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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 the Journal’s Section Editors for this issue.

Section Editors

Livia Casciola-Rosen, PhD

Dr Casciola-Rosen was born in Durban, South Africa. She is currently Associate Professor of Dermatology and Medicine at the Johns Hopkins University School of Medicine in Baltimore. She received her PhD at the University of Cape Town, South Africa in 1987, where she studied lipoprotein receptor turnover and intracellular proteolysis. She subsequently performed postdoctoral research at the Johns Hopkins University School of Medicine, initially in cell biology in the laboratory of Dr Ann Hubbard, and subsequently in Immunology (in Dr Douglas Fearon’s laboratory). She was appointed to the faculty in the Department of Dermatology at Johns Hopkins in 1993, where she was the recipient of a Dermatology Foundation Career Development Award. During this time, she initiated studies on the biochemical and cell biological fate of autoantigens during cell death, particularly in photosensitive skin diseases like SLE and dermatomyositis. Her early faculty studies, done in collaboration with her husband Antony Rosen, MD, identified apoptotic cells as a likely source of immunogen in systemic autoimmunity. She was the first to observe that autoantigens are unified by their striking susceptibility to become modified during cell death pathways by proteolytic cleavage, and other post-translational modifications.

Dr Casciola-Rosen currently directs an NIH-supported myositis research program focused on defining the biochemical events affecting autoantigens in affected muscle in autoimmune myositis. With her colleagues, she has identified regenerating muscle cells (but not mature muscle cells) as an important source of myositis-specific autoantigens in this disease. She is also an investigator in other funded programs investigating the mechanisms of scleroderma, Sjögren's syndrome, systemic lupus erythematosus (SLE) and accelerated transplant atherosclerosis.

John Varga, MD

Dr Varga was born in Budapest, Hungary. He received his undergraduate training at Columbia University in New York, McGill University in Montreal, and Glasgow University in Scotland. He received his medical degree at New York University, and completed a residency in Internal Medicine at Rhode Island Hospital, Brown University in Providence, Rhode Island, followed by a Rheumatology Fellowship at Boston University.

Dr Varga was a post-doctoral research fellow of the Arthritis Foundation in the laboratory of Sergio Jimenez at the University of Pennsylvania. He joined the faculty of Jefferson Medical College in Philadelphia, in 1987. In 1995, he was appointed Director of Rheumatology at the University of Illinois College of Medicine, and in 2004, he was named The Gallagher Professor of Medicine at Northwestern University Feinberg School of Medicine in Chicago.

Dr Varga is Chair of the Scientific and Medical Advisory Board of the Scleroderma Foundation, and of the Abbott Scholar Advisory Board. He has served on National Institutes of Health Study Section panels since 1998. He has published over 130 peer-reviews articles, along with 60 reviews and book chapters, and two books. Dr Varga directs an NIH-supported research program focusing on basic, translational, and clinical aspects of scleroderma. He has trained over 20 clinical and research fellows.
Autoimmune myositis: new concepts for disease initiation and propagation
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Introduction
Systemic autoimmune diseases are characterized by specific pathology (which is frequently focused on a distinct constellation of tissues) and a unique autoantibody response that generally targets a group of ubiquitously expressed autoantigens [1]. For example, patients with diffuse scleroderma frequently have antibodies against topoisomerase-1, a ubiquitously expressed molecule that functions to regulate the higher order structures of DNA [2]. Similarly, patients with dermatomyositis and skin rash frequently have autoantibodies against Mi-2, a component of the nucleosome remodeling and deacetylation complex that regulates gene expression [3]. The mechanisms underlying the association of immune responses against specific, apparently ubiquitously expressed autoantigens with distinct disease phenotypes remain undefined. Several recent studies have focused attention on unique properties and expression patterns of the myositis-specific autoantigens themselves as features of potential importance in myositis pathogenesis.

Domains within aminoacyl transfer RNA synthetases have chemokine activity
Initial studies by Wakasugi and Schimmel [4] on tyrosyl tRNA synthetase demonstrated that the C-terminal region of this molecule had significant homology to endothelial monocytes-activating polypeptide II. During apoptosis, mature endothelial monocytes-activating polypeptide II as well as an N-terminal interleukin-8–like chemoattractant activity was generated from tyrosyl tRNA synthetase and released to recruit inflammatory cells to the area [5]. These studies were subsequently extended by Howard et al. [6], who examined whether histidyl tRNA synthetase (HRS; Jo-1), the most frequent aminoacyl tRNA synthetase targeted in autoimmune myositis, had similar chemoattractant properties. They demonstrated that HRS induced migration of monocytes and dendritic cells through the CC chemokine receptor 5. Additionally, HRS also was able to recruit CD4 and CD8 T cells. The authors postulate that the chemoattractant properties of HRS and potentially other myositis-specific autoantigens may play a role in the feed-forward loop that typifies the autoimmune rheumatic diseases, such that autoantigens themselves may play direct roles in regulating the immune response.

