>
<td>CD27, CD154, FcγRIIa</td>
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<tr>
<td></td>
<td>Antibody working group markers</td>
</tr>
<tr>
<td></td>
<td>Erythrocyte-C4d</td>
</tr>
<tr>
<td>Specific organ (renal involvement</td>
<td>Anti-dsDNS</td>
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<tr>
<td></td>
<td>Anti-C1q</td>
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<tr>
<td></td>
<td>Anti-nucleosome</td>
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<tr>
<td></td>
<td>Urinary sVCAM and MCP-1 levels</td>
</tr>
</tbody>
</table>

This table is not meant to be exclusive. Some of the less-studies markers are not listed.

*Markers identified as priority candidates for validation by the Lupus Biomarker Working Group.

### Biomarkers for specific organ involvement

Systemic lupus erythematosus can affect virtually any tissue and organ. Not all organs are affected simultaneously, however, and involvement of a specific organ will not necessarily be manifested the same in all patients. It would therefore be beneficial if physicians could determine or predict organ-specific disease in SLE. Of the myriad manifestations of SLE, nephritis is a cause of significant morbidity and mortality. It is present in 25% to 50% of patients with SLE [74]. Anti-dsDNA has traditionally been used as a serologic indicator to follow the development and severity of lupus nephritis, although there are mixed opinions regarding its utility. Recently, investigators have explored the use of other autoantibodies in monitoring and preferably predicting renal disease in patients with SLE. Among those autoantibodies, antichromatin/nucleosome antibodies [75] and anti-C1q antibodies [76] have been extensively studied and have shown promise as new measures for renal involvement.

Chromatin, the DNA-histone complex found in the nucleus of eukaryotic cells, is organized into a repeating series of nucleosomes. Recent studies have demonstrated that the nucleosome is a major autoantigen targeted by T and B cells in SLE [77]. Antinucleosome antibodies are reportedly present in 70% to 80% of patients with SLE and have a high specificity (up to 97%) for SLE.
These antibodies have also been found in patients who tested persistently negative for anti-dsDNA antibodies. In one study [79], 60% of the patients who were tested positive for antinucleosome antibodies but negative for anti-dsDNA antibodies were shown to have renal disease, suggesting that antinucleosome antibodies may serve as a sensitive marker of renal involvement in the absence of anti-dsDNA. Several studies have shown a strong correlation between antinucleosome antibodies and SLE disease activity measured by SLEDAI or ECLAM [75,79].

Anti-C1q antibodies can be found in a small proportion of healthy individuals and have been commonly found in patients with autoimmune disorders such as hypocomplementemic urticarial vasculitis and SLE. The prevalence of anti-C1q in patients with SLE ranges from 34% to 47% [76]. Elevated serum concentrations of anti-C1q antibodies have been found in patients with SLE, compared with healthy controls [80], and a strong correlation between the presence of anti-C1q antibodies and renal involvement in SLE has been reported [81,82]. The absence of anti-C1q antibodies has been reported to exclude a diagnosis of lupus nephritis [83], and an increase in anti-C1q antibodies has been suggested to predict renal flares [81]. Positive predictive value and negative predictive value of anti-C1q antibodies for lupus nephritis have been reported to be 58% and 100%, respectively [81]. In several studies, anti-C1q antibodies were shown to correlate with active lupus nephritis with a sensitivity of 44% to 100% and a specificity of 70% to 92% [81,83]. Moreover, a pathogenic role for anti-C1q antibodies in lupus nephritis has been suggested by detection of anti-C1q antibody deposited in the kidneys [84] and by induction of renal disease in mice with anti-C1q antibodies and immune complexes [85*].

Recently, Marto et al. [86] conducted a cross-sectional study to investigate the correlation between anti-C1q titers and renal disease activity in 151 patients with SLE. They found a higher prevalence of anti-C1q antibodies in patients with active nephritis than in those with no renal disease (74% vs 32%); the anti-C1q levels were higher in patients with nephritis than in those without nephritis. Interestingly, it was also shown that anti-C1q antibodies could be detected in 39% of SLE patients without history of renal disease and that 27% of those patients developed lupus nephritis within a median interval of 9 months. These results confirm the previously reported strong correlation between the presence of anti-C1q antibodies and lupus nephritis, and they further suggest that anti-C1q determination may provide a useful means to monitor renal involvement or predict renal flares.

**Conclusion**

The greatest challenge in identifying and developing specific biomarkers for SLE is the complex etiopathogenesis and clinical heterogeneity of SLE. Reliable SLE biomarkers may be informative at different time points in the disease process, such as at diagnosis, during the course of the inflammatory phase of the disease, in assessment of end-organ damage, or in evaluation of response to treatment. Moreover, owing to the multifactorial nature of SLE, no single biomarker will emerge as 'the lupus biomarker'. The rapid progress we are witnessing in this field will most likely lead to a validated 'lupus biomarker panel' that will include assays for individual molecules as well as 'molecular biosignatures' [87].

The attempt to discover useful biomarkers for SLE has traditionally been conducted based on hypothesis-driven approaches, i.e. to investigate a single or a small number of factors (e.g. genes, autoantibodies, and cytokines) that are thought to be important in the underlying pathophysiologic mechanisms. Although these approaches have yielded many putative biomarkers, no biomarkers have been validated to date. Advances in high-throughput technology during the past decade have undoubtedly opened new avenues to efficiently discover biomarkers for SLE. In this new research arena, several fields are likely to be the main 'mines' of biomarkers for autoimmune diseases and will particularly attract investigators’ attention. These fields include gene transcription profiling using RNA extracted from cells/tissues of patients in conjunction with DNA microarray or polymerase chain reaction techniques; autoantibody profiling using autoantigen arrays, and signaling pathway and cytokine/chemokine expression profiling using DNA and protein microarrays, flow cytometry, and proteomics techniques [88*]. Several research groups have already begun to use these technologies to perform large-scale screen assays with an attempt to identify potential biomarkers or biosignatures for SLE [50,52*,59,61]. Initial results have been encouraging and point to great cause for optimism in the lupus biomarker arena.

**Acknowledgements**

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Papers of particular interest, published within the annual period of review, have been highlighted as:

- of special interest
- - of outstanding interest


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Biomarkers for systemic lupus erythematosus


An excellent review on the role of interferon in the pathogenesis of SLE. Ongoing interesting studies of interferon signature are discussed.


Another excellent review linking interferon and the pathogenesis of SLE.


An inspiring review on the use of high-throughput technology in discovering candidate biomarkers for SLE.
Rituximab anti-B-cell therapy in systemic lupus erythematosus: pointing to the future
Petros P. Sfikakis, John N. Boletis, and George C. Tsokos

Purpose of review
To discuss the clinical effects and the immunologic consequences of transient B-cell depletion using the anti-CD20 monoclonal antibody rituximab in systemic lupus erythematosus.

Recent findings
A total of 100 rituximab-treated patients with severe disease, refractory to major immunosuppressive treatment, have been reported so far. Within a median follow-up period of 12 months rituximab was well tolerated, which is compatible with the experience accumulated from its use in more than 500,000 lymphoma patients. About 80% of patients achieved marked and rapid reductions in global disease activity. Because of the clinical heterogeneity, dosing differences, and concomitant treatments, including cyclophosphamide in 35% of patients, a proper evaluation of the clinical efficacy of rituximab is difficult. Variable degrees of clinical benefit have been reported for all clinical systemic lupus erythematosus manifestations, including active proliferative nephritis. Whereas 4-weekly infusions of 375 mg/m² of rituximab result in complete B-cell depletion lasting most often from 3 to 8 months, a prolonged depletion does not always correlate with a more favorable clinical response. Total immunoglobulin levels and protective antibodies are preserved, but anti-dsDNA antibody titers decrease, often independently of the clinical response.

Summary
The findings reviewed point to a growing optimism for targeting B cells in the treatment of systemic lupus erythematosus; therefore double-blind studies comparing rituximab with existing immunosuppressive therapies are needed. Moreover, careful assessments of the effects of transient B-cell depletion on distinct autoimmune pathogenetic processes will enable optimization of therapeutic single or combined therapeutic schemes.

Keywords
B cell, nephritis, rituximab, systemic lupus erythematosus, T cell

Abbreviations
BLyS B-lymphocyte stimulator
HACA human antichimeric antibody
SLE systemic lupus erythematosus

Introduction
Systemic lupus erythematosus (SLE) B cells produce antibodies directed against self-antigens to form immune complexes that deposit to tissues and, with the help of local factors, instigate an inflammatory process [1,2,3]. SLE B cells are hyperactive following the engagement of the B-cell receptor that may be driven not only by extrinsic factors but also by intrinsic defects resulting in an excessive response to immunologic stimulation [2,4,5]. In addition to the production of autoantibodies, B cells have a central role in the activation of the immune system through the production of various cytokines and serving as potent antigen-presenting cells [6]. Therefore, a drug that specifically targets B cells may represent an ideal therapeutic approach for SLE patients. Indeed, interest is increasing in the exploration of the therapeutic potential of rituximab in SLE [7] and other autoimmune systemic diseases [8].

Rituximab is a chimeric monoclonal antibody against the B-cell marker CD20. CD20 is expressed throughout the stages of B-cell development, but not on plasma cells, and provides a stable target for rituximab because it is neither internalized nor shed [9]. Rituximab was approved for the treatment of B-cell malignancies in 1997 and has been administered to more than 500,000 lymphoma patients, with a highly acceptable safety profile. The biology of CD20 and the mode of action of rituximab have been recently reviewed in detail elsewhere [9]. Here we briefly review the information generated in murine models of SLE following B-cell depletion and present critically the published experience on the use of rituximab in patients with SLE. In addition, we present immune studies performed in patients who received rituximab that provide additional in-vivo insights on the role of B cells in the pathogenesis of human SLE.

Lessons from lupus animal models
The hypothesis that transient B-cell depletion can provide therapeutic benefit to SLE patients is supported by experiments indicating that Fas-intact, MRL lupus mice in a B-cell-deficient background do not develop...
nephritis at a time when the B-cell-intact littermates have severe disease [10]. B cells that cannot secrete circulating immunoglobulin in genetically engineered MRL/lpr lupus mice, however, can still induce glomerulonephritis, suggesting that antibodies are not required for the development of the disease [11]. Also, breaking tolerance to dsDNA and chromatin is not required for the pathogenesis of chronic glomerulonephritis in the NZM2328 lupus-prone mouse [12]. These studies confirm that aberrant interactions between autoreactive B and T cells are crucial for the development of autoimmune tissue injury and that this process is independent of the production of autoantibodies. Moreover, experiments in lupus-prone MRL-lpr/lpr mice that lack B cells indicate that expansion of activated T cells is highly B cell dependent [13]. Along these lines, experiments in rituximab-treated immunodeficient mice that were implanted with human rheumatoid synovium have shown that local T-cell activation depends on the presence of B cells [14]. These results are compatible with the notion that B cells exert an important pathogenic role as autoantigen-presenting cells supporting the activation of autoreactive T cells [6,15].

Although the lifespan of autoantibody-producing plasma cells is not known for any human autoimmune disease, recent experiments in lupus-prone mice have shown that certain plasma cells are short or long lived. Even following antiproliferative immunosuppressive therapy, the long-lived, nondividing plasma cells survive and continue to produce autoantibodies [16*]. Because CD20 is not expressed on autoantibody-producing long-lived plasma cells, rituximab may not remove such existing cells. In a murine model for human CD20 expression, in which treatment with rituximab mimics B-cell depletion observed in humans, distinct sensitivities of B-cell subsets to rituximab depend on factors derived from the cellular microenvironment, including the B-lymphocyte stimulator (BlyS) survival factor [17*]. Because an anti-BlyS monoclonal antibody (belimumab) is currently under study in patients with autoimmune disease [18*], such experiments advance our understanding on the potential of combined anti-B-cell therapy in lupus patients. We can foresee that rituximab will be the proper drug to induce remission in patients with active disease (induction phase), whereas biologic agents such as belimumab will be sufficient to consolidate the therapeutic effect (consolidation phase).

**Rituximab for difficult systemic lupus erythematosus: clinical efficacy**

Until March 2005, 90 SLE patients were reported, either in full-length papers [19,20*,23*,24–28,29*,30–35] or in abstract form [36*,37*,38,39*,40*], to have received rituximab. Three children with severe lupus [28,34] are also included among the total of 56 patients in published series. Most of the patients had severely active disease, refractory to standard treatments; about 50% were treated for proliferative lupus nephritis (Table 1). Results from three open-label, uncontrolled, prospective phase I/II studies from the Universities of London, UK [19], Rochester, NY [20*], and Athens, Greece [21*] have been published so far, and the publication of results from two additional studies is pending [36*,39*]. Of note, rituximab has already been used off label in routine clinical practice and 13 patients not participating in any study have received rituximab for refractory SLE, according to a retrospective survey of 866 rheumatologists and internists in France only [22*].

Because the rituximab-treated patients were highly heterogeneous in disease severity, organ involvement, and previous treatments, as well as because different rituximab doses were used and concomitant therapies varied, a proper evaluation of the clinical benefits of transient B-cell depletion per se is difficult. Collectively, about 40% of the patients have received rituximab at the dose that has been approved for lymphoma patients, i.e. 4-weekly infusions of 375 mg/m². Also, about 35% of the reported patients received concomitant cyclophosphamide. Clinical outcome has been assessed by standard disease activity measures (Systemic Lupus Erythematosus Disease Activity Index [SLEDAI], Systemic Lupus Activity Measure [SLAM], or British Isles Lupus Assessment Group [BILAG] Index) in most patients. Rituximab administration was associated with clinically meaningful decrease in global lupus disease activity in 80% of the patients (Table 1). The reported follow-up periods range from 3 [38] to 46 months in one patient [40*], but for most of the patients follow-up data do not exceed the 12 months. As summarized here, considerable clinical efficacy on distinct clinical manifestations has been observed, including remission of active, proliferative nephritis.

**Lupus nephritis**

Collectively, the rate of clinical remission among the reported patients who received rituximab for active, lupus nephritis is about 80% (Table 1). Following the promising results in the first five lupus patients reported by Leandro et al. [19], these investigators have treated more patients [37*,40*]. Six patients with active nephritis (five with World Health Organization class IV and one with class V) refractory to intravenous cyclophosphamide received two doses of 1000 mg of rituximab, combined with two doses of 750 mg of cyclophosphamide and high-dose corticosteroids over a 2-week period. All six patients improved, with four showing major improvement. Serum creatinine and urine protein/creatinine ratio decreased by a mean of 23% and 63%, respectively, at 3 months and by 23% and 14% at 6 months. Available follow-up data include one patient who had a relapse at 6 months and was retreated and two patients who remained nephritis free 24 months later [37*].

Looney et al. [20*] described the clinical outcome following administration of three different dosing regimens of
rituximab combined with moderate doses of corticosteroids in 16 patients with SLE, including seven with nephritis (three with World Health Organization class III, three with class IV, and one not classified). In all 10 patients who achieved complete B-cell depletion, disease activity improved, in contrast to those six whose B cells did not deplete and who did not have a clinical benefit. Creatinine levels remained within 20% of baseline values in all seven patients with nephritis throughout the 12 months of observation. One patient with class IV nephritis...
showed a major clinical improvement; complete resolution of renal proliferative changes was confirmed by histology 12 months after rituximab treatment [20*]. Similar impressive responses, also confirmed by renal biopsy, were reported in two patients with lupus nephritis refractory to standard cyclophosphamide treatment, who were treated with the full rituximab dose plus two infusions of cyclophosphamide [24]. In two other patients, complete remission of active nephritis was observed following administration of only two weekly infusions of 375 mg/m² rituximab, despite the fact that complete B-cell depletion was not achieved [23*]. Several additional patients with active lupus nephritis who were treated with rituximab alone [38,39*], or in combination with cyclophosphamide [27,29*,36*], achieved significant clinical remission.

In a recent study, 10 patients with active, proliferative nephritis (four with World Health Organization class III and six with class IV) received the full rituximab dose combined with oral prednisolone (0.5 mg/kg/d for 10 weeks, tapered by 4 mg every 2 weeks thereafter). Complete remission of nephritis was defined as normal serum creatinine and albumin levels, inactive urine sediment, and 24-hour urine protein less than 500 mg. Partial remission was defined as more than 50% improvement in all baseline abnormal renal parameters. Eight of 10 patients achieved partial remission within a median of 2 months, in five of whom complete remission was subsequently established (median of 4 months after baseline) and sustained at 12 months in four patients [21*]. Unlike in other studies [19,36*,37*], rituximab was not combined with cyclophosphamide, whereas moderate doses of corticosteroids were used with a rather fast tapering. Moreover, the median time to achieve complete remission of nephritis was 4 months, which is substantially shorter compared with patients who receive the standard combination of steroids and cyclophosphamide; therefore, it is likely that beneficial responses were indeed driven by rituximab. Although three patients relapsed after achieving remission, four patients remained completely free from active lupus disease at 12 months after treatment without immunosuppressive medication or small residual doses of prednisone [21*].

**Extrarenal manifestations**

In most patients with lupus nephritis as described here, the clinical response of extrarenal manifestations such as rash, arthritis, serositis [19,20*,21*], or central nervous system disease [23*] paralleled the nephritis response. As shown in Table 1, a beneficial effect has also been described in patients without nephritis in whom lupus disease was refractory to conventional immunosuppressive treatment [25,26,28,30,32,36*,39*,40*]. Musculoskeletal manifestations, skin ulcers, and malar rash responded well, and diffuse alopecia responded surprisingly well in one study [20*]. Thrombocytopenia [21*,25,30,34,35,40*] and haemolytic anaemia [23*,29*,33,40*] also responded in most. Notably, all of the seven patients described in published reports with central nervous system disease responded well to rituximab treatment without concomitant cyclophosphamide treatment [23*,26,30].

**Transient B-cell depletion in systemic lupus erythematosus: safety issues**

Opportunistic infections and permanent ovarian failure, occurring in 15% of women [41], are the leading causes of morbidity associated with intravenous cyclophosphamide, the current treatment standard for patients with severe lupus disease. Taking into account the experience from the extensive use of rituximab in lymphoma patients [42], as well as in patients with rheumatoid arthritis who participated in a randomized, controlled study [43*], it is clear that this treatment is far more benign than cyclophosphamide. Indeed, rituximab was very well tolerated in about 90% of lupus patients reported on so far, at least within the available follow-up extending to 12 months for the vast majority of them.

Despite standard premedication with phenylfrelamine or hydrocortisone, hypersensitivity reactions during infusion occurred in approximately one of every 10 treated patients [20*,21*]; such reactions always resolved with proper treatment. An episode of delayed-type hypersensitivity was reported in one patient [35]. During the period of B-cell depletion, serious adverse effects have been observed infrequently, which is compatible with the large safety data set accumulated from the use of rituximab in more than 500 000 patients with lymphomas so far [42]. Major infections, successfully treated with antibiotics, were observed in four patients [20*,21*], but it is not certain whether rituximab-induced B-cell depletion was entirely responsible. Perhaps the incidence of infections in lupus patients is higher than the value of less than 5% that is observed in patients treated for lymphomas [42]. Selection of patients with severe lupus disease, including patients with severely low complement levels or leukopenia, who had been previously or concomitantly treated with major immunosuppressive regimens, may represent additional confounding factors.

Safety issues regarding long-term maintenance therapy or repeated rituximab administration for patients with refractory disease, and for those who have a relapse after achieving remission, have yet to be defined. So far, fewer than 10 lupus patients have received a second course of rituximab treatment; no significant side effects were reported [26,28,40*]. Because rituximab is a mouse/human chimeric antibody, generation of anti-immunoglobulin response (human antichimeric antibodies; HACAs) may occur. Despite the fact that HACAs develop in less than 1% or 5% of patients treated for lymphomas [42] or rheumatoid arthritis [43*], respectively, 30% of lupus patients treated by Looney et al. had detectable HACAs in their serum.
This response was associated with lower levels of rituximab, less depletion of B cells, African ancestry, and higher lupus disease activity at baseline [20*]. Although such antibodies are not associated with clinical symptoms [20*,42], following repeated rituximab administration they may contribute to undesirable adverse effects or they may affect B-cell depletion. One such patient with HACAs received a new humanized anti-CD20 monoclonal antibody that is currently being used in a phase I/II trial for lymphomas, and effective B-cell depletion was achieved [29*].

Immunologic consequences of rituximab administration: correlations with clinical outcome provide insights into immunopathogenesis of lupus

Following the onset of B-cell depletion, marked changes in several aspects of the (auto)immune response have been observed. Correlating these changes with the rituximab-induced clinical benefits may lead to a better understanding of the pathogenetic mechanisms leading to tissue damage.

B cells

Rituximab destroys B cells via complement-dependent cytotoxicity, antibody-dependent effector cell-mediated cytotoxicity, and apoptosis when cross-linked by Fc-γ receptor-expressing cells [9*]. An inherited low-affinity allele of Fc-γ receptor IIIa may limit the degree of B-cell depletion in patients with SLE [44]. Complete B-cell depletion in the peripheral blood, i.e. nondetectable CD19+ cells, however, occurs almost always when the full rituximab regimen is given, even without cyclophosphamide [20*,21*,25,26,32,34,35]. Whether complete depletion of B cells occurs also in the lymphoid organs is not known at present. Depletion of splenic B cells was evident in a single lupus patient at 1 month after rituximab administration [25]; however, splenectomy was performed due to inadequate clinical response, suggesting that the cellular impact of this intervention is indeed complex [7**].

The average duration of complete depletion of peripheral B cells ranges from 3 to 8 months in the published series [19,20*,21*]. Exceptional patients achieving short periods of depletion, such as only 1 month [21*], and patients with prolonged periods exceeding 3 years [40*], have been reported. Moreover, the kinetics of repopulation differ remarkably among patients treated with rituximab monotherapy [21*,45*]. Although further study is required, it appears that lupus patients achieve shorter periods of depletion compared with patients treated for lymphomas. A more prolonged B-cell depletion seemed to influence favorably the clinical outcome of nephritis; however, no consistent pattern of correlation between the clinical outcome and the degree of depletion or kinetics of B-cell reconstitution was revealed among the 10 patients who were treated with the full rituximab dose as monotherapy [21*].

Anolik et al. [45*] reported significant improvements in several abnormalities in peripheral B-cell homeostasis following rituximab administration in their dose-escalation study. Naïve B-cell lymphopenia, expansion of CD27+ IgD+ B cells and expansion of circulating plasmablasts resolved after regeneration of the peripheral B-cell pool, whereas the frequency of memory B cells bearing VH4.34, which may have autoreactive properties, decreased 1 year after treatment. Moreover, in five patients who received half the rituximab dose and did not achieve complete B-cell depletion, a marked reduction of both CD40 and CD80 costimulatory molecules expression on B cells was found on serial phenotypic assessments of residual B cells [23*]. A sustained clinical remission in lupus patients after effective regeneration of the peripheral B-cell pool suggests that rituximab may downregulate lupus B-cell overactivity in the long term, at least in some patients.

Immunoglobulin levels and autoantibodies

Because plasma cells are spared following rituximab administration, hypogammaglobulinemia occurs rarely, and obviously it can be corrected by administration of intravenous gammaglobulin. In all studies, mean total serum IgG, IgM, and IgA levels decrease mildly to moderately but remain within normal limits [20*,21*,25,39*,40*]. Protective antibodies such as against tetanus toxoid [20*,25,43*], and antipneumococcal capsule polysaccharide [20*], or antibodies directed against measles, rubella virus, and diphtheria [25] are preserved, at least when rituximab is not given in combination with cyclophosphamide.

Reduction of autoantibody serum levels was seen in most patients treated with at least two infusions of rituximab without cyclophosphamide [20*,21*,25,26,28,30]. Increases in complement levels and associated reductions in circulating immune complexes also occurred [21*]. Among 22 such patients for whom published information is available [20*,21*,25,28,30,33,45*], increased anti-dsDNA levels at baseline dropped significantly within the first few months following rituximab administration in 17 patients (77%), suggesting that these antibodies are not produced only by long-lived, CD20+ plasma cells that escape rituximab. Similar results in patients who received rituximab treatment without cyclophosphamide have been reported, i.e. decreases in rheumatoid factors in patients with rheumatoid arthritis [43*], anticardiolipin antibodies in patients with antiphospholipid syndrome [28,46,47], as well as disappearance of antineutrophil cytoplasmic antibodies in patients with Wegener’s granulomatosis [48*].

In the study of 10 patients who were treated for lupus nephritis, the titers of anti-dsDNA antibodies decreased by twofold to several-fold in all patients, regardless of the
clinical outcome. Anti-dsDNA antibody titers became undetectable 4 months after treatment in the two patients in whom clinical remission was not achieved [21\*]. These findings are reminiscent of the results obtained in B-cell-depleted lupus mice described here and are in line with the results of a randomized, placebo-controlled trial in patients with lupus nephritis showing that the selective reduction of dsDNA autoantibodies following administration of abetimus sodium has no significant effect on renal flares [49]. As reported by Looney et al. [20\*], in rituximab-treated lupus patients, clinical responses can occur without concomitant changes in anti-dsDNA antibodies, as happened in patients who did not receive the full dose of rituximab. Taken together, these observations indicate that B cells play an important role in the expression of SLE pathology, and this effect may be clearly independent of the production of antibody and autoantibodies considered to have pathogenic value.

**T cells**

Peripheral T-cell subsets were prospectively assessed at monthly intervals for 12 months in 10 patients with lupus nephritis who received the full rituximab dose [21\*]. Within the first month, significant increases of CD8\(^+\) T cells and decreased natural killer cell numbers were observed. Also, the expression of the costimulatory molecule CD40-ligand on CD4\(^+\) T cells, which has been found overexpressed in lupus patients [50–52], decreased by fourfold and almost disappeared when partial remission was clinically evident. As suggested recently, patients with active lupus nephritis display immune abnormalities driven via CD40L / CD40 interactions [53]. Indeed, rituximab-induced downregulation of the expression of CD40L on T-helper cells preceded the clinical remission of nephritis, and it was almost blocked at the subsequent timepoints of partial remission. Moreover, this result was sustained in patients who progressed to complete remission [21\*]. In parallel, expression of the early T-cell activation molecule CD69, implicated in abnormal immune regulation in lupus [54], decreased significantly. The decrease in T-helper cell activation was further documented by downregulation of the HLA-DR late activation molecule [21\*], as well as by normalization of the increased levels of soluble interleukin-2 receptors (P.P. Sfikakis, unpublished), in patients who achieved clinical remission. In contrast, in patients who did not respond or in those who had relapses after an initial remission, such decreases were not prominent [21\*]. These findings suggest that an early downregulation of CD40L overexpression as a result of rituximab-induced B-cell depletion may prevent activation of lupus T cells through cognate interactions with CD40\(^+\) autoantigen-presenting cells.

**Conclusion**

Although the data are still limited, the findings reviewed here point to a growing optimism for targeting B cells in SLE. The accumulated evidence clearly suggests that rituximab monotherapy or rituximab used in conjunction with a classic immunosuppressive agent can be of therapeutic value in patients with severe disease. It appears that the short-term data are not shadowed by severe or morbid side effects. The high benefit/risk ratio thereby implicated may justify its use on a trial basis even if future complete studies demonstrate that only a fraction of SLE patients respond to rituximab.

We have learned also that B-cell depletion does not affect the levels of protective antibody in the short term although it decreases the levels of autoantibody. The data are quite limited to be interpreted, but they suggest that the requirements for the production of protective antibody and autoantibody are different. Although some of the clinical effect may be associated with the decrease in the autoantibody level, it also appears that B-cell depletion has a profound effect in the generation and preservation of activated T cells and, probably, in the presentation of autoantigen. Unlike lymphoma patients, SLE patients are expected to live long lives, and therefore the long-term effects of B-cell-depleting treatment should be evaluated carefully. It is possible that following repeated treatment with rituximab or other B-cell-depleting agents, the reservoir of CD20\(^-\) plasma cells will decrease with unpredictable rates of severe immunodeficiency.

It is obvious that properly controlled clinical trials are needed to determine accurately the clinical efficacy of treatment with rituximab. These trials should establish the proper drug dosage, the development of side effects, and whether certain SLE clinical subsets benefit more than others from rituximab treatment. The difficult questions that need to be addressed by trial planners are two-fold: how will the clinical efficacy be measured in view of the fact that proper surrogate endpoint SLE disease markers are still at large, and the inclusion of appropriate control treatment groups. Should rituximab be compared with cyclophosphamide treatment—at least in patients who under current practices would be treated with cyclophosphamide? Rituximab monotherapy was reported efficacious in patients with severe SLE. Should rituximab be tested for added value in patients receiving accepted treatment? Human use committees, at least in the United States, would be happier with this choice. The obvious limitation imposed by the heterogeneous nature of the disease can be overcome either by selecting clinically homogenous cohorts (e.g. only central nervous system or kidney disease) or by the inclusion of large numbers of patients to permit disease manifestation-targeted a-posteriori analysis. Finally, it should be kept in mind that rituximab may prove most efficacious when used in conjunction with other B-cell-targeted biologics used either simultaneously or sequentially.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• of special interest
•• of outstanding interest


A comprehensive review of recent data suggesting a central pathogenic role for B cells in SLE.


A literature review identifying 116 autoantibodies and describing their frequencies, clinical associations, and correlation with lupus disease activity.


A thoughtful discussion of the mechanistic aspects and the potential clinical benefits of rituximab therapy for patients with SLE.


An excellent review on the biology of CD20 and the effector mechanisms that operate following administration of rituximab.


This is an elegant demonstration of the lifespan of autoantibody-secreting cells in a lupus mouse model. A fraction of DNA-specific plasma cells are nondividing, long lived, and resistant to anti-proliferative immunosuppressive therapy.


Elegant studies demonstrating a central role of the B-cell survival factor BlyS for defective B-cell depletion in a murine model for human CD20 expression, in which treatment with rituximab mimics B-cell depletion observed in humans.


An excellent review on the role of the B-cell survival factor BlyS in SLE and on preliminary findings pointing to BlyS or its receptors as attractive therapeutic targets.


A dose-escalation study in 18 patients, demonstrating good tolerance, variability in the effectiveness of B-cell depletion, and clinical efficacy in patients who achieved complete depletion.


Full-dose rituximab given as monotherapy resulted in complete B-cell depletion and decreases in dsDNA autoantibodies in all 10 treated patients. Clinical remission in eight patients correlated with decreases in activation/costimulation status of T cells but not with autoantibody changes.


This paper describes short-term clinical efficacy and good tolerance of rituximab given off-label in routine clinical practice in France for rheumatoid arthritis, SLE, systemic sclerosis, vasculitis and polymyositis.


This pilot study describes high efficacy of rituximab as monotherapy in refractory SLE, as well as treatment-induced downregulation of CD40 and CD80 costimulatory molecules on B cells.


Effective B-cell depletion with an investigational humanized anti-CD20 monoclonal in one patient with severe resistant SLE who developed HACAs after rituximab treatment.


Successful and well-tolerated treatment in nine patients, including five with nephritis, with a median follow-up of 9 months.
   Improvement of nephritis with rituximab plus cyclophosphamide in six patients in whom intravenous cyclophosphamide failed.
   An ongoing, open-label trial showing good tolerance and substantial benefit of rituximab monotherapy-induced complete B-cell depletion in the first eight patients.
   The largest open-label study to date, showing that in patients with active refractory disease, B-cell depletion based on rituximab can be effective.
   The first randomized, double-blind, controlled study showing the efficacy and safety of rituximab in difficult rheumatoid arthritis. Large and rapid decreases in rheumatoid factor levels were observed, with preservation of IgG, IgM, and IgA immunoglobulin levels and antitetanus toxoid titers.
   Naïve lymphopenia, expansion of a CD27+ IgD+ B-cell population and expansion of circulating plasmablasts resolved after reconstitution of the B-cell pool following effective depletion with rituximab.
   An open-label study showing rituximab-induced clinical remission in all patients and significant decreases in antineutrophil cytoplasmic antibody titers.
Immunopathogenesis of primary Sjögren’s syndrome: implications for disease management and therapy

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Purpose of review
Recent studies have broadened our understanding of the etiopathogenesis and immunopathology of primary Sjögren’s syndrome. This review highlights recent advances in understanding the underlying mechanisms of the disease as well as their implications for clinical handling and therapeutic options.

Recent findings
It becomes increasingly apparent that certain disturbances of the immune system (i.e., B-cell hyperreactivity and enhanced levels of B-cell-activating factor/B-lymphocyte stimulator) play a central role in this entity. Whether this is a primary abnormality or the result of predisposing factors or infectious, e.g., viral, agents remains uncertain. New insights into the pathogenesis also provide candidates for better diagnosis and classification of disease severity, such as flow cytometric analysis, measurement of soluble cell surface molecules, autoantibodies, cytokines, and ligands (B-cell-activating factor/B-lymphocyte stimulator). Whether B-cell-directed therapies (i.e., blocking B-cell-activating factor/B-lymphocyte stimulator, anti-CD20 therapy) will have an impact on primary Sjögren’s syndrome needs to be shown in clinical trials. Alternative therapeutic approaches such as organ-targeted gene transfer are in development but must be carefully evaluated for safety and efficacy in preclinical models that resemble human primary Sjögren’s syndrome.

Summary
The pathogenesis of primary Sjögren’s syndrome is complex and the factors initiating and driving autoimmunity in this entity are largely unknown. Recent studies provide new insights into potential pathogenetic mechanisms of the disease and, thereby, the chance for improved strategies in disease management and therapy.

Keywords
animal models, immunopathogenesis, predisposing factors, serology, Sjögren’s syndrome, therapy

Abbreviations
BAFF B-cell activating factor
BLyS B-lymphocyte stimulator
pSS primary Sjögren’s syndrome
SLE systemic lupus erythematosus
TNF tumor necrosis factor

Introduction
Primary Sjögren’s syndrome (pSS) represents an autoimmune exocrinopathy characterized by both organ-specific and systemic manifestations. Chronic lymphocytic inflammation, impaired function, and finally destruction of the lacrimal and salivary glands resulting in keratoconjunctivitis sicca and xerostomia are the characteristic hallmarks of pSS. A variety of clinical and laboratory manifestations that may precede, accompany, or follow the glandular manifestations, however, emphasize that pSS is a systemic disorder. Moreover, pSS must be distinguished from Sjögren’s syndrome secondary to various other rheumatic diseases, such as rheumatoid arthritis and systemic lupus erythematosus (SLE), as well as from several other conditions presenting with sicca symptoms. Currently available immunologic data indicate that Sjögren’s syndrome may have distinct expressions. Whether this reflects different subtypes of the disease or distinct degrees of activity/severity needs consideration in further studies to improve classification for research purposes, disease management, and finally tailoring of potential immune therapies in Sjögren’s syndrome.

Environmental factors and genetic susceptibility
As with other complex multigenic and multifactorial autoimmune diseases, several infectious agents have been postulated to be involved in priming or triggering pSS [1–4, 5\textsuperscript{••}]. Associations with most of the potential candidates, e.g., the ubiquitous Epstein-Barr virus [2–4], however, remain rather weak. Conversely, reactivation of Epstein-Barr virus by chronic sialadenitis may be involved in perpetuating the entire inflammatory process in pSS [4]. Of note, a recent study has strongly implicated a possible role for coxsackieviruses (i.e., the A13 and B4 strains) in induction and maintenance of pSS [5\textsuperscript{••}]. Disturbed clearance from salivary gland epithelial cells [6,7] may lead to glandular persistence of these viruses and to chronic lymphocytic sialadenitis with subsequent formation of germinal
center-like follicles [5**]. It remains of interest whether chronic glandular infection with coxsackieviruses is primary or secondary in the development of autoimmunity in pSS patients or whether it depends on other environmental, hormonal, or hereditary factors or may also cause lymphocytic infiltrates of labial salivary glands in normal persons [8,9].

It is well established that hereditary susceptibility influences onset, development, and severity of pSS, although different genetic associations have been detected in different populations [3,10]. Recent studies aimed to detect additional potential associations between pSS and genetic polymorphisms. Homozygosity for the 168His variant of the minor histocompatibility antigen HA-1 has been recently found to be associated with reduced risk of pSS in three distinct white populations (Norwegians, Hungarians, Germans) [11*]. The −509 T genotype of the transforming growth factor-β gene, which has been associated with several disease states including rheumatoid arthritis [12], is probably not a major risk factor for pSS or SLE [13]. Moreover, apolipoprotein E polymorphism does not affect susceptibility to pSS or inflammatory indices but may be associated with early onset of pSS [14*]. Finally, a polymorphism of the tumor necrosis factor (TNF) gene (−308 A) [15,16] leading to enhanced TNF-α production might have an impact on susceptibility to renal manifestations as has been suggested by a preliminary study in pSS patients [16] but requires further evaluation. A potential role of complement (e.g. C1q) deficiency in the pathogenesis of Sjögren’s syndrome remains uncertain [17].

**Dysregulation of cellular and humoral immunity**

Although the processes that underlie autoimmunity in pSS are not known, disturbances of T and B lymphocytes as well as of glandular cells, e.g. ductal epithelial cells, are involved (Fig. 1). Several recent studies have documented a pivotal role of B cells in the ethiopathogenesis of pSS. B-cell activating factor (BAFF), also known as B-lymphocyte stimulator (BLyS), is a member of the TNF superfamily that regulates B-lymphocyte proliferation, maturation, and survival [18] but is also suggested to be an important factor in both local and systemic autoimmunity [19*,20–22, 23*,24,25*,26,27**]. Moreover, BAFF/BLyS emerged as a potent survival factor for B-cell malignancies [25*,26,27**]. Enhanced levels of BAFF/BLyS have been demonstrated in rheumatic diseases such as pSS, SLE, and rheumatoid arthritis, which are associated with abnormal B-cell function and autoantibody production [19*,20–22,23*,24,25*,26,27**]. Notably, the highest BAFF levels were found in pSS patients [20,21]. Local BAFF/BLyS expression [22] by infiltrating T cells and macrophages [23*] may be central in the progression of the entire autoimmune process by triggering B-cell hyperactivation and (auto)antibody production [21,23*]. BAFF/BLyS also appears to be intimately involved in T-cell-independent B-cell activation [25*]. The degree of this response in pSS, however, remains unclear. Studies in BAFF-transgenic mice revealed expansion of the marginal-zone B-cell population and enhanced T-cell-independent immune responses [27**] and documented that the effect of BAFF leading to autoimmunity is independent of TNF. In accordance, a recent study has
shown a clear antiapoptotic effect of BAFF/BLyS on peripheral blood B cells from pSS patients that might lead to prolonged B-cell survival in pSS [24]. Altogether, these studies strongly suggest an important role for BAFF/BLyS overproduction in the B-cell disturbances and malignant complications of pSS, although this pathogenic mechanism might not be unique for pSS.

Uregulation of CD72, a costimulatory molecule with both positive and negative effects on B-cell signaling, may reflect another feature of B-cell hyperactivity in pSS that seems to be independent of BAFF/BLyS elevation and, remarkably, has not been detected in SLE and rheumatoid arthritis patients [28]. Despite abnormalities in the surface expression of costimulatory molecules on T-cell and B-cell subsets, immune dysregulation in pSS is also indicated by abnormal levels of shed surface molecules, e.g. of soluble CD21 [29], CD27 [30], and CD28 [31]. Such abnormalities may both reflect or contribute to disturbed immune responses and have been found to exhibit distinct patterns in different autoimmune conditions [28]. Despite abnormalities in the surface expression of costimulatory molecules on T-cell and B-cell subsets, immune dysregulation in pSS is also indicated by abnormal levels of shed surface molecules, e.g. of soluble CD21 [29], CD27 [30], and CD28 [31]. Such abnormalities may both reflect or contribute to disturbed immune responses and have been found to exhibit distinct patterns in different autoimmune conditions [28].

The value of measuring plasma concentrations of such molecules in evaluating disease activity or in differential diagnosis needs further assessment.

Epstein-Barr virus-transformed B-cell lines from pSS patients displayed enhancement of stress functions in undamaged and damaged (gamma-irradiated) cells, i.e. in DNA-dependent protein kinase activity, apoptosis, and cell cycle arrest, respectively, indicating that an enhanced stress response might be a pathogenic mechanism in pSS [28]. Interestingly, DNA protein kinase activity not only functions in DNA damage response but also forms an essential part of V(D)J recombinase, mediating rearrangement of immunoglobulin and T-cell receptor genes [33]. In this regard, retention of preswitch immunoglobulin transcripts in circulating post-switch B cells has been identified in pSS patients, consistent with the conclusion that there are fundamental abnormalities in the molecular mechanisms governing expression of the B-cell receptor in patients with pSS [34].

B-cell hyperactivity in pSS is also reflected serologically by hypergammaglobulinemia and circulating autoantibodies. Various autoantibody specificities have been identified in pSS patients including antibodies to both ubiquitous autoantigens (e.g. SS-A/Ro, SS-B/La, α-fodrin) and to autoantigens that are mostly restricted to the target tissues (e.g. islet cell antigen 69, muscarinic M3 receptor) [3,35]. Although many of these autoantibodies have also been linked to other pathologic conditions [3,35,36,37-41,42,43,44,45,46], autoantibodies most frequently occurring in pSS are used to classify the disease [47]. The prevalence of autoantibodies may vary between different populations of pSS patients, however, depending on classification criteria, genetic background, treatment, laboratory, and the test system used [36,37-41,42,43,44,48]. Antibodies to cyclic citrullinated peptide are regarded as representing an efficient early and highly specific diagnostic marker for the diagnosis of rheumatoid arthritis [49,50]. Importantly, recent studies point out that anti-cyclic citrullinated peptide antibodies are rarely found in patients with pSS when using second-generation test kits (7.5% of patients), but positive test results do not rule out this diagnosis [42,43,44]. Antibodies to cyclic citrullinated peptide-positive patients should be carefully followed up, however, because they may predominantly develop rheumatoid arthritis with secondary Sjögren’s syndrome [42,44].

Cytokines are thought to play an important role in the pathogenesis of pSS by triggering and promoting several cellular and humoral autoimmune processes [51,52,53,54,55,56,57]. Notably, cytokines that have been regarded to be involved in T-helper type 1-driven immune responses may play a key role in the pathogenesis of pSS but also in other systemic autoimmune conditions [52,53,54]. For example, enhanced plasma levels of interleukin-18 have been found to correlate with IgG1 levels in both pSS and rheumatoid arthritis patients [52] as well as with anti-SSA/Ro and anti-SSB/La levels in pSS patients [53]. Moreover, interleukin-18 seems to be involved in local glandular inflammatory pathways in pSS [53]. Similar to circulating plasma interleukin-10 levels, elevated levels of interleukin-10 in the saliva have also been found to correlate with severity of pSS [54]. Conversely, local release of interleukin-10 following gene transfer in animal models of pSS caused significant decreases in glandular inflammation along with diminished circulating plasma interleukin-10 levels [55,56,57]. Altogether, these findings implicate complex immunomodulatory properties of interleukin-10 in this entity. At least, abnormal local interferon-α production by activated plasmacytoid dendritic cells in pSS salivary glands may be crucial in triggering and promoting the entire autoimmune process, despite low interferon-α serum levels seen in pSS patients [57]. Thus, it has been assumed that determination of plasma cytokine patterns, including a broader variety of cytokines, may be useful to characterize and subcategorize pSS patients by assessment of disease activity/severity along with common clinical and laboratory parameters [51].

The impact of apoptosis and aberrant expression of apoptosis-regulating proteins in pSS glandular destruction remains controversial [39,58-60]. In this context, a recent in-vitro study has suggested that Fas-mediated apoptosis of glandular epithelial cells in pSS requires costimulation via CD40, which provides a potent proapoptotic signal by diminishing Fas resistance [61]. This finding may partly explain conflicting results from previous studies. The impairment of myoepithelial laminin α2-chain/laminin-2 expression in pSS salivary glands may indicate a
characteristic defect in both the glandular acinar compartment and extracellular matrix-to-cell signaling in pSS [62].

**Improved animal models for primary Sjögren’s syndrome**

Mouse models have become an important tool for studying the pathogenesis of systemic autoimmune diseases. The availability of several mouse models, each exhibiting unique characteristics [63,64,65**,66–69], has also provided the possibility of investigating particular aspects of the etiopathogenesis of Sjögren’s syndrome [63,64,65**, 66–69,70*,71,72**]. The nonobese diabetic mouse is the most extensively studied animal model for Sjögren’s syndrome [55,63,64,69,70*]. In nonobese diabetic mice, lymphocytic sialadenitis and dacryoadenitis (coinciding with disturbed secretory function) develop independent from autoimmune diabetes but on a complex genetic background [10,64,67,69]. Two susceptibility loci for autoimmune exocrinopathy have been recently characterized in nonobese diabetic mice [67]. More recently, E2H homozygous mutant nonobese diabetic mice have been shown to be highly predisposed to the development of Sjögren’s syndrome-like exocrinopathy and diabetes, indicating that T-cell regulation based on a common genetic locus contributes significantly to both autoimmune disorders in this model [69]. In addition, the prototypical T-helper type 1 cytokine, interferon-γ, has been shown to play a critical role during both the early preimmune and the later immune phases of autoimmune exocrinopathy in nonobese diabetic mice [70*]. The NFS/sld (sublingual differentiation arrest) mouse represents another model for Sjögren’s syndrome-like exocrinopathy that shares a common candidate autoantigen with Sjo¨gren’s syndrome, 120-kD α-fodrin [63,64]. Recent studies have suggested that autoreactive CD4+ T-helper cells play a pivotal role for autoimmune glandular tissue destruction in NFS/sld mice [71,72**]. Moreover, age-related bystander T-cell activation may contribute to the development of autoimmune arthritis in this murine model [73]. Studies in aromatase knockout mice, a model for estrogen deficiency that exhibits features of lymphoproliferative autoimmune disease, underlined the possible role of estrogen deficiency in the development of pSS [60,68]. Passive transfer of patient immunoglobulin-containing antimuscarinic receptor 3 (M3R) antibodies to normal (BALB/c) mice revealed that initial cholinergic hyperresponsiveness associated with Sjögren’s syndrome may be partly autoantibody related, i.e. caused by a compensatory increase of M3Rs [74]. The Id3 knockout mouse, most recently described as a murine model for pSS [65**], spontaneously develops lymphocytic infiltrates and decreased secretory function of only the salivary and lacrimal glands as well as autoantibodies to SS-A/Ro and SS-B/La later in life. Of note, these features of pSS arise because of a single genetic lesion resulting in the absence of a single nuclear protein, Id3, on an otherwise healthy murine background [65**]. Despite new insights into the pathogenesis, these models provide the chance to study more detailed therapeutic approaches in pSS.

**Recent advances in the clinical management of Sjögren’s syndrome**

Primary Sjögren’s syndrome represents a complex, multifactorial, multistep process that shares numerous features with other related autoimmune disorders representing a similar breakdown in normal immune function and subsequent chronic stimulation of the immune system. Moreover, Sjögren’s syndrome may occur secondary to other connective tissue diseases, such as SLE, rheumatoid arthritis, and systemic sclerosis. Thus, pSS must be distinguished from secondary Sjögren’s syndrome as well as from a wide variety of conditions that can lead to sicca symptoms, such as virus infections, drug therapy, and sarcoidosis. Other extraglandular clinical or laboratory findings may precede characteristic symptoms of keratoconjunctivitis sicca and xerostomia or salivary gland enlargement in pSS patients. This implies that there will be difficulty in early diagnosis and classification. Recent articles focused on advances in diagnosis and clinical management of Sjögren’s syndrome [35**,36*,42*,43,44*,48]. Noteworthy, although Sjögren’s syndrome is a common disease (especially in perimenopausal women), it has been estimated to remain undiagnosed in more than half of the affected patients [35**], thereby predisposing to profoundly diminished quality of life as well as to local complications because of diminished salivary, lacrimal, mucous, and gland function, e.g. periodontal disease, tooth decay, keratoconjunctivitis sicca, dry nose and throat, xerorachea with chronic dry cough, accelerated local infection, pruritus, and vaginal dryness [35**]. Systemic manifestations in Sjögren’s syndrome may involve the dermis, lungs, liver, kidneys, blood, gastrointestinal tract, vasculature, and nervous system [35**,36*,42*,43,44*,48,75–79]. The association of Sjögren’s syndrome with celiac disease should be recognized because of dental and mucosal complications including an increased risk for malignant lymphoma, which warrants early diagnosis and dietary treatment [75,76]. A subgroup of pSS patients with high-risk profile for lymphoma needs particular attention [80**,81,82]. These lymphomas are almost exclusively of B-cell origin, frequently indolent at the time of presentation, and usually arising in tissues affected by the underlying chronic autoimmune disease, most frequently in the salivary and lacrimal glands [80**,81,82]. Despite a preference of affected organs, extraglandular manifestations (e.g. palpable purpura, dermal vasculitis, peripheral neuropathy) as well as gammopathy, cryoglobulinemia, or reduced C4 level may indicate an enhanced risk for lymphoma development or early lymphoma and need further work-up [35**, 80**,81,82].
Therapeutic advances for glandular and extraglandular manifestations

Local treatment of pSS (along with fastidious dental care) is mainly symptomatic and aims to prevent complications of the disease early. For patients whose sicca symptoms are not adequately controlled by moisture preservation and replacement, secretagogues, i.e. the stimulators of muscarinic receptors, pilocarpine or cevimeline [83,84,85,86], may be used when potential contraindications have been excluded. In this context, a recent study revealed that the newer selective muscarinic agonist, cevimeline, 20 mg three times daily, is safe and effective in improving symptoms of dry eye in patients with Sjögren’s syndrome [84•]. The incidence of serious adverse events was comparable in the cevimeline and placebo groups, as had been reported in previous studies also [85,86]. Although demonstration of clinical benefit is lacking [35••], hydroxychloroquine has been reported to improve clinical conditions, likely related to reduced immunologic hyperreactivity in pSS patients [87]. Dehydroepiandrosterone as an alternative approach showed no evidence of efficacy in Sjögren’s syndrome in a randomised trial of 28 patients over 24 weeks [88]. Several studies focused on therapy for pSS using biologic agents [89••,90••,91–94,95•,96–99,100•]. Recent studies have shown that short-term use of the TNF blockers infliximab [89••] and etanercept [90••], respectively, is ineffective in pSS, although a primary pilot study revealed promising results with infliximab [91–93]. By contrast, anti-CD20 treatment with rituximab exhibited benefit in both pSS and its malignant complication, mucosa-associated lymphoid tissue type lymphoma [95••, 96–99], although two pSS patients developed severe serum sickness [100•]. Because BAFF/BlyS is significantly elevated, treatment with anti-BLys monoclonal antibody or receptor constructs or targeting of BlyS/BAFF receptors is a potential therapeutic strategy in pSS that remains to be assessed [19•,25•]. Alternative therapeutic approaches at preclinical stages, such as adenovirus gene transfer of interleukin-10, have the advantage of targeting specifically affected tissue [55,56•] and, if safe, can ultimately lead to new treatments for improved saliva [55] and tear [56•] production. Promising local immunomodulatory gene transfer of human interleukin-10 by a recombinant adenovirus associated virus in the nonobese diabetic mouse that resembles human pSS [55], however, needs further studies in other preclinical models of this entity to assess both its safety and its potency in treating local symptoms of pSS [101,102]. Finally, pros and cons of gene therapy [102] in pSS, a multifactorial disease with inadequate conventional therapy but an overall normal life span, despite excess lymphoma mortality in a small subgroup of patients [80••,81], must be carefully evaluated.

Conclusion

Numerous studies provided evidence of various disease-related factors (genetic predispositions, virus infections) as well as disturbances of the immune system. In particular, B-cell disturbances appear to play a central role in the pathogenesis of pSS. Therefore, these identified changes may allow improvements in disease management (diagnosis and classification of subpopulations of patients and disease severity) as well as development of targeted therapies. If viral infections are substantiated in further studies, antiviral therapy or even vaccinations could be candidates for innovative therapies in pSS.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

• Of special interest
•• Of outstanding interest


The presence of the apolipoprotein E ε4 polymorphism seems to predispose to early onset of pSS, as has been previously reported for coronary atherosclerosis, Alzheimer’s disease, and several infectious diseases. The underlying pathway remains speculative.
Primary Sjögren’s syndrome

Hansen et al. 563


This thorough review focuses on BAFF/BLyS as a potential therapeutic target in human rheumatic diseases, such as SLE, rheumatoid arthritis, and pSS.


Notably, in addition to macrophages, monocytes, and dendritic cells, infiltrating T cells may also express BAFF/BLyS in pSS salivary glands and, thereby, could play a role in triggering the activation of autoantigen-driven B cells.


A comprehensive review that focuses on new elements of BAFF/BLyS biology as well as on the potential role of abnormal BAFF/BLyS production in autoimmunity and development of B-cell lymphomas.


This study reports on the expression of similar disease expression in BAFF/FcγRn mice with an expansion of transitional and marginal zone B cells. B-cell lymphomas were found in more than 35% of the animals at 1 year, however, indicating that TNF may have an effect on the prevention of lymphoma development under the influence of remarkably enhanced BAFF levels.


An interesting observation of these analyses is the identification of enhanced CD72 expression on B cells from pSS patients as compared to those of SLE and rheumatoid arthritis patients that may reflect a feature of B-cell hyperreactivity in pSS. The demonstration of this difference in the expression of an inhibitory surface molecule adds further to a distinct immunocytometric profile in autoimmune patients, as has been established for autoantibody profiles.


This study implicates that strong DNA-dependent protein kinase activity may be part of an enhanced stress reaction of pSS B cells, probably contributing to heightened cell cycle arrest and apoptosis.


Both B-cell hyperactivity and abnormalities in heavy-chain switch recombination have been indicated at the single-cell mRNA level in peripheral memory B cells from pSS patients.


This comprehensive review highlights the recent classification criteria for Sjögren’s syndrome in the context of its usefulness for rheumatologists, general practitioners, dentists, and ophthalmologists, in order to determine diagnosis early, and considers carefully the heterogeneity of pSS. Current treatment options are discussed.


This study in a cohort of 26 SLE-Sjögren’s syndrome patients among 283 unselected SLE patients describes a distinct clinical, serologic, pathologic, and immunogenetic profile of this SLE-Sjögren’s syndrome subgroup, allowing further insight into the spectrum of patients with systemic autoimmunity.


Follow-up in 184 patients in whom Sjögren’s syndrome or Sjögren’s syndrome-like disease was diagnosed according to the revised criteria of the American-European consensus group revealed a high diagnostic value of anti-cyclic citrullinated peptide antibodies to identify patients who have or will develop rheumatoid arthritis.


This study in a cohort of 134 pSS patients (none of whom fulfilled the American College of Rheumatology classification criteria for rheumatoid arthritis) revealed that most patients (92.5%) were negative for anti-cyclic citrullinated peptide antibodies when using second-generation test kits, but positive test results did not definitively rule out this diagnosis.


Systemic lupus erythematosus and Sjögren’s syndrome


Interleukin-18, an immunoregulatory and proinflammatory cytokine, has been strongly implicated to be involved in both local and systemic autoimmunity in pSS by this thorough study.


In a first attempt, interleukin-10 gene transfer on induced autoimmune dacyoadenitis has been shown to improve tear production and suppress features of auto-immune exocrinopathy.


Local overproduction of interferon-α in pSS salivary glands has been suggested to be involved in a vicious circle-like mechanism that promotes the entire autoimmune process, with formation of endogenous interferon-α inducers, e.g. RNA-containing immune complexes. Virus infection may initiate this process.


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Sankar V, Brennan MT, Kok MR, et al. Etanercept in Sjögren’s syndrome: a twelve-week randomized, double-blind, placebo-controlled pilot clinical trial. Arthritis Rheum 2004; 50:2240–2245. A randomised, double-blind, placebo-controlled pilot study in 28 patients with Sjögren’s syndrome, most of them with pSS, suggests that short-term treatment with the soluble TNF-α receptor, etanercept, at a dosage of 25 mg twice weekly, is clinically efficacious in this entity, although a larger study is necessary to definitively address this. Combined data from studies on systemic TNF blockade in pSS, however, made a beneficial effect unlikely.


Gottenberg JE, Guillevin L, Lambotte O, et al. Tolerance and short-term efficacy of rituximab in 43 patients with systemic autoimmune diseases. Ann Rheum Dis 2005; 64:913–920 [epub ahead of print]. A retrospective study on off-label use of anti-CD20 antibody (rituximab) in patients with various refractory autoimmune diseases, including six with pSS, revealed that swelling of the parotid gland, arthralgias, and cryoglobulinemia-related vasculitis were sensitive to rituximab in five pSS patients. No conclusion could be drawn, however, regarding an effect on sicca symptoms. Importantly, one pSS patient developed serum sickness-like reaction.


Pediatric and heritable disorders

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Abbreviation

SLE systemic lupus erythematosus

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The contributions to this section of Current Opinion in Rheumatology review diverse topics in the pediatric rheumatic diseases, ranging from clinical outcomes in systemic lupus erythematosus (SLE) to the use of microarrays to monitor the expression of tens of thousands of genes in complex biologic samples that may provide insight into better classification of disease states. Advances in our understanding of the genetic and pathogenic basis of rarer disorders such as the granulomatous arthritides and familial autoinflammatory diseases are being made at a rapid pace, and appreciation is growing of the potential therapeutic uses of immunomodulatory peptides in rheumatic diseases.

Ravelli et al. (pp. 568–573) discuss outcomes in juvenile-onset SLE, underscoring improvements in both 5-year and 10-year survival rates that are accompanied, perhaps not surprisingly, by an increased accumulation of organ damage. They emphasize the importance of new treatments and treatment strategies that not only control disease activity but also minimize organ damage. An important advance is the development of outcome measures suitable for evaluating therapeutic response in children. A particular area of interest in regard to long-term damage risk is the prevention of cardiovascular disease in this population.

Graham (pp. 574–578) reviews recent advances in imaging juvenile arthritis. These are largely focused on the use of magnetic resonance imaging to improve early detection of bone and cartilage destruction as well as inflammatory changes. Graham also notes that ultrasound has shown promise in detecting synovial changes in patients whose disease is thought to be inactive clinically. Additional studies will be needed to determine whether these synovial changes are predictive of ongoing inflammation and the potential for further joint damage and destruction.

Rose and Martin (pp. 579–585) have reviewed advances in rheumatic disorders associated with mutations in the CARD15/NOD2 gene. A significant proportion of patients with familial (Blau's syndrome) or sporadic granulomatous arthritis have been found to have novel mutations in CARD15/NOD2. Interestingly, mutations in this gene were first discovered as a cause of Crohn's disease, but it appears that the mutations that result in granulomatous arthritis are distinct. The CARD15/NOD2 protein is expressed in the cytosol and can trigger the activation of nuclear factor-kB after encountering certain bacterial products that gain access to the interior of the cell. Elucidating the mechanisms that lead from this cellular abnormality to chronic inflammation, and understanding why some mutations lead to inflammatory bowel disease whereas others result in arthritis, will undoubtedly be instructive.

Stojanov and Kastner (pp. 586–599) have provided an update on the exciting advances in our understanding of the genetic basis for the systemic autoinflammatory diseases, focusing on hereditary recurrent fevers. This area will likely continue to advance rapidly, contributing greatly to our understanding of the innate immune response.

Koffeman et al. (pp. 600–605) summarize recent developments in immunomodulatory peptides and their implications for the treatment of juvenile rheumatic diseases. The concept encompasses groups of peptides that may stimulate the innate immune system or have antibacterial action, as well as those stimulating adaptive responses through B or T cells. The authors succinctly review these concepts and discuss selected examples for which evidence suggests that immunomodulatory peptides might be of benefit as epitope-specific therapy in rheumatic diseases.

Jarvis (pp. 606–611) comments on the use of microarray-based gene expression profiling in pediatric rheumatic disease. The power of this technology has become apparent from studies of peripheral blood gene expression profiles in patients with SLE, in whom the importance of type I interferons and the cells producing them has been emphasized. Other studies in juvenile arthritis and dermatomyositis have been limited to relatively small numbers of patients, and given the complexity of the data generated, clearly must be expanded. Jarvis emphasizes important limitations in the interpretation of data generated using this
technology. Despite caveats, the ability to comprehensively evaluate gene expression in an unbiased fashion promises to yield important discoveries.

Adams and Lehman (pp. 612–616) summarize recent advances in the treatment of systemic-onset juvenile rheumatoid (or idiopathic) arthritis. This disease has been difficult to treat and appears to be less responsive to tumor necrosis factor-α inhibitors (etanercept [Enbrel, Immunex Corp., Thousand Oaks, CA] and infliximab) than other forms of childhood or adult-onset inflammatory arthritis. The authors highlight the use of new biologic agents such as the interleukin-1 receptor antagonist (anakinra) and a monoclonal antibody that blocks the interleukin-6 receptor (monoclonal receptor antibody [MRA]), thus targeting the actions of two important cytokines in this disease. Thalidomide, which decreases the production of several cytokines, has also been shown to be of benefit.

Yeung (pp. 617–623) has written about advances in our understanding of the pathogenesis and treatment of Kawasaki’s disease. The debate about a potential infectious etiology continues, but fortunately this has not thwarted advances in therapy. Animal models have proven useful in studying early pathogenic events and may help to identify useful biomarkers and thus have complemented translational studies with human cells and tissues that are more difficult to obtain. Emphasis is still on the importance of early recognition and intervention for favorable outcomes.
Introduction

Systemic lupus erythematosus (SLE) is a multisystem, inflammatory, autoimmune disease that is characterized by widely variable clinical manifestations and unpredictable course. If left untreated, SLE is often progressive and has a significant fatality rate. It is estimated that 15–20% of patients with SLE have their onset before 16 years of age [1*]. SLE that begins in childhood has been considered more severe than SLE with onset during adulthood [2]. Furthermore, children diagnosed with SLE may need high-dose corticosteroids and immunosuppressive agents for disease control more often than do their adult counterparts [3].

Prognosis in juvenile-onset SLE (JSLE) has improved considerably over the past 2 decades, probably secondary to earlier diagnosis, recognition of mild forms, and better approaches to treatment of the disease and its complications. Consequently, children and adolescents with SLE are living longer and enter adult life with a chronic disease and morbidity secondary to the sequelae of disease activity, side effects of medications, and comorbid conditions, such as recurrent infections [4,5], accelerated atherosclerosis [6**,7,8**,9], osteoporosis [10*,11], and hypertension. This morbidity may affect their health-related quality of life (HRQL), raising problems related to the physical and psychological adaptation to a chronic illness.

For these reasons, the treatment of patients with JSLE is now directed not only at preventing death but also at reducing permanent damage to involved organ systems resulting from the disease, its therapy, or complications. Furthermore, the traditional outcome of patient survival has become too insensitive to assess the effect of medical care [12*]. In recent years, it has been increasingly recognized that the optimal clinical assessment of children and adolescent with SLE requires not only the measurement of disease activity but also the estimation of accumulated damage and the understanding of the effect of the disease and prescribed therapies on physical and psychosocial well-being [13**]. Health status, disease activity, and accumulated damage are regarded as important independent

**Abbreviations**

HRQL health-related quality of life
JSLE juvenile-onset systemic lupus erythematosus
SDI Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index
SLE systemic lupus erythematosus
SLEDAI Systemic Lupus Erythematosus Disease Activity Index

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**Purpose of review**

Over the past 2 decades, there has been a marked improvement in survival among patients with juvenile-onset systemic lupus erythematosus. As a result of the increased life expectancy, children and adolescents with systemic lupus erythematosus are now faced with considerable morbidity resulting from sequelae of disease activity, side effects of medications, and comorbid conditions. This morbidity affects physical and psychosocial well-being. Therefore, the need is increasing for monitoring the development of irreversible organ damage and the effect of the disease and its treatment on daily life. This review summarizes the recent advances in the investigation on survival, accumulated damage, and health-related quality of life in patients with juvenile-onset systemic lupus erythematosus.

**Recent findings**

The 5-year survival rate of patients with juvenile-onset systemic lupus erythematosus approaches 100%, and the 10-year survival rate is close to 90%. The development of cumulative organ damage has been observed in 50–60% of patients. Children and adolescents with systemic lupus erythematosus have been found to have poorer health-related quality of life, particularly in the physical domain, and lower socioeconomic achievements than their healthy peers.

**Summary**

The prolongation of the life span of patients with juvenile-onset systemic lupus erythematosus has been accompanied by a substantial risk of damage accumulation and has not been paralleled by an improvement in health-related quality of life. This problem highlights the need of measuring cumulative organ damage and health-related quality of life in the long-term follow-up of patients with juvenile-onset systemic lupus erythematosus and of designing new treatments and treatment strategies that are aimed not only at improving control of disease activity but also at minimizing the development of nonreversible damage.

**Keywords**

disease damage, health-related quality of life, mortality, systemic lupus erythematosus
outcome measures in lupus [14] and are recommended for use in longitudinal follow-up studies [15].

This review summarizes the recent developments and current understanding of prognosis and outcome of JSLE, examined in terms of long-term survival, accumulated damage, and HRQL.

**Long-term survival**

A marked improvement in survival over time among patients with JSLE has been documented in the literature [2,16–29,30] (Table 1). The application of life-table methods, where possible, to published data from the 1950s and 1960s yields 5-year survival probabilities of only 17.5–69% [26]. In contrast, series from the 1980s and 1990s report survival rates of 59–93% at 5 years [2,21–28] and 28–86% at 10 years [22–25,27,29]. In the 2000s, the 5-year survival rate approaches 100%, and the 10-year survival rate is close to 90% [30,31]. Although these data reveal a very encouraging trend, they may not provide generalizable information on survivorship in JSLE. Studies are hampered by the relatively small number of patients included (mainly tertiary care center patients followed in single units), the disparity in the age limits adopted and in the selection criteria for patient inclusion, and the frequent lack of patient stratification by major disease manifestations (e.g. with/without renal disease, with/without central nervous system involvement), by ethnic group (it is known that SLE tends to be a more serious disease among non-white populations), and by other known prognostic risk factors; furthermore, investigations have rarely used life-table analysis, and differences in treatment have been seldom taken into account. The prognosis of lupus nephritis in children has been reviewed recently [32•].

To obtain generalizable figures on survivorship in JSLE, a great deal of effort should be directed toward assembling multicenter, multinational cohorts of patients that must be assessed through a standardized and comprehensive protocol that incorporates all major risk factors. To minimize any possible selection bias, the study should recruit all eligible prevalent and incident patients with SLE with onset in childhood, including those seen in inpatient and outpatient services, and in public and private settings from the study areas. Population-based studies are very difficult to perform in most countries, however, so referral center-based populations would be enrolled in most centers. The resultant cohorts must represent all major ethnic groups and cover the whole clinical and socio-demographic spectrum of patients with JSLE.

**Organ damage**

In the last 10 years, there has been considerable interest in the development of appropriate instruments for measuring cumulative organ damage in patients with SLE. This effort has led to the development of the Systemic Lupus International Collaborative Clinics/American College of Rheumatology Damage Index (SDI) [33,34], which has been shown to be a valid and reliable tool in adults with SLE [35]. The SDI measures accumulated damage that has occurred since the onset of SLE resulting from either the disease process or its treatment. Damage is defined as an irreversible change in an organ or system that has been present for at least 6 months. The SDI records damage in 12 organs or systems and has a score range of 0 to 47. Recently, the amount of accumulated damage has been assessed through the SDI in some series of patients with JSLE (Table 2).

Brunner et al. [36] investigated retrospectively disease activity and damage in 66 patients with a mean follow-up of 3.3 years and sought potential risk factors for damage. At the end of the follow-up period, 40 patients (61%) had damage in at least one organ or system, with the mean SDI score 1.8. Damage occurred primarily in the ocular, musculoskeletal, neuropsychiatric, and renal domains. Avascular necrosis of bone and cataract were the most frequently observed specific damage items. Cumulative disease activity over time, as measured by the Systemic Lupus Erythematosus Disease Activity Index (SLEDAI), was the single best predictor of damage ($R^2 = 0.30$). Other likely important risk factors for damage were cumulative corticosteroid treatment, the presence of antiphospholipid antibodies, and history of acute thrombocytopenia. Duration of therapy

**Table 1. Survival rates in published series of juvenile-onset systemic lupus erythematosus**

<table>
<thead>
<tr>
<th>Authors, year</th>
<th>Patients, n</th>
<th>Percent</th>
<th>5 years</th>
<th>10 years</th>
<th>15 years</th>
</tr>
</thead>
<tbody>
<tr>
<td>Meislin and Rothfield, 1968 [16]</td>
<td>42</td>
<td>42/72a</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Garin et al., 1976 [18]</td>
<td>25b</td>
<td>61</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>King et al., 1977 [19]</td>
<td>108</td>
<td>78</td>
<td>–</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Fish et al., 1977 [20]</td>
<td>49</td>
<td>–</td>
<td>86</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Abeles et al., 1980 [21]</td>
<td>67</td>
<td>99/100a</td>
<td>–</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Ciceiro et al., 1981 [22]</td>
<td>42</td>
<td>59/83a</td>
<td>48/76a</td>
<td>–</td>
<td>–</td>
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<tr>
<td>Morris et al., 1981 [23]</td>
<td>36b</td>
<td>–</td>
<td>77</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>Platt et al., 1982 [24]</td>
<td>70</td>
<td>90</td>
<td>85</td>
<td>77</td>
<td>–</td>
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<tr>
<td>Lacks and White, 1990 [26]</td>
<td>32</td>
<td>85</td>
<td>–</td>
<td>–</td>
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<td>McCurdy et al., 1992 [27]</td>
<td>71</td>
<td>78</td>
<td>28</td>
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<td>Yang et al., 1994 [28]</td>
<td>127b</td>
<td>91</td>
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<td>Tucker et al., 1995 [2]</td>
<td>39</td>
<td>93</td>
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<tr>
<td>Baqi et al., 1996 [29]</td>
<td>56b</td>
<td>–</td>
<td>29</td>
<td>–</td>
<td>–</td>
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<td>Candell Chalom et al., 2004 [30]</td>
<td>64</td>
<td>94</td>
<td>87</td>
<td>79</td>
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<tr>
<td>Miettunen et al., 2004 [31]</td>
<td>51</td>
<td>100</td>
<td>86</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*aPercentage with renal disease/percentage without renal disease.

*bAll patients had lupus nephritis.
with immunosuppressive agents was determined to have a protective effect against damage.

In a multicenter, multinational study, Ravelli et al. [37] determined accumulated damage in 387 patients seen in pediatric rheumatology centers from Europe (Italy and Greece), the United States, Mexico, and Japan. Overall, 195 (50.5%) patients had damage within a mean of 5.7 years after disease onset. Renal and neuropsychiatric system involvement was observed most frequently, followed by musculoskeletal, ocular, and skin system involvement, with a mean SDI score of 1.1. Nephrotic-range proteinuria was the most commonly recorded item. In multivariate models, neuropsychiatric manifestations at diagnosis, longer disease duration, and use of cyclophosphamide pulses yielded the strongest association with damage.

Miettunen et al. [31*] examined retrospectively the associations of sex and ethnic origin with long-term outcome in 51 patients followed for a median of 7.2 years (minimum follow-up, 5 years) at a single institution. Fifteen patients were white, 14 Chinese, nine East Indian, and 13 of other ethnic background. The SDI was the main outcome measure, with a score of 2 or greater assigned a poor outcome. The median SDI score at last follow-up was 2.1, with 69% of patients showing damage in at least one organ or system. Half of the patients (51%) had a poor outcome. Damage occurred primarily in the musculoskeletal domain, followed by the neuropsychiatric, cardiovascular, peripheral vascular, skin, ocular, and renal domains. No association was found on logistic regression between sex, ethnicity, age at diagnosis, or length of follow-up and final SDI scores.

In a cross-sectional study of 71 patients with a mean disease duration of 10.8 years, Lilleby et al. [38*] found evidence of damage in 61% of patients, with the mean SDI score 1.3. The most frequent areas of damage were in the neuropsychiatric, renal, and musculoskeletal domains. The most common recorded SDI items were cognitive impairment or major psychosis and muscle atrophy or weakness. Hypertension, longer disease duration, and use of cyclophosphamide were significantly related to an increased SDI score in multiple regression analysis.

Recently, Bandeira et al. [39] investigated the relation between damage accrual (SDI score increase ≥ 1), flares of disease activity, and cumulative drug therapies in 57 patients who were first seen within 1 year after disease presentation and were followed for 3 years or more. Disease flare was defined as an increase in SLEDAI score of 3 points or more from the previous visit and was classified as slight (SLEDAI = 6), moderate (SLEDAI = 7–11), and severe (SLEDAI ≥ 12). Damage accrual was significantly correlated with the frequency of severe flares in the first 3 years of follow-up, but not with the cumulative duration of corticosteroid and immunosuppressive therapies.

Taken together, these studies show that cumulative organ damage is common in patients with JSLE. The mean SDI score (1.1–2.1) and the frequency of damage (50.5–61%) recorded in pediatric patients are in the range of those reported in series of patients with adult-onset SLE with disease duration of 3.8 to 12.9 years, which vary from 1.0–2.4 and from 56–86%, respectively [40–47]. This seems to disprove the idea that JSLE is a more severe disease than that occurring in adults. The prevalence of damage by organ system is variable among JSLE cohorts, although musculoskeletal, renal, and neuropsychiatric systems tended to be most frequently affected. The search for risk factors for damage has yielded diverging results among studies. Discrepancies may depend on differences in socio-demographic characteristics, clinical features and ethnic composition of patient populations, length of follow-up, and treatment regimens. A potential limitation of the existing studies is their retrospective nature. Better insights in the development of long-term damage and its prediction can be obtained by studying cohorts of newly diagnosed patients followed over time and assessed at standard time points.

Although the SDI has been shown to be a valid and reliable instrument in JSLE, it does not cover all forms of damage that children or adolescent with lupus may develop over time, particularly effects on growth and development [35]. It has been suggested that growth retardation and

Table 2. Summary of studies on cumulative organ damage in juvenile-onset systemic lupus erythematosus

<table>
<thead>
<tr>
<th></th>
<th>Brunner et al. [36]</th>
<th>Ravelli et al. [37]</th>
<th>Miettunen et al. [31*]</th>
<th>Lilleby et al. [38*]</th>
</tr>
</thead>
<tbody>
<tr>
<td>Patients, n</td>
<td>66</td>
<td>387</td>
<td>51</td>
<td>71</td>
</tr>
<tr>
<td>% Caucasian/white</td>
<td>27</td>
<td>36</td>
<td>29</td>
<td>93</td>
</tr>
<tr>
<td>Mean/median disease</td>
<td>3.3</td>
<td>5.7</td>
<td>7.2</td>
<td>10.8</td>
</tr>
<tr>
<td>duration with SDI ≥ 1</td>
<td>61</td>
<td>50.5</td>
<td>59</td>
<td>61</td>
</tr>
<tr>
<td>Mean SDI score</td>
<td>1.8</td>
<td>1.1</td>
<td>2.1</td>
<td>1.3</td>
</tr>
<tr>
<td>% of patients with</td>
<td>16%</td>
<td>44%</td>
<td>66%</td>
<td>50.5%</td>
</tr>
<tr>
<td>damage by organ/system</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ocular</td>
<td>44</td>
<td>11</td>
<td>12</td>
<td>4</td>
</tr>
<tr>
<td>Neuropsychiatric</td>
<td>11</td>
<td>16</td>
<td>14</td>
<td>28</td>
</tr>
<tr>
<td>Renal</td>
<td>9</td>
<td>22</td>
<td>10</td>
<td>13</td>
</tr>
<tr>
<td>Pulmonary</td>
<td>3</td>
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<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Cardiovascular</td>
<td>2</td>
<td>3</td>
<td>12</td>
<td>6</td>
</tr>
<tr>
<td>Peripheral vascular</td>
<td>3</td>
<td>6</td>
<td>12</td>
<td>9</td>
</tr>
<tr>
<td>Gastrointestinal</td>
<td>5</td>
<td>4</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Musculoskeletal</td>
<td>26</td>
<td>12</td>
<td>27</td>
<td>13</td>
</tr>
<tr>
<td>Skin</td>
<td>6</td>
<td>10</td>
<td>12</td>
<td>3</td>
</tr>
<tr>
<td>Premature gonadal</td>
<td>0</td>
<td>4</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>failure</td>
<td>3</td>
<td>0.5</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Malignancy</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>1</td>
</tr>
</tbody>
</table>
Health-related quality of life

The assessment of HRQL in patients with SLE has been increasingly recognized as one of the most important measures to appraise how much the disease process and its treatment are affecting their lives and to determine the need for social, emotional, and physical support during illness. HRQL measurement has been recommended for inclusion in SLE clinical trials because it addresses aspects of HRQL and its effects that are not fully captured by other endpoints [54,55]. As a result, HRQL assessment has been incorporated in the core set of outcome measures for the evaluation of response to therapy in SLE [56**,57**]. A number of HRQL tools are available for children and adolescents with chronic rheumatic diseases [58–60], although none of them has been designed specifically for JSLE. In recent years, a few studies have evaluated the HRQL of patients with JSLE.

Ruperto et al. [13**] investigated the HRQL of 297 patients with JSLE using the Child Health Questionnaire (CHQ) [58,61]. A greater impairment in the physical than in the psychosocial health domain was observed. The instrument scores were in the low range of those observed in juvenile idiopathic arthritis (JIA) patients, but much lower than those found in healthy children [62]. The most impaired CHQ subscales were global health, general health perceptions, and parent impact-emotional. Higher disease activity scores on the SLEDAI were associated with lower CHQ scores in the physical ($r = -0.29$; $P < 0.0001$) and psychosocial ($r = -0.25$; $P < 0.0001$) domains, whereas the amount of damage on the SDI had a significant effect only on physical functioning ($r = -0.23$; $P < 0.0001$). Overall, the greater impairment of HRQL occurred in patients with involvement of the central nervous, renal, and musculoskeletal organ/systems.

Moorthy et al. [66*] employed qualitative techniques to identify domains that are critical in determining HRQL in patients with JSLE. Patients and their parents were asked a single open-ended question related to lupus. Themes derived from patients’ responses focused primarily on coping and maintaining control of their lives despite SLE. Themes from patients’ responses emphasized efforts to cope with their children having SLE and appreciation/sadness in connection with their children’s coping process. The authors concluded that qualitative exploration of different facets of HRQL in patients with JSLE is critical to understand the specific factors that facilitate the coping process and to formulate interventions for improving children’s/family’s self-efficacy and disease management.

Altogether, these studies suggest that children and adolescents with SLE have poorer HRQL and lower socioeconomic achievements than their healthy peers. Similarly to what has been observed in patients with adult-onset disease [67], the available data indicate that the disease has its most profound effect on physical well-being, suggesting that social, mental, emotional, and behavioral health may adapt and improve over time. The difficulties in
Pediatric and heritable disorders

Copies with the chronic illness and its consequences remain a primary concern for both patients and their parents, however. Additional data and, perhaps, a disease-specific measure of HRQL are needed to characterize fully the effect of JSLE.

Conclusion

Although the life span of children and adolescents with SLE has improved dramatically over the past 20 years, many of them still develop organ damage and experience significant deterioration of their HRQL. This emphasizes the need of continuing a careful long-term follow-up of currently treated patients to understand the overall effect of the disease and its therapy. Future treatments and treatment strategies should be aimed not only at better controlling disease activity but also at reducing the development of nonreversible organ damage.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as: • of special interest •• of outstanding interest

Juvenile onset systemic lupus erythematosus Ravelli et al. 573


These authors report on the frequency of organ damage and its risk factors in 71 patients with JSLE.


49 Gladman DD, Urowitz MB. The SLICC/ACR damage index: progress report and experience in the field. Lupus 1999; 8:632—637.


This review highlights the recent achievements obtained by the international research networks in pediatric rheumatology.


This study describes the large-scale data collection and validation procedures that have led to the development of a core set of clinical measures for the evaluation of response to therapy in JSLE.


This study illustrates the consensus formation and statistical processes that were used to generate a preliminary definition of improvement for JSLE clinical trials.


The aim of this study was to identify the domains of quality of life that are more affected by SLE in children.

Imaging in juvenile arthritis
T. Brent Graham

Purpose of review
The purpose of this review is to highlight recent developments in imaging in juvenile arthritis.

Recent findings
The developments in imaging in juvenile arthritis are primarily focused on evaluation of destructive changes and inflammatory changes in joints. Plain radiography can demonstrate destructive changes in juvenile arthritis. The most validated instrument for assessing destructive changes in juvenile arthritis is the Poznanski index, and this index is being used more in studies to understand the natural history and clinical correlates of destructive disease. Magnetic resonance imaging has been shown to be superior to plain radiography in demonstrating destructive changes. Further work is proceeding to detect earlier, biochemical changes in articular cartilage prior to the development of thinning or erosion. Magnetic resonance imaging and ultrasound can demonstrate both inflammatory and destructive changes. Utilization of these techniques to show inflammatory changes can provide information about joints that can supplement physical examination, particularly in difficult joints to examine, such as the hips, temporomandibular joints, small joints of the feet, and tenosynovial locations. This information may help to guide therapy.

Summary
Imaging provides useful information to supplement clinical and laboratory examination in the optimal treatment of patients with juvenile arthritis.