Enhanced myositis autoantigen expression in regenerating cells in myositis
The source of myositis autoantigens that drives the ongoing immune response in myositis has remained unclear. In recent studies performed in our laboratory, we examined myositis antigen expression in control and myositis muscle and were surprised to observe that control muscle expresses very low levels of several myositis autoantigens, including HRS, Mi-2, and DNA-dependent protein kinase [7]. In contrast, autoantigen levels were strikingly elevated in muscle obtained from patients with myositis. Enhanced expression was observed in muscle cells themselves as well as in infiltrating inflammatory cells. Interestingly, this enhanced autoantigen expression was a feature of regenerating cells in myositis muscle rather than mature myotubes. We observed that Mi-2, an autoantigen targeted in dermatomyositis and not polymyositis, was expressed at high levels only in dermatomyositis, demonstrating the correlation of a dermatomyositis-specific pattern of autoantigen expression and antibody response.

Of note, the expression of myositis autoantigens was also found to be robustly increased in several cancers that are associated with autoimmune myositis relative to the levels detected in the corresponding normal tissues. This finding indicates that undifferentiated myoblasts and tumor cells are antigenically similar and has led us to propose that in cancer-associated myositis, the autoimmune response directed against cancer cross-reacts with regenerating muscle cells to produce a feed-forward cycle of tissue damage and antigen selection [7]. This hypothesis awaits experimental validation.

Taken together, these new studies focus attention on the expression and inflammatory properties of myositis autoantigens and the roles that they may play in initiating and driving this autoimmune process. Antigen expression is
very low in control muscle, so it is unlikely that control muscle is the site of initiation of autoimmunity. Rather, it is more likely that damaged muscle, in which there are areas of regeneration, provides a source of antigen, which itself may also influence the local inflammatory context.

Implications for initiation and therapy
The studies reviewed raise important questions about disease initiation and propagation in myositis. First, mature healthy tissue may not be the primary target of autoimmunity, but rather injured and repairing tissue in which stem cells or differentiating cells are replacing injured cells. Such a response against regenerating muscle cells may be a critical principle that enables a feed-forward loop of tissue damage and antigen selection. Second, the phenotype-specific antigen fingerprint and phenotype-specific autoantibody response are related. Third, regenerating muscle cells and tumor cells share similarities in their antigenic composition, which differs from that present in mature, differentiated tissues [7]. Fourth, it is possible that initiation of the immune response and development of the amplifying, self-sustaining phenotype in myositis are separated in time, with the latter event stimulated by nonspecific muscle damage occurring in the setting of a prior anti-tumor immune response.

Although available therapies target the immune effector components in myositis, inhibiting antigen expression during muscle regeneration may decrease the amplitude of the amplifying loop and be of therapeutic significance.

References
Interstitial lung disease in polymyositis and dermatomyositis
Maryam Fathi\textsuperscript{a} and Ingrid E. Lundberg\textsuperscript{b}

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**Purpose of review** & **Abbreviations**
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The purpose of this review is to discuss current concepts regarding management of interstitial lung disease in polymyositis and dermatomyositis. & BAL bronchoalveolar lavage  
BOOP bronchiolitis obliterans with organizing pneumonia  
DAD diffuse alveolar damage  
DLco diffusing capacity for carbon monoxide  
FEV\textsubscript{1} forced expiratory volume in 1 second  
FVC forced vital capacity  
HRCT high-resolution computerized tomography  
ILD interstitial lung disease  
KL-6 Krebs von den lungen-6  
NSIP nonspecific interstitial pneumonia  
TNF-\alpha tumor necrosis factor \alpha  
UIP usual interstitial pneumonia 
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**Recent findings**
Interstitial lung disease seems to be a more frequent manifestation in patients with polymyositis and dermatomyositis than previously reported. Modern technology, including high-resolution computerized tomography in combination with pulmonary function tests provides sensitive tools to detect early signs of interstitial lung disease. By systematic use of these investigations in newly diagnosed polymyositis and dermatomyositis, up to two thirds of patients were discovered to have signs of interstitial lung disease in a recent study. Clinical symptoms such as cough and dyspnea may not be sensitive enough to detect interstitial lung disease. Awareness of this complication in patients with myositis is important, because early diagnosis and management of interstitial lung disease may prevent development of chronic pulmonary fibrosis and thereby prolong patient survival and improve quality of life. Treatment recommendations of interstitial lung disease in polymyositis and dermatomyositis are still limited by absence of controlled trials and could only be based on experiences from small case series and case reports. At least some patients with interstitial lung disease improve with immunosuppressive treatment, but data are limited, and longitudinal studies are needed.