Keywords
imaging, juvenile idiopathic arthritis, juvenile rheumatoid arthritis

Introduction
Imaging has been a useful method of measuring outcome at the joint level in patients with inflammatory arthritis for many years [1–3]. Plain radiography has traditionally been used to detect structural damage in both adult rheumatoid arthritis and juvenile arthritis (juvenile rheumatoid arthritis [JRA] and juvenile idiopathic arthritis [JIA] as used as specified in each individual article in this review). The evolution of newer techniques of ultrasonography and magnetic resonance imaging has enabled delineation of inflammatory changes in joints as well as more sensitive depiction of destructive disease [4–11]. With improved therapies showing great potential to control disease activity, more sensitive techniques to detect inflammatory changes as well as early changes in articular cartilage are important in assessing patients.

Destructive changes by plain radiography
Plain radiography continues to be a useful and readily available modality. Recent studies have added to understanding of the natural history of destructive disease as well as clinical correlations.

Van Rossum et al. [12] described plain radiographic features of JIA using 67 patients with oligoarticular, extended oligoarticular, and polyarticular JIA. All joints thought to be affected by arthritis and the contralateral joints were radiographed. A radiologist and pediatric rheumatologist read these films in consensus and then read the films again 4 years later to assess intra-reader reliability. Median duration of disease was 24 months. Joint space narrowing was found in 28% of patients, and erosions were found in 15% of patients. The joints most likely to be affected by joint space narrowing or erosions were the hands (wrist included) and feet. Some of the joints with radiographic abnormalities were normal on clinical examination, but history of involvement of these joints clinically is not included in this article. Risk factors for radiographic abnormalities, excluding soft tissue swelling, in the univariate analysis included onset of arthritis at age greater than 10 years, overall articular severity score, absence of antinuclear antibody (ANA), erythrocyte sedimentation rate (ESR) greater than 20 mm/h, presence of human leukocyte antigen (HLA)-B27, presence of immunoglobulin A (IgA) rheumatoid factor, and presence of immunoglobulin M (IgM) rheumatoid factor. Disease onset type and disease duration were not significant predictors. In the multivariate analysis, the predictive variables were presence of IgM rheumatoid factor and HLA-B27. Intra-reader reliability was very good for joint space narrowing, subchondral cysts, and erosions (Cohen's $\kappa$, 0.79–0.86).
Reliability was significantly less strong with respect to sub-chondral osteopenia (Cohen’s k, 0.40).

This article adds to previous information about destructive disease. Wallace and Levinson [13] found destructive changes in approximately 50% of patients with polyarticular or systemic JRA, with a median time to development of changes of approximately 2 years. One can interpolate that these findings are quite similar, although the patient populations are different. The study by van Rossum et al. [12] adds the analysis of reliability in assessing destructive changes, joint distribution, and clinical correlations.

Magni-Manzoni et al. [14] also used the Poznanski score to assess radiographic progression in patients with JIA with bilateral wrist involvement. Poznanski score is derived by dividing the radiometacarpal width by the length of the second metacarpal [15]. A standardized score is calculated. A negative score reflects decreased carpal width for the patient’s size, an indicator of destructive disease. The authors followed 94 patients with bilateral wrist arthritis by yearly wrist radiographs and clinical assessments every 6 months for a median of 4.5 years. The primary outcome variables were Poznanski score, yearly change in the Poznanski score, and Childhood Health Assessment Questionnaire (CHAQ) score. The authors found that progression was greatest during the first year of follow-up and that radiographic progression during the first year was the best predictor of all three long-term outcomes. The authors point out the limitations of the Poznanski score. Severe erosive disease with destruction of bony margins can make measurement of carpal length impossible. In addition, if the second metacarpal growth plates have closed, the M2 cannot be measured.

Recognizing the lack of applicability to certain patients, this study highlights the connection between radiographic outcome and functional status. It also demonstrates that radiographic progression in the wrist can be early and rapid. This study highlights the need for early aggressive treatment. Although this was not a treatment study, the vast majority of patients received second-line therapy. It is possible that aggressive therapy led to slowing down of radiographic damage in later years of the study, as has been demonstrated previously using the method of Poznanski [15,16].

Mason et al. [17] found that 28% of patients with polyarticular JRA who had wrist radiography performed at diagnosis had joint space narrowing or erosions present at diagnosis. This group is subject to selection bias because not all patients had wrist radiographs performed, but it again shows the high prevalence of destructive disease in this joint area.

Cassone et al. [18*] evaluated bilateral wrist radiographs in patients with JIA and clinical evidence of arthritis in at least one wrist annually beginning in 1986. They examined this group of 250 patients representing a cohort of approximately 400 patients biannually and performed wrist radiography yearly. This study looked at a subgroup of six patients with unilateral destructive wrist synovitis. They were notable for having oligoarticular onset in five patients and seropositivity for rheumatoid factor in only one patient, yet aggressive destructive disease. All patients developed a polyarticular disease course. Poznanski scores ranged from −2.39 to −8.50. CHAQ scores ranged from 0.375–1.6. This study highlights a subgroup of patients with oligoarticular disease, generally more benign, who are at risk for polyarticular disease with destruction, at least locally.

Inflammatory changes by magnetic resonance imaging and ultrasonography

Ultrasound has been relatively newly studied in the evaluation of inflammatory changes in juvenile arthritis. To this point, ultrasound in rheumatoid arthritis for assessment of synovitis has not been validated with histologic correlation. It has been validated indirectly by comparison with magnetic resonance imaging and plain radiography in rheumatoid arthritis [9,11,19].

Doria et al. [20] evaluated contrast-enhanced ultrasound in evaluation of the knee in 31 knees in 22 patients with JRA and 10 knees in five controls. They evaluated patients with active disease, inactive disease, and equivocal disease activity. Patients with equivocal disease activity were defined as patients with no active arthritis on examination but with elevated ESR. Patients with equivocal disease activity demonstrated increased mean pixel intensity values of synovium compared with patients with inactive disease and control patients both before and after contrast administration. This study showed that ultrasound can add to the value of clinical examination in the knee joint in patients in whom disease activity is unclear.

Frosch et al. [21] performed ultrasound of 15 hips with clinically active arthritis in eight patients with JRA and 38 knee joints with clinically active arthritis in 25 patients with JRA. These joints were examined three times in intervals of 4–6 weeks. Synovial thickening persisted, as 81% of knees felt clinically to have inactive arthritis had synovial thickness greater than 2 mm. In addition, knees with clinically inactive arthritis did not differ from knees with clinically active arthritis with respect to mean synovial thickness (5.2 mm; SD, 2.1 mm; compared with 5.8 mm; SD, 1.9 mm, respectively). In the hip joint, 43% of hips thought to be clinically inactive demonstrated abnormal synovial joint space (>6 mm).

This article highlights the fact that abnormally thickened synovium may be persistent, even in patients with patients thought to have clinically inactive arthritis. We have seen
Küseler et al. [24••] evaluated the temporomandibular joint (TMJ) by magnetic resonance imaging in patients with JIA. Patients were 8 years and older at the time of the first imaging evaluation and had disease duration of not more than 3 years. Patients were evaluated with contrast-enhanced magnetic resonance imaging of bilateral TMJs at intervals of 6–8 months for a total of four imaging evaluations. Signs and symptoms of TMJ involvement were not required for entry into the study.

Disease onset was oligoarticular in nine of 15 patients, polyarticular in four of 15, and systemic in two of 15. Clinical examination of signs and symptoms relative to the TMJ was performed, resulting in a clinical score that was a modified version of the Helkimo index. Magnetic resonance imaging score included both inflammatory and destructive changes. In this study, 93% of patients demonstrated synovial enhancement, and 71% demonstrated condylar erosions. No correlation was found between total clinical score and total magnetic resonance imaging score.

Two findings are most relevant to clinicians. One is the prevalence of TMJ involvement in this group of patients. The second is that there was no correlation between clinical score and magnetic resonance imaging findings. As with most magnetic resonance imaging studies in children, the population is skewed toward older patients. Selection bias is possible because patients with TMJ symptoms may be more likely to participate in such a study.

The patients who did not participate did not differ from those who did participate with respect to subtype or disease activity, but signs and symptoms referable to the TMJ were not reported in patients who did not participate.

This study is an example of using magnetic resonance imaging to detect local inflammatory changes not always fully appreciated by clinical assessment. Given the prevalence of TMJ involvement and the important functional and cosmetic consequences of uncontrolled disease in these joints, the clinician should be aggressive in seeking and treating TMJ synovitis.

Adib et al. [25•] performed a clinical and imaging series of isolated inflammatory coxitis in children. JIA has traditionally been thought to occur rarely as isolated hip arthritis, except as an early manifestation of spondyloarthropathy. This case series examines patients diagnosed with protrusio acetabuli, Otto pelvis, or idiopathic chondrolysis of the hip for clinical and radiologic characteristics, including histology and response to therapy.

Imaging was useful in this study for confirming the prevalence of involvement of the contralateral, often asymptomatic, hip. Ten of 14 patients had physical examination and/or radiographic evidence of contralateral hip involvement, although none of these patients or referring physicians reported symptoms in the contralateral hip.

In addition, magnetic resonance imaging of the hip with gadolinium demonstrated synovial enhancement in seven of seven patients. These findings correlate well with histologic findings, available in six patients. Two patients demonstrated chronic inflammatory cell infiltrate: one patient demonstrated fibrous changes of synovium, and one patient demonstrated edema of the synovium. No synovium was available from biopsy specimen in two additional patients.

These patients had aggressive, destructive disease localized to one or both hips. The authors propose that this is a subtype of JIA and propose diagnostic criteria. The contribution of imaging to this study was largely in demonstrating synovial enhancement. This may justify consideration as an inflammatory arthritis and treatment as such.

This study is a rare one in children in which histologic confirmation is included.

Tynjälä et al. [26•] used magnetic resonance imaging of the ankle and foot to assess synovitis in preparation for corticosteroid injection into 22 ankles/feet in 19 patients. These patients had failed to respond to previous corticosteroid injection into this region without prior magnetic resonance imaging. The striking finding in this study was the presence of multiple sites of active synovitis in these
patients. More than one affected joint was found in 13 of 22 ankles/feet. Frequent active inflammation of subtalar and inter-tarsal joints was found. Synovitis with accompanying tenosynovitis was found in 17 of 22 ankles/feet.

This study shows that extent of synovitis can be better appreciated, particularly in the midfoot and tenosynovial regions surrounding the ankle and midfoot, with magnetic resonance imaging. Given the number of joints involved and potential difficulty with local corticosteroid injections in these regions, magnetic resonance imaging is quite useful prior to consideration of injection in this region. Remedios et al. [27] have previously demonstrated sensitivity of magnetic resonance imaging in the ankle and subtalar joints with improved response to fluoroscopically-guided injection.

Magnetic resonance imaging and ultrasonography in assessment of damage
Argyropoulou et al. [28] evaluated 28 patients with JIA with magnetic resonance imaging of bilateral hips before and after contrast. They used a magnetic resonance grading scale that encompassed both synovitis and articular cartilage and bony damage. Patients had a mean disease duration of 6.9 years. In this study, patients with active arthritis of the hip joint on examination demonstrated higher magnetic resonance imaging scores, reflecting more active disease and/or damage than patients without active arthritis of the hip clinically. Patients with systemic disease had higher magnetic resonance imaging scores than patients with either polyarticular or oligoarticular disease. This study does not describe how these patients were selected, and a wide range of disease duration is included (1–21 years). The magnetic resonance scoring system does not differentiate active synovial disease from residual damage. Nevertheless, the demonstration of greater hip disease in systemic patients is helpful.

In the study by El-Miedanny et al. [22] involving the knee, the increased sensitivity of contrast-enhanced magnetic resonance imaging in detecting cartilage destruction was striking. By magnetic resonance imaging, 22 of 38 patients were found to have articular cartilage destruction, compared with seven of 38 patients with ultrasound and five of 38 patients with plain radiography.

Kight et al. [29*] in our group evaluated T2 mapping in the assessment of articular cartilage in the weight-bearing portion of the femur in female patients age 4–11 years with JRA, history of knee arthritis, and disease duration of 2–7 years. T2 mapping maps T2 relaxation time in cartilage compared with normalized distance from subchondral bone. T2 relaxation time measures mobility of water within the collagen-proteoglycan matrix. In this study, T2 relaxation time was found to be increased in patients with JRA compared with healthy age-matched and sex-matched controls. This increase may reflect loss of integrity of the collagen matrix [30]. The implications of these findings for long-term health of articular cartilage in children with JRA are unknown.

Conclusion
Imaging remains an important tool in the assessment of patients with juvenile arthritis. With improved treatment options, imaging must be very sensitive to detect both inflammatory and destructive changes. Currently, the only validated way to assess destructive disease is the Poznanski index. Recent research has shown that magnetic resonance imaging in particular can detect synovitis quite sensitively and adds significant information to the clinical examination, particularly in the temporomandibular joint and the foot. Recent data on ultrasound in juvenile arthritis hold promise but need further validation. Future work should focus on validating imaging outcome measures to be used in clinical trials and clinical practice. In addition, more sensitive identification of the disease process with respect to both inflammatory and destructive changes is needed.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest


24 Küsele A, Pedersen T, Gelineck J, et al. A 2 year followup study of enhanced magnetic resonance imaging and clinical examination of the temporomandibular joint in children with juvenile idiopathic arthritis. J Rheumatol 2005; 32: 162–169. This study gives information about a joint that is difficult to examine. Lack of correlation between clinical and magnetic resonance imaging findings highlights the importance of magnetic resonance imaging in this joint.


Caspase recruitment domain 15 mutations and rheumatic diseases
Carlos D. Rose\textsuperscript{a,b} and Tammy M. Martin\textsuperscript{c,d}

Purpose of review
The purpose of this article is to review the foundational work and current developments on a group of rheumatic disorders associated with mutations in the caspase recruitment domain 15/nucleotide oligomerization domain 2 gene.

Recent findings
To date, there are at least 10 arthritic conditions for which specific genetic mutations have been demonstrated. They include familial Mediterranean fever; tumor necrosis factor receptor associated periodic syndrome; hyper immunoglobulin D syndrome; neonatal onset multisystemic inflammatory disease; pyogenic arthritis pyoderma gangrenosum and acne; Muckle-Wells syndrome; familial cold autoinflammatory syndrome; immunodysregulation, polyendocrinopathy, enteropathy, X-linked syndrome; Crohn’s disease; and familial and sporadic sarcoid granulomatous arthritis. This review focuses on recent progress in the last two diseases and the caspase recruitment domain 15 genetic defects with which they are associated. Up to 50% of patients with familial granulomatous arthritis (Blau’s syndrome), 90% of those with sporadic granulomatous arthritis (early-onset sarcoidosis), and 40% of individuals with Crohn’s disease have documented mutations in the caspase recruitment domain 15 gene.

Summary
Although histologically, Crohn’s disease and familial and sporadic sarcoid granulomatous arthritis are distinct from rheumatoid arthritis because of the defining presence (albeit in not all cases) of non-caseating granulomata in the synovial and intestinal tissues, respectively, they still represent a promising model of both chronic synovitis and uveitis. In addition, once the actual mechanism is discovered by which defects of the caspase recruitment domain 15 gene product lead to chronic arthritis, it may uncover unsuspected biologic targets for therapeutics.

Keywords
Blau’s syndrome, caspase recruitment domain 15, Crohn’s disease, sarcoidosis

Introduction
Caspase recruitment domain (CARD) 15, also called nucleotide oligomerization domain (NOD) 2, was catapulted into recognition in 2001 when two research groups independently discovered that mutations altering this protein conferred increased risk of Crohn’s disease \cite{1,2}. Amazingly, mutations in this same gene were then found to cause a rare autosomal-dominant form of granulomatous arthritis, Blau’s syndrome \cite{3}. In this review, we explore the relation between Blau’s syndrome and associated conditions now known to be associated with \textit{CARD15} genetic defects.

Caspase recruitment domain 15
CARD15 is expressed as a cytoplasmic monomer 1040 amino acids in length. CARD15 has two isoforms, a full-length product (isoform 1) and isoform 2, which uses an alternative initiation site corresponding to amino acid position 28. While isoform 1 is more abundant, both have the ability to activate the transcription factor nuclear factor \textit{k}B (NF\textit{k}B). Activation of NF\textit{k}B is a key step in the up-regulation of many genes involved in inflammatory cascades.

CARD15 is composed of two amino-terminal CARDs, a central neuronal apoptosis inhibitor protein, CIITA, HET-E, and TP1 (NACHT) domain (which contains a potential ATP binding site), and a leucine-rich repeat (LRR) region at the carboxy-terminus (Fig. 1) \cite{4}. The CARD domain structure is highly conserved across species, is found in many proteins, and is involved in protein interactions via CARD-CARD binding. One example of this is the mediation of NF\textit{k}B activation resulting from CARD-CARD interactions between CARD15 and a pivotal signaling molecule, the adaptor kinase receptor interacting protein-like interacting caspase-like apoptosis regulatory protein kinase (or RICK; this is also referred to as the receptor interacting protein 2 [RIP2] or the CARD-containing interleukin-1 beta converting enzyme [ICE] associated kinase [CARDIAK]) \cite{5,6}. The NACHT...
domain is also referred to as a NOD or a nucleotide-binding site. This domain of the protein is likely to be involved in homodimerization of CARD15 and in binding nucleotides. NACHT domains are known to function as ATPases and are found in proteins involved in apoptosis and in proteins involved in transcriptional activation of the major histocompatibility genes. The LRR region is structurally related to those of the Toll-like receptors (TLRs), which are molecules of the innate immune system indispensable for sensing molecular motifs specific to pathogens, such as lipopolysaccharide. In fact, early research conducted on CARD15 was thought to demonstrate that lipopolysaccharide was its LRR ligand. Subsequent studies demonstrated, however, that the moiety recognized by CARD15 was actually muramyl dipeptide, a building block of peptidoglycan found in essentially all bacterial cell walls (i.e. not restricted to Gram-negative bacteria as is lipopolysaccharide) [7,8].

A commonly quoted observation from the initial CARD15 studies is that expression is restricted to monocytes [4]. Even though monocytes, granulocytes, and dendritic cells do indeed express CARD15, non-hematopoietic cells also express CARD15. One of the particular implications for Crohn’s disease is that CARD15 is expressed in intestinal epithelial cells [9–11], including Paneth cells located at the base of the villous crypts [12].

The role of CARD15 in autoimmune or inflammatory disease has been the subject of several recent reviews [13–16]. The emerging picture indicates that CARD15 serves as an intracellular sensor of bacteria through recognition of muramyl dipeptide, and that it participates in an inflammatory signaling cascade involving RICK, triggering the activation of NFκB. Research on this fascinating molecule is far from complete, and a comprehensive look at the detailed investigations of CARD15 is beyond the scope of this review. It is worthwhile mentioning a few recent advances in our understanding of CARD15 function, however. Tanabe et al. [17*] analyzed a large series of CARD15 mutants to dissect the amino acid residues essential for response to muramyl dipeptide and activation of NFκB. They demonstrated that the full-length LRR region was needed for response to bacterial components. In addition, a regulatory region encompassing part of the LRR (with increased NFκB basal activity) was identified. Furthermore, a region at the amino-terminus spanning both CARDs was necessary for activity. As a result of the cytoplasmic localization of CARD15 and its potential for participating in various signaling complexes, it will be important to determine the array of proteins that CARD15 interacts. One such study has identified a new binding partner of CARD15, the gene associated with retinoid-interferon mortality 19 (GRIM-19), which may be an integral component of CARD15-mediated responses [18**].

The mutations in CARD15 which are associated with Crohn’s disease are found near or in the LRR region [1,2,19]. The effect of these mutations is a defect in the ability of CARD15 to sense muramyl dipeptide. Given that the response of CARD15 to muramyl dipeptide is the activation of an inflammatory cascade, the effect of these mutations in predisposing to inflammatory disease seems counter-intuitive. This means that the role of CARD15 in NFκB activation is complex, with both inflammatory and anti-inflammatory consequences. Recent insights into this complexity have been provided. Abbott et al. [20*] report that the modulation of NFκB release from its inhibitor complex is dependent on RICK and involves ubiquitination of a protein called the nuclear factor-kappa B essential modulator (NEMO) (ubiquitination targets a protein for degradation). Interestingly, the CARD15 Crohn’s disease mutations are defective in this NEMO ubiquitination [20*]. Another mechanism by which CARD15 may promote regulation of inflammation was recently reported by Watanabe et al. [21*], who examined the intersection between the CARD15 and the TLR2 signaling cascades and found evidence for CARD15-dependent down-regulation of the TLR2 response to peptidoglycan. Thus, CARD15 is involved in a coordinated effort to sense bacterial components, although downstream effects of its activation appear complex and are still not well understood. The relation between CARD15 mutations and granulomatous disease are even more intriguing.

**Blau’s syndrome**

In 1985, Jabs et al. [22] and Blau [23] reported two separate families showing a similar phenotype including early-onset polyarticular ‘boggy’ synovitis (Fig. 2), rather severe pan-uveitis and an autosomal-dominant pattern of inheritance. Subtle differences were seen between the two descriptions. The family reported by Blau [23] also presented with a tan-colored maculo-papular rash, absent in the Jabs et al. [22] report, while the latter included cranial neuropathies. These patients generally lack antinuclear antibodies and characteristically show non-caseating
granulomata on tissue biopsy (synovium, conjunctiva, and dermis) in association with a chronic inflammatory infiltrate (Fig. 3). A family reported subsequently by Pastores et al. [24] was phenotypically analogous to the Blau phenotype, leading to the lingering idea that Blau’s syndrome was strictly limited to the classic triad of boggy synovitis, uveitis, and rash. This strict definition was later challenged by the publications of two families: one with associated liver involvement [25] and another with renal disease [26]. These and other observations depicting an expanded phenotype allowed a more inclusive definition. Most authors currently accept cranial neuropathies, systemic involvement (fever), and arteritis as part of the disease spectrum. Hence, the family reported by Jabs et al. [22] and earlier by Hafner and Vogel [27] and by Rotenstein et al. [28] can be considered examples of Blau’s syndrome or, as some prefer, familial granulomatous arthritis- uveitis or familial juvenile systemic granulomatosis [25,29].

To keep the historical record straight, we should note that the first family with Blau’s syndrome was published by Jabs et al. [22] 5 months prior to Blau’s [23], albeit without the description of the conspicuous rash recognized by Blau [23]. Perhaps if the current nomenclature is to be kept, the disease should be renamed Jabs-Blau syndrome. The series by Hafner and Vogel [27] is the largest sporadic series published to that date and includes a mother-daughter pair (familial form).

The first important contribution to the elucidation of the mutation is owed to the linkage analysis work on the original pedigree. The gene in this region that was responsible for the observed linkage to Crohn’s disease was found to be \textit{CARD15} [1,2,19]. Moreover, in 2001, a seminal work by Miceli-Richard et al. [3] demonstrated that \textit{CARD15} mutations different than those associated with Crohn’s disease were responsible for Blau’s syndrome. Wang et al. [32], working on the largest collection of Blau’s syndrome pedigrees (10 families), reported \textit{CARD15} mutation in 50% of them, while our own experience with four families showed \textit{CARD15} mutation in each family. In all of the families we reported with \textit{CARD15} mutation, there is a 100% correlation between phenotype and mutation [33].

The \textit{CARD15} mutations that cause Blau’s syndrome identified thus far are all amino acid substitutions within or near the NACHT domain (Fig. 1). This is in contrast to the substitutions associated with Crohn’s disease, which cluster around the LRR region. Functional studies indicate that cells carrying mutations identified in Blau’s syndrome exhibit an increased basal NFkB activity, that is, a gain of function consistent with the autosomal-dominant nature of this disease.

**Early-onset sarcoidosis**

In parallel with the evolution of the understanding of Blau’s syndrome but dating to the 1960s and 1970s, pediatric rheumatologists have been aware of a similar clinical entity characterized by granulomatous boggy synovitis and tenosynovitis, uveitis, and rash clinically indistinguishable from Blau’s syndrome albeit without family history [34]. This condition is known as early-onset or preschool sarcoidosis (EOS) to distinguish it from the adult form. Remarkable clinical differences are seen between the two, with EOS presenting before age 5 years and almost never associated with hilar adenopathy. The difference between

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**Figure 2. Blau’s syndrome**

Typical boggy synovitis of the wrist. Note the scaly rash on the dorsum of hand and wrist.

**Figure 3. Granulomatous synovitis**

Synovial biopsy from carpal arthritis from a patient with Blau’s syndrome. Note intense synovial inflammatory infiltrate, increased subsynovial vascularity, and a multinucleated giant cell at the bottom of the picture.
these two diseases has been the topic of multiple reviews and is beyond the scope of this article.

Few population-based studies of EOS are available. In the Danish Sarcoid Registry between 1979 and 1994, only 48 confirmed cases out of 5536 reported had onset before age 15 years and only three before age 5 years [35*]. Of these three, two were twins, and although family history is not provided, one could assume autosomal-dominant inheritance in this case (Blau’s syndrome). According to this study, sarcoidosis in the pediatric age has an incidence of 0.29/100,000 person-years and EOS (familial and sporadic) of 0.06/100,000 person-years [34].

Similar to the example of Blau’s syndrome, the cardinal symptoms of the EOS disease triad has been expanded to include large vessel vasculitis [36–39] and visceral involvement including granulomatous infiltration of the lungs, heart, liver, and kidneys [40].

The long debate about the identity of the two forms of the disease – sporadic and familial (Blau) – has received renewed attention this year thanks to recent developments in the molecular basis of the sporadic form. Miceli-Richard et al. [3] reported in their original report two patients with sporadic EOS who tested negative for the CARD15 mutations; however, investigators from Kyoto University [41**] and our own group [42**] confirmed the presence of previously identified substitutions of CARD15 in two patients with EOS. Further, the investigators from Kyoto tested 10 Japanese patients with documented EOS and found a mutation in nine of them [43**]. A patient with a double CARD15 mutation (one involved in Blau’s syndrome and one associated with Crohn’s disease) was reported by the French authors [44]. Together, these findings corroborate the impression that Blau’s syndrome and EOS are the same disease, with an inherited mutation in the familial form and a de-novo mutation in the sporadic form.

Granulomatous arthritis-uveitis (sporadic or familial) is the first disease with a known genetic cause in which rheumatic and ocular manifestations constitute the bedrock of the clinical picture and are the source of most of the morbidity. Notwithstanding the differences, it is a promising field for those interested in the mechanisms of chronic synovitis in both adult and juvenile-onset rheumatoid arthritis.

** Crohn’s disease-associated arthritis**

Classically, two arthritic patterns have been recognized in association with inflammatory bowel disease: peripheral and axial (spondylitic). The latter, characterized in children by sacroiliitis and peripheral arthritis, is not as strongly associated with HLA-B27 as other forms of spondyloarthropathies. Peripheral arthritis is more common, tends to be intermittent, is usually non-erosive, and at times precedes the intestinal symptoms. In those cases, it commonly presents as bilateral ankle synovitis in the experience of one of the authors (CDR).

Reviews on the clinical manifestations of Crohn’s disease arthritis are abundant in the literature.

**Granulomatous arthritis: an emerging common pattern?**

The arthritis associated with Blau’s syndrome and EOS on the one hand and that seen in Crohn’s disease and adult sarcoidosis on the other hand rarely are considered together. Using strict clinical criteria, such a position is understandable, since the first two are polyarticular, unremitting, and massively proliferative, with sacroiliac sparing and with invariable pediatric onset. Crohn’s disease arthritis tends to be oligoarticular, tends to be easy to control, affects all ages, and can be associated with sacroiliitis. Despite this discrepancy, these two groups of arthritic conditions share two major common features: (1) granulomatous inflammation universally in Blau’s syndrome and EOS and in at least some cases of Crohn’s disease, and (2) presence of CARD15 mutation with frequencies of 40% in Crohn’s disease, 50% in Blau’s syndrome, and 90% in EOS. The granulomatous synovial histology is of particular interest since in all these conditions, granulomas are found in synovial tissue with different frequency. While the finding of granulomas in synovial tissue is the rule in Blau’s syndrome and EOS, the fact that granulomatous synovitis can be found in Crohn’s disease is probably less known. At least two reports exist of synovial granulomata in Crohn’s disease synovium, with one case in which the articular findings led the investigations to confirm the diagnosis of Crohn’s disease by colonoscopy [45,46]. The actual frequency of granuloma in Crohn’s disease synovium may be underestimated since very few histologic studies are available. Similarly, granulomatous inflammation in intestinal samples is not universal and is seen at onset in about 25% of patients with Crohn’s disease. Two recent studies specifically addressed this issue in light of the association between granulomatous disease and CARD15 mutation. Investigators from Belgium reviewed 161 patient specimens and found epithelioid granulomas in 68.9% of the specimens, with a higher incidence in younger patients and distal location (90% of rectal biopsies). No correlation, however, was found with mutations in either CARD15 or TLR4 genes [47**]. Investigators from Rennes, France, reviewed the cases of 188 consecutive patients with Crohn’s disease. Granulomas were found in 37% of the specimens, with 25% at presentation. In this group of patients, site was not important, but the number of specimens correlated directly with the frequency of granuloma [48]. It appears that the interplay between CARD15 mutation and development of synovitis (granulomatous in particular) among patients with and without gut
granulomas needs to be investigated to see whether the granulomatous variant of Crohn’s disease and arthritis constitutes a Blau’s syndrome-like subset.

The case of adult sarcoid arthritis is intriguing. Two patterns exist of arthritis in adult sarcoidosis. The most common is seen in association with hilar adenopathy, erythema nodosum (also seen in Blau’s syndrome; personal observation, July 2002), known as Löfgren’s syndrome. The disease is febrile, acute, and accompanied by uveitis and arthritis. The overwhelming majority of patients show arthritis of the ankles at presentation. In a recent study, ankle involvement at presentation was seen in 95% of the patients [49]. It is not known how many patients in this subset present granulomatous synovitis, nor is the frequency of CARD15 mutation known, although in adult sarcoidosis as a whole, such a mutation is not seen [50,51].

A more indolent form of sarcoid arthritis has been recognized in association with chronic sarcoidosis. The disease affects predominantly the knees and presents with variable degree of destructive potential. Early work by Sokoloff and Bunim [52] demonstrated typical non-caseating granulomas in the synovium, but later work Palmer and Schumacher [53] with needle biopsy in seven patients with chronic sarcoid arthritis revealed proliferative synovitis without granuloma. Hence, the finding of synovial granulomas in adult sarcoid arthritis in which CARD15 mutations are not found does not appear to be a constant finding in the more current and systematic studies.

Other diseases
Several genetic analyses have been conducted to search for mutations in CARD15 associated with other inflammatory conditions. The vast majority of these studies have been negative, including investigations of psoriasis [54–58], systemic lupus erythematosus [59,60], and rheumatoid arthritis [61,62]. No association between CARD15 and primary ankylosing spondylitis has been demonstrated [63–67]; however, a recent study has demonstrated an association of CARD15 mutations with sacroiliitis in patients with Crohn’s disease [68•]. For psoriatic arthritis, studies on Italian and German patient cohorts have been negative [58,69], whereas an association was demonstrated with a Newfoundland patient cohort [70]. These studies have to be interpreted with caution, because most analyses tested only the three mutations commonly seen in patients with Crohn’s disease and did not systematically look at all the CARD15 exons. It may also be possible that mutations exist in association with inflammatory disease that confer changes to regulatory regions of CARD15. Future studies will undoubtedly uncover such mutations, if they exist.

Conclusion
It is clear now, given the tremendous clinical overlap and the sharing of the mutation, that Blau’s syndrome and early-onset sarcoidosis are the same disease. Perhaps, as suggested before, the disease should be renamed granulomatous arthritis, recognizing that there are two forms: sporadic and a familial [29]. This idea was suggested by Miller and supported by us and others as clinical similarities between the two continued to emerge.

Some families with familial granulomatosis still have not had the mutation established (50%), lending legitimacy to current efforts by the authors and others to establish an international registry and DNA repository. The fact that the sporadic form seems to carry the mutation more frequently that the familial form needs confirmation, another of the goals of the registry. Waiting in line is the subset with large vessel involvement. Do they represent a genetically distinct group with a second facilitating mutation? Would work with those patients provide a clue for large vessel vasculitis in general? And finally, CARD15 is the first solidly established mutation for a disease with acceptable prevalence depicting chronic deforming arthritis as a major manifestation. Would downstream or upstream mutations in the same metabolic pathway prove important for other conditions like rheumatoid arthritis? In other words, is the CARD15 mutation granuloma-togenic or arthritogenic?

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest

Pediatric and heritable disorders

13 Philpott DJ, Girardin SE. The role of Toll-like receptors and Nod proteins in bacterial infection. Mol Immunol 2004; 41:1099–1108.
17 A heroic mutational effort to dissect the CARD15 functional requirements.

This study used the powerful technique of yeast two-hybrid screening to identify a unique binding partner of CARD15. This binding is specific for CARD15 as no binding was seen with a closely related protein (NOD1). GRIM-19 co-localizes with CARD15 and was found to be expressed in colonic biopsies, with markedly decreased levels in involved tissues from patients with Crohn's disease. Furthermore, the activation of NFκB was dependent on GRIM-19.


An intriguing study illustrating the complexity of regulation of signaling cascades. This and related future works may lead to new ideas for therapeutic targets.


The most extensive epidemiologic study on childhood sarcoidosis. A large study carried out to address the importance of this particular form of inflammation. CARD15 mutation in nine of 10 patients. Four previously unidentified mutations were described and all demonstrated in-vitro up-regulation of NFκB activation. A finding not yet replicated by other investigators. A commendable, well-orchestrated national effort.


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Familial autoinflammatory diseases: genetics, pathogenesis and treatment
Silvia Stojanov and Daniel L. Kastner

Purpose of review
The systemic autoinflammatory diseases are characterized by seemingly unprovoked inflammation, without major involvement of the adaptive immune system. This review focuses mainly on a subset of these illnesses, the hereditary recurrent fevers, which include familial Mediterranean fever, the tumor necrosis factor receptor-associated periodic syndrome, the hyperimmunoglobulinemia D with periodic fever syndrome, and cryopyrin-associated periodic syndromes. This review elucidates how recent advances have impacted diagnosis, pathogenesis, and treatment.

Recent findings
More than 170 mutations have been identified in the four genes underlying the six hereditary recurrent fevers. Genetic testing has broadened the clinical and geographic boundaries of these illnesses, given rise to the concept of the cryopyrin-associated periodic syndromes as a disease spectrum, and permitted diagnosis of compound heterozygotes for mutations in two different hereditary recurrent fever genes. Genetics has also advanced our understanding of amyloidosis, a complication of the hereditary recurrent fevers, and suggested a possible role for common hereditary recurrent fever variants in other inflammatory conditions. Recent advances in molecular pathophysiology include the elucidation of the N-terminal PYRIN domain in protein-protein interactions, the description of the NALP3 (cryopyrin) inflammasome as a macromolecular complex for interleukin-1β activation, and the identification of signaling defects other than defective receptor shedding in patients with tumor necrosis factor receptor-associated periodic syndrome. These molecular insights form the conceptual basis for targeted biologic therapies.

Summary
Advances in molecular genetics extend our ability to recognize and treat patients with systemic autoinflammatory diseases and inform our understanding of the regulation of innate immunity in humans.

Keywords
genetics, hereditary recurrent fevers, inflammasome, systemic autoinflammatory diseases, therapy

Abbreviations
ASC  apoptosis-associated specklike protein with a caspase-recruitment domain
CAPS  cryopyrin-associated periodic syndromes
CARD  caspase-recruitment domain
CINCA  chronic infantile neurologic cutaneous and articular syndrome
FCAS  familial cold autoinflammatory syndrome
FMF  familial Mediterranean fever
HIDS  hyperimmunoglobulinemia D with periodic fever syndrome
HRF  hereditary recurrent fever
LRR  leucine-rich repeat
MIM  Mendelian inheritance in man
MWS  Muckle—Wells syndrome
NACHT  domain present in neuronal apoptosis inhibitory protein, CIITA, HET-E, and TP1
NALP  NACHT, leucine-rich repeat- and PYRIN domain-containing protein
NOMID  neonatal-onset multisystem inflammatory disease
PAPA  pyogenic arthritis with pyoderma gangrenosum and acne
PSTPIP1  proline serine threonine phosphatase interacting protein 1
SAA  serum amyloid A
TNFRSF1A  p55 tumor necrosis factor receptor
TRAPS  tumor necrosis factor receptor-associated periodic syndrome

Introduction
The concept of autoinflammatory disease was first proposed in 1999 to describe a group of inherited disorders characterized by episodes of seemingly unprovoked inflammation that, in contrast to the traditionally defined autoimmune diseases, lack high-titer autoantibodies or antigen-specific T cells [1]. Two hereditary recurrent fevers (HRFs), familial Mediterranean fever (FMF; Mendelian inheritance in man [MIM] 249100) and the then newly recognized tumor necrosis factor receptor-associated periodic syndrome (TRAPS, MIM 142680), were the prototypes for this diagnostic category. The following year, this concept was extended to subsume several mendelian disorders, including other HRFs, the familial ucticarial syndromes (now included among the HRFs), familial Mediterranean fever, and granulomatous disorders such as Blau’s syndrome (MIM 186580) [2]. Several illnesses with a complex mode of inheritance, such as Behcet’s disease (MIM 109650) and idiopathic pulmonary fibrosis (MIM 178500), were also included among the proposed autoinflammatory diseases, and it seems reasonable to suggest that some apparently acquired disorders of inflammation, such as the syndrome of periodic fever with aphthous stomatitis, pharyngitis, and cervical adenopathy (PFAPA) [3], may also properly fall under this rubric.

Subsequent advances in molecular genetics have vindicated the notion of autoinflammatory disease as a unifying concept, at both the structural and functional levels [4].
**Table 1. Characteristics of the hereditary recurrent fevers and the autoinflammatory PAPA syndrome**

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<tr>
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<th>FMF</th>
<th>HIDS</th>
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<td>Mutations cause increased PSTPIP1 binding to pyrin, leading to increased IL-1β secretion</td>
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<td>Reported in 25% of cases</td>
<td>Reported in a minority of patients who reach adulthood</td>
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</table>

PAPA, pyogenic arthritis with pyoderma gangrenosum and acne; FMF, familial Mediterranean fever; HIDS, hyperimmunoglobulinemia D with periodic fever syndrome; TRAPS, tumor necrosis factor receptor-associated periodic syndrome; FCAS, familial cold autoinflammatory syndrome; MWS, Muckle-Wells syndrome; NOMID/CINCA, neonatal-onset multisystem inflammatory disease/chronic infantile neurologic cutaneous and articular syndrome; −, approximately; MK, Mevalonate kinase.
This is particularly well illustrated among the HRFs, salient genetic and clinical features of which are summarized in Table 1. FMF, the most common and probably most thoroughly studied HRF, is caused by mutations in \textit{MEFV}, encoding the pyrin/marenostrin protein [5,6]. Mutations in the related protein cryopyrin (alternatively called NALP3 [because it belongs to a family of proteins containing a NACHT domain, leucine-rich repeat, and PYRIN domain] or PYPAF1) give rise to the so-called cryopyrin-associated periodic syndromes (CAPS): familial cold autoinflammatory syndrome (FCAS, MIM 120100), Muckle–Wells syndrome (MWS, MIM 191900), and neonatal-onset multisystem inflammatory disease (NOMID, also called chronic infantile neurologic cutaneous and articular syndrome or CINCA, MIM 607115) [7–9]. Both pyrin and cryopyrin share an N-terminal motif, the PYRIN domain, that facilitates cognate protein-protein interactions (reviewed by Kastner and Aksentijevich [10*]). The PYRIN domain is, in turn, a member of a larger family of protein motifs, the death domain-fold superfamily [11]. Another member of this superfamily, the death domain, is found at the N-terminus of the protein mutated in TRAPS, the \textit{p55 TNF} receptor (TNFRSF1A) [1]. As discussed here, through their respective PYRIN and death domains, cryopyrin, pyrin, and the \textit{p55 TNF} receptor play an important role in regulating cytokine secretion, nuclear factor-kB activation, and apoptosis, and thereby the innate immune system.

Although the gene mutated in the hyperimmunoglobulinemia D with periodic fever syndrome (HIDS, MIM 260920) [12,13] does not encode such a motif, recent data suggest that it may also impinge on the innate immune system through the regulation of interleukin-1B secretion. There are also structural and functional relationships between the HRF proteins and the proteins mutated in several other autoinflammatory disorders, including Blau’s syndrome and the syndrome of pyogenic arthritis with pyoderma gangrenosum and acne (PAPA, MIM 604416).

Increased awareness of the systemic autoinflammatory diseases, coupled with the widespread availability of genetic testing, has catalyzed the evolution of our concepts of diagnosis, genotype-phenotype interaction, and the broader role of the causative genes and proteins in health and disease, while concomitant advances in our understanding of pathophysiology have allowed dramatic breakthroughs in targeted biologic therapy. This review focuses on significant advances of the past year.

**Clinical genetics**

Although no new HRF genes have been identified over the past year, mutational studies of cohorts of affected patients have substantially advanced our understanding of the biologic role of the relevant genes and proteins. Areas of progress include refinement of the relationships between gene mutations and specific disease-associated clinical manifestations; analysis of the role of specific mutations and modifier factors in the risk of amyloidosis; and delineation of the relationship between common gene variants and the broader spectrum of inflammatory disease.