**Summary**
Interstitial lung disease seems to be a common manifestation in patients with polymyositis and dermatomyositis already at diagnosis of the muscle disease. When present, interstitial lung disease has a major effect on morbidity and mortality and should be looked for in these patients using high-resolution computerized tomography and pulmonary function tests early in the disease course, because immunosuppressive treatment may change the course of the lung disease.

**Keywords**
high resolution computerized tomography, histopathology, interstitial lung disease, myositis

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**Introduction**
Interstitial lung disease (ILD) is a heterogeneous group of noninfectious, nonmalignant disorders of the lower respiratory tract, characterized by infiltration of inflammatory cells and interstitial fibrosis. Since the original description of ILD in a case of dermatomyositis by Mills and Mathews [1] in 1956, the association of ILD with polymyositis/dermatomyositis has been widely accepted. In another subset of inflammatory myopathies, inclusion body myositis, ILD seems to be infrequent; we found only a few reports of ILD in this subset [2]. The presence of ILD in patients with myositis affects the prognosis and contributes substantially to morbidity and mortality [3–5]. Although this review focuses on current concepts regarding ILD associated with polymyositis/dermatomyositis, it should be emphasized that not only the myositis itself but also the immunosuppressive treatment and secondary infections caused by immunosuppressive therapy used in these patients might lead to development of interstitial pneumonia and cause diagnostic and therapeutic dilemmas.

**Incidence**
The reported incidence of ILD in polymyositis/dermatomyositis varies between 5\% and 46\% in earlier cross-sectional studies, depending on whether clinical, radiologic, functional, or pathologic criteria have been used [4–8]. In a recent prospective study of 17 patients with newly diagnosed polymyositis/dermatomyositis, 11 patients (65\%) were diagnosed with ILD at onset of diagnosis [9**]. In this study, ILD was defined as the occurrence of radiographic signs of ILD on chest radiograph and/or high-resolution computerized tomography (HRCT) and/or restrictive ventilatory defect with reduced lung volumes. This frequency was higher than that reported in previous studies despite its restriction to newly diagnosed cases and excluding patients with cancer and overlap syndromes. The high incidence of ILD in this study could
be a result of the systematic use of sensitive detection methods such as HRCT and pulmonary function tests. Moreover, all new patients with myositis were investigated regardless of clinical lung symptoms, and some asymptomatic patients were detected with signs of ILD using these tests. It is likely that the reported incidence of ILD will increase even further with the increasing use of other sensitive diagnostic methods such as bronchoalveolar lavage (BAL), as has been reported in patients with other connective tissue diseases [10,11]. Adult patients with polymyositis and dermatomyositis seem to be equally predisposed to develop ILD. Data on prevalence of ILD in juvenile dermatomyositis are limited. According to one study, about half of the patients in a small case series of 12 patients with juvenile dermatomyositis had asymptomatic lung disease detected by pulmonary function tests [12].

Clinical features of interstitial lung disease
The clinical manifestations of ILD in patients with polymyositis or dermatomyositis may vary from asymptomatic to severe, rapidly progressive dyspnea with pulmonary insufficiency and fatal outcome. Patients with polymyositis and dermatomyositis with ILD have been described with three different clinical patterns of the ILD based on the clinical symptoms: those with an acute onset of symptoms, those who present with chronic, slowly progressive symptoms, and those without pulmonary symptoms but abnormal chest radiographs or pulmonary function tests [13]. Cough and dyspnea are the most commonly reported symptoms, although ILD is also reported to occur in patients without any clinical overt signs of pulmonary involvement. In one study, 27% of the patients with myositis with ILD were asymptomatic; inversely, two-thirds of the patients without any signs of ILD on radiograph/HRCT or reduction of lung volumes had either cough or dyspnea [9**].