**Population genetics and genotype-phenotype relationships**

Given the relative accessibility of DNA diagnostics and the absence of reliable biochemical markers for FMF, TRAPS, and CAPS, genetic testing has become an important adjunct in the diagnosis of the HRFs. The growing list of mutations and polymorphisms of these mendelian disorders is frequently updated in INFEVERS [14*], a mutational database accessible on the World Wide Web at http://fmf.igh.cnrs.fr/infevers. To date more than 50 disease-associated mutations are listed for FMF, more than 40 for TRAPS, more than 35 for CAPS, and more than 30 for HIDS. It is interesting to note that, among HRF-associated mutations, nearly all are missense mutations, and, with two exceptions in FMF, nearly all spare the death domain-fold motif, where present, in the respective HRF proteins.

Implementation of genetic screening has extended the diagnosis of specific HRFs to a wider range of ethnicities than originally appreciated. The recognition of FMF among Greeks, Italians, and some non-Mediterranean populations [15*] and of TRAPS in an even more global distribution [16] is already established. A recent report from Italy extends the geographic distribution of HIDS, formerly regarded as occurring primarily in individuals of northern European ancestry, to the south, with a total of 14 mutation-positive cases from Italy and Albania [17**].

Several recent reports also address specific mutations in HRF genes. A Spanish group has reported a novel \textit{HIDS} \textit{MEFV} variant associated with prolonged fevers, dominant joint involvement, colchicine resistance, and an autosomal dominant mode of inheritance in a three-generation family [18*]. This severe \textit{MEFV} variant joins two others, \textit{ΔM694V} and the M694I-E148Q complex allele, with an apparent dominant inheritance [19]. Perhaps at the opposite end of the spectrum of severity is the \textit{MEFV} variant E148Q, which is present at sufficiently high frequency in several Middle Eastern control populations to be considered a low-penetrance variant [20] or perhaps even a benign polymorphism [21]. A recent Turkish series reported clinical features on 26 individuals homozygous for E148Q, all but four of whom were symptomatic [22*]. With the reservation that these patients did not undergo complete \textit{MEFV} sequencing, and therefore could possibly harbor other unknown mutations, E148Q homozygotes had a distribution of symptoms similar to that of patients with other FMF-associated genotypes and a similar
responsiveness to colchicine. These data suggest that, at least under certain as-yet undefined genetic and environmental conditions, this *MEFV* variant may be associated with the FMF phenotype.

Especially in the case of TRAPS, recent publications point to a possible broadening of the clinical phenotype. The question of neurologic involvement in TRAPS has been raised in several case reports, including the description of one woman with the T50K *TNFRSF1A* mutation with abnormal findings on magnetic resonance imaging [23*], although the causal relation is not completely clear due to concomitant etanercept treatment. A second paper reported panniculitis in individuals with the T50M and R92Q mutations [24*]. A third report presented the case of an African American boy with the P46L *TNFRSF1A* variant and myocardiitis and sacroiliitis, two previously unrecognized manifestations of TRAPS [25*]. Although P46L is the only *TNFRSF1A* variant that we have seen among African American TRAPS patients in our clinic, it should be noted that it is also seen in approximately 4% of African American control individuals [26], and in an even higher percentage of West African controls [27*], indicating that P46L is frequently not fully penetrant, at least for the TRAPS phenotype.

Considerable recent attention has also been focused on mutations in *CIAS1*, which can cause FCAS, MWS, and the NOMID/CINCA syndrome. According to accepted clinical definitions, FCAS is characterized by cold-induced episodes of fever and urticarial skin rash, without evidence of hearing impairment [28**]. MWS presents with febrile episodes not necessarily induced by cold, but often with sensorineural hearing loss and systemic amyloidosis [10**]. NOMID/CINCA manifests urticarial rash regardless of temperature, with central nervous system involvement (papilledema, cerebrospinal fluid pleiocytosis, or sensorineural hearing loss) and a characteristic arthropathy [29*]. Recent case reports and clinical series confirm earlier impressions of a more continuous spectrum of phenotypes [30–32], including MWS patients with features of FCAS [33*], families in which various members exhibit manifestations of FCAS, MWS, or NOMID/CINCA [34**,35*], and patients with unique variant phenotypes [36*], one of which is associated with the first mutation to be described in the cryopyrin leucine-rich repeat (LRR) domain [37*]. Moreover, several mutations have been identified in both FCAS and MWS [7,29**,38,39,40*] and in both MWS and NOMID [8,9,29**,32,38,40*,41].

Genetic screening for mutations in the HRF genes has also revealed the coexistence of mutations of two different autoinflammatory disease genes in a single subject. In one case, a 7-year-old girl was found to have the V377I mutation at the HIDS-associated mevalonate kinase (*MVK*) locus, as well as the R92Q variant at *TNFRSF1A*, and presented with mild features of HIDS but responded to steroids in a way more characteristic of TRAPS [42*]. A second patient with compound heterozygosity for V377I/S378P *MVK* was also found to have the R92Q *TNFRSF1A* variant and manifested disproportionately severe biochemical mevalonate kinase deficiency relative to her mild clinical phenotype [43*]. Another patient with V377I and G211A mutations in *MVK* and the P46L *TNFRSF1A* variant had more severe symptoms that partially responded to the TNF inhibitor etanercept [44]. Yet another patient of Chinese ancestry with prolonged episodes of fever and abdominal pain was found to have compound heterozygosity for the Y20D *TNFRSF1A* mutation and the E148Q variant of *MEFV* [45*]. Given the relatively high frequency of R92Q in the white population [26] and E148Q in the Chinese [46], it is not altogether surprising that compound heterozygosity involving these variants would be observed. Longitudinal follow-up over many years may be needed to define the phenotypic ramifications of these gene interactions.

Several important questions in the genetics of the HRFs must be resolved. Substantial numbers of patients meeting clinical criteria for FMF, HIDS, or the cryopyrinopathies, or who have clinical features resembling TRAPS, do not have demonstrable mutations at any of the known causative genes. Although noncoding mutations remain a logical possibility, it is also possible that there are additional HRF genes yet to be found. A second major area of interest is defining the factors affecting penetrance. Population-based estimates of the frequency of *MEFV* mutations among several ethnic groups [10**], of the V377I mutation in *MVK* in the Netherlands [47], and of the R92Q [26] and P46L [27*] variants of *TNFRSF1A* in whites and African Americans, respectively, all point to the likelihood of reduced penetrance of the respective mutations. Finally, for the case of the recessively inherited FMF, it remains a puzzle why as many as one third of patients with clinical disease have only one demonstrable mutation [10**]. The answer to this latter question may be tied to the resolution of the first two.

**Amyloidosis in the hereditary recurrent fevers**

Systemic amyloidosis is one of the most serious manifestations of the HRFs and is the result of the tissue deposition of misfolded fragments of serum amyloid A (SAA), one of the acute-phase reactants produced by the liver in response to systemic inflammation [48]. Most frequently, deposition occurs in the kidneys, gastrointestinal tract, adrenals, spleen, testes, and lung and sometimes in the liver, heart, and thyroid. In the precolchicine era, amyloidosis was a frequent cause of death in patients with FMF, particularly north African Jews, Turks, and Armenians. Amyloidosis in FMF can sometimes precede the development of febrile attacks (phenotype II), a phenomenon that
is probably due to the persistent subclinical inflammatory state seen even in the absence of symptoms in some HRF patients [16,49–52,53*].

A substantial body of literature indicates an increased risk for amyloidosis among Jewish, Arab, and Armenian patients who are homozygous for the M694V mutation [55–59]. In a series of more than 1000 Turkish patients for whom mutational analysis was available [60*], however, there was no statistically significant association between this genotype and the risk of amyloidosis. Although other smaller series from Turkey have come to the same conclusion [61,62], the explanation for the difference from other populations is not clear but could involve either differences in the frequency of modifier genes or environmental effects.

One apparently important modifier factor in amyloidosis risk in FMF is the SAA1 precursor isoform, with the α/α variant conferring increased risk [63,64]. In a recent series from Turkey, seven of 23 FMF patients with this genotype had amyloidosis vs one of 51 patients with other SAA1 genotypes [65*]. Significant differences were also observed in a recent study of 70 Arab patients [66*]. The mechanism by which this SAA1 variant increases amyloid risk is unknown, but current speculation focuses on differences in macrophage processing or intrinsic potential for fibril formation [63].

Amyloidosis also occurs relatively frequently in patients with MWS and NOMID/CINCA, as well as TRAPS. In TRAPS, susceptibility to amyloidosis appears to be increased among patients with mutations at cysteine residues [26], although patients with noncysteine mutations, most notably T50M, have been reported [67*]. Amyloidosis is extremely rare in HIDS, with the first case having been reported only within the past year [68*]. It is not clear whether the rarity of amyloidosis in HIDS, relative to FMF, TRAPS, MWS, and NOMID/CINCA, is due to an overall lower SAA burden in HIDS, to less amyloidogenic alleles at modifier genes, or to environmental factors.

Role of hereditary recurrent fever genes in inflammation
Given the relatively high frequency of certain HRF alleles in the general population, there has been considerable speculation that some of these variants may also predispose to other inflammatory phenotypes [26]. It goes without saying that in situations such as this, in which common genetic variants of HRF genes are sought in other relatively common illnesses, controls that are appropriately matched, particularly for ethnic background, are essential. Particularly striking are the results of a study of the R92Q variant in a large European study of cardiovascular disease [69*]. Among 62 cigarette smokers with carotid plaque, 9.7% had R92Q, vs 2.1% of 338 smokers without plaque, for an odds ratio of 5.97 (95% confidence interval 1.64–15.63,  P = 0.0048). Other less dramatic associations were also noted between R92Q and carotid intima-media thickness. R92Q and the E148Q MEFV variant have also been recently associated with increased susceptibility for reactive systemic AA amyloidosis in other chronic inflammatory disorders [70*].

Two studies have noted an increased incidence of Crohn’s disease in patients or families with FMF [71,72]. Recently, another investigative group examined the frequency of MEFV mutations in a cohort of 209 Israeli patients with Crohn’s disease [73*]. In this study, there was no increase in the frequency of specific MEFV mutations in cases relative to controls, although the E148Q variant was associated with perianal disease, with an odds ratio of 3.26 (95% confidence interval 1.2 – 8.8,  P = 0.02).

Finally, associations have been drawn between FMF and Behçet’s disease. Increased frequencies of MEFV mutations have been reported in Behçet’s patients [74], and, conversely, Behçet’s disease has been reported at an increased frequency among Israeli patients with FMF [75]. A recent paper from Turkey found MEFV mutations in 15 of 42 Behçet’s patients, but in only seven of 66 controls (  P = 0.0034) [76*].

Although the HRF genes may, in some circumstances, conspire with other genetic and environmental factors to cause a broader spectrum of inflammatory diseases, certain disorders may actually be less common in the HRFs. Recently a group from Turkey drew attention to the complete absence of systemic lupus erythematosus among their cohort of more than 1000 FMF patients [77]. The authors speculated that high levels of C-reactive protein typically seen in FMF patients might increase clearance of apoptotic cells and autoantigens. Although this remains an intriguing hypothesis, it underscores the potentially complicated and even reciprocal interactions among autoinflammatory and autoimmune disorders, which represent respective aberrations of the innate and adaptive arms of the immune system. The suggestion of a possible positive correlation between systemic lupus erythematosus and TRAPS in the Japanese population [78] awaits confirmation.

Pathogenesis
The elucidation of the molecular basis of the HRFs has focused attention on a group of genes encoding proteins (Fig. 1) that regulate several critical inflammatory and apoptotic pathways. Much of the past 2 to 3 years’ work has concentrated on further delineating these pathways and understanding how specific disease-associated genes cause autoinflammation.
Pyrin and family

Signal transduction and protein oligomerization in inflammation and apoptosis are often mediated by a group of protein-protein interaction domains, the so-called death domain-fold superfamily [11]. This family currently comprises four members, the death domain, the death effector domain, the caspase-recruitment domain (CARD), and the PYRIN domain. Each motif has an antiparallel arrangement of six $\alpha$-helices that allows binding of cognate domains (death domains with death domains, etc.) through electrostatic charge interactions [11,79–81].

The pyrin protein is the prototype for the death domain-fold motif that bears its name. The recognition of the PYRIN domain, an N-terminal 92-amino-acid motif, in pyrin set the crucial cornerstone for further insights into the underlying mechanisms of the HRFs. Of the approximately 20 PYRIN domain-containing human proteins currently known [82••], pyrin and cryopyrin have been shown to harbor HRF-associated mutations. A third member of this family, apoptosis-associated specklike protein with a CARD (ASC), is a bipartite adaptor protein consisting of an N-terminal PYRIN domain, through which it can interact with pyrin [79,83–85] or cryopyrin [84,86,87], and...
a C-terminal CARD, through which it can interact with several downstream molecules. Although no disease-associated ASC mutations have been identified in HRF patients to date, it is a pivotal molecule in the pathogenesis of these diseases.

Recent biochemical evidence indicates that cryopyrin (NALP3) and ASC participate in a larger macromolecular complex termed the NALP3 inflammasome [88••,89••] that mediates the activation of interleukin-1β and interleukin-18. The NALP3 inflammasome activates interleukin-1β by bringing molecules of caspase-1 (interleukin-1β-converting enzyme) zymogen into proximity, thus allowing autocatalysis of its p20 and p10 subunits, which, when released, cleave prointerleukin-1β into its biologically active form. As depicted in Figure 2, interaction of the LRR domain of cryopyrin/NALP3 with the NACHT domain (so named because it was first observed in neuronal apoptosis inhibitor protein, CIITA, HET-E and TP1) ordinarily inhibits the interaction of cryopyrin/NALP3 with Cardinal, another protein in the complex. Stimuli that 'open' the cryopyrin/NALP3 structure permit this interaction, through which one molecule of caspase-1 is recruited to the complex. A second caspase-1 molecule is recruited through the interaction of the PYRIN domain of cryopyrin/NALP3 with ASC.

From the foregoing analysis, it would appear that the LRR – NACHT domain interaction in cryopyrin/NALP3 is a critical control point in the activation of the inflammasome. Just as extracellular LRRs of the Toll-like receptors can interact with various pathogen-associated molecular patterns, intracellular muramyl dipeptide, a common pathogen-associated molecular pattern, can activate the NALP3/cryopyrin inflammasome [89••], presumably by binding the LRR. CAPS-associated mutations are almost exclusively in the NACHT domain, and macrophages from a patient with MWS showed increased interleukin-1β secretion in the presence of muramyl dipeptide. In some cases, CAPS-associated cryopyrin/NALP3 mutations may even permit constitutive interleukin-1β maturation [88••,90••,91*] without the requirement for exogenous muramyl dipeptide. It is also possible, although not proven, that FCAS-associated cryopyrin/NALP3 mutations destabilize the NACHT-LRR interaction in the cold, thereby permitting interleukin-1β activation.

Pyrin itself also appears to play an important role in regulating interleukin-1β activation. In-vitro data suggest that pyrin competes with both cryopyrin and caspase-1 for binding to ASC [83,84]. Mice expressing a truncated, hypomorphic pyrin variant exhibit heightened sensitivity to endotoxin challenge, with increased activation of both caspase-1 and interleukin-1β. These data suggest that one function of wild-type pyrin is the suppression of inflammasome-mediated interleukin-1β production and that FMF-associated mutations may interfere with this process (Chae et al., unpublished observations). Mutations in proline serine threonine phosphatase interacting protein 1 (PSTPIP1), a protein recently shown to bind pyrin, appear to exert a dominant negative effect on this pathway [92]. Two PSTPIP1 mutations (Fig. 1) have been associated

![Figure 2. Schematic of the molecular mechanisms defining the cryopyrin (NALP3) inflammasome](image)
with increased pyrin binding, excessive interleukin-1β production, and a severe autoinflammatory disorder, the PAPA syndrome.

Both cryopyrin and pyrin also appear to regulate another process important in inflammation: apoptosis. The aforementioned pyrin-deficient mice exhibit a defect in leukocyte apoptosis through an interleukin-1β-independent, caspase-8-dependent pathway [83], suggesting a pro-apoptotic role for the wild-type protein, although in certain transfection systems it exerts an antiapoptotic effect [79,84,85]. Enforced expression of cryopyrin in HEK293T cells also induces apoptosis [84].

Depending on the cellular context, both pyrin and cryopyrin can either activate or suppress nuclear factor-κB [84,86,87,93,94], a family of transcription factors involved in the initiation and resolution of inflammation. Although the precise mechanism is still under investigation, this appears to be ASC dependent and, under some conditions, involve the inhibitor of nuclear factor-κB kinase complex [93]. Because endogenous pyrin has recently been shown to localize in the nucleus in several cell types, including synovial fibroblasts, neutrophils, and dendritic cells (but not monocytes) [95**], it is also possible that pyrin may associate with one or more components of the nuclear factor-κB complex. Moreover, in the absence of ASC, a relatively rare isofrom of pyrin with an inframe deletion of exon 2 also localizes in the nucleus, regardless of FMF-associated mutations [96*].

**TRAPS: the plot thickens**

Stimulation through the p55 TNF receptor can lead either to nuclear factor-κB activation or apoptosis, depending on the balance of several contextual factors. Upon receptor activation through TNF, metalloprotease-induced cleavage of the extracellular TNFRSF1A domain can limit continuous signaling at the cell surface while simultaneously creating a pool of potentially antagonistic soluble receptor (Fig. 3A). Initial studies of a family with the C52F mutation indicated impaired activation-induced apoptosis of receptor ‘shedding’ [1], thereby possibly explaining the inflammatory phenotype.

Subsequent studies indicate a more complex picture, with defects in TNF receptor cleavage varying with mutation [26,97] and cell type [98**]. Moreover, in transfection experiments, certain TNFRSF1A mutants exhibit impaired intracellular trafficking and TNF binding, although their ability to signal through the death domain is unimpaired [99**]. Conceivably, the conformational changes in the p55 receptor that lead to altered intracellular trafficking could also impair metalloprotease-induced cleavage of mutant receptors that do reach the surface. Studies of dermal fibroblasts and monocytes from a patient with the newly identified C43S mutation suggest yet another possible mechanism for TRAPS: a defect in TNF-induced apoptosis, leading to an inappropriately prolonged inflammatory response [100**].

TRAPS-associated p55 mutations might also cause constitutive activation, perhaps by permitting intermolecular disulfide homodimerization and ligand-independent activation. This possibility was considered for patients with the C52F mutation in the initial description of TRAPS but appeared not to be operative [1]. Moreover, such a mechanism would appear to be inconsistent with the therapeutic effects of TNF inhibitors (vide infra). It may be fruitful, however, to reexamine this issue for a broader sampling of patients, given the heterogeneity of cleavage defects for different mutations, the observation of biochemical inflammation in TRAPS patients even between attacks [16], and the discovery of ligand-independent noncovalent interactions mediated by the first cysteine-rich domain of the p55 receptor [101]. Yet another conceptually attractive possibility relates to the recent finding that the predominant form of TNFRSF1A in human plasma is full length, probably the result of exosome-linked release of receptor [102*]. In light of the aforementioned defects in receptor trafficking, it is intriguing to hypothesize that TRAPS mutations might impair such a process.

From the foregoing, it appears clear that there may be multiple mechanisms leading to the TRAPS phenotype and that the pathophysiology may be heterogeneous among patients. Clarification of these issues will undoubtedly require triangulation between studies of primary cells from patients, transfected cell lines, and knock-in animal models.

**Hyperimmunoglobulinemia D with periodic fever syndrome: nature’s elaborate deception?**

Perhaps the most enigmatic of the HRFs is HIDS. The enzyme mutated in HIDS, called mevalonate kinase, is the only HRF protein that does not include a death domain-fold motif. Mevalonate kinase catalyzes the conversion of mevalonic acid to 5-phosphomevalonic acid in the synthesis of sterols, including cholesterol, vitamin D, bile acids, and steroid hormones (Fig. 3B). Evidence is strong that HIDS is not due to excessive IgD, because there are well-documented patients who have the HIDS phenotype and MVK mutations but persistently normal IgD levels [12,103–105], and, even among patients with increased serum IgD, the levels do not predictably fluctuate with attacks [106]. Moreover, the HIDS phenotype appears not to be due to a defect in cholesterol synthesis, because patients have cholesterol levels in the low-normal range, and more severe disorders of cholesterol biosynthesis do not have an autoinflammatory phenotype [107].

Currently there are two major hypotheses on the pathogenesis of HIDS: that the inflammatory attacks could result from the accumulation of mevalonic acid, the
substrate for the mevalonate kinase enzyme [108], or that the autoinflammation is caused by a shortage of isoprenoids, which are normally synthesized through the mevalonate pathway [109]. These latter compounds are involved in the post-translational prenylation (farnesylation or geranylation) of several important intracellular signaling molecules, including the Ras, Rho/Rac, and Rab families of small guanosine triphosphate-binding proteins. In an in-vitro system, accentuated interleukin-1β secretion by leukocytes from HIDS patients can be reversed by the addition of farnesol or geranyl-geraniol, lending support to the second hypothesis [109].

Both the isoprenoid deficiency and mevalonate accumulation hypotheses predict a worsening of symptoms with decreased mevalonate kinase enzymatic activity. In-vitro studies of cell lines harboring wild-type or HIDS-mutant MVK indicate that the mutant enzyme functions best at 30°C, with a diminution at 37°C and further decreases at 39°C [110]. This finding may account for the triggering

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Figure 3. Schematic of the proposed pathogenic mechanisms of TRAPS (a) and HIDS (b)

(a) The mechanisms suggested to be involved in the pathogenesis of TRAPS are shown from left to the right and include defects of TNFRSF1A intracellular trafficking with pathologic storage in the Golgi apparatus and subsequent reduced cell surface expression of TNFRSF1A, defects of the TNF-α induced signaling through TNFRSF1A with subsequent alterations of NF-κB activation and apoptosis, as well as a TNFRSF1A cleavage defect from the cell surface with subsequent reduced levels of soluble TNFRSF1A. The green circles represent the metalloproteinases that induce the receptor shedding at the cell surface. The blue curved lines represent the four extracellular domains of TNFRSF1A, followed by the transmembrane region marked as a black line and the intracellular death domain drawn as brown diamond. TNFRSF1A forms a homotrimer at the cell surface. The punctuated red lines represent the cell membrane with the area above representing the extracellular and the area below the intracellular space. The orange stars highlight the various sites for which defects in the TRAPS pathogenesis have been described so far.

(b) The mevalonate pathway. Patients with HIDS show markedly reduced mevalonate kinase activity, which leads to an increase of mevalonic acid and decrease of isoprenoids. Both consequences may lead to IL-1β activation with subsequent inflammation in HIDS.
of HIDS attacks by immunizations and infections and may also account for the increased urinary mevalonate levels seen during HIDS attacks.

**Treatment**

Advances in our understanding of the biology of HRFs, coupled with the expanded armamentarium of new targeted therapies, have led to new approaches to the treatment of these disorders. Therapeutic goals include suppression of acute attacks, which are usually not life threatening but can be very disabling, and preventing long-term sequelae, such as amyloidosis and long-term neurologic/intellectual impairment in CAPS.

The most promising results of the past year involve the use of anakinra, a recombinant human interleukin-1β receptor antagonist, in patients with CAPS. FCAS patients who were pretreated with interleukin-1β receptor antagonist before cold challenge did not develop clinical symptoms or increase in acute-phase reactants [111]. Serum levels of interleukin-1β and cytokine mRNA in peripheral blood mononuclear cells were normal but highly elevated in affected parts of the skin, implicating differences in the distribution of cells contributing to disease phenotype. A complete cessation of clinical symptoms and biochemical changes was also reported in MWS patients following administration of interleukin-1β receptor antagonist [112,113]. Even children with the more severe phenotype of NOMID/CINCA responded to anakinra doses of 1–2 mg/kg per day with resolution of uveitis, rash, and fever and a significant decline in cerebrospinal fluid pressure [114–117]. The dramatic nature of the response of CAPS patients to interleukin-1 inhibition is, in a way, surprising, given the apparent role of cryopyrin in other inflammatory processes, such as nuclear factor-κB activation and apoptosis. Given the reduced life expectancy of NOMID/CINCA patients, who have a death rate of about 20% before the age of 20, it will be important to follow a larger series of these children on anakinra to monitor long-term outcome with regard to mental and physical development, as well as to determine whether early treatment can prevent joint deformities.

As noted in the previous section, interleukin-1β also appears to play a role in the pathogenesis of FMF, PAPA syndrome, and HIDS and may also be involved indirectly in the pathogenesis of TRAPS. Interleukin-1 inhibition could therefore represent a possible option as first-line or second-line treatment in these diseases. Anakinra has been reported effective in the treatment of one patient each with TRAPS and PAPA syndrome [118,119].

There is also a substantial experience with TNF inhibitors in the HRFs, most notably the use of etanercept, the p75 TNFR:Fc fusion protein, in TRAPS. The administration of 50–75 mg per week in adults, or 0.8–1.2 mg/kg/wk in children, is effective in reducing, although not usually eliminating, clinical and laboratory evidence of inflammation [4,16], thereby allowing a dose reduction in nonsteroidal anti-inflammatory drugs or glucocorticoids. In some patients, etanercept appears to prevent amyloid formation or even reduce proteinuria in patients with amyloid nephropathy [120,121]. Unfortunately, development of amyloidosis can occur even when symptoms are controlled by etanercept [122], and it is likely that monitoring of SAA levels is necessary to titrate the optimal dosage [120,121].

Although HIDS very rarely leads to systemic amyloidosis, and does not share the neurologic sequelae of CAPS, attacks are frequently severe enough to warrant treatment, particularly in childhood and adolescence. To date there is no accepted therapy for HIDS, other than antipyretics and palliative measures, but pilot studies have been conducted in two areas. First, a small trial has been conducted with simvastatin, an inhibitor of 3’-hydroxy-3’-methylglutaryl – coenzyme A reductase, the enzyme immediately preceding mevalonate kinase in the mevalonate pathway (Fig. 3B). It appears safe, and preliminary data suggest a possible benefit [108]. A pilot study of etanercept showed substantial symptomatic improvement in two mutation-positive children with HIDS [105], although a third HIDS patient who did not respond to etanercept was recently reported by another group [123]. Interleukin-1 inhibition may represent yet another possible therapeutic strategy.

Daily oral colchicine therapy has been established as effective in preventing both the acute attacks of FMF and the development of amyloidosis. In the subset of patients who are poorly responsive to colchicine, lower colchicine concentrations were found in mononuclear cells [124], suggesting that differences in responsiveness may be due to polymorphisms in transporters that control intracellular drug concentrations, such as the MDR-1-encoded P-glycoprotein pump. In such patients, several adjunctive approaches are under investigation, including subcutaneous interferon-α [125,126,127] and biologic therapies aimed at TNF [128] or interleukin-1β. Allogeneic bone marrow transplantation has recently been proposed as a treatment for refractory FMF [129], based on the predominant expression of MEFV in leukocytes [5]. Although it is possible that this approach could be effective, in nearly all cases other options exist, and the risks outweigh the potential benefits [130].

**Conclusion**

Identification of the genes mutated in the HRFs has led to great strides in our approach to patients with these disorders. Although substantial numbers of patients with clinical recurrent fever syndromes do not have mutations in the respective genes, the availability of genetic testing as an adjunct has led to more widespread and earlier identification of patients with these syndromes. The ability to diagnose patients before age 20 years, and the availability of targeted therapies to treat and prevent complications, has transformed the care and outcomes of these patients.
recognition of these conditions, and recognition of important pathogenic and therapeutic differences among patients who, 10 years ago, were largely lumped together as FMF variants. Exciting advances in molecular biology have defined new families of motifs and proteins relevant to inflammation and apoptosis, but important questions remain regarding the role of the products of the mevalonate pathway. Perhaps most notable are the great strides in therapy brought about by the happy confluence of breakthroughs in molecular pathogenesis and the new availability of targeted biologic agents. Fascinating areas for further investigation include the possible identification of additional genes that might account for patients who are currently mutation negative, the elucidation of modifier genes, the more thorough understanding of molecular pathogenesis and mechanisms of specific mutations, and a careful comparative analysis of various available treatments in multicenter trials.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


A very comprehensive current review of the HRFs.


This thorough paper extends the geographic distribution of HIDS to Italy and describes several new mutations and clinical phenotypes.


An interesting clinical report describing a new dominantly inherited MEVF variant associated with a severe phenotype.


A close examination of the clinical features of Turkish patients with the E148Q/ E148Q genotype.


First report of panniculitis in TRAPS.


This paper reports two new manifestations in an African American patient with the P46L mutation and severe TRAPS.


This paper reports a P46L allele frequency of approximately 10% in west African populations, suggesting that this substitution frequency is not associated with symptoms of TRAPS. Additional genes that may affect the phenotype associated with this variant remain to be identified.


A comprehensive review of the urticarias, including detailed clinical and diagnostic features.


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This paper outlines a possible risk of TNFRSF1A 92Q allele carriers for atherosclerosis.


The E148Q variant of MEFV and the R92Q variant of TNFRSF1A may contribute to amyloidosis susceptibility in a small number of patients.


Analysis of MEFV mutations in an Israeli cohort of Crohn’s disease patients. Although MEFV mutations were not associated with an increased risk of Crohn’s disease, the E148Q variant was associated with perianal disease.


A survey of MEFV mutations in a Turkish Behçet’s disease cohort suggests that MFM-associated mutations may be implicated in the pathogenesis of Behçet’s disease.


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First report of a NOMID / CINCA patient with favorable response to anakinra.


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First report of anakinra treatment of a patient with TRAPS.


First report describing the effect of anakinra treatment in PAPA patients.


Clinical trial supporting the treatment of renal amyloidosis in TRAPS patients with etanercept.


Recent developments in immunomodulatory peptides in juvenile rheumatic diseases: from trigger to dimmer?
Eva C. Koffeman\textsuperscript{a,b,c}, Berent Prakken\textsuperscript{d} and Salvatore Albani\textsuperscript{a,b,c}

\textbf{Purpose of review}
Current therapy for juvenile rheumatic diseases is based on general immune suppression or blocking inflammatory pathways. These treatments do not induce long-term disease remission and have a risk of side effects; this is especially unfavorable in children. It is better to focus on induction of tolerance mechanisms than on suppression of inflammation. This promotes epitope specific immunotherapy as a possible safe treatment option.

\textbf{Recent findings}
In the search for specific peptides for immunotherapy in autoimmunity, the focus is shifting from purported triggers of disease to peptides that regulate the ongoing inflammation. These so-called ‘immunomodulatory peptides’ are important in every healthy immune system. Several juvenile rheumatic diseases have been linked to certain immunomodulatory peptides. In juvenile dermatomyositis, peptides from human skeletal myosin play a role in the perpetuation of the disease. In systemic lupus erythematosus, the focus is mostly on DNA-derived peptides and peptides from anti-DNA antibodies. In juvenile idiopathic arthritis, heat shock proteins have been shown to contain important immunomodulatory epitopes.

\textbf{Summary}
Immunomodulatory peptides play an important role in juvenile rheumatic diseases. Promising candidates for immunotherapy have been identified. This opens the possibility of clinical testing in rheumatic diseases of childhood.

\textbf{Keywords}
immunomodulatory peptides, juvenile rheumatic disease, therapy, tolerance mechanisms

\textbf{Abbreviations}
- **AMP**: antimicrobial peptides
- **APCs**: antigen presenting cells
- **DMARDs**: disease modifying antirheumatic drugs
- **hsp**: heat shock proteins
- **JDM**: juvenile dermatomyositis
- **JIA**: juvenile idiopathic arthritis
- **SLE**: systemic lupus erythematosus
- **T reg**: regulatory T cells
- **TCR**: T cell receptors

\textbf{Introduction}
In this review, current knowledge about immunomodulatory peptides and its implications on the treatment of juvenile rheumatic diseases are discussed.

A peptide is a fragment of protein, self or non-self, that is immunologic relevant. The peptides described here do not automatically induce a pro inflammatory response. The term ‘immunomodulatory’ stands for the fact that these peptides affect the intensity and quality of the immune reaction of the innate and adaptive immune system.

Juvenile rheumatic diseases are defined, as ‘inflammatory diseases of the connective tissue in children [1] not associated with infectious agents or immunodeficiencies’. Juvenile idiopathic arthritis (JIA), systemic lupus erythematosus (SLE), and juvenile dermatomyositis (JDM) are the focus in this review.

\textbf{Immunomodulatory peptides in the healthy immune system}
Immunomodulatory peptides play an important role in the physiology of the healthy immune system. They can be divided into two major groups: peptides that initiate a reaction through the innate immune system, and peptides that directly induce an adaptive response, from either B cells or T cells.

The first group of immunomodulatory peptides consists of antimicrobial peptides (AMP). These are small and evolutionary ancient cationic peptides, found in the gastrointestinal, respiratory, and urinary tracts and in the skin of most vertebrates. AMP are important immunomodulatory peptides, because besides acting as natural antibiotics, they serve in a sentinel role as multifunctional effectors of innate immunity, initiating, mobilizing, and amplifying adaptive immune responses [2]. The two major families
Immunomodulatory peptides are true immunomodulatory peptides. Research has shown that changes in AMP are associated with a variety of pathologic processes [3].

The immunomodulatory peptides of the second group serve as antigens to the T cells and B cells of the adaptive immune system, inducing long-lasting memory. They are recognized by T cell receptors (TCR) or B cell derived immunoglobulins, which can function as B cell receptors. Immunoglobulins and TCRs are related in their protein structure and in their genetic mechanism, which produces their great variability. However, their function is different. Igs can recognize and bind antigen directly, thus neutralizing the antigen and recruiting other cells and molecules to destroy the pathogen. T cells react to antigens when they are presented as peptides on MHC molecules on antigen presenting cells (APCs). Binding of the T cell receptor by the MHC-peptide complex triggers a cascade of signaling that ultimately leads to cell proliferation and cytokine production. The peptides for the adaptive immune system can be divided into pathogenic peptides and self-derived peptides.

Pathogenic antigens, from bacteria and viruses, induce an active response from antigen specific lymphocytes. This response is coordinated by the T effector cells. Because the defense to different pathogens requires specific responses, different peptides induce different T cell types to respond. T helper (Th) cells orchestrate the decision between a cellular response (Th1), or a humoral response (Th2) to the pathogen [4]. Th1 and Th2 type cells differ in their chemokine receptors and cytokine production. The fate of a Th precursor cell to become Th1 or Th2 type antigen specific cells depends on the avidity of the antigen, the nature of the co-stimulatory molecules, and the cytokine environment. If the Th1/Th2 balance has been skewed to one arm, this arm usually stays dominant in the response, because induction of one cell type involves inhibition of the other.

Because the nature of the peptide defines which side of the spectrum will be involved, peptides from bacteria and viruses are true immunomodulatory peptides.

Antigen presenting cells also continuously present peptides from the body’s own proteins, and every healthy system contains self-reacting T effector cells. Proinflammatory reactions to self-peptides result in destruction of the body’s own tissues, called autoimmunity. Tolerance mechanisms exist to avoid autoimmune reactions [5]. The tolerance process starts in the thymus, where peptides from endogenous proteins are presented to the immature T cells. T cells with high affinity to these self-antigens undergo clonal deletion by apoptosis, a process called ‘negative selection’. However, not all self-antigens are presented in the thymus and some self-reactive T cells escape negative selection. Peripheral tolerance is the system that keeps these autoreactive T cells under control. It works through different pathways: deletion by apoptosis, ignorance of the antigen, and active regulation through anergy and suppression by regulatory T cells (T reg). These relatively recently described cells suppress autoreactive T cells and play a role in infection [6]. Different groups of T regs have been described, with different phenotypes and cytokine expression. One group is called ‘naturally occurring’ T reg [7]. They are highly enriched in the CD4+ CD25+ population. They are antigen specific, but exert their function in a nonantigen specific way and are able to induce tolerance to other antigens [8,9]. Another group of T reg is identified by their production of IL10 and TGFβ [10].

Self-peptides are immunomodulatory peptides that are probably integral to normal immune function. The resulting induction of tolerance mechanisms possibly has a role in keeping immune homeostasis [11]. In infection and inflammation, the expression of self peptides from destroyed tissue probably induces a feedback tolerance mechanism that prevents further damage (Fig. 1).

Within the self-peptides, ‘idiotypic’ peptides play a special role. These are peptides derived from the Igs and TCRs themselves, expressed on autoreactive CD4+ T cells. Interestingly, the body has a specific physiologic response to these self-antigens. After recognition of TCR peptides by CD4+ T cells B cells and CD8+ regulatory T cells can delete clones of autoreactive lymphocytes or induce a cytokine shift from Th1 to Th2 and so prevent autoimmunity [12]. This is another mechanism of peripheral tolerance. Through similar mechanisms, B cell populations are kept under check by T cells through recognition of the Ig derived antigens B cells present on their surface [13]. Idiotypic antigens are real immunomodulatory peptides because the anti-idiotypic T cell reaction has a role in the homeostasis of the immune system.

Immunomodulatory peptides in juvenile rheumatic diseases

Juvenile rheumatic diseases are autoimmune diseases. Autoimmunity is the term used to describe when tolerance to self is broken, with the result of a proinflammatory immune reaction against self-antigens. The reaction to self and non-self should probably be seen as a continuum, with the induced reaction being dependent on the level of immune regulation [14••]. Therefore, autoimmunity could be seen as a misbalance of the tolerance mechanisms.

For a long time the focus in autoimmunity research has been to find the specific self-antigen that is the cause of the disease. Autoantigens have often been identified through studies in animal models that were transposed
to humans. Based on animal models and molecular mimicry anticollagen antibodies have been associated with juvenile rheumatic diseases [15]. This led to trials of antigen specific therapy with collagen in JIA [16,17]. Chromatin has also been proposed as an important antigen in JIA [18]. In SLE (adult and juvenile form), 116 auto-antigens have been described [19].

This search for a unique, disease specific trigger is probably futile in the light of treatment options. Human autoimmune diseases may, unlike animal models, start out as a reaction to, probably multiple, self-antigens. However, in humans once the disease progresses and becomes more destructive, the immune reactions spread out over many more targets, a process called ‘epitope spreading’ [20]. Once tolerance has been broken and the immune system has evolved into a proinflammatory mode, the process amplifies itself [Fig. 1]. Induction of tolerance to a single, by then elusive triggering peptide would not change the ongoing inflammation in this later stage. Hence, it may be advantageous to focus on antigens that play an immunomodulatory role in the perpetuation of the inflammation [Fig. 2]. Candidate antigens should be present and possibly over-expressed at the site of inflammation. They should be immunologically relevant, meaning that they trigger T cell responses and cytokine production that contribute to modulating the inflammatory reaction locally and systemically. The concept of molecular mimicry may also be important. This concept implies that antigens can be structurally so similar that a lymphocyte cannot distinguish between them. Resulting cross-reactivities between pathogenic and self-peptides can be responsible for autoimmunity [20]. Recently, several of these immunomodulatory peptides have been identified in juvenile rheumatic diseases [Table 1].

In JDM a relation with streptococcal infection has been suggested that has led to the identification of epitopes

**Figure 1. In infection and inflammation, the expression of self peptides from destroyed tissue probably induces a feedback tolerance mechanism that prevents further damage.**

![Healthy and autoimmune reactions](image)

**Figure 2. Therapy approaches to autoimmunity**

![Therapy mechanisms](image)
Table 1. Self-peptides that could be candidates for immunomodulatory therapy in juvenile rheumatic diseases

<table>
<thead>
<tr>
<th>Protein</th>
<th>Epitopes of interest</th>
<th>Origin</th>
<th>Disease</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Human skeletal myosin</td>
<td>114–122</td>
<td>Molecular mimicry with Strept. M5</td>
<td>Juvenile dermatomyositis</td>
<td>Massa et al. [21]</td>
</tr>
<tr>
<td>Histones</td>
<td>H4 16–39, 71–93</td>
<td>DNA-derived</td>
<td>Systemic lupus erythematosus</td>
<td>Monneaux et al. [22**]</td>
</tr>
<tr>
<td>Spliceosomal proteins</td>
<td>SmD1 83–99</td>
<td>DNA-derived</td>
<td>Systemic lupus erythematosus</td>
<td>Schotte et al. [23]</td>
</tr>
<tr>
<td>VH region anti-DNA antibodies</td>
<td>pCONS</td>
<td>Idiotypic</td>
<td>Systemic lupus erythematosus</td>
<td>Monneaux et al. [22**]</td>
</tr>
<tr>
<td>HLA-DRB1*1101</td>
<td>EYWNSQKDL</td>
<td>Molecular mimicry with EBV</td>
<td>Juvenile idiopathic arthritis</td>
<td>Kalsi et al. [20]</td>
</tr>
<tr>
<td>HLA-DRB1*0801</td>
<td>TYWQNLQNL</td>
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<tr>
<td>HLA-DRB1*0201</td>
<td>TELGRPSAEYL SLTRDDAEYL ATEEEEEAV</td>
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<td></td>
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<tr>
<td>Human HSP 60</td>
<td>290–294</td>
<td>Heat shock protein</td>
<td>Juvenile idiopathic arthritis</td>
<td>Kamphuis et al. [43**]</td>
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<td>242–256</td>
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... (rest of the text)
interleukin-6. They have brought a more specific and effective treatment for autoimmune diseases. However, they are not immunoregulatory and do not induce sustained disease remission. Life-long treatment with these agents is needed and long-term side effects may result [45]. The safety and value of these agents need more study [46], particularly in a pediatric setting.