Other clinical or laboratory signs should also raise the awareness of a concomitant ILD in patients with polymyositis or dermatomyositis. The strongest predictive factor for ILD in patients with myositis is the presence of positive anti-aminocarboxyl tRNA synthetase antibodies, of which the anti-histidyl tRNA synthetase antibody (anti-Jo1) is the most frequently found, in approximately 20% of patients with myositis. The reported frequency of ILD in patients with anti-Jo1 antibodies is more than 70% [3,9**,14,15]. Clinical manifestations other than skin and muscle involvement that occur frequently in patients with ILD and myositis are arthralgia/arthritis, fever, Raynaud’s phenomenon, and mechanics hands, known as manifestations of the antisynthetase syndrome [14,15]. In addition to positive anti-Jo1 antibodies, elevated levels of Krebs von den lungen-6 (KL-6), a glycoprotein expressed on type II alveolar pneumocytes and bronchiolar epithelial cells, and serum surfactant protein D are suggested to be useful as markers for ILD in patients with polymyositis/dermatomyositis [16–18].

It has been established that ILD can appear concomitantly with, before, or after the onset of skin or muscle manifestations [3,6,19]. Case reports even exist of ILD and polymyositis or dermatomyositis ‘sine myositis’ at presentation in some patients with an acute-onset, rapidly progressive interstitial lung disease [20**,21,22]. The most frequently reported physical finding is bibasilar crepitate rales.

Diagnosis of interstitial lung disease
Clinical respiratory symptoms are not reliable signs to detect ILD in patients with myositis, because neither cough nor dyspnea may be present as early signs. The most useful tests to diagnose ILD are pulmonary function tests, which typically show a restrictive ventilatory defect with decreased total lung capacity, functional residual capacity, residual volume, forced expiratory volume in 1 second (FEV1), and forced vital capacity (FVC), but with a normal or elevated FEV1/FVC ratio and a decreased diffusing capacity for carbon monoxide (DLco) (Fig. 1). Not all of these abnormalities may be found in every patient, however. The most sensitive test seems to be the DLco, but a decreased DLco is not specific for ILD and can also be seen in pulmonary hypertension, for example.

![Figure 1. Pulmonary function test with a restrictive pattern.](image)
Chest radiographs, including serial examinations, are highly useful as screening tests and for detection of complications of ILD such as pneumothoraces, but are rarely useful in detecting early ILD. A normal chest radiograph has been found in approximately 10% of the patients with biopsy-proven diffuse lung disease [23]. Compared with chest radiograph, HRCT of the lungs has a higher sensitivity to detect ILD (Figs. 2 and 3). HRCT is now widely used not only for detection of ILD but also for identifying the extent and severity of the disease as well as to discriminate between fibrotic disease and active inflammation in the lungs [24–26]. The most common findings on HRCT are irregular linear opacities with areas of consolidation and ground glass attenuation, suggesting active inflammation (Fig. 3). Honeycombing indicative of ‘end-stage lung’ has not been a common finding in patients with myositis [27••,28–30].

Lung biopsies are not routinely performed in patients with myositis with signs of ILD for diagnostic purposes because of the potential morbidity associated with surgical lung biopsy [7]. Transbronchial biopsies are rarely helpful in the diagnostic procedures, although they are usually abnormal, because the histopathologic findings are non-specific. Furthermore, previous studies have shown that the HRCT could predict the histologic appearance of ILD in open lung biopsy specimens [24–26,31]. A lung biopsy could be helpful to determine prognosis, however, because different histopathology features predict response to corticosteroid treatment.

Figure 2. Chest radiograph.

Bronchoalveolar lavage is a safe, noninvasive, and generally well tolerated procedure. BAL is useful in identifying other causes of interstitial lung disease such as infections, drug-induced pneumonitis, and sarcoidosis. Moreover, the BAL fluid cell profile may have a supportive role in the assessment of disease activity and prognosis and guiding of therapy in patients with myositis-associated ILD [3,19]. Anti-Jo1 antibodies could not be used as a diagnostic tool for ILD, but because these auto-antibodies are highly associated with ILD, their presence requires careful evaluation of lung involvement using lung function tests and HRCT.

Pathogenesis and histopathology of interstitial lung disease in myositis

The mechanisms that cause ILD in patients with myositis could be several on the basis of the various histopathologic features that have been observed. Studies describing the histopathology of ILD in polymyositis/dermatomyositis have shown several patterns, including bronchiolitis obliterans with organizing pneumonia (BOOP; or cryptogenic organizing pneumonia), diffuse alveolar damage (DAD), nonspecific interstitial pneumonia (NSIP), and usual interstitial pneumonia (UIP) [3,7,30,32••]. These are not specific for ILD with myositis but are identical to those found in idiopathic pulmonary fibrosis. That the mechanisms could vary is further supported by the varying responsiveness to immunosuppressive therapy, which is correlated with different histopathologic patterns. Thus, BOOP responds favorably to corticosteroids. Histopathology compatible with DAD, UIP, or acute interstitial pneumonia responds poorly to corticosteroids and other immunosuppressive therapies and has a poor prognosis [33]. The histopathologic subgroup NSIP encompasses...
a varying degree of alveolar wall inflammation or fibrosis, and the response to corticosteroids depends on the degree of inflammation or fibrosis [7,34]. Nonspecific interstitial pneumonia followed by organizing pneumonia was the most commonly observed histologic pattern in patients with myositis with ILD [30,32**]. In a retrospective review of 54 lung biopsies from 37 patients with a variety of connective tissue diseases, 13 of 37 patients had diagnosis of polymyositis or dermatomyositis. In several biopsies, there was more that one pattern of disease [32**].

**Prognosis and prognostic markers of interstitial lung disease in myositis**

Although the prognosis for patients with ILD and myositis varies, ILD is considered to be a major risk factor for premature death in patients with myositis. According to two recently reported studies, 1-year survival of patients with polymyositis and dermatomyositis with ILD was 85.8% in one study and 94.4% in the other, 3-year survival was 74.4% or 90.4%, and 5-year survival was 60.4% or 86.5% [3,30]. Negative prognostic factors for survival in patients with ILD were Hamman–Rich-like syndrome (acute interstitial pneumonia), initial diffusing capacity of carbon monoxide less than 45%, neutrophil alveolitis, and histopathologic features of UIP [3].

Other reports have considered the histopathology of lung biopsy to be of prognostic value in patients with ILD. Patients with BOOP and cellular NSIP tend to have the best prognosis and response to corticosteroid treatment, whereas those with DAD have the worst prognosis. Patients with UIP tend to have an intermediate course [3,7].

High-resolution computerized tomography could also be helpful as a prognostic tool, because certain patterns of ILD observed on HRCT correlate well with findings on open lung biopsy [24–26,31]. Thus, a reticular pattern on computed tomography (CT) scan of the lungs correlated with a histologic finding of fibrosis, whereas a ground glass pattern correlated with reversible inflammatory disease and a better prognosis in patients with fibrosing alveolitis [35].

The cellular composition of BAL fluid could have a prognostic value. In idiopathic pulmonary fibrosis, a high number of lymphocytes in BAL fluid represents a favorable outcome, while neutrophils and/or eosinophils in BAL fluid were associated with a poor outcome [36]. Reembling idiopathic pulmonary fibrosis, polymyositis/dermatomyositis-associated ILD with neutrophil alveolitis had a progressive, deteriorating course [3,19]. In a study by Schnabel et al. [19], all patients with progressive ILD had an increased number of neutrophils in BAL fluid and also tended to have a higher eosinophil count than nonprogressive patients.

Whether serum markers could have a prognostic value is less clear. Once ILD has developed, the presence of anti-Jo1 antibodies does not appear to have a prognostic value for outcome of ILD [3,30,37**]. The overall prognosis seems to be worse in patients with anti-Jo1 antibodies, however, compared with patients without these antibodies [15].

A new interesting serum marker with a potential of carrying prognostic value is KL-6. Elevated serum concentrations of KL-6 correlated not only with presence of ILD but also with severity of disease [16,17]. In a study of 42 adult patients with polymyositis and dermatomyositis, elevated serum KL-6 levels correlated with presence of ILD and decreased percentage diffusing capacity of carbon monoxide and percentage vital capacity [16]. Bandoh et al. [17] demonstrated that KL-6 concentrations in sera of six patients with polymyositis or dermatomyositis were associated with interstitial pneumonia and changed according to the progression or improvement of interstitial pneumonia. Although this marker needs to be tested in larger patient cohorts over time, and serum levels need to be compared with appropriate outcome measures for lung function, it is interesting as a possible future serum marker for prognostic evaluation.