To avoid side effects of immune suppression and to ensure effective treatment of autoimmune diseases, it may be preferable to control just those cohorts of lymphocytes that are responsible for the specific inflammation in the autoimmune disease. This would translate in immune deviation instead of immune suppression. Specifically targeting the antigen specific T cells that are responsible for the disease perpetuation can be done by inducing mucosal tolerance. In this form of therapy, tolerance mechanisms to a certain antigen are induced by mucosal administration of the peptide. Because the mucosa is a tolerogenic environment, this induces suppression of injurious immune responses to the administered self-antigen [47]. Antigen specific interleukin-10 producing Tregs probably play a role in this tolerance induction [48]. In this review, several immunomodulatory peptides that can be very successful antigens in mucosal tolerance therapy in juvenile rheumatic diseases have been described. Promising peptides for this purpose are human myosin derived peptides in JDM and hsp peptides in JIA. In SLE, a DNA derived peptide or anti-DNA antibody derived epitope could be a candidate. In a phase I and phase II clinical trial in rheumatoid arthritis, the PI peptide from the hsp dnaJ has shown to be an effective immune modulator for oral tolerance induction [49]. Based on the results in this clinical trial in RA (phase II will soon be concluded), a clinical trial in JIA with an hsp peptide will soon be begun. Such an approach may be based on the concept of immunomodulatory peptides acting as ‘dimmers’ of inflammation instead of putative single disease triggers.

Epitope specific therapy has the potential to be complementary to current biologics. A recent study has shown the effectiveness of a combination of lower dose anti-TNFα and epitope specific immunotherapy in a adjuvant arthritis [50]. The immune modulation induced by nasal tolerance induction was complemented by the effect of anti-TNFα therapy on restoration of Treg function. Antigen specific therapy in a more regulatory environment, built by the anticytokine therapy, may result in a more long-term regulation of the disease [10]. This new approach would significantly reduce the costs and undesirable effects of current first generation biologics. This would be especially favorable in children.

Conclusion
The dramatic evolution in molecular immunology and molecular therapeutics is enabling a shift of focus concerning immunomodulatory peptides from individual purported triggers to members of regulatory networks. This evolution may have significant implications for our understanding of the complex pathogenesis of juvenile rheumatic diseases and open new perspectives for therapy.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


Here is a good review of peptides in SLE.


Gene expression profiling in pediatric rheumatic disease: what have we learned? what can we learn?

James N. Jarvis

Purpose of this review
Gene expression profiling is emerging as a promising methodology in pediatric rheumatology research. There is considerable interest in using this technology as the basis for diagnostic assays. This review will summarize the new knowledge.

Recent findings
Most gene expression studies of children have been exploratory in nature. However, preliminary gene expression studies in juvenile dermatomyositis, systemic lupus erythematosus, and chronic forms of arthritis demonstrate both the promise and limits of this technology. It seems likely that gene expression profiling will significantly enhance our understanding of the immunopathology of childhood-onset rheumatic diseases; however, considerable impediments must be overcome before these assays move into the clinical arena.

Summary
Gene expression profiling carries considerable potential to provide novel insights into the rheumatic diseases of childhood. Future developments will determine whether these technologies provide new clinical diagnostic or prognostic tools.

Keywords
gene expression profiling, juvenile dermatomyositis, juvenile rheumatoid arthritis, systemic lupus erythematosus

Introduction
Gene expression profiling is one of the most interesting by-products of the efforts to sequence the human genome. This technology, sometimes referred to by the term, gene microarray has great potential to transform how we understand, diagnose, and treat a broad range of rheumatic diseases in both children and adults. Indeed, gene expression profiling is already advancing both the research and clinical agendas of specific pediatric subspecialties, most notably pediatric oncology. This article will provide an overview of gene expression profiling as it’s been used thus far in pediatric rheumatology, and provide a critical analysis of the promise and pitfalls of moving this technology from the research to the clinical arena.

General considerations
A gene microarray is a potentially powerful tool that allows the investigator to examine expression levels of multiple genes in a specific cell population or tissue. A typical array consists of a solid platform (glass or nylon) where oligonucleotide representations of multiple genes have been placed (e.g. see Fig. 1). Though specific methodologies differ, the end product of a gene array analysis is a comparison of the levels of expression of hundreds, thousands, or even tens of thousands of genes in compared samples (e.g. experimental versus control cells; patients versus healthy children). Obviously, the technology has considerable promise to unravel some of the baffling questions about rheumatic diseases in adults and children.

One of the challenges of understanding the pathogenesis of rheumatic disease is the sheer complexity of the inflammatory process. Inflammation involves multiple cell types, cell-surface receptors, and secreted protein and non-protein mediators that all change over time. Furthermore, longstanding practice, encouraged by NIH study sections, directed investigators toward narrowly focused, in-depth investigation of specific aspects of a given scientific or clinical problem. A great deal of highly informative science was generated by this approach; however, our practical understanding of rheumatic diseases in children remained elusive. Indeed, pediatric rheumatology investigators found themselves in a predicament rather like that of the blind men with the elephant. JRA, for example might seem like a T-cell disease, a B cell disease, or a monocyte-driven disease, depending on the investigator’s interest. Each of the observations might be relevant in themselves: There was no effective way of seeing the ‘whole elephant’. Gene arrays may finally provide such a method.
There are a few key points that need to be made about the limits of gene arrays as a research tool. The use of gene arrays to unravel disease pathogenesis rests on two assumptions; neither of them is necessarily true. The first assumption is that the relevant mediators for a disease are, in fact, proteins. This seems a bit obvious, yet it is necessary to point out that we already know that for most chronic forms of arthritis, non-protein mediators play a critical role. The extensive use of non-steroidal anti-inflammatory drugs in arthritis is based on their capacity to limit production of eicosanoids (albeit through interference with protein enzymes). The second assumption is that, for key protein mediators, mRNA accumulation is the critical step regulating protein production. This is also not necessarily the case, and exceptions to the general rule include such key mediators as IL-1β and many of the soluble factors released by neutrophils. Examining gene expression patterns will likely prove enormously helpful to our understanding of the immunopathogenesis of rheumatic diseases in children; but they are unlikely to prove to be the ‘Rosetta Stone’ that completely unravels the undecipherable complexity of these diseases.

The emergence of gene expression technologies was initially hailed as a major milestone that would allow investigators to unravel complex diseases such as juvenile rheumatoid arthritis (JRA) and might even allow for the development of specific diagnostic assays in this disease that has so far defied such a development. However, enthusiasm soon turned to disappointment as early experiments invariably produced large numbers of false positive identifications. This large number of false positive identifications occurred, in part, because one of the strengths of this technology, its capacity to provide enormous amounts of data from a single sample or experiment, is also its
Juvenile dermatomyositis

Juvenile dermatomyositis (JDMS) is the most common inflammatory muscle disease in children. The disease is characterized by a diffuse vasculopathy and possibly intense vasculitis affecting the skeletal muscles and other tissues. Indeed, the vascular abnormalities are so prominent that at least one early study referred to the illness as, ‘Systemic Angiopathy of Childhood’ [8**]. Children typically present with insidious onset of weakness and a characteristic rash. Partly because of its relative rarity (compared with other rheumatic diseases in children), JDMS has been particularly vexing to understand and presents considerable challenges to treatment.

In 2002, T ezak et al. published the first gene microarray study in children with rheumatic disease [9**]. The investigators examined muscle biopsies from four HLA-DQA1*0501-positive children with JDMS, comparing expression profiles with those seen in a non-inflammatory myopathy. Although the studies were done on a small number (n = 4) of highly selected patients, the attention to data reproducibility and laudable statistical rigor allowed the authors to reach some interesting conclusions. Not surprisingly, the JDMS muscle samples expressed genes that were clearly associated with inflammation, including complement components (C1 and C4). Of special interest were the over-expression in JDMS muscle of genes under the regulation of IFN-αβ and the resemblance of the expression profile to an in-vitro model of viral inflammation. This was another tantalizing piece of evidence of what has been long suspected: There is a strong link between preceding viral infection and the onset of JDMS [10]. The study left unanswered the specific roles of the vascular endothelium, complement, and the adaptive immune system in this process. Such answers are likely to be forthcoming as array data are generated from a broader spectrum of patients with disease; the data would include the study of peripheral blood leukocytes during different phases of the disease process.

Systemic lupus erythematosus

In 2003, Bennett et al. published an intriguing study: Gene expression profiling was used to examine peripheral blood mononuclear cells (PBMC) of children with systemic lupus erythematosus (SLE) [11**]. The gene chip they used was a commercially available, 12,561 gene array, sufficiently comprehensive to obtain a broad overview of gene expression in the cells and patients of interest. These authors found strong evidence for over-expression of interferon (IFN)-regulated genes in all patients with active disease. These findings corroborated what was reported about the same time [12,13] in adult lupus populations, and the ‘IFN signature’ in SLE is now regarded as one of the tantalizing, but as yet incompletely explained, hints into disease pathogenesis garnered from gene expression studies. It is critical to note that this IFN signature is closely related to clinically relevant immunopathology, as it is abrogated by standard therapies and disappears as SLE disease activity index (SLEDAI) scores improve.

A less-noted, but potentially revolutionary, finding from the Bennett study was the co-existence of a ‘granulopoiesis signature’ in the peripheral blood of children with SLE. This pattern is not described in the two papers on adult SLE that appeared at the same time, and it is unclear whether this finding represents a unique aspect of pediatric SLE or (equally likely) different statistical and analytic approaches from Dr. Virginia Pascual’s research group [11]. Under any circumstances, the findings from this study should alert investigators that our existing concepts of SLE pathogenesis are too simplistic. Although we are inclined to see SLE as a classic example of ‘autoimmunity’, driven by a breakdown in the mechanisms that distinguish ‘self’ from ‘non-self’ within the adaptive immune system, the Bennett study reminds us that innate immunity likely plays a critical role in how autoimmunity is expressed and regulated. Although there are likely to be attempts to develop new therapies for SLE based on the recognition of the IFN signature, the presence of the granulopoiesis signature may suggest equally fruitful targets of intervention.
Juvenile rheumatoid arthritis

Two studies have been published describing gene expression profiles in children with juvenile rheumatoid arthritis, one from the Oklahoma University College of Medicine [14], and the other from the pediatric rheumatology group at Cincinnati Children's Hospital Medical Center [15]. Both these studies were preliminary in nature and neither yielded the potentially revolutionary insights that emerged with publication of the JDMS and SLE studies. Several useful concepts have emerged, however. The most obvious is that one can, indeed identify differentially expressed genes (compared with control populations) in the peripheral blood of children with JRA. This is useful to know in that the site of immunopathology in JRA, the synovium, is not easily accessible to investigators. Work from our group suggests that many of these overexpressed genes are under control of IFN, but the significance of this finding remains uncertain. Furthermore, this pattern was not identified by Barnes et al. [15], although absence of this pattern may simply reflect how they analyzed and mined their data sets. Work from the Cincinnati group suggests some subtle differences between expression patterns of children with polyarticular versus pauciarticular JRA, but these findings await confirmation in a larger study. However, these data strongly support the long-accepted view that pauciarticular and polyarticular JRA are clinically and immunogenetically distinct diseases. There is every reason to believe that more-detailed studies of larger, phenotypically homogeneous groups of patients will allow investigators to clearly define the distinct, relevant immunopathology of these two illnesses. Similar studies should also allow for a clear distinction between classic JRA and childhood-onset spondyloarthopathies, a distinction that can sometimes be challenging on clinical grounds.

A particularly interesting finding from our work has been the suggestion that, using discriminant function analysis (DFA) of gene expression patterns that emerge within 2 months of therapy, physicians might identify those children unlikely to respond to their current therapy and, thus, in need of more aggressive treatment approaches. This, of course, would be an extraordinarily useful clinical tool to have, and it seems likely that this sort of analysis will enter the clinical arena before microarrays are used as a diagnostic modality (see below).

Mining data: what can we learn from newer methods of data analysis?

It is obvious that much of what one gets out of a gene expression study is dependent on how one analyzes the data and what one is looking for in that analysis. The ‘end product’ of a gene array is a table listing expression levels (relative to background) of hundreds, thousands, or tens of thousands of genes. How one turns such lists into a pathogenic ‘story’ is partly investigator-driven. Various commercial software programs are available to assist this process, some develop beautiful, graphic representations of the functional interactions between the differentially expressed genes.

In addition to examining differences in expression levels in gene array experiments, we have become interested in gene expression as a manifestation of cellular dynamics, and have examined how those dynamics may be disrupted during the course of disease. For example, all genes in all cells (even so-called ‘housekeeping genes’) show predictable levels of variability. For some genes, the degree of variability between specific organisms or between specific times of the day is quite small. For others, considerable variability can be shown. This degree of variability can be plotted along a standard Gaussian curve. We have observed that, in the context of chronic inflammatory disease (in this case, JRA) specific genes show greater-than-expected variability in expression levels, even if these genes are not identified as specifically ‘over-expressed’ using conventional statistical analysis techniques. Examination of these ‘hyper-variable genes’ may provide interesting clues in pathologic disruption of normal cellular dynamics [3].

Interesting aspects of JRA emerge by approaching gene array data as a window on cell dynamics. One of the salient characteristics of the genes is that the levels of each of the specific genes transcribed by a specific cell are not random, independent events. Expression levels of gene A are dependent on genes B-D; for example, cellular IL-1β mRNA levels may be directly tied to cellular TNFα. This connectivity of gene expression levels can be analyzed and mapped visually. Studies from our group have demonstrated that, whatever the fundamental pathologic process that may be at work, JRA leukocytes display an obvious disruption of that normal connectivity (Fig. 2).

We are just scratching the surface of what we can learn even from the small number of data sets that have been generated from gene expression arrays in children with rheumatic disease. Investigators should be encouraged to return frequently to their existing data, especially as new analytic methodologies emerge, to cull them further for pathogenic clues and as a basis for designing additional in-silico, in-vitro, or human subject studies.

Design, controls, and interpretation

The different studies described in this review have all taken slight variations in approach and study design, as well as in methods for analyzing the data. For future purposes, however, it may be helpful to establish some minimal standards of design and interpretation that will allow investigators in one group to relate their own findings with investigators in other institutions. Editorial boards of peer-reviewed journals in the field have set some
standards for published work [1], but these standards relate specifically to statistical analysis and independent corroboration. Because of the relatively small number of pediatric rheumatology centers, and the even smaller number of research groups, collaborative, inter-institutional research activities will remain a critical element of pediatric rheumatology investigation. It is useful to comment here on elements of design that should be considered in future studies to facilitate such inter-institutional cooperation.

It may seem obvious from the start, but it is important to note that control populations for pediatric studies will almost certainly need to use age- and sex-matched control populations. At least one report reveals important age- and sex-associated differences in gene expression patterns in PBMC [16]. Recruiting perfectly normal, matched controls may present some tactical and ethical challenges. For example, our own IRB (institutional review board) interdicts drawing blood from perfectly healthy children unless there is a reasonable chance that the child will benefit from the information gained by the procedure. Children with mechanical musculoskeletal problems (e.g., patello-femoral pain syndromes) make up a large number of the children seen in rheumatology clinics [17], but such children rarely, if ever, require phlebotomy as a part of their evaluation. In the end, different IRB rules at different institutions are likely to make different populations of ‘healthy children’ available to different investigators, and it is yet to be determined whether these subtle population differences will affect our ability to interpret studies from different institutions.

Another group of patients consistently lacking from adult or pediatric rheumatology studies, and the one that is most likely to be informative, is patients with chronic inflammation of non-rheumatic origin. Certainly, some information can be gained by studying patients with other rheumatic diseases; in Bennett’s study, children with JRA did not show the IFN signature now associated with SLE. For JRA, this is more problematic. How do we know, for example, that any of the described immunologic abnormalities in JRA are actually specific to the disease process? Is it really the case that any of the described immunologic abnormalities in JRA are actually specific to the disease process? It is useful to comment here on elements of design that should be considered in future studies to facilitate such inter-institutional cooperation.

Clinical application: will it ever happen?

One of the critical barriers to clinical application that will have to be overcome is that there is no standard, validated method for interpreting a single array on a single patient. Published array studies have examined groups of patients, and biostatistical considerations demand that, the larger the number of genes queried, the larger the number of replicates that have to be done to achieve statistical reliability. Although Dr. Olsen’s work suggests that specific profiles can be quite reproducible between individual patients, a great deal will need to be done to establish and validate assays for individual patients. Given the present scarcity of the appropriate expertise in biostatistics and bioinformatics, it is likely to be several years before reliable assays suitable for individual patients emerge.

The next issue has both ethical and practical implications, and that is the issue of appropriate control populations. As I noted above, it is becoming increasingly clear that pediatric gene arrays, particularly gene arrays using peripheral blood leukocytes, will require comparison of the disease group with age- (and probably sex-) matched controls. Given that patient-based arrays are likely to be a highly lucrative market, it is easy to envision ferocious competition between commercial enterprises to develop libraries of RNA from healthy pediatric tissues or peripheral blood. Where such pediatric subjects will be recruited and how their well-being will be protected is anyone’s guess, but it is unlikely that such considerations will be examined with the same scrupulous attention to personal protection in the business domain, as they are by academic institutional review boards. At the same time, it is almost as disturbing to consider the possibility that the ethical and practical considerations associated with the creation of such RNA ‘banks’ will be so daunting that pediatric patients will be left behind if and when gene expression arrays enter widespread clinical use.

Use of gene expression arrays for prognosis may be more promising and may move more quickly into the clinical arena. Promising data in this regard has already been published [14*], and the establishment of 2–3 national reference centers where such analyses can be performed routinely on patients from across North America seems quite possible, at least within the context of a collaborative research agenda, within the next 3–5 years.
Conclusion
Gene expression arrays represent an extraordinarily promising tool in the over 50-year search to unravel the pathogenesis of the rheumatic diseases of children. Larger array platforms, automated labeling and hybridization procedures, and novel bioinformatics approaches will continue to broaden what can be learned (and confirmed) from this approach. Gene expression and proteomic discovery science is likely to dominate the pediatric rheumatology research agenda for several years, but these new tools are unlikely to eliminate the need to focused, hypothesis-driven work.

Whether clinical applications will emerge from gene expression technologies remains uncertain. The promise is certainly there; it is anticipated that use of these technologies will very soon affect our delivery of medical care to children with these devastating diseases.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

Update on the pathogenesis and treatment of systemic onset juvenile rheumatoid arthritis
Alexa Adams and Thomas J.A. Lehmana,b

Purpose of review
Although systemic onset juvenile rheumatoid arthritis accounts for only about 20% of most reported series, children with systemic onset juvenile rheumatoid arthritis are often the most difficult to treat. Many children with persistent systemic onset juvenile rheumatoid arthritis have marked physical and emotional disability as a result of both disease and treatment-related morbidities. This review highlights recent studies that better elucidate the etiopathogenesis of systemic onset juvenile rheumatoid arthritis. New therapies derived from better understanding of cytokines, cytokine gene expression, and their complex interactions, which result in inflammation, are improving our ability to control active disease while reducing or reliance on corticosteroids.

Recent findings
Recent advances in our understanding of the etiopathogenesis of systemic onset juvenile rheumatoid arthritis have led to therapies that specifically target the cytokines found in abnormal quantities in children with active disease. Biologic agents that directly target interleukin-1a, interleukin-6, and tumor necrosis factor α are currently in use, and additional agents that modulate interleukin-18, myeloid-related proteins 8 and 14, natural killer cell function, and macrophage migration inhibitory factor production are under investigation.

Summary
Anakinra, monoclonal antibody to interleukin-6 receptor, and thalidomide each have led to significant clinical improvement with fewer side effects than resulted when corticosteroids were the mainstay of therapy.

Keywords
anakinra (Kineret), interleukin-6, juvenile idiopathic arthritis, juvenile rheumatoid arthritis, macrophage activation syndrome, macrophage migration inhibitory factor, systemic onset juvenile idiopathic arthritis, systemic onset juvenile rheumatoid arthritis, thalidomide (Thalomid), tumor necrosis factor α

Introduction
Systemic onset juvenile rheumatoid arthritis (SoJRA) is an inflammatory condition of unknown etiology manifested by fever, rash, and arthritis. Significant laboratory abnormalities include marked leukocytosis, thrombocytosis, and anemia with often dramatically elevated acute phase reactants such as C-reactive protein and ferritin, which lead to a dramatic rise in the erythrocyte sedimentation rate. The clinical manifestations of SoJRA bear little resemblance to those of the other subtypes of juvenile rheumatoid arthritis (JRA). SoJRA differs in the abruptness of its onset, the presence of fever and rash, the patterns of cytokine and other laboratory abnormalities, and a near equal ratio of affected male patients to female patients. These findings suggest that it is in fact an entirely distinct entity. It is therefore necessary to consider the etiology, pathogenesis, and therapy of children with SoJRA separately from those of children with other forms of JRA.

Children severely affected by SoJRA are at significant risk for life-long disability. Steroid dependence and the associated morbidity occur in nearly one-third of patients [1–3]. Macrophage activation syndrome (MAS) is an additional complication seen most often in children with SoJRA. For children affected with SoJRA to have the best possible quality of life, the clinical manifestations of active SoJRA must be controlled, and the morbidities associated with long-term usage of corticosteroids must be avoided [4–7].

Many different immunosuppressive regimens have been used for the treatment of children with SoJRA in hopes of minimizing corticosteroid usage. Pulse methylprednisolone, cyclophosphamide, methotrexate, sulfasalazine, etanercept, and intravenous immunoglobulin all have been used singly and in varying combinations [8–14]. None of these regimens has proven consistently successful.

Recognition of the distinctive cytokine profile associated with SoJRA has led investigators to develop therapies that
target the specific cytokine abnormalities found in children with active disease. These therapies may allow us to control the clinical manifestations of SoJRA without excessive use of corticosteroids. Substantial advances have been made in our understanding of the etiopathogenesis and treatment of SoJRA as investigators have begun to recognize the importance of recognizing it as a distinct entity rather than considering it simultaneously with other forms of JRA.

Etiopathogenesis
The etiology and pathogenesis of SoJRA remain unclear. No reliable evidence points to a single initiating infection or other event. Once SoJRA has become established, there is a distinctive pattern of abnormal cytokine production. In children with active SoJRA, tumor necrosis factor (TNF)-α is significantly overproduced, while interferon-γ levels are markedly decreased [15–17]. Significantly altered levels of interleukin-6, interleukin-8, monocyte chemoattractant protein-1 (MCP-1), E-selectin, and intracellular adhesion molecule (ICAM-1) are also found as noted below [18–20].

Tumor necrosis factor α
Tumor necrosis factor α is a pro-inflammatory cytokine known to be increased in all subtypes of JRA. Polymorphisms in the 5'-flanking promoter/enhancer region of the TNF-α gene were found in an increased numbers of children with SoJRA compared with children with other JRA subtypes and compared with healthy controls [17]. These polymorphisms may play a role in TNF-α overproduction in SoJRA, and their presence may be a risk factor for the development of SoJRA [17]. Despite the success of the anti-TNF agents etanercept (Enbrel, Amgen/Wyeth, Philadelphia, PA), infliximab (Remicade, Centocor, Horsham, PA), and adalimumab (Humira, Abbott, Abbott Park, IL) in the treatment of other rheumatic diseases, however, TNF-α blockade has not proven consistently effective for the treatment of SoJRA [13].

Interleukin-6
Interleukin-6 is a pyrogen and stimulates hepatocyte production of acute phase reactants. Recently, the role of interleukin-6 in the pathogenesis of SoJRA has received increased attention. Serum interleukin-6 levels parallel fever spikes and correlate with clinical and laboratory measures of disease activity [21,22]. Interleukin-6 levels are increased in patients with SoJRA with active disease and decrease with remission [21]. Soluble interleukin-6 receptor (an interleukin-6 agonist) is elevated in children with SoJRA compared with children with other JRA subtypes, and preliminary data suggests an imbalance in interleukin-6 homeostasis in children with SoJRA [23]. A recent study confirmed an association between SoJRA and interleukin-6-174 nucleotide polymorphism [24*]. This association was seen in SoJRA patients older than 5 years at the time of disease onset, but there is at present no clear evidence that children with onset of SoJRA before 5 years of age are a distinct subgroup.

Animal model work has demonstrated that transgenic mice over-expressing interleukin-6 develop growth retardation similar to that seen in children with SoJRA. The pathogenic effects of interleukin-6 in this model are partially reversed by administration of monoclonal antibody to murine interleukin-6 receptor [25]. Early studies suggest a similar efficacy for antibody to the interleukin-6 receptor in children with SoJRA.

Other cytokines
Other cytokines in addition to TNF-α and interleukin-6 are also being recognized to play an important role in the pathogenesis of SoJRA. The triggers for the overproduction of pro-inflammatory cytokines remain under investigation. Interleukin-1 is known to be important in the pathogenesis of adult-onset rheumatoid arthritis [26]. Evidence is increasing that this is also true for SoJRA as demonstrated by the efficacy of anakinra.

A variety of additional associations between the development of SoJRA and cytokine genetics have been demonstrated. Interleukin-4-1098 T/G polymorphisms were associated with JRA in one study in which 15 of 130 patients with JRA had systemic onset disease [27*]. Interleukin-18 has been identified in the bone marrow of a patient with SoJRA on autopsy [28*]. A role for myeloid-related protein (MRP)-8 and MRP-14 in the acute phase of SoJRA has been proposed as well. A 120-fold increase in serum concentrations of these proteins was found when SoJRA sera were compared with sera from healthy controls. A 12-fold increase was noted compared with patients with other inflammatory diseases [29*]. Skin biopsy samples taken from the SoJRA skin rashes of the same patients showed infiltration of leukocytes expressing MRP-8 and MRP-14 [29*]. Whether these findings are pathogenetically significant or epiphenomena remains to be determined.

Macrophage migration inhibitory factor (MIF) levels are increased in children with JRA, and the highest levels are found in children with SoJRA [30]. Serum MIF levels correlate both with systemic symptoms and joint involvement. A fall in synovial fluid MIF levels correlates with extended remission after intra-articular steroid injections [30]. Among patients with SoJRA, those carrying the 173 single nucleotide G to C polymorphism of MIF have higher serum and synovial fluid MIF levels. For children with SoJRA, the presence of the MIF-173C allele is associated with more severe disease [31,32*].

Etiopathogenesis of macrophage activation syndrome
As seen in familial hemophagocytic lymphohistiocytosis, the clinical manifestations of MAS result from uncontrolled
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Activation and proliferation of macrophages and T cells. Among seven patients with SoJRA complicated by MAS, four had decreased natural killer cell number and activity. These children were also found to have decreased perforin expression by CD8+ and CD56+ cytotoxic cells [33]. The three remaining children were reported to have decreased natural killer cell activity and low perforin levels in all cytotoxic cells [33]. This study suggests similarities between MAS and hemophagocytic lymphohistiocytosis that may share a common pathogenesis involving natural killer cell dysfunction and perforin deficiency [33].

Treatment of systemic onset juvenile rheumatoid arthritis

Corticosteroids have been the standard therapy for children with persistent SoJRA, but the prolonged use of corticosteroids results in an unacceptable morbidity. Therapeutic interventions that target specific patterns of abnormal cytokine production seen in children with SoJRA have been very effective in limited numbers of children. Placebo-controlled trials remain impractical in the small numbers of children with severe SoJRA with divergent age, race, and sex. At present, non-steroidal anti-inflammatory drugs supplemented with corticosteroids in limited amounts remain the appropriate initial therapy [34].

Pulse methylprednisolone alone or in combination with other agents may be helpful [8,35]. Despite their efficacy in other subtypes of JRA, most other disease-modifying agents are without consistent efficacy for children with SoJRA. Although occasionally effective, methotrexate seems to be less effective for children with SoJRA compared with other subtypes [36]. The newer, more specifically targeted agents with a direct effect on cytokine levels appear to offer the best hope for an improved outcome for children with persistent disease.

Tumor necrosis factor α blockade

Although it has been widely used, etanercept has been disappointing in the treatment of SoJRA [13,37]. In one study, 15 patients with SoJRA refractory to methotrexate were treated with a combination of methotrexate and etanercept. Despite early apparent benefit, SoJRA flares occurred during treatment, and etanercept was discontinued in seven [13]. One child with SoJRA has been reported who developed MAS following the initiation of etanercept therapy [38].

Infliximab has also been used for children with SoJRA. An early case report described improvement in systemic manifestations of SoJRA, but there was no improvement in the articular manifestations [39]. Anecdotal reports note that very high-dose infliximab may be effective, but this is associated with increased risk of infection and other complications. At present, there are only anecdotal reports regarding the use of the TNF blocker adalimumab for SoJRA, with varied results.

Monoclonal antibody to interleukin-6 receptor

MRA, a recombinant, humanized, monoclonal antibody to the IL-6 receptor, has been successfully used for the treatment of SoJRA in pilot studies [40]. In one case, a child who had failed to respond to multiple medications experienced resolution of systemic symptoms, normalization of laboratory parameters, and significant catch-up growth [40]. Phase II and III trials of the use of MRA for SoJRA are underway. Similar successes have been reported using MRA for the treatment of adult patients with Still’s disease, Castleman’s disease, and rheumatoid arthritis [41–43]. Initial reports of the success of MRA are very promising, but much more information is needed regarding the toxicity of the agent and the durability of the response.

Anakinra

Anakinra is an interleukin-1 receptor antagonist whose efficacy for the treatment of SoJRA has been noted in several case reports [44–46]. Although there are occasional failures in clinical experience, many children with SoJRA experienced significant clinical improvement accompanied by a reduction of prednisone dosage.

Other mediators

At this time, there are no available agents specifically directed at abnormal production of interleukin-4, interleukin-18, MRP-8, MRP-14, or MIF. Whether these mediators will ultimately prove important in some or all of the children with SoJRA is unknown. As our understanding of SoJRA improves, we may find that there are a variety of defects in the regulation of inflammatory cytokine pathways, each of which can be specifically targeted with appropriate therapy. Specifically targeted therapy may allow better control of the clinical manifestations of SoJRA without the increased risk of infection accompanying many of the current regimens that block cytokines.

Thalidomide

Thalidomide is a unique immunomodulatory agent that is known to reverse many of the cytokine disturbances seen in SoJRA. Thalidomide decreases TNF-α, interleukin-1, nuclear factor-κB, and interleukin-6, and has known antiangiogenic effects [47]. A multi-center study of the use of thalidomide for treatment-refractory SoJRA included 13 children who received 2–5 mg/kg/day [48]. After 6 months, 11 of the 13 children had significant reduction in prednisone dosage, reduction in erythrocyte sedimentation rate, increased hemoglobin, and decreased disease activity. Mild side effects included sedation and constipation. Transient paresthesias were seen but resolved, and in no patient was medication discontinued because of side effects.
Thalidomide is a potent teratogen, and all patients must be made aware of this risk. Adolescent patients must be monitored for appropriate contraceptive use, and pubertal female patients require monthly pregnancy tests to minimize the risk of inadvertent conception while on therapy. Thalidomide is rapidly cleared with the cessation of therapy, however, and there is not believed to be a lingering risk of teratogenicity after therapy is discontinued. Like all of the agents used for persistent SoJRA, thalidomide may have severe side effects, but it is a useful option in refractory cases.

It is noteworthy that unlike the other newer agents that block the action of cytokines either directly or at the receptor level, thalidomide seems directly to effect transcription of the mRNA necessary for cytokine synthesis. This different mode of action may be important in allowing reversibility in the face of infection. Infection is the most common major complication associated with most agents that block cytokines, but it does not appear to be a complication of thalidomide therapy, despite thalidomide’s frequent use in patients with human immunodeficiency virus and other populations vulnerable to infection. Newer thalidomide analogues with substantially improved safety profiles are in phase I and II clinical trials.

**Statins**

A single case report has been published describing the successful use of Atorvostatin for a 9-year-old boy with SoJRA who previously had failed to respond to autologous stem cell transplantation and other therapies [49**]. Drugs of the statin class are known to have a variety of immunomodulatory effects. This may represent a new approach to the treatment of inflammatory conditions such as SoJRA, but further experience with the use of these drugs in children is needed. The side effect profile of these medications is not insignificant in the adult population, and their safety has not yet been investigated in children.

**Autologous stem cell transplantation**

In severe and treatment-refractory cases of SoJRA, autologous stem cell transplantation may be effective. Long-term follow-up of more than 40 patients with JRA (25 of whom were classified as SoJRA) transplanted since 1997 has been published [50]. Dramatic successes have been reported, but many children have relapsed (although often with less severe disease). Unfortunately, the procedure carries a significant risk of mortality and is therefore reserved for only the most severe refractory cases.

**Conclusion**

The past year has seen important new developments both in our understanding of the etiopathogenesis of SoJRA and its therapy. Key to these improvements has been recognition of SoJRA as a unique disease entity that must be studied separately from other forms of JRA. The pathogenesis of SoJRA remains under investigation. It is clear that disease expression is associated with complex alterations in the cytokine profiles of affected children. The variety of genetic variations associated with an increased susceptibility to SoJRA suggests that there may not be a single predisposing genetic abnormality.

The past year has seen further refinement of a variety of new therapeutic agents including MRA, anakinra, and thalidomide. Appropriate use of these agents may make it possible to avoid the morbidity associated with the long-term use of corticosteroids. As our knowledge of the regulation of inflammatory processes increases and our ability to regulate the effects of specific cytokines evolves, the key role of these processes in many different diseases is becoming increasingly evident. As our understanding of the etiopathogenesis and treatment of SoJRA expands, there is an important opportunity to gain further insight into many other inflammatory illnesses. The next decade should be a time of rapid evolution in the emergence of newer agents that continue to target specific cytokine abnormalities. With early initiation of aggressive therapy, corticosteroid-related morbidity can be minimized, and the outlook for children with persistent SoJRA can be dramatically improved.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

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** of outstanding interest

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Pathogenesis and treatment of Kawasaki’s disease

Rae S.M. Yeung

Purpose of review
Coronary artery damage resulting from Kawasaki’s disease is the leading cause of acquired heart disease in children in the developed world. This review highlights advances in our understanding of the etiology of Kawasaki’s disease, the immune response leading to vascular damage, potential biomarkers, and insights into mechanisms of disease addressed by an animal model. Clinical dilemmas are discussed in the context of the new American Heart Association recommendations for the diagnosis and treatment of Kawasaki’s disease.

Recent findings
Improved understanding of the mechanisms of disease will assist in identifying predisposed individuals and in development of more effective therapy. Most investigators agree that an infectious trigger leads to massive activation of the immune system, resulting in a prolonged self-directed immune response at the coronary arteries. The etiology debate has centered on the nature of and mechanisms involved in immune activation. Genetic studies have not provided conclusive answers to these questions. Mechanistic studies done in animal models have pointed to specific biologic factors critical for coronary artery damage and together with studies in children may lead to more rationally conceived biologically based interventions. Increasingly, the questions regarding clinical management address timing of therapy and the management of children presenting with atypical Kawasaki’s disease. New guidelines and management algorithms have been proposed by the American Heart Association to address these concerns.

Summary
Biochemical and phenotypic characterization of Kawasaki’s disease continues to improve. Answers are closer on etiology, reliable biomarkers, valid predictors of coronary outcome, and improved treatment of this syndrome.

Keywords
aneurysm, animal model, etiology, immune response, Kawasaki’s disease, therapy

Introduction
Kawasaki’s disease is the commonest cause of multisystem vasculitis in childhood. Kawasaki’s disease affects children of all nationalities and its incidence appears to be increasing worldwide. Because of its predilection for the coronary arteries, Kawasaki’s disease is now recognized as the number one cause of acquired heart disease in children in the developed world [1]. One of the most pressing areas of research in Kawasaki’s disease is unraveling the etiology and pathogenesis of disease towards improving therapy and coronary outcome. Our current standard therapy of high-dose intravenous immunoglobulin (IVIG), together with aspirin during the acute phase of Kawasaki’s disease, still results in 5% of affected children developing coronary artery aneurysms [2]. When adjusted for body surface area, this number increases to 20% to 30% of children [3]. Persistent structural changes coupled with new findings of functional abnormalities in both the coronary arterial and systemic arterial vasculature lead to concerns regarding the risk for premature atherosclerosis in children with Kawasaki’s disease. I review here the recent advances in our understanding of the etiology, the immune response, the vascular damage, correlates to disease models, their clinical implications, and therapeutic interventions during the past year in Kawasaki’s disease research.

It is important to note the limitations of many of the investigations reported in children with Kawasaki’s disease. Small study numbers have limited power and may not have the ability to detect clinically important differences. Additionally, Kawasaki’s disease is an endemic disease with epidemics. The clinical phenotype may be outbreak dependent and contribute to the different results from different investigators at different times in different locales. Biologic specimens, especially affected coronary arteries, are not readily available and much data have been generated from a limited number of autopsy specimens from these extreme clinical presentations.

Etiology
Since the original description of 50 children with mucocutaneous lymph node syndrome by Kawasaki [4,5] in 1967, much work has centered on identifying the etiologic agent or agents responsible for disease. Despite the widely held belief that Kawasaki’s disease is an infectious disease, its etiology remains elusive. The epidemiology of the disease suggests infections: endemic disease with epidemics, seasonal predominance in late winter and early spring, geographic clustering of outbreaks, and cases within clusters sharing similar clinical features. With each new geographic outbreak, reports of serologic, tissue culture, and molecular evidence of specific bacteria and viruses populate the
literature. Kawasaki’s disease has been linked with many different etiologic agents ranging from bacteria such as Propionibacterium, Staphylococcus, Streptococcus, and Chlamydia to viruses such as Epstein-Barr, parvovirus, and retroviruses, but no one causative agent has been consistently demonstrated (reviewed by Yeung [6]). The etiology debate has centered on the mechanism of immune activation. Is the responsible infectious agent a conventional antigen or a superantigen? Evidence supporting both hypotheses continues to accumulate.

Some investigators have focused their work on identifying one specific pathogen or family of pathogens responsible for disease. One group has identified oligoclonal IgA antibodies present in arterial tissue from three fatal cases of Kawasaki’s disease [7]. After cloning several prevalent IgA genes from these samples, synthetic human antibodies were produced. Using these synthetic antibodies as detecting antibodies in immunohistochemical assays on tissue from fatal cases of Kawasaki’s disease, positive staining was found in the respiratory epithelium of 10 of 13 Kawasaki’s disease cases and none of nine control subjects, as well as in a subset of macrophages in various inflamed tissue from 17 children with Kawasaki’s disease [8]. The authors conclude that a conventional antigen-driven response leads to Kawasaki’s disease and the respiratory track is the site of entry for this pathogen [9]. Intense interest and debate have recently centered on a novel human coronavirus found by one group of investigators in the respiratory secretions of some children with Kawasaki’s disease. In the original report on the New Haven coronavirus, reverse transcriptase polymerase chain reaction was used to detect the virus in eight of 11 (72.7%) of children with Kawasaki’s disease but in only one of 22 (4.5%) of control specimens. Others investigators have not been able to confirm this data (reported at the 8th International Kawasaki Disease Symposium, San Diego, February 2005), echoing the outbreak-dependent nature of this syndrome. The longer the search for a single infectious agent responsible for Kawasaki’s disease, the longer the list of diverse infectious agents found. The more plausible underlying principle is a shared property, common to multiple infectious agents and resulting in the same pathogenic process leading to the clinical syndrome of Kawasaki’s disease. One such common feature of many infectious agents is the presence of superantigenic activity.

Evidence of a superantigen-mediated disease process in Kawasaki’s disease includes identification of superantigen-producing organisms in isolation of bacterial superantigens from, or finding the hallmarks of superantigen activation in the immune system of affected children. Investigators have isolated superantigen-producing bacteria from children with acute Kawasaki’s disease, with a focus on toxic shock syndrome toxin 1 producing Staphylococcus aureus and pyrogenic exotoxin producing Streptococcus [11–13]. Investigators have also found increased titers of specific antisuperantigen antibodies, including those directed against toxic shock syndrome toxin 1 [14], streptococcal pyrogenic exotoxin A [13], and streptococcal pyrogenic exotoxin C [12] in children with Kawasaki’s disease.