Serum surfactant protein D was also found to be increased in patients with ILD associated with polymyositis/dermatomyositis. The level of serum surfactant protein D was inversely correlated with vital capacity and DLco in those patients [18]. Whether this marker is sensitive to changes with time and thus could be used as a prognostic marker in patient treatment needs to be investigated.

Whether the prognosis of ILD in patients with polymyositis is different from that in patients with dermatomyositis is uncertain. In a recent study by Fujisawa et al. [38*], patients with dermatomyositis and associated ILD were less responsive to corticosteroid therapy compared with patients with polymyositis-associated ILD, resulting in a poor prognosis. An aggressive course of ILD, often with a fatal outcome, was also reported in a few patients with amyopathic dermatomyositis, a variant of dermatomyositis that is characterized by the typical skin rash but without myositis [20**,21,22]. Digital infarcts with microangiopathy in patients with dermatomyositis were also suggestive of severe pulmonary involvement and poor prognosis [39*].

**Treatment**

The optimal treatment for myositis-associated ILD is not known. No published controlled trials exist on the effects of different therapies in polymyositis/dermatomyositis with associated ILD. Thus, available information on treatment efficacy is based on small case series or case reports. Corticosteroid therapy is often used as a first-line treatment, as in the case of patients with myositis without...
ILD. Initial therapy usually recommended is prednisolone with a dosage of 0.75–1 mg/kg/day for 6–8 weeks and subsequent tapering depending on clinical and laboratory evaluation. Corticosteroid treatment as a single agent is often not sufficient to cause improvement of ILD. Furthermore, the high doses required over a long period are often associated with severe side effects. Thus, other immunosuppressive agents are often required. The most frequently used drugs with reported beneficial effects on lung function are cyclophosphamide, cyclosporine A, azathioprine, and methotrexate [3,19,20,40–42]. Pulse methylprednisolone in combination with intravenous cyclophosphamide has also been reported to be beneficial in patients with rapidly progressive ILD [19,43]. Why only a limited number of patients respond to these therapies is unknown, but according to some reports, it could be related to different histopathology of ILD, suggesting different disease mechanisms. Furthermore, the response rate may be higher when treatment is initiated early in the course of the disease, before irreversible changes have developed.

One interesting case series suggested a beneficial effect of tacrolimus in polymyositis-associated ILD [44]. In this preliminary study, tacrolimus led to stabilization or improvement of ILD in four of five anti-Jo1 antibody-positive patients with polymyositis. These observations were confirmed in a larger patient cohort of 13 patients with myositis and anti-aminocyl-tRNA synthetase antibodies-associated ILD, in which significant improvement was observed in all pulmonary parameters, including FVC, FEV1, and DLco after an average treatment for 51.2 (range, 6–120) months [45].

Tumor necrosis factor α (TNF-α) inhibitors have become promising agents for treatment of many rheumatic diseases. Whether TNF-α inhibitors are effective in patients with myositis with ILD is not known. Notably, there have been a few case reports in which ILD developed in patients with rheumatoid arthritis during treatment with TNF-α inhibitors [46*].

Whether these immune-modulating agents really have an effect in myositis-associated ILD requires confirmation by prospective, randomized controlled trials. For patients who develop severe end-stage ILD, refractory to medical therapy, lung transplantation could be considered as a possible therapy, but there are no published reports to date on the outcome of lung transplants in patients with myositis and ILD.

Careful follow-up of lung function and HRCT of the lungs is important to assess course and treatment response in individual patients who have signs of ILD. In idiopathic pulmonary fibrosis, if response occurs, improvement of lung function and regression of changes on the radiograph/HRCT is usually noted within 3 months. Thus, in patients with myositis with ILD, follow-up assessment including pulmonary function tests and radiograph and/or HRCT after approximately 3 months of therapy is recommended to evaluate treatment response. It is not known how long immunosuppressive treatment is required, but the beneficial effects need to be balanced with the risk of serious side effects inevitably associated with long-term immunosuppressive therapy. When cyclophosphamide is used as induction therapy, replacement with a less toxic immunosuppressive agent such as azathioprine, methotrexate, or cyclosporine A should be considered later during the treatment, but when the replacement could be made is uncertain and should be based on clinical assessment in combination with evaluation of pulmonary function tests and HRCT of the lungs.