Although the debate continues regarding the mechanism of initial immune activation, the more likely scenario is that there is cooperation between different mechanisms and a final common pathway of immune activation responsible for this clinical syndrome [6]. All are in agreement with the massive immune activation observed in the acute phase of Kawasaki’s disease. A unifying model proposes that a microbe with superantigenic activity initiates the massive activation of the developing immune system. A subpopulation of the superantigen-responsive T cells is rescued from apoptosis because of interaction with an antigen-presenting cell presenting a conventional peptide antigen and providing costimulation. This peptide antigen may be derived from self or an infectious mimic of self. The immune response is perpetuated locally where the self-antigen is found, in this case, the coronary vessel wall. A superantigen-initiated immune response lasts 10 to 14 days. Untreated, the acute phase of Kawasaki’s disease lasts 10 to 14 days. T cells rescued by peptide antigens mediate a persistent, low-grade inflammatory response. Children with Kawasaki’s disease continue to have evidence of systemic inflammation for up to 6 weeks and evidence of ongoing microvascular inflammation in affected cardiac tissue for up to 23 years after having Kawasaki’s disease [15]. Self-antigens in the coronary arteries direct the localized inflammatory response, resulting in damage and aneurysm formation. This model accounts for all the findings by the proponents of the superantigen and conventional antigen camps. Thus, superantigens and conventional peptide antigens work together to direct a persistent immune response leading to coronary artery damage. Kawasaki’s disease fits nicely in the spectrum between an infectious disease and a true autoimmune disease, with an infectious trigger leading to a prolonged self-directed immune response.

**Immune response**

The immune response in Kawasaki’s disease is wide ranging, encompassing aspects of both innate and adaptive immunity (reviewed by Yeung [6]). Activation of the two arms of adaptive immunity is present during the acute phase of Kawasaki’s disease, with evidence showing activation of T cells and B cells together with significantly increased proinflammatory cytokine production. Most prominent and most studied among these proinflammatory cytokines is TNF-α. Many groups have found elevated levels of TNF-α in children with acute Kawasaki’s disease irrespective of presence or absence of coronary artery lesions. Several groups have studied promoter polymorphisms controlling TNF-α production as possible
The role of interferon-γ in Kawasaki’s disease is more controversial. Early studies demonstrated high serum levels of interferon-γ during the acute phase of Kawasaki’s disease. Some of the same investigators later reported a decrease in interferon-γ-producing CD4+ T cells during the acute phase of Kawasaki’s disease. This is in accord with our own data demonstrating a lack of interferon-γ message by reverse transcriptase polymerase chain reaction during the acute and subacute phases of Kawasaki’s disease in children. By day 5 of disease, when children present to hospital and the diagnosis of Kawasaki’s disease is entertained, interferon-γ may have already come and gone. Interest in the exaggerated peripheral immune response has also led to investigation of mechanisms involved in the regulation of the immune response. Regulatory T cells appear to be decreased in children with Kawasaki’s disease [21*]. Affected children have decreased levels of CD4+CD25+ Treg and Foxp3, a transcription factor associated with this subgroup of regulatory T cells, in the peripheral circulation, compared with controls, suggesting that decreased regulatory/suppressive activity may contribute to the exaggerated immune response seen in the acute phase of Kawasaki’s disease.

Other groups have focused on the innate immune response and have found that specific polymorphisms in the promoter of the CD14 toll-like receptor gene are associated with coronary outcome in Kawasaki’s disease [22]. Mannose-binding lectin is another molecule associated with neutrophils and the innate immune response. Mutations in mannose-binding lectin are increased in children with Kawasaki’s disease compared with healthy children and may be associated with coronary artery lesion development [23]. One study also suggests a relation between mannose-binding lectin genotype and vascular stiffness following Kawasaki’s disease [24]. Another marker of innate immunity is S100A12 (EN-RAGE), a neutrophil-derived factor that is increased during the acute phase of Kawasaki’s disease and decreases after clinical response to IVIG therapy [25,26]. The systemic immune response localizes to target tissues with the help of leukocyte migration signals upregulated by proinflammatory cytokines. Studies have found increased levels of chemokines and chemokine receptors [27], as well as localized increased expression of adhesion molecules [28] in the coronary artery lesions during evolution of Kawasaki’s disease.

**Vascular damage**

The endothelium plays a central role in the regulation of normal arterial function. Endothelial dysfunction is the earliest precursor in the atherosclerotic disease process, and its presence and persistence in Kawasaki’s disease patients remain a concern. Nitric oxide is an important mediator of endothelial function, which can be impaired by oxidative stress. Several groups have evaluated the role of nitric oxide and nitric oxide synthase in vascular damage and in particular endothelial cell function. Some have found association of this family of molecules with progression of coronary artery lesions [29*], but others were not able to confirm these findings [30,31]. Vascular endothelial growth factor (VEGF) is a marker of endothelial activation and may be elevated in the presence of vascular damage and the subsequent repair process. VEGF is elevated in acute-phase sera and appears to be related to the development of coronary artery lesions in Kawasaki’s disease. There also appears to be a relation between VEGF and VEGF receptor gene polymorphisms and coronary outcome in Kawasaki’s disease [32].

Increased vascular permeability, as measured by a decrease in serum albumin, increase in water retention, decrease in serum sodium concentration, and increase in total body weight, appears to be associated with resistance to IVIG treatment and poor coronary outcome [33,34]. Functional abnormalities of the coronary vessels include altered endothelium-dependent vascular reactivity both at the coronary vessels [35] and in systemic muscular arteries [36,37] together with decreased fibrinolytic capacity, another measure of endothelial function [38].

The renin-angiotensin system has also been studied. There appears to be an association of angiotensin-1-converting enzyme gene polymorphisms and predisposition to Kawasaki’s disease [39,40] but no relation between genetic polymorphisms and coronary outcome [39]. Taken together, structural changes from vascular damage coupled with persistent functional abnormalities in both the coronary arterial and systemic arterial beds lead to concerns as to whether children with Kawasaki’s disease are at risk for premature atherosclerosis (reviewed by McCrindle [41*]).

The extracellular matrix scaffolding is a critical component of the coronary artery. Breakdown of the extracellular matrix allows reshaping of tissue. There is intense research activity on enzymes involved in regulating the extracellular matrix. The balance between synthesis and degradation of the extracellular matrix involves families of proteases and their inhibitors and influences whether...
a physiologic or pathologic state prevails. Elastin breakdown is the hallmark of aneurysm formation, making the elastase family of enzymes of particular interest. These include matrix metalloproteinases with elastolytic activity, such as matrix metalloproteinase-2 and matrix metalloproteinase-9, and serine elastases including neutrophil elastase. Matrix metalloproteinase-2 and matrix metalloproteinase-9 expression have been detected in coronary artery aneurysms from fatal cases of Kawasaki’s disease [42]. Interestingly, increased local matrix metalloproteinase activity at the coronary artery is not reflected in the peripheral blood, where there is no difference in active matrix metalloproteinase-9 in children with Kawasaki’s disease compared with febrile or afebrile controls, although counterintuitively, the tissue inhibitor of matrix metalloproteinase-9 is elevated in acute-phase serum in children with Kawasaki’s disease [43]. Interestingly, plasminogen activator inhibitor 1, a non-specific matrix metalloproteinase inhibitor, is elevated in the peripheral blood of children with Kawasaki’s disease, and its level appears to be related to poor coronary outcome [44]. Cystatin C is an inhibitor of elastolytic cysteine proteases, and decreased activity of cystatin C is associated with abdominal aortic aneurysms in adults. Children with Kawasaki’s disease have lower serum levels of cystatin C, first detected in the acute phase and persisting into the subacute phase of Kawasaki’s disease, compared with controls [45].

Animal model of Kawasaki’s disease

Strains of inbred mice develop coronary arteritis in response to intraperitoneal injections of lactobacillus casei cell wall extract (LCWE) [46]. The resultant vasculitis is similar to Kawasaki’s disease in children and demonstrates identical histologic changes on light microscopy and a similar time course to coronary artery disease [47] and response to IVIG, the same therapeutic agent effective in children [48]. We discovered a novel superantigen found within LCWE responsible for development of vascular disease [47]. This superantigen possesses all the hallmarks of a superantigen-mediated response, and more importantly, superantigenic activity directly correlates with the ability to induce coronary arteritis in mice.

What distinguishes this novel superantigen from other bacterial superantigens is the presence of localized production of interferon-γ in affected vascular tissue. Ablation of interferon-γ confirmed that interferon-γ regulates the immune response and surprisingly is not necessary for the induction of coronary artery disease [49]. TNF-α usually works synergistically with interferon-γ, potentiating the others’ cellular responses. Mice with absence of TNF-α activity, however, do not develop coronary disease after LCWE stimulation. Despite a robust systemic T-cell response, there is neither local inflammation nor vessel wall breakdown in the absence of TNF-α activity [50]. Interferon-γ and TNF-α play important but divergent roles in the development of coronary arteritis in our animal model of Kawasaki’s disease. My team has identified two important TNF-α-mediated functions participating in local inflammation and tissue damage, leukocyte recruitment, and extracellular matrix breakdown. Recent data demonstrate an important role for T-helper type 1-associated chemokines and adhesion molecules in leukocyte migration to the coronary artery as well as matrix metalloproteinase-9 as a central player in extracellular matrix breakdown [51]. These data are exactly in accord with the studies in children with Kawasaki’s disease. Affecting cardiac tissue is not readily available in children with Kawasaki’s disease. In addition to providing diseased coronary arteries, the animal model is a valuable resource for answering mechanistic questions and dissecting the path from systemic inflammation to arterial wall damage.

Diagnosis and management

Increased physician awareness of Kawasaki’s disease has led to early diagnosis of the disease and treatment of children presenting with the classic signs and symptoms. Increasingly, the questions regarding clinical management address timing of therapy and the treatment of children presenting with fever and fewer than four of the clinical criteria for the diagnosis of Kawasaki’s disease. Several studies have addressed the question of early treatment with IVIG in children presenting with all the classic criteria of Kawasaki’s disease but fewer than 5 days of fever. All studies found that therapeutic intervention before day 5 or after day 5 of fever does not affect coronary outcome [52,53]. A recent large Japanese study [54], with 4731 children treated between days 1 and 4 of fever, compared with 4020 children treated between days 5 and 9 of fever, found a slightly increased rate of IVIG retreatment in those treated early. More importantly, this study, as in the previous studies, found no difference in coronary outcome between the two groups. The current American Heart Association (AHA) recommendations suggest that in the presence of four of five of the classic criteria for Kawasaki’s disease, the diagnosis can be made and the treatment initiated on day 4 of fever, with the first day of fever counting as day 1 [55].

Treatment of the child with fever and some but not all the diagnostic features of Kawasaki’s disease continues to be an area of clinical concern. Increased recognition of children with atypical or incomplete Kawasaki’s disease and the implications for coronary outcome have led to new guidelines for the treatment of these children put forward by the AHA [55]. Algorithms were proposed to help guide clinical management of suspected Kawasaki’s disease in children. The absence of a gold standard for diagnosis of Kawasaki’s disease clearly represents a problem for evidenced-based guidelines. Therefore, the management algorithm proposed by the AHA committee of experts represents informed opinion rather than evidence.
The guidelines stress a high index of suspicion for Kawasaki’s disease in any child presenting with prolonged fever and incomplete clinical features of Kawasaki’s disease. If two or three clinical criteria are present together with supportive laboratory findings of Kawasaki’s disease, the AHA recommends performing an echocardiogram as well as continued clinical observation, reevaluation, and treatment as warranted.

**Treatment**

The efficacy of high-dose IVIG administered as a single dose in the acute phase of Kawasaki’s disease in reducing coronary artery lesions is well established [56,57] and is reviewed by Dunmer and Newburger [58]. Recently, a meta-analysis confirmed that high-dose IVIG, 2 g/kg, administered before day 10 was the optimal therapeutic regimen [59]. Despite appropriate treatment with IVIG, approximately 5% of children with Kawasaki’s disease develop coronary artery aneurysms and 1% develop giant aneurysms [2]. When adjusted for body surface area, the number increases to 20% to 30% of children with Kawasaki’s disease who develop coronary artery abnormalities [3].

Historically, aspirin has been used at high doses for its anti-inflammatory effects and at low doses for antiplatelet properties. High-dose aspirin was the sole therapy for Kawasaki’s disease in the early years, prior to IVIG. During the acute phase of Kawasaki’s disease, high-dose aspirin (80–100 mg/kg per day) is administered together with high-dose IVIG. High-dose aspirin and IVIG appear to have additive anti-inflammatory effects. Although the duration of high-dose aspirin therapy varies from center to center, most North American institutions administer high-dose aspirin until the child has been afebrile for 48 to 72 hours, reducing to antiplatelet dose (3–5 mg/kg/d) at that time. There has been much debate about the role of aspirin in the acute treatment of Kawasaki’s disease. Recently, investigators have questioned the effectiveness of high-dose aspirin during the acute phase of Kawasaki’s disease [60,61]. On a mechanistic level, high-dose and not low-dose aspirin inhibits nuclear factor-κB nuclear translocation [62]. Nuclear factor-κB is a critical transcription factor in signaling the downstream effects of TNF-α and many proinflammatory cytokines. AP-1, another transcription factor involved in inflammatory cytokine signaling, is also inhibited by aspirin. Additionally, high-dose aspirin inhibits dendritic cell maturation and decreases expression of costimulatory molecules [63], all of which are important in propagating and intensifying the immune response. Of recent interest is the role of aspirin in inhibiting matrix metalloproteinase activity [64].

Although corticosteroids are the mainstay of treatment in systemic vasculitis, their use in Kawasaki’s disease is limited. An early study suggested potential deleterious effects of corticosteroids when used in the acute phase of Kawasaki’s disease [65]. In recent years, investigators have ventured again to study this powerful immunosuppressive agent. Investigators found in a retrospective review that inclusion of corticosteroids in the initial treatment regimen was associated with a significantly shorter fever duration and lower rate of coronary artery lesions [66]. These findings are supported by recent studies showing that steroids rapidly decrease systemic inflammatory markers and proinflammatory cytokines [67] with no adverse effect on coronary outcome [68,69].

**Conclusion**

Kawasaki’s disease is a syndrome complex resulting from massive activation of the developing immune system by infectious agents, which initiate a self-directed immune response in genetically predisposed children. TNF-α mediates leukocyte migration to the coronary arteries and degradation of the vessel wall, leading to aneurysm formation. The long-term effects of endothelial damage and continuing impairment of vascular reactivity in all children affected with Kawasaki’s disease, regardless of whether they have developed coronary artery lesions, are of concern. Encouraging progress has been made in our understanding of Kawasaki’s disease. Harnessing the power of molecular biology and genetics, together with increased collaboration of interdisciplinary research teams, will help answer the many remaining questions in the diagnosis and treatment of children with Kawasaki’s disease.

**References and recommended reading**

Papers of particular interest, published within the annual period of review, have been highlighted as:

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** of outstanding interest

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Kawasaki’s disease

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The sources of pain in knee osteoarthritis
David T. Felson

Introduction
Pain is the most prominent and disabling symptom of osteoarthritis. Whereas much of the focus of osteoarthritis has been on cartilage loss, there are no pain fibers in cartilage and cartilage loss may occur without any accompanying symptoms. To optimally treat this common disease, an understanding of causes of pain is needed.

There are four essential elements to understanding knee pain: what structures produce pain inside the knee; how the nervous system reacts to pain impulses emanating from the joint; which external stimuli trigger the articular stimuli and why; and how psychosocial features of the person affect pain severity and its impact. Although psychosocial factors clearly contribute to pain sensitivity, perception, and magnification, their contributions to pain are complex and a comprehensive discussion of their relation to pain is beyond the scope of this review. The interested reader can get insights elsewhere [1,2]. I focus here on reviewing the first three elements: nociceptive structures in the joint, nervous system reactions, and person-related factors that mechanically trigger nociceptors.

Nociceptors in the knee
Four different types of nerves innervate the joint ranging from large type 1 and type 2 fibers, smaller myelinated type 3 fibers, and lastly tiny and slowly conducting non-myelinated type 4 or C fibers. Group 3 and 4 are high threshold fibers, are the primary nociceptors, and are activated by noxious movements or manipulation of the joint [3**]. There are two predominant nociceptive neuropeptide neurons, the isolectin-positive and the calcitonin gene-related peptide (CGRP)-containing neurons. Other neuropeptides can be identified in each of these kinds of neurons; substance P is present in half of the CGRP-positive neurons [4]. Infrequent stimulation of a single fiber does not produce the conscious sensation of pain. Rather, both temporal and spatial summation in a population of nerve fibers produces the sensation of pain and correlates with its magnitude. Sensory neurons are excited in this manner, and in the dorsal horn of the spinal cord they release CGRP, substance P, or other neuropeptides.

Where in the joint are these nociceptive fibers? There are four sources of information about the location of sensory fibers. First, dissections of different animal species have looked for the anatomic structures of unmyelinated fibers or tiny myelinated ones. Second, in some studies structures have been stained for neuropeptides such as...
substance P. Third, two unusual ‘awake’ explorations of unanesthetized joints have been done with the subject providing feedback about structures that were sensitive; and fourth, in recent studies, MRIs of those with and without joint pain have been compared.

In the anatomic studies, although types 3 and 4 fibers have been found in most joint structures, they have not been consistently found in the synovium nor in the inner avascular portion of the meniscus. Also, studies have not found such fibers in hyaline articular cartilage [5]. Immunohistochemistry staining has shown that fibers containing substance P are present in most joint structures including the periosteum, the subchondral bone, and the marrow underneath it, the fat pad, the capsule, and even in osteoarthritic knees at the junction between the bone and cartilage where erosion channels may connect the bone marrows with clefts through the tidemark of the cartilage into the cartilage substance. That seems to be the only mechanism by which cartilage would itself be innervated [6].

In young adults with anterior knee pain, substance P-containing fibers have been found in the lateral retinaculum of the patella [7], a structure implicated as the source of pain in these subjects. The nerve status of this retinaculum in controls without knee pain is not well described.

Two studies of awake subjects undergoing arthrotomy [8] or arthroscopy [9] whose knees remained unanesthetized have suggested that the most pain-sensitive structures in the knee are ligament site insertions, synovium, and the fat pad deep to the patella. Cartilage was not tender. One investigator actually inserted a probe inside the patella (which was not terribly painful), but inserting liquid into the patella caused great pain, suggesting that bone pain may be caused in osteoarthritis by an increase in bone pressure.

Magnetic resonance imaging findings and pain: bone marrow lesions
Magnetic resonance imaging provides the opportunity to evaluate all structures in a joint including those innervated by nociceptive fibers. Abnormalities in these structures can be identified and their relation to the existence and severity of the pain tested. Several studies have compared MRI findings in persons with joint pain vs those without. Usually all subjects have evidence of osteoarthritis. Bone marrow lesions consisting of poorly delineated lesions of increased signal intensity on fat suppressed T2-weighted images have been identified as more frequent in those with pain than in those without it in a study by Felson et al. [10]. Also, a study of women in their 40s with and without knee pain (Sowers et al. [11]) showed that, whereas tiny bone marrow lesions were not associated with pain, larger lesions similar to those identified in the study by Felson et al. were strongly correlated with the presence of knee pain. A cross-sectional study in persons with temporomandibular joint pain, including subsets with osteoarthritis and with internal derangement, showed that the presence of bone marrow edema identified joints most likely to painful [12].

Why bone marrow lesions are associated with pain is an enigma. Bone marrow lesions could reflect intraosseous hypertension due to poor venous drainage seen in many patients with osteoarthritis [13]. Indeed, fenestration of the bone in patients with this syndrome not only lowers intraosseous hypertension but may lessen pain [14]. Histologically, given their brightness on T2 images, suggestive of water, bone marrow lesions should show pathologic evidence of increased water, blood, or other fluid inside bone. On histologic examination, however, bone marrow lesions show surprising little edema [15,16] but rather show abnormal bone with excessive fibrosis, small areas of osteonecrosis, and extensive bony remodeling. This is a picture most consistent with ongoing bone trauma and would explain the association of bone marrow lesions with malalignment [17].

Outside of knee osteoarthritis, a variety of pathologic processes generate bone marrow edema findings on MRI [18] including osteonecrosis, Sudek’s atrophy (reflex sympathetic dystrophy), bone contusion after major trauma, microfracture, and stress fracture. These lesions are also found in inflammatory arthritides, where they are thought to be caused by intraosseous inflammation.

Despite this prior cited evidence, not all studies have reported an association of bone marrow lesions with knee pain. One recent study focused only on those with knee osteoarthritis and pain, and found that the severity of pain was not significantly correlated with the severity of bone marrow edema in the painful knee. There were only 50 patients in this study, however, and the pain score increased with increasing size of bone marrow lesions, suggesting that the failure to find a significant association between bone marrow edema and the severity of pain might have been due to an inadequate number of subjects. Other studies of MRI findings in those with and without painful knee osteoarthritis [19] have not reported such clearcut findings, suggesting that many structural abnormalities are present in painful knees with osteoarthritis and that bone marrow lesions are only one of these.

Other magnetic resonance imaging features associated with pain
Other MRI features have been linked to pain. The presence and size of knee effusions are correlated with the occurrence of pain; synovial thickening on the MRI not only tends to occur more often in those with pain but has also been associated with increased pain severity.
[20]. Recently, about 15% of patients with painful knee osteoarthritis were found to have perarticular lesions such as tendonitis or bursitis, lesions that were absent in almost all persons with knee osteoarthritis who did not have knee pain. This suggests that such soft tissues lesions may account for a small proportion of pain [21].

One method of evaluating synovitis on MRI was validated by Fernandez-Madrid et al. [22] and evaluates synovitis on the sagittal image in the Hoffa’s fat pad area deep to the patellar tendon. Synovial proliferation at this site has been found to be associated with knee pain severity [23] and with effusion.

**From articular nociceptor to the central nervous system**

The processing of nociceptive input in the spinal cord is complex and not completely understood. Two different functional types of neurons produce nociception. One is a neuron that produces only nociceptor sensation. Most nociceptive-specific neurons that extend to the spinal cord have relatively small receptor fields in the periphery that bridge both the joint and the deep soft tissue, such as muscle. The other functional type of neuron that can produce pain is a wide-dynamic-range neuron that fires in response to both innocuous pressure and noxious pressure and is interpreted as one or the other based on the frequency with which it fires. The receptor fields for wide-dynamic-range neurons include both the joint and the overlying skin and cover a wide territory of skin and joint.

Inflammation plays a critical role in processing nociceptive input, both peripherally and centrally. In the presence of inflammation, the input from these nociceptors initially increases [4]. It is likely that a variety of inflammatory cytokines participate, including prostaglandins. The net result of this increased stimulation and the activation of cytokines is to change the sensitivity of peripheral receptors to nociceptive input. The changes that occur are twofold: the receptor field of given sensory neurons enlarges so that nociceptive input can be produced in a nerve by a stimulus that previously did not produce nociceptive input there. Plus, the threshold for nociceptive activation drops. Thus, a stimulus that was previously innocuous becomes painful. A clinical example would be the tendency for persons with gout to note during a gout attack that mild skin pressure is painful. Occasionally, the receptor field expands to include the equivalent site on the other limb, introducing the possibility of bilateral sensitivity. The central nervous system’s inhibitory influences appear to increase during inflammation, possibly in an attempt by the central nervous system to quash the crescendo of nociception.

The relevance of this model of inflammation to osteoarthritic pain is not clear. Although some of the tested animal models are ones in which there is marked inflammation, others show only mild levels of inflammation and these can also produce increases in nociceptive sensitivity. It is not clear whether the modest and sometimes inconsistent levels and focality of inflammation that occurs in osteoarthritis are sufficient to produce this enhanced receptor sensitivity.

There is evidence that in osteoarthritis there is abnormal sensitivity to pain and noxious stimulation. In a series of experiments, Le Bars and Villanueva [25] and Ordeberg [26**] examined 15 patients with painful hip osteoarthritis and a similar number of age-matched controls and found that the threshold for pain induction was lower for those with osteoarthritis. This was present in both unaffected limbs and affected limbs when blood pressure cuffs were blown up to a painful level. There was also a tendency to note sensitivity to innocuous warmth and cold in patients with painful hip osteoarthritis. These differences between patients and controls were eliminated by 3 months after the patients underwent hip replacement. Repeat quantitative sensory testing showed there were no longer any differences in pain thresholds, and a marked increase in the thresholds required to induce pain was seen among those who had hip osteoarthritis, suggesting that whatever neurologic abnormality existed as a result of hip osteoarthritis could be eliminated by replacement of the joint [26**]. Bradley et al. [27] have confirmed and extended this work, showing that patients with painful knee osteoarthritis have greater pain sensitivity at sites far distant from the knee than do age-matched healthy controls.
Factors triggering articular nociceptors: activity, loading, and knee pain

Evidence is overwhelming that most knee pain, including that in osteoarthritis, is mechanically based. Pain occurs with certain activities and not with others, e.g. walking up and downstairs but not lying in bed. Runners may note pain running up and down hills but not on level ground. Tibial osteotomy serves as a natural experiment in which high levels of loading in a localized area of the joint are relieved by realigning the bones, distributing load. This operation often has a dramatic pain-lowering effect, proving the importance of loading in causing pain. On a less dramatic level, studies have suggested that persons likely to develop knee pain have dynamic overloading of the medial compartment of the knee [28*], an increased varus moment. Also, those with knee pain adopt strategies to lessen or prevent pain: limping (unloading the painful limb) and externally rotating their feet during walking, thereby decreasing their dynamic varus moment [29,30]. Another strategy to lower varus moment is to walk slowly and this may also lessen knee pain [31].

Factors that increase loading and pain

Although excess loading clearly induces symptoms of knee pain, not all persons are equally susceptible to pain. Personal factors that produce excess loading across a joint tend to increase the likelihood of pain. Obesity has been related to the occurrence of pain [32]. In a recent population-based survey of knee pain, Webb et al. [33*] found not only that obese persons more often had knee pain but also that the knee pain they experienced was more often disabling. Of the disabling knee pain in the community, 36% was attributable to obesity.

Recent data [34] suggest that tall persons are also at higher risk of getting knee pain than short persons even given the same amount of structural disease and adjusting for weight. This is probably because of the increased length of the lever arm in the legs of tall people. The relation of both obesity and height to knee pain suggests that factors that increase transarticular loading are likely to produce pain.

Conclusion

Noicception in the knee is complex, and the nociceptive stimuli are related to but fundamentally different from those producing cartilage loss. Better appreciation for these processes will facilitate the development of new treatments.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


A comprehensive review of how the spinal cord receives and processes peripheral nociceptive input. Includes description of spinal and descending inhibitory effectors and a focus on effects in the cord of inflammation and its mediators.


A review of a series of experiments using quantitative sensory testing in persons with hip osteoarthritis before and after replacement and controls. Authors report that pain thresholds are lower in hip osteoarthritis patients even at sites distant from their hips and that this altered sensitivity resolves after the hip replacement.


Polymorphism in signal transduction is a major route through which osteoarthritis susceptibility is acting

John Loughlin

Purpose of review
In the last year there has been considerable success in the identification of genes harbouring susceptibility for primary osteoarthritis. This report brings the reader up-to-date by focusing on three of the more compelling finds.

Recent findings
A UK group reported an association of the FRZB gene with hip osteoarthritis in females. FRZB codes for secreted frizzled-related protein 3, an antagonist of Wnt signalling. The Wnt signal transduction pathway is critical for normal development and is also active in adult tissues. Secreted frizzled-related protein 3 helps to maintain articular cartilage and the associated alleles at FRZB reduce the activity of this important protein. A Japanese group has reported an association of the asporin gene ASPN with knee and hip osteoarthritis and an association of the calmodulin 1 gene CALM1 with hip osteoarthritis. Asporin is a cartilage extracellular protein that regulates the activity of transforming growth factor-β. Calmodulin is an intracellular protein that interacts with a number of proteins involved in signal transduction. The associated alleles at ASPN and CALM1 reduce the ability of chondrocytes to express the genes encoding aggrecan and type II collagen. Since these are essential structural components of articular cartilage, the ASPN and CALM1 associations are predicted to adversely affect the maintenance of cartilage.

Summary
The FRZB, ASPN and CALM1 results are compelling and highlight that polymorphism in signal transduction pathways is a major component of osteoarthritis susceptibility. This is an exciting observation since signal transduction pathways are malleable and therefore potentially amenable to intervention and modification.

Keywords
asporin, calmodulin, genetic risk, osteoarthritis, secreted frizzled-related protein 3

Introduction
There has long been a perception in the primary osteoarthritis research community that a major proportion of the genetic susceptibility for this common disease is acting through non-synonymous variation in genes encoding cartilage extracellular matrix (ECM) structural proteins [1]. The logic is that amino acid substitution in cartilage ECM structural proteins, such as type II collagen, subtly affects the capacity of these proteins to provide adequate physical resilience for the cartilage. This belief principally evolved from an extrapolation of the genetic causes of the rare chondrodysplasias. These Mendelian diseases often present with severe, early onset osteoarthritis as one of their phenotypic components. De-novo missense mutations within cartilage ECM structural protein genes are often the cause of these diseases [2–5]. The hypothesis, therefore, is that amino acid substitution that has a less severe effect on the functioning of the structural protein could be a risk factor for common, late-onset primary osteoarthritis. Considerable effort has been exercised in testing the cartilage ECM structural protein genes for linkage and for association to osteoarthritis. Although some successes have been reported [6], most reports have provided little or no evidence to support a major role for amino acid substitutions in these genes as risk factors for osteoarthritis [7,8]. This has led to a rethink on what genes are likely to encode for susceptibility to primary osteoarthritis. Many investigators are now focusing their efforts on genes that encode for proteins responsible for the development and maintenance of articular cartilage: there has been a shift away from genes encoding proteins with structural function towards genes encoding proteins that have a cartilage regulatory function. This has proven to be a fruitful move and this review concentrates on three of the more compelling recent finds: an association to the secreted frizzled-related protein 3 (SFRP3) gene, FRZB; an association to the asporin gene, ASPN; and, finally, an association to the calmodulin 1 gene, CALM1. These reports highlight that polymorphism in signal transduction is a major component of osteoarthritis genetic susceptibility.
\textbf{FRZB}\n
In 2000, a UK group reported a linkage to chromosome 2q [9], with subsequent finer mapping narrowing the linkage to an 8.6 cm interval [10]. The linkage was restricted to affected sibling pair families concordant for hip osteoarthritis, with the ascertainment criteria being the need for elective hip replacement surgery due to primary osteoarthritis. The linkage encompassed eight candidate genes: the tumor necrosis factor \(\alpha\)-induced protein 6 gene, \textit{TNFAIP6} (chromosome 2q23.3); the activin A receptor gene, \textit{ACVR1} (2q24.1); the fibroblast activation protein \(\alpha\) gene, \textit{FAP} (2q24.2); the integrin alpha 6 gene, \textit{ITGA6} (2q31.1); the activating transcription factor 2 gene, \textit{ATF2} (2q31.1); the integrin alpha 4, \textit{ITGA4} (2q31.3); the SFRP3 gene, \textit{FRZB} (2q32.1); and the integrin alpha V gene, \textit{ITGAV} (2q32.1). The candidates were chosen based on reports of expression in joint tissue during development or in the adult. Polymorphic microsatellite markers within or near to each candidate were genotyped in the 378 probands from the hip families that had provided the finer linkage and in 760 age-matched controls. All probands and controls were UK Caucasians. Those microsatellites targeting \textit{TNFAIP6}, \textit{ITGA6} and \textit{FRZB} were associated at \(P < 0.05\) [11**]. The \textit{TNFAIP6} association was in males and females whereas the \textit{ITGA6} and \textit{FRZB} associations were restricted to females. Subsequent database searches identified a nonsynonymous single nucleotide polymorphism (SNP) in \textit{TNFAIP6}, two nonsynonymous SNPs in \textit{ITGA6} and two nonsynonymous SNPs in \textit{FRZB}. Initial tests revealed that the \textit{TNFAIP6} and \textit{FRZB} SNPs were genuine, whereas the \textit{ITGA6} SNPs were not real. Genotyping of the \textit{TNFAIP6} and \textit{FRZB} SNPs in the probands and in the controls revealed association to one of the two \textit{FRZB} SNPs, with a \(P\) value of 0.04 in the female probands. An additional cohort of 338 female hip cases also showed association to this SNP \((P = 0.04)\). The \textit{FRZB} SNPs encode for the substitution of highly conserved arginine residues. The first SNP is in exon 4 and is a C to T transition coding for Arg200Tip. The second SNP is in exon 6 and is a C to G transversion coding for Arg324Gly. It was the G-allele of the exon 6 SNP that was associated with osteoarthritis. Females who possessed a copy of both substituted arginines were at an increased osteoarthritis risk, with an odds ratio of 3.6 (95% confidence interval [CI] 1.6–8.3). This risk increased slightly in those females who had both arginines substituted in the same protein molecule, with an odds ratio of 4.1 (95% CI 1.6–10.7). A re-examination of the linkage data revealed that those families harbouring arginine-substituted \textit{FRZB} alleles accounted for the chromosome 2q linkage. The investigators had therefore identified a convincing association of nonsynonymous SNPs in \textit{FRZB} with female hip osteoarthritis.

\textit{FRZB} codes for SFRP3, an antagonist of extracellular Wnt ligands [12–14]. Wnts are secreted glycoproteins that bind to membrane-spanning frizzled receptors. The Wnt signalling pathway has a crucial role in chondrogenesis [15–17] and SFRP3, which is synthesized by adult articular chondrocytes, has been shown to control chondrocyte maturation [18]. Wnt signalling regulates the accumulation of cytoplasmic \(\beta\)-catenin. In the absence of Wnt, the \(\beta\)-catenin is phosphorylated, ubiquitylated and finally degraded in proteasomes, whereas in the presence of Wnt, the \(\beta\)-catenin accumulates, is translocated to the nucleus and instigates gene transcription. To assess whether the two \textit{FRZB} SNPs had any impact on the functioning of SFRP3, the investigators compared the ability of wild type SFRP3 and of the Arg200Tip and Arg324Gly substituted proteins to antagonize Wnt-signalling by the transient transfection of HEK293 cells [11**]. The wild type protein efficiently inhibited Wnt activity. However, the Arg324Gly substitution and the Arg200Tip/Arg324Gly double substitution showed diminished activity. Furthermore, HEK293 cells transfected with the plasmid containing the Arg324Gly substitution required higher levels of expressing plasmid to modestly decrease cytosolic and nuclear levels of \(\beta\)-catenin. These results clearly demonstrated that the conserved arginines are functionally important, with their substitution reducing the ability of SFRP3 to antagonize Wnt signalling.

A Netherlands group very recently genotyped the two \textit{FRZB} SNPs in a sample of 1,369 subjects from a population-based cohort scored for radiographic osteoarthritis in the hip, hand, spine and knee and in a patient population of 191 affected sibling pairs with symptomatic osteoarthritis at multiple sites [19**]. Neither SNP demonstrated association in subjects with hip osteoarthritis. The G-allele of the Arg324Gly SNP, however, was associated \((P < 0.05)\) in individuals with a generalized osteoarthritis phenotype. This phenotype constituted osteoarthritis in at least two of four joint sites (hand, knee, hip and spine). This is potentially a very important report, as it represents an independent replication, albeit in generalized osteoarthritis rather than in hip osteoarthritis, of the original \textit{FRZB} association. Replicating associations for complex traits is extremely important in that it not only helps to distinguish true positives from false positives but also provides information regarding the global relevance of a reported find.

\textbf{ASPN}\n
Asporin is an ECM protein belonging to the small leucine-rich proteoglycan (SLRP) family, other members of which include decorin, biglycan, fibromodulin, epiphycan and chondroadherin [20–23]. SLRP family members are able to bind to other structural components of the ECM, such as collagen. These protein–protein interactions appear to be mediated by the leucine-rich repeat [24]. Some SLRPs, such as decorin and biglycan, are also able to bind to growth factors that reside in the ECM, including transforming growth factor \(\beta\) (TGF\(\beta\)) [20,23]. Based upon amino acid sequence and gene organization, the SLRPs...
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have been subdivided into four classes [25,26]. Asporin is most similar to decorin and biglycan and is therefore considered to be a member of class I. Asporin is expressed in a number of tissues, including adult articular cartilage, and is coded for by the gene \textit{ASPN}, which resides on chromosome 9q22.31. This region of the genome has not previously been reported to harbour osteoarthritis susceptibility in any of the osteoarthritis genome-wide linkage scans [27\textsuperscript{*}]. Based purely on the functional properties of asporin and without any prior genetic information regarding a role for \textit{ASPN} in osteoarthritis susceptibility, a Japanese group tested \textit{ASPN} for association to osteoarthritis [28\textsuperscript{**}].

The group initially demonstrated that \textit{ASPN} was expressed in osteoarthritic articular cartilage. They then sequenced the eight \textit{ASPN} exons and its flanking regions. Twenty-one DNA variants were detected, eight of which had frequencies greater than 5% and were therefore considered common and potentially able to code for osteoarthritis susceptibility. The 8 common variants were genotyped in a population-based cohort of 371 Japanese individuals, 137 of whom were diagnosed as having radiographic knee osteoarthritis (mean age of 75.3, 72% female) whilst 234 were diagnosed as radiographically unaffected (mean age of 73.6, 61% female). One of the variants demonstrated association; a triplet repeat within exon 2 coding for a polymorphic stretch of aspartic acid residues in the amino-terminal region of asporin. This repeat polymorphism was titled the D-repeat after the one-letter code for aspartic acid and had ten alleles encoding 10–19 D residues. The D14 allele was more common in knee osteoarthritis individuals than the unaffecteds 10–19 D residues. The D14 allele was again associated with osteoarthritis (7.8% versus 4.7% respectively, \(P = 0.0013\), odds ratio = 2.29, 95% CI 1.4–4.4). The D-repeat was subsequently genotyped in a Japanese case-control cohort of 393 cases with knee osteoarthritis (mean age of 72.5, 84% female) and 374 controls (mean age of 28.8, 56% female). The D14 allele was again associated with osteoarthritis (7.8% in cases versus 4.8% in controls, \(P = 0.018\), odds ratio = 1.66, 95% CI 1.1–2.5). Combining the two cohorts generated a \(P\) value of 0.00024 (odds ratio = 1.87, 95% CI 1.3–2.6). The investigators finally genotyped the D-repeat in 593 Japanese individuals with hip osteoarthritis (mean age of 58.3, 93% female), which revealed that the D14 allele was also associated with hip disease (\(P = 0.0078\), odds ratio = 1.70, 95% CI 1.1–2.5). As well as the association to D14, the investigators also noticed that allele D13, which has one aspartic acid residue less than D14, was under-represented in the knee osteoarthritis and in the hip osteoarthritis individuals. An osteoarthritis-susceptibility allele (D14) and an osteoarthritis-protective allele (D13) at \textit{ASPN} had therefore been detected.

They initially demonstrated that asporin inhibited the expression of the aggrecan gene \textit{AGC1} and of the type II collagen gene \textit{COL2A1}: aggrecan and type II collagen are the principal structural components of cartilage. The investigators also demonstrated that TGF\(\beta\) induces transcription of \textit{AGC1} and \textit{COL2A1} and that asporin interacts with and inhibits TGF\(\beta\) signalling. This inhibition was particularly strong for asporin coded for by a D14 allele and significantly less so for asporin coded for by a D13 allele. These functional studies provided a model of how the D-repeat could influence osteoarthritis susceptibility: asporin naturally inhibits TGF\(\beta\) signalling and therefore regulates the synthesis of aggrecan and type II collagen, critical structural components of articular cartilage; this inhibition is strongest for the D14-coded asporin, leading to insufficient quantities of aggrecan and type II collagen and therefore a structurally compromised cartilage; D13-coded asporin has a weakened TGF\(\beta\) inhibitory effect resulting in a more structurally resilient cartilage. An important question that emerges from the Japanese study is whether asporin modulates the signalling of other members of the TGF\(\beta\) superfamily, such as the bone morphogenetic proteins. These proteins also regulate cartilage development and can influence the maintenance of joint tissues [29–32]. Another outstanding issue, which the Japanese investigators highlight, is how exactly does the size of the D-repeat influence asporin activity? Is the effect mediated by alterations in the conformational structure of the protein or does the repeat itself bind directly to TGF\(\beta\)?

Overall, the asporin study is genetically and functionally highly compelling. The association was replicated in independent Japanese cohorts and the subsequent studies in to the activity of D14-coded and D13-coded asporin provided a logical model through which the \textit{ASPN} gene can influence osteoarthritis risk.