**Conclusion**

Investigations to detect interstitial lung disease should be performed during the initial evaluation as well as during follow-up of patients with myositis, because ILD is a frequent manifestation in patients with polymyositis or dermatomyositis and because ILD is associated with increased morbidity and mortality. This evaluation should include chest radiograph, HRCT of lungs, pulmonary function tests including diffusing capacity, and serum levels of anti-Jo1 antibodies. In the patients with ILD, clinical or subclinical, treatment with high doses of corticosteroids in combination with other immunosuppressive therapy should be initiated. Some histopathologic features including DAD, UIP, neutrophil alveolitis, digital infarcts showing microangiopathy in dermatomyositis, and amyopathic dermatomyositis have all been reported as risk factors for poor outcome. Presence of these factors suggests the use of aggressive immunosuppressive therapy and careful monitoring of lung function.

**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

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 788).

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A prospective study of 17 patients with PM/DM who were investigated by radiograph/HRCT of the lungs and pulmonary function tests, regardless of clinical symptoms, at the time of diagnosis.


14 Miller FW. Myositis-specific autoantibodies: touchstones for understanding symptoms, at the time of diagnosis.


A case report of a 49-year-old man with intestinal pneumonia (NSIP) associated with amyopathic DM who died of respiratory failure.


Retrospective study of the pattern, distribution, and extent of changes on HRCT of the lungs in patients with PM/DM with ILD.


This is an important new contribution to histopathologic characterization of interstitial lung disease in patients with myositis.


A study looking at the response to treatment and the long-term outcome of 12 patients with PM/DM with anti-Jo-1 antibodies who were evaluated over a mean period of 66.4 months.


A study of differences in clinical picture and prognosis of 16 patients with PM and 12 patients with DM with ILD.


Report on three cases of DM with multiple digital infarcts with histopathological evidence of microangiopathy in the early course of the disease who developed rapid progressive ILD.

40 Kashiwabara K, Ota K. Rapidly progressive interstitial lung disease in a dermatomyositis patient with high levels of creatine phosphokinase, severe muscle symptoms and positive anti-Jo-1 antibody. Intern Med 2002; 41:584–588.


Report on three fatal cases of RA who developed signs of fibrosing alveolitis after three or fewer doses of infliximab. All three had asymptomatic fibrosing alveolitis before treatment with infliximab.
Damage and inflammation in muscular dystrophy: potential implications and relationships with autoimmune myositis
James G. Tidball\textsuperscript{a,b} and Michelle Wehling-Henricks\textsuperscript{a}

Purpose of review
This review provides an updated evaluation of the role of inflammation in muscular dystrophy, and presents findings which suggest that non-immunological factors promote idiopathic inflammatory myopathies. Recent findings are summarized which indicate that immune-targeted interventions may provide useful approaches to treat muscular dystrophy.

Recent findings
Elevated expression of the cytotoxic T-lymphocyte derived cytolytic molecule, perforin, and the inducible costimulatory molecule have been identified in muscles of Duchenne muscular dystrophy patients, which strengthens evidence that a cellular immune response contributes to dystrophinopathy. Conversely, new findings implicate non-immune factors in inflammatory myopathy pathogenesis. Muscles from healthy individuals expressed autoantigens typically present in inflammatory myopathies, and autoantigen expression increased along with elevated major histocompatibility complex class I expression at sites of muscle regeneration in inflammatory myopathies. Those observations suggest that regeneration could render conditions sufficient for an autoimmune response in inflammatory myopathies. Further studies of corticosteroids or tumor necrosis factor blockade in treating dystrophinopathy indicate that immunological interventions may yield improved therapies for muscular dystrophy. In addition, advancements in understanding the involvement of chemokines in muscular dystrophy and inflammatory myopathies suggest that targeting specific chemokines has potential therapeutic value.

Summary
Our developing understanding of the pathogenesis of muscular dystrophies and inflammatory myopathies shows complex interactions between immunological and non-immunological features of these diseases that can affect disease onset and course. Among the muscular dystrophies, the best evidence for an immunological component to disease pathogenesis exists for dystrophinopathies. Conversely, muscle damage leading to regeneration may promote some inflammatory myopathies, although much remains to be learned concerning the identity and pathological significance of non-immunological features of inflammatory myopathies.