\textbf{CALM1}

The group that conducted the asporin study have also carried out a genome-wide association analysis to identify hip osteoarthritis susceptibility loci in the Japanese population [33\textsuperscript{**}]. They initially genotyped and tested for association 71,880 SNPs in 94 individuals with symptomatic hip osteoarthritis and 633 controls. Two thousand two hundred and nineteen SNPs demonstrated association at \(P < 0.01\). These positive SNPs were subsequently genotyped in an independent cohort of 334 hip cases and 375 controls. Several SNPs demonstrated strong association, including a SNP located in intron 3 of the calmodulin 1 gene \textit{CALM1} (chromosome 14q24-q31). Calmodulin is an intracellular protein that binds to Ca\(^{2+}\) and interacts with a number of cellular proteins [34,35]. The association at the intron 3 SNP was particularly significant in those hip cases that had inherited two copies of the associated allele (i.e. a recessive effect), with a \(P\) value of 0.00065 and an odds ratio of 2.40 (95% CI 1.43–4.02).
The association was present in both male and female cases, although most of the cases (94%) were female. The investigators subsequently analyzed all other common variants within CALM1 to assess whether the intron 3 SNP was in linkage disequilibrium with other polymorphisms. This revealed that the SNP was in strong linkage disequilibrium with four other SNPs: an SNP in the core promoter region of CALM1, two SNPs in intron 1 and a SNP in the 3’UTR. All these SNPs demonstrated strong association (P ≤ 0.00065) to hip osteoarthritis.

Of the 5 associated SNPs that were in linkage disequilibrium, the core promoter SNP was considered the most likely to have a functional effect on calmodulin 1. The investigators initially demonstrated that CALM1 was expressed in human articular chondrocyte. They also showed higher levels of CALM1 expression in osteoarthritis cartilage compared with normal cartilage. The investigators subsequently assessed the effect that the 2 alleles of the core promoter SNP had on the expression of CALM1. This revealed that the associated allele (the T allele) resulted in reduced transcriptional activity relative to the unassociated allele (the C allele). The investigators then demonstrated that calmodulin 1 increases the expression of the aggrecan gene, AGC1, and of the type II collagen gene, COL2A1. These functional studies provide a model of how the core promoter SNP of CALM1 could influence osteoarthritis susceptibility: calmodulin 1 naturally increases the synthesis of aggrecan and type II collagen in articular cartilage; this synthesis is reduced for the T-allele, particularly in those individuals who are TT homozygotes, leading to insufficient quantities of aggrecan and type II collagen and therefore a structurally compromised cartilage.

Because calmodulin 1 and asporin both regulate the expression of AGC1 and COL2A1, the investigators finally assessed whether the associated allele at CALM1 (the T allele of the core promoter SNP) and the risk allele at ASPN (the D14 allele of the D-repeat) had a combinatorial effect. This revealed that individuals who had inherited two copies of the T allele and at least one copy of the D14 allele were at a particularly high risk of developing hip osteoarthritis, with an odds ratio of 13.16 (95% CI 1.66–104.06). This makes sense, since both the T allele of CALM1 and the D14 allele of ASPN lead to a reduction in expression of AGC1 and COL2A1.

Conclusion
Identifying genes that encode for susceptibility to complex traits is a daunting task, especially for very common and clinically heterogeneous diseases such as primary osteoarthritis; however, a number of breakthroughs have occurred in recent years. This review has focused on three. What distinguishes these three from other reports is that the genetic results have been supported by functional studies. Nevertheless, it is important that each result be tested in additional cohorts to assess more accurately the contribution each gene is making to osteoarthritis risk and to determine whether this risk is restricted to particular ethnic groups. For FRZB, there has been a second positive report. It will be particularly interesting to determine whether the ASPN and CALM1 associations detected in Japanese have a role in osteoarthritis development outside of Asia. The osteoarthritis community is fortunate in that a number of cohorts have been collected throughout the world [36*]. It is a priority that each association be tested in each cohort.

An important factor that links FRZB, ASPN and CALM1 is that these osteoarthritis susceptibility genes are mediating their effects through signal transduction pathways. It is time, therefore, for the research community to update its thoughts regarding the genetic basis of primary osteoarthritis: polymorphisms in genes whose proteins regulate the development and maintenance of joint tissue are major components of osteoarthritis genetic risk. This is an exciting observation since signal transduction pathways are amenable to modification, opening the door to new treatment development.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
** of outstanding interest

11 Loughlin J, Dowling B, Chapman K, et al. Functional variants within the secreted frizzled-related protein 3 gene are associated with hip osteoarthritis in females. Proc Natl Acad Sci USA 2004; 101:9767–9772. The FRZB association — one of the three studies that constitute the focus of this review.


The Netherlands study that also reported association of FRZB with OA.


A good clinical and genetics overview of OA.


The ASPN association — the second of the three studies that this review focuses on.


The CALM1 association — the final study of the three that this review has concentrated on.


A brief overview of the OA patient collections currently available to the research community.
Exercise as a treatment for osteoarthritis
Kim Bennell and Rana Hinman

Purpose of review
This review highlights recent important research, future directions, and clinical applications for exercise and osteoarthritis. It focuses on knee osteoarthritis because of its prevalence and the dearth of research involving other joint osteoarthritis. The review covers exercise prescription for symptomatic relief, and its potential role in reducing development and slowing progression of osteoarthritis.

Recent findings
Meta-analyses support recommendations that exercise is important in osteoarthritis management. Benefits appear to be additive when exercise is delivered with other interventions such as weight loss. Mode of exercise delivery has cost implications and may influence overall outcome. It appears that supervised exercise sessions are superior to home exercises for pain reduction. The challenge remains to increase the proportion of patients exercising. Areas of emerging interest are exercise to prevent disease or slow its progression and recognition of patient subgroups that may respond differently to treatment. Based on studies showing a relation between weaker quadriceps strength and increased risk of developing knee osteoarthritis, particularly in women, strength training may be able to prevent knee osteoarthritis. Novel exercise programs that strengthen hip muscles or alter impairments in knee neuromuscular control may also influence disease progression.

Summary
Future studies must identify cost-effective exercise modes, strategies to maximize exercise compliance and optimal treatment combinations. The role of muscle strength and altered neuromuscular control in the prevention and development of osteoarthritis must be evaluated with the view to devising and testing novel exercise interventions.

Keywords
compliance, exercise, knee, muscles, osteoarthritis

Introduction
Osteoarthritis is a chronic joint disorder with the knee most frequently affected [1]. Patients often report pain, muscle weakness, stiffness, and instability, as well as reduced physical functioning. Ultimately, these lead to a loss of independence and a reduction in quality of life. In the past, studies have evaluated the role of exercise as a tertiary prevention strategy (treating pain and disability) but more recently, its potential role in primary (reducing disease incidence) and secondary (slowing progression to serious disease) prevention is receiving increasing attention.

The purpose of this review is to highlight recent important research, future directions, and the clinical application of research findings in the area of exercise and osteoarthritis. This review focuses on knee osteoarthritis because of its prevalence [1] and the dearth of research involving hip and other joint osteoarthritis. This review will cover: exercise prescription for symptomatic relief of osteoarthritis, the role of exercise in reducing development of osteoarthritis, and the role of exercise in slowing disease progression.

Exercise prescription for symptomatic relief of osteoarthritis
As there is currently no cure for osteoarthritis, most research continues to evaluate the use of exercise as a treatment to alleviate symptoms of the disease. Recent common themes in the literature will be explored in this section, and the results of studies discussed in relation to clinical practice.

Clinical guidelines and meta-analyses
Clinical guidelines have been developed by leading bodies (American College of Rheumatology and the European League Against Rheumatism) to aid health practitioners in treating osteoarthritis [2,3]. These recommend exercise therapy to reduce pain and improve function, based largely on expert opinion and the results of large randomized controlled trials evaluating exercise [4–6].

Recent meta-analyses support these recommendations [7–9]. Two published in 2004 focus specifically on the efficacy of strengthening [8•] and aerobic exercise [9•] for osteoarthritis. Twenty-two trials of strengthening exercise were identified and included isometric, isotonic, isokinetic, concentric, concentric–eccentric, and dynamic modalities. Improvements in strength, pain, function, and quality of life were noted with muscle strengthening;
however, there was no evidence that the type of strengthening exercise influences outcome. Findings suggest that the effectiveness of joint-specific strengthening is maximized when combined with general strength, flexibility, and functional exercises. Regarding aerobic exercise, 12 trials were identified including walking programs, aquatic exercises, jogging in water, yoga, and T’ai Chi. Results indicated that aerobic exercise alleviates pain and joint tenderness, and promotes functional status and respiratory capacity. While strengthening appears superior to aerobic exercise in the short-term for specific impairment-related outcomes (e.g. pain), aerobic exercise appears more effective for functional outcomes over the longer term. Different exercise types have different effects; thus, an individualized approach to exercise prescription is recommended, based on presenting symptoms, problems and the needs of the patient.

Roddy et al. [10••] published evidence-based recommendations for exercise in managing hip and knee osteoarthritis. These recommendations are unique in that they combine and differentiate expert opinion and research evidence, as well as address important clinical questions such as adherence, predictors of response, need for individualized exercise prescription, and mode of exercise delivery. Recommendations include the following:

1. Both strengthening and aerobic exercise can reduce pain and improve function and health status.
2. There are few contraindications to prescription of strengthening or aerobic exercise.
3. Prescription of both general (aerobic fitness training) and local (strengthening) exercises is an essential, core aspect of management.
4. Exercise therapy should be individualized and patient-centered taking into account factors such as age, co-morbidity, and overall mobility.
5. To be effective, exercise programs should include advice and education to promote a positive lifestyle change with an increase in physical activity.
6. Group exercise and home exercise are equally effective and patient preference should be considered.
7. Adherence is the principle predictor of long-term outcome from exercise.
8. Strategies to improve and maintain adherence should be adopted.
9. Effectiveness of exercise is independent of presence or severity of x-ray findings.
10. Improvements in muscle strength and proprioception gained from exercise programs may reduce the progression of osteoarthritis.

Unfortunately, many of the recommendations proposed by expert opinion have not been researched; however, they provide a valuable framework to guide clinical practice and future research directions.

Developing specific and novel exercise programs
Osteoarthritis is heterogeneous with regards to associated symptoms, impairments, and changes in the local mechanical environment [11]. This has implications for the development of novel exercise programs and for tailoring standardized programs to suit specific patient subgroups. Traditionally, exercise therapy for knee osteoarthritis has centered on quadriceps strengthening; however, it appears that such an exercise strategy may not be the optimal choice for all patients. For example, functional instability, the symptom of buckling, slipping, or giving way of the knee during functional activities, has recently been identified as a problem in a significant proportion of individuals with knee osteoarthritis. In a cohort of 105 people, 44% reported instability that affected their ability to function [12•]. Instability is likely to be multifactorial, resulting from factors such as passive joint laxity, structural damage, muscle weakness, pain, and altered neuromuscular control. Further research needs to examine the extent these factors contribute to the symptom of instability in knee osteoarthritis as this will influence treatment strategies utilized. Fitzgerald et al. [13] suggest an agility and perturbation training program may be effective by exposing the individual to potentially destabilizing loads in a controlled manner allowing the neuromuscular system to adapt to such conditions. The effect of such an exercise program requires formal evaluation before widespread implementation clinically.

Muscle strengthening is a key component of exercise for osteoarthritis because of the relation between muscle weakness and pain and function [14,15]. However, traditional muscle strengthening exercises may be inadequate in the subgroup of patients whose strength loss is largely because of central-mediated or reflex-mediated inhibition [16]. Instead, more specialized interventions may be needed to supplement volitional exercise programs. Identifying muscle inhibition is currently difficult in a clinical setting; it may be worth considering other interventions if strength gains with exercise are not as great as would be expected.

Evaluating the interaction of exercise with other interventions
Clinical practice generally implements more than one treatment concurrently for patients with osteoarthritis. Past research has tended to evaluate exercise therapy in isolation or in combination with other treatments. However, for the latter, study design precludes the elucidation of interaction effects. It is likely that exercise, when combined with other efficacious treatments, maximizes clinical outcome. This is supported by the recent 18-month randomized, single-blind ADAPT trial [17••]. The results in 316 overweight and obese individuals with knee osteoarthritis showed that the combination of modest dietary weight loss and moderate exercise (aerobic and
strengthening exercise three times per week) provides better overall improvements in function, pain, and mobility than either intervention alone. A similar study design evaluating exercise and drug therapies in particular is required.

**Mode of delivery**

Rising healthcare costs necessitate delivering exercise in the most cost-effective and efficacious method. A Cochrane Review compared the effect size of exercise delivered as individual treatments, supervised group classes, and home exercise [18]. For pain, comparable medium effect sizes were observed with individual treatments and group classes, while a small effect size was evident for home exercise. Small effect sizes were reported for all modes of delivery regarding physical function. This suggests that supervised group or individual treatments are superior to independent home exercise.

A ‘minimalist’ approach to exercise intervention seems ineffective in patients with hip and knee osteoarthritis [19\*]. In a large study, rheumatologists randomly delivered one of four interventions: patient-administered assessment tools, home exercise, patient-administered assessment tools plus home exercise, or usual care. The exercise program aimed to improve joint mobility and increase muscle power. The rheumatologist provided an oral explanation of the importance of exercise and a booklet illustrating the exercises as well as a videotape. There was a limited capacity to expand the exercise program. There was no difference in outcome between interventions. Numerous factors likely contribute to the ineffectiveness of exercise in this study. Patients were poorly compliant (only 29–33% met the specified criterion of adherence) and a standardized exercise program and dosage was used that may have been ineffective for such a heterogenous patient group. While a videotape demonstration of the exercises was provided, it would appear that technology is no substitute for personal demonstration and instruction in correct exercise technique. It is quite possible that many patients were performing the exercises incorrectly, further reducing their effectiveness.

Others have studied the effects of supplementing home exercise with a class-based program [20\*]. Patients were taught home exercises with their intensity individualized at baseline and reassessed and increased at 4-week and 8-week reviews. In addition, half of the patients were randomly allocated to undertake an 8-week physiotherapist-supervised class exercise program. Results demonstrated that supplementation of a home program with exercise classes lead to greater improvements in pain and locomotor function at 12 months follow-up. Importantly, this study demonstrates that the short-term addition of exercise classes to an ongoing home exercise program results in significant symptomatic benefits in the longer term. Economic analyses demonstrated that the additional cost of the group exercise classes was offset by reductions in resource use elsewhere in the healthcare system [21]. Thus, exercise class supplementation represents a relatively cost-effective method of maximizing the benefits of a home exercise program that would otherwise result in only small benefits of questionable clinical effectiveness.

**Improving uptake of exercise in the osteoarthritis population**

Despite evidence of the benefits of exercise in osteoarthritis, the challenge remains to increase the proportion of patients exercising. While there are many barriers to the uptake of exercise in the osteoarthritis population, two are of particular importance: recommendation of exercise to patients by medical practitioners and appropriate referral to exercise professionals, and compliance by patients with prescribed exercise programs.

Exercise is under-used by medical practitioners as a treatment strategy for osteoarthritis. A French study surveyed 3,000 general practitioners as to their treatment of a hypothetical knee osteoarthritis patient. [22\*]. Less than 15% of general practitioners reported that they would prescribe exercise as a first-line therapeutic approach. Perhaps even more alarming is the finding that bed rest was recommended (2% of general practitioners for less severe osteoarthritis and 24% for more severe disease), despite the lack of evidence for this outdated treatment method. A survey of osteoarthritis patients in Canada revealed that only one-third had been advised to use exercise for their condition [23]; however, 73% reported that they had tried exercise in the past. Given the large number of patients who chose to try exercise independently, it is possible that many failed to consult a professional regarding the most appropriate exercise to commence. Thus, it is likely that many patients selected inappropriate exercise programs or dosages. Coupled with the risk of incorrect technique, many patients may have failed to achieve any therapeutic benefit from exercise.

Patient compliance is a key factor in determining outcome from exercise therapy in osteoarthritis patients. Ettinger et al. [4] demonstrated a dose-response relation between compliance and exercise effects. The strongest and most consistent predictor of compliance in this study was exercise behavior in the previous months of the trial [24], suggesting that the ‘habit’ of exercise is important in determining future exercise behavior. Similarly, effect sizes with home exercise drop from medium to small as self-reported adherence falls [25]. Unfortunately, the beneficial effects of exercise in osteoarthritis last only as long as the patient exercises, as demonstrated in a follow-up of 183 patients 6 months after completion of a 12-week exercise program [26]. Although exercise resulted in
significant improvements in pain and disability, these effects were lost 6 months after the exercise program had ceased.

Another study evaluated the factors that motivate patients with knee osteoarthritis to join a 12-month trial comparing strength training and flexibility exercise [27]. Results indicated that social support, the presentation of an organized exercise opportunity conducted by professionals, having a partner exercise alongside the patient, familiarity with the exercise task, and having positive outcome expectations of exercise were all important. Campbelle et al. [28] interviewed knee osteoarthritis patients who had participated in a physiotherapy trial consisting largely of home-based strengthening exercises. Patients were most compliant in the initial period while still seeing the physiotherapist. However, compliance declined once therapist contact ceased. Reasons cited as affecting motivation to comply included attitude towards exercise, perceived severity of symptoms (those with more severe symptoms were most likely to comply), ideas about the cause of arthritis (those who thought arthritis was the result of age or ‘wear and tear’ were less compliant) and the perceived effectiveness of the intervention (high levels of continued compliance were related to the perception that physiotherapy is effective and an improvement in symptoms was experienced).

The role of exercise in preventing disease development

Whether exercise can prevent osteoarthritis is unclear but from a public health perspective, primary prevention is an area worthy of investigation. It is thought that contraction of the quadriceps muscle helps to absorb shock at heel strike [29]. A direct link between impact loading and development of osteoarthritis has only been shown in animal models [30,31]. A potential relation between muscle strength and disease development was first reported by Slemda et al. [32] who found that in women, but not men, stronger quadriceps muscles reduced the risk of developing radiographic knee osteoarthritis. Two recent studies support these results. Hootman et al. [33*] evaluated 3081 community-dwelling adults free of osteoarthritis, joint symptoms, and injuries. Women with a moderate to high isokinetic quadriceps strength had a 55% and 64% reduced risk of developing hip or knee osteoarthritis, respectively. The results were less conclusive for men. The relationships between body composition measured using dual energy x-ray absorptiometry, and changes in tibial cartilage volume over 2 years were measured by magnetic resonance imaging (MRI) in 86 healthy middle-aged adults [34]. Increased muscle mass was strongly associated with medial tibial cartilage volume as well as a reduction in the loss of both medial and lateral tibial cartilage volume. Body fat was not an independent risk factor for cartilage loss. These results are consistent with another study also showing a positive association between muscle size and knee-cartilage volume [35]. However, co-inheritance rather than local muscle hypertrophy arising from exercise, may explain these relationships. In fact, a common genetic link for these parameters was established in a recent sib-pair study [36]. Furthermore, in the study by Cicuttini et al. [34] the relation between tibial cartilage volume and muscle mass was significant for multiple sites (the lower limbs, all four limbs, and total body muscle mass) rather than being isolated to a particular limb that would support a co-inherited mechanism. Randomized controlled trials are needed to establish whether exercise interventions designed to increase muscle mass do, in fact, protect against future hip and knee osteoarthritis.

Role of exercise in slowing disease progression

Knee load plays a role in disease progression. The external knee adduction moment generated during walking forces the knee laterally into varus, compressing the medial joint compartment. It is one of a few factors known to predict progression of osteoarthritis in humans [37]. Therapeutic exercise could have a disease-modifying effect by altering...
knee joint load but whether this effect is positive or negative is unclear as only indirect evidence exists.

A potential effect of strengthening exercise on disease progression was first explored in 1999. However, this longitudinal cohort study failed to find a relation between quadriceps strength and disease progression possibly because of limited statistical power [38]. Controversy was later triggered by the unexpected results of another study [39] where higher baseline quadriceps strength was associated with a greater risk of disease progression in knee osteoarthritis patients with malalignment and laxity. In neutrally aligned and stable knees, there was no relation between strength and progression. This suggests that exercise may have differential effects depending on patient presentation and highlights the need for specificity of exercise prescription.

Though the focus has been on quadriceps strength, it may be that other muscles also influence knee load and hence disease progression. During walking, the abductor and adductor muscles stabilize the pelvis on the hip joint and thus weakness may influence pelvic level or toe out angle during gait thereby increasing the knee adduction moment [40]. An abstract of a longitudinal cohort study found that those with a lower external hip adduction moment (indicating less use of the hip abductor muscles) had more rapid knee osteoarthritis progression [41]. Greater toe out is associated with a lower adduction moment and reduces the odds of radiographic progression [42]. Less is known about the hip adductor muscles in relation to knee osteoarthritis but they may assist in resisting the knee AM, particularly in a varus malaligned knee. By virtue of their attachment to the distal medial femoral condyle, the adductors could eccentrically restrain the tendency of the femur to move into further varus. Yamada et al. [43] found that patients with knee osteoarthritis had stronger hip adductors compared with age-matched controls, and that those with more-severe osteoarthritis had even stronger adductors than their less-severe counterparts. They hypothesized that this increased strength may be because greater use of the hip adductors in an attempt to lower the knee adduction moment. Given that hip mechanics can alter knee load, hip strengthening could be a novel intervention for rehabilitation of knee osteoarthritis patients. This requires further evaluation.

Reductions in muscle strength and proprioception, and abnormal gait patterns are well described in knee osteoarthritis [44–46]. However, there is growing recognition that other aspects of neuromuscular control, such as muscle activation strategies, are also altered. Recent cross-sectional electromyographic studies have identified differences in both the timing and amplitude of muscle activity in patients with knee osteoarthritis compared with healthy controls [47•, 48, 49••,50•]. Childs et al. [47] found that the lower limb muscles were activated for 1.5 times longer in osteoarthritis compared with controls during walking and stair ascent and descent. Greater muscle co-activation between the vastus lateralis and the medial hamstrings was also evident in the osteoarthritis group. Others have similarly reported increased levels of co-contraction with greater activation of the hamstrings [49,50]. Lewek et al. [49] reported that differences in co-contraction levels comparing osteoarthritis patients and controls were confined to the medial side with similarly high levels of lateral muscle co-contraction in the two groups. This was associated with greater medial joint laxity, a higher adduction moment and more self-reported instability in the osteoarthritis group compared with controls. The authors suggest that frontal plane laxity is localized to the medial side of the joint in osteoarthritis and that the greater medial co-contraction is an attempt at stabilization. However, this strategy is likely to result in greater medial joint compressive loads and thus may hasten disease progression.

These neuromuscular alterations in knee osteoarthritis represent coping strategies to combat pain, weakness, or local mechanical changes including joint laxity. While such strategies may have short-term benefits, they may have long-term negative consequences by altering the distribution and increasing the magnitude of load and potentially speeding disease progression. This has led to the recommendation that novel exercise approaches designed to reduce levels of co-contraction should be developed to address these neuromuscular changes [47••,50•]. Further research is needed to identify the patient subgroups most likely to present with these changes, how these changes can be identified in the clinical setting, and to develop and test novel interventions.

**Conclusion**
To maximize overall patient outcome, an exercise program incorporating strengthening and aerobic elements together with other specific exercises based on individual requirements is most appropriate. In the future, it is likely that novel exercise regimes will be developed to address recently identified neuromuscular changes and impairments associated with osteoarthritis and having the potential to influence disease progression. Maximizing compliance is a key element dictating success of exercise therapy. This could be enhanced by the use of supervised exercise sessions (possibly in class format) in the initial exercise period to allow appropriate patient education regarding benefits of exercise, ensure safe and correct exercise technique, and provide a supportive social environment for exercise (Fig. 1). At this early stage, prescription and instruction in tailored home exercises by a suitably trained professional is also important. Bringing patients back for intermittent consultations with the exercise practitioner, or attendance at ‘refresher’ group exercise classes may assist long-term compliance.
References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest
** of outstanding interest


A report from an expert committee details consensus recommendations describing key clinical points regarding the role of exercise for hip and knee osteoarthritis. The evidence-base to support each recommendation is described. In contrast to previously published recommendations that focus on the efficacy of exercise for treating symptoms, this article also addresses key clinical issues such as adherence, mode of exercise delivery, the need for individualized prescription, and the role of exercise with regard to disease progression. Gaps in available research evidence are identified and should guide future research.


This study of 105 individuals with knee osteoarthritis is the first to formally report the prevalence of self-reported knee instability and highlight this as an under-recognized symptom that appears to correlate with physical function. It raises questions about the causes of the symptom and the type of exercise program that might best address it.


A well-designed clinical trial showing that exercise has additive benefits to weight loss in 316 overweight and obese individuals with knee osteoarthritis. This is one of the few studies to evaluate the effect of exercise in isolation and in combination with another treatment intervention.


A 24-week randomized clinical trial evaluating the clinical efficacy of patient administered assessment tools and an unsupervised home-based exercise program in hip and knee osteoarthritis. The ‘minimalist’ approach to exercise utilized in this study resulted in no significant change in symptoms over time; this has implications for the delivery and prescription of exercise to patients in clinical practice.


This randomized controlled trial compares two methods of providing exercise therapy to knee osteoarthritis patients. Supplementing a home-based program with supervised classes for 8 weeks was more effective in improving pain and function at 12 months than the home-based program alone.


This is a large-scale survey of general practitioners regarding their treatment of patients with knee osteoarthritis. Results were compared with the 2000 EULAR recommendations on the management of knee osteoarthritis. Findings demonstrate the under-utilization of many non-pharmacological treatment strategies (including exercise) as a first-line approach to management; this is in direct contrast to the guidelines.


Osteoarthritis


This is one of several recent cross-sectional studies focusing on alterations in muscle activation patterns in knee OA [49••,50•]. It found that the muscles were activated at a higher level and for longer during gait and stairs in people with knee OA. The results have implications for disease progression.


This cross-sectional study reported greater medial joint laxity in people with medial knee OA than in controls that tends to be opposite to traditional thinking that joint laxity is found laterally. They also showed greater self-reported knee instability, knee adduction moments, and medial quadriceps-medial gastrocnemius co-contraction in the knee OA group. The authors suggest that while the neuromuscular changes are an adaptation to joint laxity they may, in fact, be detrimental to long-term cartilage integrity. They claim that medial joint laxity should thus be the focus of interventions.


This cross-sectional study also found higher hamstring and gastrocnemius muscle co-contraction in people with knee OA. The authors suggest that exercise interventions should not just focus on quadriceps strengthening but on improving muscle balance at the knee.
Biomarkers in osteoarthritis
Virginia Byers Kraus

**Purpose of review**
Biomarker discovery and validation for osteoarthritis have accelerated over the past several years, coincident with an evolving understanding of joint tissue molecules and their complex interactions, and the compelling need for improved osteoarthritis outcome measures in clinical trials. This review highlights advances in osteoarthritis-related biomarker research within the past year.

**Recent findings**
The studies in the past year involving biochemical markers in humans can be assigned to one of four categories: new approaches and new biomarkers, exploratory studies in specialized disease subsets, large cross-sectional validation studies, and longitudinal studies, with and without an intervention.

**Summary**
Most these studies have examined the association of a biomarker with some aspect of the natural history of osteoarthritis. As illustrated by the six studies reviewed here that included therapeutic interventions, however, several biomarkers are emerging that display credible association with disease modification. The expanding pool of informative osteoarthritis-related biomarkers is expected to positively impact the development of therapeutics for this disease and, it is hoped, ultimately clinical care.

**Keywords**
biochemical markers, biomarkers, magnetic resonance imaging, metabolomics, osteoarthritis, proteomics, rheumatoid arthritis, validation

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**Introduction**
The availability of useful biomarkers for osteoarthritis detection and monitoring is dependent on successful discovery and successful validation. The discovery component, long delayed due to the challenges related to cartilage biochemistry [1], has accelerated over the past several years, coincident with the slow deciphering of joint tissue molecules and their complex interactions. The merits and utility of a biomarker are commensurate with the precision and clinical relevance of the gold standard criteria used to validate it. The current standard criteria are radiographic and are generally regarded as inadequate for biomarker validation [2**]. Therefore, new tools and criteria for diagnosing and measuring the progression of osteoarthritis are currently under investigation [3], in particular, magnetic resonance imaging (MRI) modalities [4]. The development of alternative diagnostic technologies is being impelled by the urgent need arising from efforts to develop therapeutic agents for osteoarthritis [5]. For this reason, it is not yet possible to fully appreciate the potentialities of biomarkers for osteoarthritis, although steady progress is apparent.

Although a biomarker is classically thought of as a component of a single protein, the genomics revolution has expanded this vision to include RNA, DNA, their fragments, or a combination or multiplicity of these, to form a distinct ‘fingerprint’ of a disease state. The osteoarthritis-related biomarker studies in humans in the past year primarily reflect a diversity of protein markers, but increasingly it is to be expected that new candidate biomarkers will arise from complex proteomic and microarray approaches [6]. For each osteoarthritis-related biomarker study highlighted here, the specific number of osteoarthritis patients included (n), is provided to convey the general scope of the study.

**New approaches and new markers**
Several studies in the past year are noteworthy for approaching osteoarthritis biomarker discovery and validation in a novel way. The various approaches used include metabolomics, proteomics, quantification of post-translational modifications, evaluation of autoantigenicity of cartilage-related proteins, principal-components analysis (a form of multivariate analysis), and validation against osteoarthritis features by MRI.

In an approach termed metabolomics, a profile of urinary metabolites specific to individuals with knee and hip osteoarthritis (n = 45) has been discerned using nuclear MR spectra coupled with principal-component discriminant analysis [7**]. This technique demonstrated lower concentrations of histidine and methylhistidine, and...
elevations in urinary hydroxybutyrate, pyruvate, creatine/creatinine and glycerol, suggesting altered energy utilization in osteoarthritis.

In a study using a proteomics approach in which osteoarthritis (n = 23) was designated the ‘control’ condition and compared with rheumatoid arthritis, the S100A8/A9 heterocomplex (referred to also as ‘calprotectin’) distinguished rheumatoid arthritis from osteoarthritis patients [8]. Plasma levels of this inflammatory protein complex from neutrophils were higher in rheumatoid arthritis and only in osteoarthritis synovial fluids were synovial fluid to plasma ratios <1.

In evaluations of post-translational protein modifications, the glycosylation patterns of two acute-phase proteins, α1-acid-glycoprotein and α1-antichymotrypsin, have been shown to differ in patients with active (n = 37) vs inactive (n = 24) osteoarthritis, although the bases for this clinical distinction were not reported [9•]. The total concentrations of these two acute-phase glycoproteins did not differ between the two groups, however. The glycosylation of these acute-phase glycoproteins takes place in the liver and is a process controlled by cytokines. Taken together, these data suggest that the microheterogeneity of acute-phase glycoproteins, which reflects a difference in glycosylation patterns, is potentially a more sensitive indicator of systemic inflammatory activity than the absolute concentration of these proteins.

Another novel approach has been to look for antibodies to cartilage-related proteins [10••]. Using whole cell lysates from articular chondrocytes, a total of 19 autoantigens unique to osteoarthritis (n = 20) and 22 autoantigens common to osteoarthritis and rheumatoid arthritis were detected. One of the autoantigens unique to osteoarthritis was identified as a triosephosphate isomerase glycolytic enzyme. Serum antibodies to triosephosphate isomerase were five times more common in osteoarthritis (n = 93, 25% antibody prevalence) than in rheumatoid arthritis, systemic lupus erythematosus, or control patients. The presence of anti-triophosphate isomerase antibodies was associated with lower radiographic grades of osteoarthritis, suggesting that these autoantibodies were related to early phases of the disease. Moreover, the presence of antibodies in synovial fluid appeared to be unique to osteoarthritis. This is a very promising study of autoimmune profiles in osteoarthritis samples and warrants further investigation of extracellular matrix proteins, in addition to the intracellular chondrocyte proteome probed here.

In a subset of the ECHODIAH cohort of hip osteoarthritis patients (n = 376), principal-component analysis of baseline data was effectively used to identify independent clusters of osteoarthritis-related biomarkers [11••]. The 10 biomarkers segregated into five different clusters, which the authors speculated were representative of different pathophysiologic processes in osteoarthritis: cartilage degradation and bone turnover (urinary C-terminal cross-linking telopeptide of collagen type II or uCTX-II, N-propeptide of collagen type I, and urinary C-terminal cross-linking telopeptide of collagen type I); synovitis (cartilage oligomeric matrix protein or COMP, N-propeptide of collagen type III, and hyaluronic); systemic inflammation (high-sensitivity C-reactive protein or hsCRP, matrix metalloproteinase-1, and matrix metalloproteinase-3. In addition, pain was significantly associated with uCTX-II and hsCRP, joint inflammation with COMP, and uCTX-II with radiographic signs of joint damage. This type of approach has been used successfully in osteoarthritis model systems previously [12] and, as illustrated here, offers an effective approach to using biomarkers in combination in studies in humans.

King et al. [13•] investigated associations of structural characteristics of articular cartilage by MRI and biomarkers. COMP was negatively associated with cartilage volume, and serum collagenase-generated neoeptitope of type II collagen (C3C) was positively correlated with cartilage T2 (a measure of cartilage network organization and water content). Although it was a small pilot study of knee osteoarthritis patients (n = 16), it suggests a relation of these osteoarthritis-related biomarkers and the molecular processes of cartilage degeneration. It also surely heralds many more studies to come assessing alternatives to plain radiography.

New markers

Three new markers, all related to collagen II, are highlighted here: coll2-1 and its nitrated form [14,15•], urinary type II collagen helical peptide (HELIIX-II) [16•], and serum procollagen type IIA amino terminal propeptide (PIIANP) [17•,18]. In a study of knee osteoarthritis patients (n = 75), an increase in urinary collagen II epitope and its nitrated form over 1 year correlated negatively with the 3-year change in medial joint space width, although the mean values for osteoarthritis and controls were not given [14]. The nitrated form of collagen II epitope in serum of osteoarthritis patients (n = 217) correlated with hsCRP, suggesting that this marker may reflect systemic inflammation. Overall, these studies provide a comprehensive example of the evaluation of the performance metrics of a biomarker [15•]. The new HELIX-II peptide biomarker, indicative of type II collagen degradation, was increased 56% in patients with knee osteoarthritis (n = 90) compared with healthy controls [16•]. Patients with increased levels of both urinary HELIX-II and uCTX-II had the highest risk of progression in a parallel rheumatoid arthritis group (odds ratio 17.5).

A third collagen biomarker for which a specific immunoassay has been recently developed is PIIANP, which reflects
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synthesis of a collagen II splice variant expressed in fetal cartilage, fracture callus, and osteophytes. Recent studies of this biomarker ($n = 43$) [17,18] confirmed previous indications [19] of a state of low collagen II synthesis in knee osteoarthritis patients compared with controls.

Exploratory studies

To date, attempts to differentiate specialized disease subpopulations of osteoarthritis patients using biomarkers have met with varied success. These studies are generally small and lack assessment of statistical power with which to judge the veracity of negative results. In addition, few of these studies account for confounding of biomarker levels by osteoarthritis at other sites. Conrozier et al. [20] readily assert these limitations in their study showing no difference in serum biomarkers (hsCRP, COMP, tissue inhibitor of matrix metalloproteinase-1, hyaluronan, Type-1 procollagen or CP-1, serum c-terminal cross-linking telopeptide of type N collagen or S-CTX-I, and matrix metalloproteinase-1) in atrophic ($n = 25$) compared with hypertrophic ($n = 25$) hip osteoarthritis. Silvestri et al. [21] compared erosive and nonerosive hand osteoarthritis ($n = 59$), and although erosive osteoarthritis showed wider variation in degenerative biomarkers (Col2-3/4 short and C2C) and a narrower range of a repair epitope (CS846) compared with nonerosive disease, differences between subsets were not significant. Although the patients were free of clinical hip or knee osteoarthritis, osteoarthritis of the spine was not assessed. Similar problems are encountered in a recent study of temporomandibular joint osteoarthritis ($n = 12$) demonstrating a higher pyridinoline/deoxypyridinoline ratio in osteoarthritis patients [22], but no association of these bone turnover biomarkers with bilaterality of disease or specific disease features. Although systemic osteoarthritis was said to be excluded, the criteria on which exclusions were based were not given.

Cross-sectional studies

Several well-controlled cross-sectional studies, establishing solid relationships of particular biomarkers with various aspects of osteoarthritis, have been published [23,24,25,26] or reviewed [27] in the past year. These studies demonstrate the importance of assessing a biomarker for osteoarthritis in a diverse population to evaluate effects of age, gender, and ethnicity as well as osteoarthritis status. A role for serum hyaluronan as a potential biomarker of radiographic changes was recently supported in a population-based study ($n = 455$) [23]. In addition to associations with osteoarthritis, serum hyaluronan levels varied by ethnicity, sex, and age and were not explained by radiographic osteoarthritis, body mass index, or comorbidities.

Studies of uCTX-II in patients with advanced osteoarthritis ($n = 88$) confirmed elevated levels due to knee osteoarthritis and, for the first time, elevated levels due to hip osteoarthritis compared with clinically unaffected elderly controls [25]. This study is a nice demonstration of the use of receiver operator characteristic curves for establishing optimal biomarker cutoff values, demonstrating that the sensitivity and specificity of uCTX-II were higher in the hip osteoarthritis group. Another uCTX-II study illustrated the impact of the total body burden of osteoarthritis on the levels of a systemic biomarker, uCTX-II ($n = 267$) [24]. Although hip osteoarthritis was not quantified, osteoarthritis in each of three other sites — lumbar disks, the knees, and hands — contributed independently and additively to uCTX-II levels. This study establishes the potential for additive contributions of different joint sites to the levels of a biomarker and illustrates the potential for improvement in biomarker validation provided by precisely accounting for the total body burden of osteoarthritis.

Severity of pain, but not extent of osteoarthritis, was associated with higher hsCRP levels in a group of patients with advanced osteoarthritis ($n = 770$), although use of nonsteroidal anti-inflammatory drugs (NSAIDs) was very common in this cohort [26] and may have influenced cartilage degeneration, as shown in a study by Gineyts et al. [28]. Although, hip, knee, and hand radiographs were obtained, the spine was not assessed and may have confounded the biomarker analyses.

Longitudinal studies

No interventional longitudinal trials can provide valuable insights regarding the natural history of osteoarthritis progression and the dynamic range of a biomarker. Interventional trials provide information on the potential for change in a biomarker and its relation to a primary outcome measure. Several biomarkers have been associated with osteoarthritis progression including hsCRP, hyaluronan, COMP, and collagen II fragments (reviewed by Lohmander and Felson [29]). Studies in the past year that have contributed to this understanding are highlighted here.

The population-based Rotterdam study convincingly established that high uCTX-II levels were associated with the prevalence and progression of hip ($n = 123$) and knee ($n = 237$) osteoarthritis tracked over an average of 6.6 years in the context of a population-based study ($n = 1235$) [30]. A second study of knee osteoarthritis patients ($n = 115$), in which COMP was measured serially every 6 months, provided evidence for episodic rather than linear progression of osteoarthritis [31]. The authors showed that the probability of knee osteoarthritis progression increased as the mean of two COMP values, obtained 6 months apart, also increased. They suggested that baseline COMP data could provide an indication of likely progression over the next few years. In addition,
COMP levels rose dramatically and remained elevated for up to 1 year following joint replacement surgery \((n = 16)\). Although characterized as unexpected, this finding may have reflected enhanced expression of COMP by osteoblasts, cells known to express COMP \([32]\), during the course of bone remodeling and healing. A third study measured biochemical markers in the serum (hyaluronan, COMP, pentosidine, YKL-40, matrix metalloproteinase-13, matrix metalloproteinase-9, and tissue inhibitor of matrix metalloproteinase-1) in knee osteoarthritis patients \((n = 89)\) twice over an average of 2.9 years. This study demonstrated that baseline serum hyaluronan and pentosidine correlated with joint space narrowing \([33]*\).

Nonpharmacologic intervention

The Arthritis, Diet, and Activity Promotion Trial (ADAPT) included evaluation of serum leptin \([34]**\) and other serum biomarkers \([35]**\) during the course of an 18-month intervention in overweight or obese individuals with knee osteoarthritis \((n = 316)\) \([36]**\). This carefully done, randomized controlled clinical trial demonstrated improvement in function, pain, and mobility in response to diet-induced weight loss and exercise \([36]**\). Baseline serum leptin level predicted weight loss, and the lower the baseline leptin level, presumably indicating less leptin resistance, the greater the subsequent weight loss in response to the intervention. Moreover, the amount of weight loss correlated with the magnitude of change in serum leptin. Significant reductions were also noted for hSCRP, interleukin-6, and soluble tumor necrosis factor receptor 1 in response to the weight loss or weight loss and exercise interventions. None of the biomarkers changed in response to exercise alone, illustrating the inherent difficulty of finding the appropriate biomarkers for a particular intervention. The fact that ‘one size does not fit all’ points out the necessity of understanding the treatment pathway to develop appropriate biomarkers of response.

In a naturally occurring intervention, human T-lymphotropic virus-1 carriers with knee osteoarthritis \((n = 22)\) demonstrated increased inflammatory activity in synovial fluid relative to noncarriers \((n = 58)\), including elevations in C-terminal parathyroid hormone-related peptide, soluble interleukin-2 receptor, interleukin-6, and deoxypyridinoline \([37]\). It was not possible to determine whether the synovial fluid biomarkers originated from the joint or the circulation. Because symptoms were not reported, it was not possible to assess the clinical relevance of these biomarker alterations.

Pharmacologic intervention

The current hope is that biomarkers will facilitate the process of drug development by providing cost-effective and sensitive early indicators of a drug’s effectiveness. Highlighted here are four recent trials incorporating biomarkers as secondary outcomes.

In a glucosamine trial for knee osteoarthritis \((n = 212)\), there was no significant difference in the uCTX-II response in the placebo and glucosamine-treated groups \([38]\). A considerable overlap (extent not reported) in uCTX-II values between knee osteoarthritis patients and healthy controls was attributed to probable subclinical osteoarthritis in the control group despite lack of clinical symptoms. Patients defined as high risk (uCTX-II levels 1 SD above the mean of a control group), however, showed the greatest diminution in uCTX-II over a 3-year period in response to glucosamine. In another study, albeit in rheumatoid arthritis patients, early diminution in uCTX-II in response to therapy reflected the long-term preservation of hyaline cartilage \([39]\). These studies indicate that an osteoarthritis biomarker may facilitate the identification of patients with high cartilage turnover and the potential for a greater therapeutic response to disease-modifying agents.

In a second trial, supplementation with soy protein for 3 months, compared with milk protein, decreased knee osteoarthritis \((n = 135)\) pain and limitation to exercise, decreased serum YKL-40, and increased serum insulin-like growth factor-1 \([40]**\). These responses were confined to the men, who comprised half the study participants. These interesting biomarker alterations lent credence to the subjective clinical outcomes and provided objective evidence in support of further studies of this intervention.

Although NSAIDs have not necessarily been considered disease-modifying agents for osteoarthritis, a 6-week trial of ibuprofen \((2400 \text{ mg})\) prevented significant elevations in uCTX-II compared with placebo treatment for knee osteoarthritis \((n = 201)\) \([28]**\). A similar trend was observed for the urinary biomarker, Glc-Gal-pyridinoline, reflecting synovial degradation. It was not possible to determine whether the ability of NSAIDs to prevent uCTX-II elevations resulted from a direct effect on joint tissue metabolism, or an alteration of synovial or systemic clearance, or a combination of both mechanisms. These findings underscore the potential for significant alterations in at least one osteoarthritis-related biomarker by commonly prescribed NSAID therapy, suggesting that NSAID therapy may be a potential confounder in clinical trials testing other therapeutic agents for osteoarthritis.

Finally, a fourth trial evaluated biomarkers proximal to the signal osteoarthritis joint by measuring synovial fluid biomarkers in response to intra-articular injections of hyaluronic acid \([41]\). The injections reduced synovial fluid levels of both intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 in patients with knee osteoarthritis \((n = 40)\), providing a possible rationale for anti-inflammatory effects of hyaluronan therapy in knee osteoarthritis \([41]\).
Conclusion
The advent of improved and novel diagnostic technologies, and the insights provided by osteoarthritis-related biomarker studies, constitute a paradigm shift in the method of defining osteoarthritis. The burgeoning of a rich osteoarthritis-related biomarker pool is cause for optimism that biomarkers will be able to facilitate the validation of therapeutic interventions in clinical trials and speed the approval and adoption of new therapies.

References and recommended reading
Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest

This is an excellent review of a multitude of osteoarthritis-related biomarkers by an author with a command of the literature.
A very promising osteoarthritis-related biomarker, now able to be quantified by immunoradiometric assay.
•• of outstanding interest
Nuclear MR spectroscopy coupled with multivariate analysis yields a urinary profile in this study that is unique to osteoarthritis patients.
An intriguing study demonstrating autoantigenicity of chondrocyte proteins in osteoarthritis revives an area of past interest in the field with potential import in the pathophysiology of osteoarthritis.
This intriguing study demonstrating autoantigenicity of chondrocyte proteins in osteoarthritis revives an area of past interest in the field with potential import in the pathophysiology of osteoarthritis.
This principal-components analysis provides evidence that osteoarthritis-related biomarkers cluster into categories reflecting different aspects of the osteoarthritis process.
This is a small but promising pilot study in which MRI outcomes are associated with levels of osteoarthritis-related biomarkers.
This study provides an excellent example of a standardized method of testing the performance of a biomarker.
The clinical performance of this new collagen II marker is assessed in patients with osteoarthritis or rheumatoid arthritis.
A very promising osteoarthritis-related biomarker, now able to be quantified by immunoradiometric assay.
A large cross-sectional trial establishing that serum hyaluronan is a robust biomarker of osteoarthritis status, despite its relatively ubiquitous expression in the body.
This is one of the successful first attempts to associate the level of an osteoarthritis-related biomarker to the burden of radiographic disease.
This study provides a fine example of receiver operator characteristic curves for determining an optimal cutoff level for maximizing sensitivity and specificity of a biomarker.
This is a large study of hsCRP in a population with advanced osteoarthritis showing an association of hsCRP with joint pain.
An excellent review of the evidence supporting COMP as an osteoarthritis-related biomarker.
This study demonstrates that NSAIDs in common use can alter osteoarthritis-related biomarker levels. It is left to determine whether these changes are a result of structural modification or changes in joint or systemic clearance.
This is the first large longitudinal study investigating uCTX-II as a biomarker of osteoarthritis disease progression.
This is one of the only studies to demonstrate that a biomarker can change phasically and coincidentally with osteoarthritis disease progression.


This longitudinal study evaluates the prognostic capabilities of osteoarthritis-related biomarkers for predicting knee joint space narrowing.


Outstanding investigation of serum leptin as a secondary outcome in a weight loss trial.


An outstanding trial that incorporated osteoarthritis-related biomarkers to very good effect. To better interpret the lack of response of the biomarkers to the exercise intervention, it would have been advantageous to report when the last bout of exercise occurred relative to body fluid sampling.


The report of the clinical outcomes of the ADAPT trial.


This study provides an example of stratification by a biomarker wherein high levels of uCTX-II define a high-risk group, demonstrating the largest biomarker response to glucosamine.


This is an example of a clinical trial in which biomarkers provided evidence in support of subjective clinical outcomes, thereby increasing confidence in the trial result.

Nutritional factors and osteoarthritis: recent developments
Timothy E. McAlindon and Beth Anne Biggee

Purpose of review
The role of nutrition and nutritional supplements in the development and progression of osteoarthritis is now a topic of considerable public, industry, and academic interest. This review focuses on how the evidence for a role of nutritional factors or nutritional supplements in the management of knee osteoarthritis has been changed by recent research.

Recent findings
Recent studies include clinical trials of weight loss and exercise as interventions for osteoarthritis of the knee, the elucidation of mechanisms of oxidative stress on the chondrocyte genome, further study of vitamin C supplementation in an animal with spontaneous osteoarthritis, and further clinical and pharmacodynamic evaluations of glucosamine and chondroitin sulfate. Perplexing findings among these studies include the deleterious effects of vitamin C on osteoarthritis in the Hartley guinea pig, the low levels of glucosamine achieved in serum after an oral dose, recent negative clinical studies of glucosamine, and the heterogeneity of results among glucosamine trials.

Summary
With an intensification of research in this field come new clinical and basic science data, sometimes with surprising results. These confirm the considerable potential for a role of nutritional interventions for osteoarthritis, but they emphasize the need for systematic scientific evaluation of the claims made for such products.

Keywords
antioxidants, arthritis, glucosamine, nutraceuticals, nutrition, nutritional factors, osteoarthritis

Introduction
There is enormous public interest in the relation between diet and arthritis. The over-the-counter consumption of nutritional remedies remains substantial, with glucosamine and chondroitin together ranking third among all top-selling nutritional products in the United States, and annual sales amounting to $369 million [1]. At the same time, there has been considerable development of the research agenda with progress in diverse fields ranging from the clinical efficacy of weight loss to the genomic effects of oxidative stress in chondrocytes.

Weight loss
Excessive body weight places people at considerably increased risk for osteoarthritis, especially in the knee joints [2]. Although much of the risk is likely to be mediated by biomechanical factors, there are data to suggest that other systemic effects, perhaps dietary or metabolic, may mediate some of this association [3,4].

On the basis of these observations, weight loss is considered to be a priority in the management of overweight individuals with osteoarthritis; however, relatively few rigorous studies have tested weight loss as a therapeutic intervention. The recent Arthritis, Diet, and Activity Promotion Trial is therefore significant in examining whether long-term exercise and dietary weight loss are effective interventions for functional impairment, pain, and decreased mobility in older overweight individuals with knee osteoarthritis [5**]. It showed that diet-induced weight loss needs to be combined with exercise as an effective intervention for knee osteoarthritis.

The investigators recruited 316 adults with knee osteoarthritis and a body mass index of at least 28 kg/m², and randomized them to one of four interventions: healthy lifestyle, diet only, exercise only, and diet plus exercise. The primary outcome was self-reported physical function as measured by the Western Ontario and McMaster Universities Osteoarthritis Index (WOMAC). Secondary outcomes included weight loss, 6-minute walk distance, stair-climb time, and WOMAC pain and stiffness scores. In all, 80% of the participants completed the 18-month study. Adherence ranged from 60% (exercise only) to 73% (exercise plus diet). The main finding of the trial was that only the combination of dietary weight loss with exercise resulted in significant long-term benefits for pain and physical function. The diet-only group lost more weight than did the exercise-plus-diet group (4.9% compared with 1.2%) but did not improve more on the functional or mobility measures.
A more recent study by Christensen et al. [6**] contrasts with the Arthritis, Diet, and Activity Promotion Trial in both its design and its conclusions. On the basis that weight loss might relieve knee osteoarthritis symptoms through both biomechanical effects and influences on body fat, these authors set out to test the effectiveness of a rapid diet-induced weight loss intervention on overweight individuals with knee osteoarthritis [7]. They enrolled 96 people (mostly women) with knee osteoarthritis into a comparison of a low-energy diet intervention (3.4 MJ/day) with a control diet (5 MJ/day). The low-energy-diet intervention consisted of a nutrition powder taken as six daily meals. The control intervention consisted of a traditional hypo-energetic high protein diet. The low-energy group also had weekly dietary sessions, whereas the control group was given a booklet describing weight loss practices. The primary outcome was self-reported pain and physical function limitation measured by the WOMAC index. The authors also examined changes in body weight and body composition as independent predictors of changes in knee osteoarthritis symptoms.

There were 9 dropouts, mainly resulting from noncompliance; however, this seemed to be nondifferential, so the authors performed an analysis based on completers. The low-energy-diet group lost considerably more weight than did those in the control group (11.1 compared with 4.3%), with a mean difference of 6.8% (95% CI 5.5–8.1%). The low-energy-diet group also lost 2.2% more body fat (95% CI 1.5–3.0%).

These results demonstrated substantially greater falls in WOMAC scores among the low-energy intervention group. The mean between-groups difference for the total WOMAC index was 219.3 mm (P = 0.005). Oddly, this was not reflected in the Lequesne Index assessment, which detected no between-groups difference. In subsidiary analyses the authors estimated that the ‘Number Needed to Treat’ to obtain an improvement in WOMAC score of 50% or greater in at least one patient was 3.4. They also found that the changes in WOMAC score were best predicted by reduction of body fat, with a 9.4% improvement in WOMAC score for each percent of body fat reduced (P = 0.0005).

These results indicate that rapid and substantial weight loss may, by itself, translate into reduced pain and improved function in overweight patients with knee osteoarthritis; however, some caution needs to be exerted in interpreting the results. The long-term effectiveness of this short-term intervention is uncertain. The participants were very heavy (mean body mass index 36 kg/m²), and the results may not be generalizable to a less overweight population. The effect of censoring from the analysis the participants who discontinued the intervention is also uncertain, notwithstanding the authors’ assertion that the groups remained balanced. The study was also essentially unblinded, which may also have led to between-groups biases.

Nevertheless, these data are of considerable interest and underscore a need for further research into potential benefits from more extreme weight reduction interventions. For example, preliminary results from a study of musculoskeletal symptoms among morbidly obese patients undertaking gastric bypass surgery showed a 52% reduction in the number of symptomatic sites, and an approximately 50% reduction in WOMAC score, 6 to 12 months after the procedure [8*].

Antioxidants
Recent work on the impact of oxidative stress on cartilage has also added insights into the biologic mechanisms of osteoarthritis progression. Yudoh et al. [9] studied this issue from the viewpoint of genomic instability and replicative senescence in human chondrocytes. They isolated chondrocytes from the articular cartilage of patients with knee osteoarthritis and measured oxidative damage histologically by immunohistochemistry for nitrotyrosine. They then assessed cellular replicative potential, telomere stability, and glycosaminoglycan production both under conditions of oxidative stress and in the presence of an antioxidant (ascorbic acid). Similarly, in the tissue cultures of the articular cartilage explants, they measured the presence of oxidative damage, chondrocyte telomere length, and loss of glycosaminoglycans in the presence or absence of reactive oxygen species, or ascorbic acid.

They found lower antioxidative capacity and stronger staining of nitrotyrosine in the osteoarthritic regions than in the normal regions within the same cartilage explants. This correlated with the severity of histologic damage. During continuous culture of the chondrocytes, the telomere length, replicative capacity, and glycosaminoglycan production were all decreased in the presence of oxidative stress. By contrast, treatment with ascorbic acid resulted in greater telomere length and replicative lifespan in the cultured chondrocytes. In the tissue cultures of the cartilage explants, chondrocyte telomere length and glycosaminoglycan production in the cartilage tissue subjected to oxidative stress were lower in than in the control groups. Conversely, chondrocytes cultured with ascorbic acid showed a tendency to maintain the chondrocyte telomere length and glycosaminoglycan production. These results suggest that oxidative stress induces chondrocyte telomere instability and catabolic changes in cartilage matrix structure and composition. This may be a contributory process in the development or progression of osteoarthritis.

Vitamin C
There are numerous reasons to expect that vitamin C might have beneficial effects in osteoarthritis, so the
results of a recent study of the effects of ascorbic acid supplementation on the expression of spontaneous osteoarthritis in the Hartley guinea pig are surprising [10\textsuperscript{a}]. This rigorous investigation tested the effects of three doses of ascorbic acid on the in-vivo development of histologic knee osteoarthritis. The low dose represented the minimum amount needed to prevent scurvy. The medium dose was the amount present in standard laboratory guinea pig chow and resulted in plasma levels comparable with those achieved in a person consuming five fruits and vegetables daily. The high dose was the amount shown in a previous study of the guinea pig to slow the progression of surgically induced osteoarthritis.

The authors found a positive association between ascorbic acid supplementation and the severity of spontaneous osteoarthritis. In fact, there was a dose-dependent increase in all elements of the knee joint histologic scores across the three arms of the study. Furthermore, there was a significant correlation of histologic severity score with plasma ascorbate concentration ($r = 0.38, P = 0.01$). They found a positive correlation of osteoarthritis severity with synovial fluid cartilage oligomeric matrix protein (a cartilage biomarker) and with collagen content. Transforming growth factor (TGF)-\(\beta\) is implicated in the pathophysiology of osteoarthritis, and ascorbate may function on an activator of this cytokine; therefore, the authors also immunostained the histologic sections by use of a TGF-\(\beta\)-specific antibody. There was evidence of active TGF-\(\beta\), predominantly expressed in marginal osteophyses.

Thus, there was a deleterious effect of prolonged ascorbic acid exposure in the Hartley guinea pig model of spontaneous osteoarthritis, possibly mediated by TGF-\(\beta\). The authors suggest that ascorbic acid intake in humans should not be supplemented above the currently recommended dietary allowance (90 mg/day for men and 75 mg/day for women) [10\textsuperscript{a}].

Although these findings are compelling, it remains uncertain to what extent they can be generalized to the human situation. It is paradoxical that an apparently beneficial effect of dietary vitamin C was found in the Framingham cohort study, notwithstanding the problems associated with such observational studies [11]. Also, the effects observed in this experiment may be unique to vitamin C and do not preclude the possibility of a therapeutic role for other antioxidants. Thus, the situation predicates a need for further studies of antioxidants and vitamin C in humans.

**Vitamin D**

Bone is not structurally normal in osteoarthritis. The periarticular bone exhibits increased turnover, decreased bone mineral content and stiffness, and decreased numbers of trabeculae. The increased turnover of collagen is reflected in alterations in biomarkers and bone mineral density. High bone mineral density at nonjoint sites is associated with an increased risk of osteoarthritis; however, low bone mineral density and high bone turnover seem to be associated with more rapid progression [12].

These factors predicated a recent study of the relation of antiresorptive drug use to structural findings and symptoms of knee osteoarthritis [13]. This study examined the cross-sectional association between use of medications that have a bone antiresorptive effect with structural features and symptoms of knee osteoarthritis among participants in the Women in the Health, Aging and Body Composition Study. The investigators found that the use of alendronate, estrogen, or both was associated with decreased structural lesions and lower pain scores [13]. As pointed out by Demarco and Constantinescu [14], however, they did not discuss in the original report what influence vitamin D supplement use had on these associations. Carbone et al. [15] therefore reanalyzed their results to adjust for a possible effect of vitamin D. They did not include serum 25(OH)D levels, however, and were therefore confined to an analysis of vitamin D supplement use as the exposure variable. This variable was not associated with structural changes of osteoarthritis or pain severity, nor did its inclusion as a covariate in the statistical models change the formerly observed associations.

Thus, despite a fairly compelling biologic rationale, there are conflicting results from observational studies about the role of vitamin D in osteoarthritis progression. Therefore, a clinical trial of a vitamin D intervention for knee osteoarthritis will commence at Tufts-New England Medical Center in the fall of 2005.

**Glucosamine and chondroitin**

Glucosamine and chondroitin sulfate are cartilage extracellular matrix components that are widely promulgated as a remedy for osteoarthritis. The mechanisms by which they might act remain something of a conundrum, however, especially in the absence of quantitative data on the extent to which either substance enters the human circulation after the recommended oral dose. Preliminary data from two recent studies indicate that levels of approximately 1600 ng/ml are achieved after standard oral dosing [16\textsuperscript{a},17]. Given that this concentration seems incompatible with the original supposition that glucosamine acts as a substrate for cartilage biosynthesis, researchers now need to demonstrate the biologic effects of this compound at appropriate concentrations. Current explorations include its effects on inflammatory mediators such as interleukin-1 [18].

The body of evidence concerning the clinical efficacy of glucosamine has also been altered by the publication of
several independently funded clinical trials that had null results.

Primarily as a test of an internet-based clinical trial approach, McAlindon et al. [19] performed a prototypical 12-week, double-blind, randomized, placebo-controlled trial of glucosamine among 205 individuals with knee osteoarthritis who were recruited and monitored entirely over the internet. The primary outcome measure was the pain subscale of the WOMAC questionnaire, completed online every 2 weeks. This study found no difference between groups in terms of change in any of the outcomes (e.g. pain subscale 2.0 ± 3.4 compared with 2.5 ± 3.8, P = 0.4). Stratification by osteoarthritis severity, glucosamine product, and use of a nonsteroidal antiinflammatory drug, as well as exclusion of opiate users, did not alter the results. The number and type of adverse events reported were similar between the groups. The authors concluded that glucosamine seems to be no more effective than placebo in treating the symptoms of knee osteoarthritis.

Cibere et al. [20*] performed an innovative glucosamine withdrawal trial in 137 people with knee osteoarthritis who were already using the product and reported at least moderate benefit. The design was a four-center, 6-month, randomized, double-blind, placebo-controlled glucosamine discontinuation trial in which enrollees were randomly assigned to continue taking glucosamine sulfate, or to placebo.

The primary outcome was the proportion of disease flares among the groups analyzed according to an intent-to-treat analysis. Secondary outcomes included time to flare, analgesic use, severity of flare; and changes in pain, stiffness, function, and quality of life. Ultimately, disease flares occurred in 28 (42%) of the placebo arm and 32 (45%) of the glucosamine arm (difference −3%; 95% CI 19–14). In the Cox regression analysis, after adjustment for sex, study site, and osteoarthritis radiographic severity, time to disease flare was not significantly different in the glucosamine group than in the placebo group (hazard ratio of flare = 0.8; 95% P = 0.4). At the final study visit, acetaminophen was used in 27% and 21% of placebo and glucosamine patients, respectively (P = 0.4), nonsteroidal antiinflammatory drugs in 29% and 30% (P = 0.9), and both in 20% and 21% (P = 0.8). No differences were found in severity of disease flare or other secondary outcomes between the placebo and glucosamine patients. Thus, in patients with knee osteoarthritis and at least moderate subjective improvement with previous glucosamine use, this study provides no evidence of symptomatic benefit from continued use of glucosamine sulfate. The same authors also analyzed samples for type II collagen degradation biomarkers as a proxy for osteoarthritis progression but found no statistically significant effect of glucosamine sulfate on type II collagen fragment levels over the 6-month observation period [21].

This study represents an interesting and innovative approach to testing such products and is subject to a new set of potential flaws and limitations. For example, if the duration of effectiveness of glucosamine is prolonged, the period of follow-up in the above study might have been insufficient. Also, the heterogeneity of glucosamine products on the market could have biased any differences to the null.

Michel et al. [22] recently reported the results of a 2-year randomized, double-blind, controlled trial of 800 mg chondroitin sulfate or placebo once daily among 300 patients with knee osteoarthritis. The primary outcome was joint space loss over 2 years as assessed by a posteroanterior radiograph of the knee in flexion, a better-validated technique. Secondary outcomes included pain and function. The participants in the placebo arm showed a mean cumulative joint space loss of 0.14 mm compared with no change in the chondroitin arm. In the intent-to-treat analysis, the between-groups difference in mean joint space loss was 0.14 ± 0.57 mm; P = 0.04. By contrast, the differences in the symptom outcomes between the groups were trivial and nonsignificant. Chondroitin was well tolerated, however, with no significant differences in rates of adverse events between the two groups. Although the authors inferred evidence of structure damage modification by chondroitin, vexing questions remain about the internal validity of joint space width as a measure of cartilage loss, and its relevance to the clinical state of the patient with knee osteoarthritis, especially in the absence of any overt impact on symptomatic outcomes.

Motivated in part by the heterogeneity of glucosamine trial results, Towheed et al. [23*] recently updated the Cochrane review of available data with the intent of investigating what might predicate the differences. Interestingly, an analysis restricted to the eight studies that reported adequate allocation concealment showed no benefit of glucosamine for pain and WOMAC function. Collectively, however, the pooled data from 20 eligible studies favored glucosamine over placebo with a 28% improvement in pain and a 21% improvement in function according to the Lequesne index. The results were not uniformly positive, however, and the reasons for this remain unexplained. For example, the WOMAC pain, function, and stiffness outcomes did not reach statistical significance. In the subset of trials that tested the Rotta preparation of glucosamine (n = 10), glucosamine was superior for pain and function according to the Lequesne index. The pooled results for pain and function according to the WOMAC index in those trials in which a non-Rotta preparation of glucosamine was compared with placebo did not reach statistical significance. In the four trials in
which the Rotta preparation was compared with a nonsteroidal anti-inflammatory drug, glucosamine was superior in two and equivalent in two. The authors concluded that studies that tested a non-Rotta preparation, or used inadequate allocation concealment procedures, failed to show benefit in pain and WOMAC function, whereas those evaluating the Rotta preparation showed benefit in the treatment of symptomatic osteoarthritis. It should be noted, however, that the WOMAC outcomes of pain, stiffness, and function did not show superiority of glucosamine over placebo for either Rotta or non-Rotta preparations [23**].

Another issue about glucosamine, recently highlighted in a report from the Institute of Medicine, is the uncertainty about its potential for adverse effects on insulin regulation among individuals predisposed to such problems. These concerns are based on the known ability of glucosamine to bypass the glutamine:fructose-6-phosphate amidotransferase step of hexosamine biosynthesis and desensitize glucose transport [24]. The issue is especially pertinent for individuals with osteoarthritis because they often share the factors that put them at risk for insulin resistance and diabetes. Although the effects of glucosamine have been well documented in animal models, less is known about its effects on glucose metabolism in humans. Preliminary studies have been reassuring, but their interpretation has been limited by the considerable variability in measures and the small numbers of participants [25,26].

### Resveratrol

Resveratrol is a phytoalexin found in high concentrations in the skins of grapes and red wines that has anti-inflammatory and antioxidant properties. Elmali et al. [27] tested the effects of daily intra-articular injections of this product over 2 weeks using a cruciate ligament transection rabbit model of osteoarthritis. Histologic evaluation showed reduced cartilage destruction (score of 1.7 compared with 2.8, \( P = 0.02 \)) and proteoglycan loss, but no difference in synovial inflammation. A characteristic parameter in arthritis is the progressive loss of articular cartilage. The authors suggest that intra-articular resveratrol at the onset of osteoarthritis may protect from further cartilage damage; however, these results should be viewed as highly preliminary [27].

### S-adenosylmethionine

Trials of S-adenosylmethionine also have had apparently positive results, albeit somewhat limited by adverse effects and high dropout rates [28–32]. Najm et al. [33] recently compared its efficacy with that of celecoxib, a cyclooxygenase-2 inhibitor, in a 16-week randomized double-blind crossover study among 61 individuals with osteoarthritis of the knee. The celecoxib arm experienced greater pain reduction than the S-adenosylmethionine group during the first month, but by the second month, there was no significant difference between the groups. The authors inferred that S-adenosylmethionine has a slower onset of action but is as effective as celecoxib in the management of symptoms of knee osteoarthritis. An alternative explanation is that the benefits of celecoxib decayed over time in such a manner that the groups converged.

### Conclusion

Over the last few years, there has been a gratifying intensification of research into potential nutritional interventions for osteoarthritis. This has included two recent clinical trials that demonstrated benefits from weight loss and exercise, and further elucidation of mechanisms of oxidative stress on the chondrocyte genome. Other investigations have resulted in more perplexing conclusions, such as the deleterious effects of vitamin C on osteoarthritis in the Hartley guinea pig, and the low levels of glucosamine achieved in serum following an oral dose. The diversity of such findings illustrate the importance and need for continuing research in this field.

### References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:

* of special interest  
** of outstanding interest

Osteoarthritis


Footwear alterations and bracing as treatments for knee osteoarthritis
Kelly Krohn

Purpose of review
The biomechanical aspects of gait and the impact of alignment have been recognized as important in the development and progression of knee osteoarthritis. Improving malalignment and altering the dynamic forces on the involved compartment of the knee during gait have the potential to improve the symptoms of knee osteoarthritis. This review examines the use of foot orthoses and knee braces to change the biomechanical forces on the knee joint and to reduce pain and improve function in patients with existing symptomatic knee osteoarthritis.

Recent findings
Malalignment has been shown to have an impact on the development and progression of knee osteoarthritis. Patients with medial compartment knee osteoarthritis who have a visible varus thrust will also progress at a more rapid rate than patients without a varus thrust. Lateral wedge foot orthoses have been shown in biomechanical studies and clinical studies to reduce the load on the medial compartment and improve the symptoms of medial compartment knee osteoarthritis. Knee braces that stabilize the knee joint and provide a valgus stress have been shown to improve pain and function in patients with medial compartment knee osteoarthritis.

Summary
The development of symptomatic knee osteoarthritis and the progression of joint space loss is in part a biomechanical process. To improve patients’ function and possibly reduce disease progression, a biomechanical approach should be included in the treatment plan for patients with knee osteoarthritis. Foot orthoses and knee braces have been shown in selected patients to have a role in the management of unicompartmental knee osteoarthritis.

Keywords
biomechanics, foot orthoses, gait, knee braces, malalignment, osteoarthritis, unicompartmental knee osteoarthritis

Introduction
Symptomatic knee osteoarthritis is found in approximately 10% of the population over age 65. In addition to the growing population of elderly patients with knee osteoarthritis, an increasing number of former athletes with previous knee injuries experience post-traumatic knee osteoarthritis. Patients with previous complete meniscectomies or partial meniscectomies are at higher risk for the development of osteoarthritis in the involved knee compartment. Younger patients with early to moderate knee osteoarthritis often express an interest in continuing their participation in lifetime sports and an active lifestyle. Elderly patients with knee osteoarthritis are often not candidates for surgery because of comorbid illness. Patients are interested in nonpharmacologic approaches to help manage their knee osteoarthritis symptoms. An understanding of the biomechanical aspects that lead to the development and progression of knee osteoarthritis is important for the clinician taking care of these patients.

Many patients present clinically with unicompartmental knee osteoarthritis, usually of the medial compartment. A strategy to improve their symptoms is to reduce the load on the diseased medial compartment and redistribute some of the load to the lateral compartment. A similar approach can be applied to patients with isolated lateral compartment disease. It is important to understand that this approach of attempting to shift the load from one compartment to another does not work if patients have significant involvement of both the medial and lateral compartments. Patients with patellofemoral involvement and anterior knee pain may benefit from improvement of the femoral-tibial alignment. Patellar stabilizing sleeves may be worn in addition to foot orthoses or knee braces for patients with mildly symptomatic anterior knee pain.

Surgical approaches to unicompartmental knee osteoarthritis include tibial and femoral osteotomies. These procedures are fairly technically demanding and are associated with defined morbidities such as infection, nerve injury, nonunion, and the risks of anesthesia. If the patient goes on to have a total knee arthroplasty in the future, it may be a more difficult procedure compared with the same surgery on a knee that has not undergone osteotomy. Unicompartmental knee replacements have become more popular again after being nearly abandoned for many years because of early failure. Unicompartmental knee replacements may be associated with some of the known surgical complications of knee surgery.
Nonsurgical approaches to change the alignment and biomechanical forces on the knee joint include foot orthoses and knee bracing. In many respects, the ideal patient for surgery such as osteotomy or unicompartamental knee replacement may be the ideal patient to try a knee brace, foot orthoses, or both.

This review of the literature on alignment and knee osteoarthritis will include the biomechanical and gait laboratory studies as well as some of the recent clinical trials using foot orthoses and knee braces. Also, some practical pearls will be given about patient selection and the process of getting the proper foot orthoses or knee brace for patients.

**Normal gait, malalignment, and previous trauma**

During normal gait, the medial compartment of the knee is loaded more than the lateral compartment. It is estimated that 60 to 80% of the load during the midstance phase of gait is distributed to the medial compartment in a normal knee [1]. This is due to the external varus moment (or adductor moment), which is the torque generated from the ground reaction force during stance phase as a result of the body’s center of gravity falling medial to the knee joint. This, in part, is why medial compartment osteoarthritis is more prevalent than lateral compartment disease. A visible varus thrust during gait increases the odds of progression among varus-aligned osteoarthritis knees [2]. One theoretical reason why osteoarthritis knee braces and lateral wedge orthoses may help patients with medial compartment disease is a reduction in the external varus moment.

Malalignment has been associated with the progression of radiographic joint space loss and deterioration in function [3]. Varus alignment increases the risk of medial osteoarthritis progression, and valgus alignment increases the risk of lateral osteoarthritis progression [4]. An alignment of more than 5° (in either direction) in both knees at baseline is associated with significantly greater functional deterioration than an alignment of 5° or less in both knees, after adjustment for age, sex, body mass index, and pain [3]. The effect of obesity as determined by body mass index on the progression of knee osteoarthritis is limited to knees in which moderate malalignment exists, presumably because of the combined impact of the increased load from malalignment and the excess load from increased weight [5]. Varus and valgus malalignment will influence the risk for the development of patellofemoral osteoarthritis and which compartment of the patellofemoral joint is involved [6]. Changes in the alignment of the knee may have the potential to reduce the symptoms of knee osteoarthritis and theoretically may affect the rate of progression.

Complete meniscectomy in animal models is associated with development of knee osteoarthritis [7]. Previous meniscectomy in patients has been associated with premature development of osteoarthritis in the involved knee compartment [8]. Partial meniscectomy may also be associated with the development of osteoarthritis, depending on the type of tear and the degree of resection [9–11]. Patients with previous meniscal injuries and surgical procedures may be good candidates for knee bracing or shoe orthotics because they often have unicompartamental knee osteoarthritis with a fairly normal asymptomatic contralateral compartment.

**Foot orthoses and knee osteoarthritis**

Lateral heel wedges or lateral-wedge insoles have been shown to be effective in reducing the symptoms of medial compartment knee osteoarthritis [12–14]. A review of the published literature on the efficacy of laterally wedged foot orthotics for improving these symptoms indicates a strong scientific basis for applying wedged insoles in an attempt to reduce pain in patients with medial compartment knee osteoarthritis [15]. Subtalar elastic strapping in addition to the standard lateral-wedge insole may have an additional impact on the valgus correction of the femorotibial angle and the symptomatic improvement at 6 months, compared with a lateral-wedge insole alone [12,16]. The degree of change in femorotibial angle with the insole with subtalar strapping was affected by the tilt of the lateral wedge. The higher tilt resulted in more improvement of the femorotibial angle but also resulted in more adverse events reported by patients.

Biomechanical gait laboratory studies were done on the effects of lateral-wedge insoles on the gait and medial compartment load of 17 healthy individuals. Three-dimensional gait analysis was performed for each individual with and without wearing a 5° lateral-wedge insole. The external varus moment and estimated medial compartment load at the knee were reduced significantly with the addition of the lateral-wedge insole [17]. These results suggest that the pain relief and improvement in function reported by patients with osteoarthritis using lateral-wedge insoles may be achieved by a reduction in external varus moment and medial compartment load.

In another gait study, patients with medial compartment knee osteoarthritis were studied while they walked wearing their comfortable shoes with a 5° lateral wedge compared with a nonwedged, 1/8-inch, even-thickness control insole; and with a 10° lateral wedge compared with a nonwedged 1/4-inch, even-thickness control insole. In comparison with no insole, the 5° wedge reduced the peak knee varus torque values by approximately 6%, and the 10° wedge reduced the peaks by approximately 8%. The thicker nonwedged insole and the 10° wedge were associated with varying degrees of discomfort [18].
The summary of these clinical and gait laboratory data suggest that there is a role for the use of lateral wedge orthoses for medial compartment knee osteoarthritis. Both the efficacy at the knee and the foot discomfort associated with wearing the orthoses seems to have dose effects as determined by the angle (or height) of the lateral wedge.

**Knee braces in osteoarthritis**

The basic rationale for a knee brace for unicompartmental knee osteoarthritis is to improve function by reducing the patient’s symptoms. This can be accomplished, in theory, by reducing the biomechanical load on the affected compartment of the knee, by reducing the external varus moment and by improving the patient’s perception of instability. Some patients with osteoarthritis have true instability (e.g. a torn anterior cruciate ligament), and an osteoarthritis knee brace may provide some functional stability in a fashion similar to that of the typical sports-medicine functional knee brace used in athletes with ligamentous injuries. Most companies that manufacture osteoarthritis knee braces will have a similar line of functional knee braces that are used primarily in younger athletes with ligamentous injuries. Functional braces are used to improve function of the injured knee and to protect the ligamentous repair postoperatively. The major difference between functional knee braces and osteoarthritis knee braces is the addition of a valgus angle (for medial compartment osteoarthritis) or a varus angle (for lateral compartment osteoarthritis). Most osteoarthritis knee braces will have a mechanism for adjusting the angle of the hinge for patient comfort.

In a survey of 105 individuals with knee osteoarthritis, 63% reported knee instability during activities of daily living, and 44% reported that instability affected their ability to function [19]. Exercises designed to improve the feeling of instability, and orthotics devices like knee braces that improve proprioception and provide mechanical support, may improve the feeling of instability that often accompanies knee osteoarthritis.

Gait laboratory studies have shown that osteoarthritis bracing can reduce the net varus moment about the knee and the estimated medial compartment load [20]. Increasing valgus alignment with the adjustable hinge had a greater effect on the medial compartment load than did increasing the strap tension, but both were important for the three-point design of this brace. Another brace design was studied by looking at varus moments for the braced and unbraced knees compared during gait at 15%, 20%, 25%, and 30% of stance. This brace significantly reduced the varus moment at 20% and 25% of stance [21]. Gait symmetry was significantly improved when patients with unicompartmental medial osteoarthritis of the knee wore a valgus brace, as measured by analysis of time spent on each limb during gait [22]. The joint space (condylar separation) can be visible and objectively measured by a fluoroscopic digital radiograph performed during the gait cycle. The average change in condylar separation was 1.2 mm when patients with medial compartment osteoarthritis wore an off-loading knee brace [23]. The nice radiographic images provided by this study support the concept that a properly designed osteoarthritis knee brace can change the alignment of the limb and reduce the load on the medial compartment enough to result in radiographic separation of the medial femoral-tibial joint space.

Clinical trials are generally small and difficult to adequately control because of the nature of knee braces and the difficulty in designing a trial with a true placebo. Simple neoprene sleeves, which have no significant biomechanical effect, may have efficacy for knee osteoarthritis because of changes in proprioception. Hinged rigid osteoarthritis braces have also been shown to improve proprioception, which may account for a small amount of their perceived clinical benefit [24]. The largest osteoarthritis knee brace study published to date (n = 119 total in three groups) showed that a neoprene sleeve is superior to simple analogesics. The group with the valgus knee brace experienced significant improvement, compared with the neoprene sleeve group, in standard pain and functional outcome measures [25]. Several additional small trials have shown improvements in pain and function with various brace designs [21,23,26–29].

A review of the published literature on knee bracing for osteoarthritis points out some of the limitations of the clinical trials to date but acknowledges the limited evidence for improvement in pain and function in patients using osteoarthritis braces compared with medical treatment or neoprene sleeves [30].

A comprehensive summary of the knee bracing literature for both clinical and gait analysis studies by Pollo et al. [31**] has reviewed the individual knee brace trials and will be published in 2005. Hillstrom et al. [32] have reviewed both foot orthoses and knee bracing alone as well as in combination for the treatment of knee osteoarthritis.

**Conclusion**

With the growing evidence for the role of malalignment in the development and progression of knee osteoarthritis, foot orthoses and knee braces are potentially an important modality to improve function and theoretically affect disease progression. Patients are interested in nonpharmacologic approaches to managing their knee osteoarthritis. There are very few well-controlled clinical studies in this field to date. More than 10 companies manufacture and promote osteoarthritis knee braces. Each brace has a unique design and may have features that make it more or less acceptable to the patient. Many of the braces have the ability to adjust the angle of the hinge and provide the
orthotist and patient with some flexibility in the varus or valgus angulation. Clinical trials done with one brace design may not be applicable to all osteoarthritis knee braces. The fitting of the brace and the instruction to the patient on the proper donning of the brace are crucial to the success of the osteoarthritis brace. The orthotist is a vital part of the team and must understand knee osteoarthritis and the basic biomechanics of the knee. The orthotist must also be familiar with each brace’s proper fitting and adjustment protocols. The patient must be motivated to avoid surgery and minimize the use of pharmacologic therapies. It is helpful to have the patient see an example of the brace and determine whether he or she would wear it before ordering one. Considerable work needs to be done to further validate the effectiveness of osteoarthritis knee braces and to assist the clinician in choosing the correct patient to order a knee brace. Custom knee braces can be relatively expensive ($1000–1500). If a patient does well with the brace, however, the cost can often be amortized over several years and may be less expensive than surgery or pharmacologic therapy. Newer brace designs and materials are being developed, and less expensive off-the-shelf braces ($400–1000) are becoming considerably better than a few years ago. Clinicians should be aware of these nonpharmacologic treatments for some of their patients with knee osteoarthritis.

References and recommended reading

Papers of particular interest, published within the annual period of review, have been highlighted as:
• of special interest
•• of outstanding interest


This paper highlights the importance of the combination of body mass index and malalignment on the progression of knee osteoarthritis.


This study suggests that an insole with a subtalar strap maintained the valgus correction of the femorotibial angle in patients with varus knee osteoarthritis for 6 months, indicating longer-term clinical improvement with the strapped insert than with the traditional lateral wedge insert.

This is a nice review of the clinical literature on foot orthoses for the treatment of knee osteoarthritis.

This important study highlights the concept of the perception of joint instability in patients with knee osteoarthritis and suggests that specific rehabilitation programs and orthotics like knee braces may improve this feeling of instability.

This is a comprehensive review of the literature on knee bracing in osteoarthritis and highlights both gait studies and clinical studies.
This bibliography is compiled by clinicians from the journals listed at the end of this publication. It is based on literature entered into our database between 1 May 2004 and 30 April 2005 (articles are generally added to the database about two and a half months after publication). In addition, the bibliography contains every paper annotated by reviewers; these references were obtained from a variety of bibliographic databases and published between the beginning of the review period and the time of going to press. The bibliography has been grouped into topics that relate to the reviews in this issue.

- Papers considered by the reviewers to be of special interest.
- Papers considered by the reviewers to be of outstanding interest.

The number in square brackets following a selected paper, for example [7], refers to its number in the annotated references of the corresponding review.

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