Keywords
idiopathic inflammatory myopathy, inflammation, muscular dystrophy, skeletal muscle

Introduction
A distinct line has traditionally been drawn between the pathophysologies of idiopathic inflammatory myopathies (IIMs) and the genetic muscular dystrophies. Polymyositis, the most common of the IIMs, is a T-cell-mediated pathology in which a cellular immune response is a key feature in promoting muscle pathology [1]. Dermatomyositis pathology appears to derive primarily from a humoral immune response [1]. In contrast, the muscular dystrophies primarily result from mutation of a functionally diverse group of muscle proteins, including cytoskeletal proteins, basement membrane proteins, transmembrane proteins that are possibly involved in signaling, and nuclear envelope proteins or proteases [2]. However, the distinction between immune-mediated and nonimmunemediated muscle diseases has become less sharp, however, as more is learned of the complex, underlying pathogenic mechanisms in both IIMs and the muscular dystrophies. For example, a growing body of evidence shows that the immune system is a contributor to the pathology of some muscular dystrophies. In addition, exciting new findings indicate that key events initiating the pathology of IIMs may be independent of immune cell interactions with muscle. Although the IIMs and muscular dystrophies remain functionally meaningful and clinically valuable distinctions, learning more about the contributions of noninflammatory factors to the pathogenesis of IIMs and the contributions of...
inflammatory factors to the muscular dystrophies will help us understand better the relationships between skeletal muscle and the immune system in health and disease.

Immune cell involvement in dystrophinopathies

Mutation of the membrane-associated protein, dystrophin, causes the progressive muscle wasting and weakness that occurs in Duchenne muscular dystrophy (DMD), the milder Becker muscular dystrophy, and the mdx mouse model of DMD [2]. Dystrophin deficiency causes a weakening of the sarcolemma that can be mechanically damaged during muscle use [3]. This muscle membrane damage is expected to produce dysregulation of a broad spectrum of structural and regulatory genes in muscle [4] that eventually leads to muscle fiber death that is accompanied by muscle inflammation. Although inflammation has long been recognized as a feature of dystrophinopathy, it was generally dismissed as an epiphenomenon that reflected a nonspecific inflammatory response to muscle damage. However, the inflammatory infiltrate plays a major role in promoting the pathology of dystrophin-deficient muscle, at least in mdx mice. Depletions of macrophages from mdx mice prevent most muscle membrane lysis that is present at the peak of pathology [5], suggesting that most muscle membrane lysis is attributable to macrophage cytotoxicity rather than mechanical lesions of the membrane.

Other observations have shown that a specific, cellular immune response can also promote dystrophinopathy. Most patients with DMD assayed expressed a highly conserved peptide in the hypervariable domain of the T-cell receptor in cytotoxic T lymphocytes (CTLs), which indicated that a specific immune response to a common antigen occurs in many patients with DMD [6]. Although the contribution of CTLs to the DMD pathology remains unexamined, recent findings show that expression of the CTL-derived cytolytic protein, perforin, is elevated in DMD muscle [7*]. In previous studies using the mdx mouse model, depletion of CTLs produced a reduction in muscle pathology, and null mutation of perforin eliminated apoptosis and reduced necrosis in dystrophic muscle, suggesting that CTLs may also play a role in DMD [8]. However, more recent investigations indicate that perforin-mediated apoptosis may not be an important feature of dystrophinopathy because overexpression of the anti-apoptosis protein B-cell leukemia/lymphoma 2 (BCL-2) in mdx muscle produced no reduction in the histopathology of mdx muscles [9**].

If T-cell-mediated pathology is a feature of dystrophinopathies or other muscle diseases, antigen presentation would be required for T-cell activation. Although expression of major histocompatibility complex (MHC) class I or II on dystrophic or IIM muscle has been long established [10,11], the expression of costimulatory molecules necessary for the stimulation and clonal expansion of auto-reactive T cells has been demonstrated only recently. Inducible costimulator ligand (ICOSL), a member of the B7 family of costimulatory molecules, can promote CTL activation by binding its receptor inducible costimulator (ICOS) present at the CTL surface [12*]. ICOSL expression was originally demonstrated on the surface of polymyositis, dermatomyositis, and inclusion body myositis (IBM) fibers [13], which was consistent with a role for muscle fiber activation of auto-reactive T cells in IIMs. Surprisingly, more recent findings also show that ICOS and ICOSL are expressed in DMD muscle tissue at levels that are usually higher than the median expression level in IBM muscle [7*]. However, unlike polymyositis and dermatomyositis muscle, ICOSL is expressed primarily by mononucleated cells in the connective tissue in DMD muscle and expressed only weakly at the muscle fiber surface [7*]. Furthermore, ICOS-expressing CTLs are commonly observed invading the cytosol of IIM muscle fibers that express ICOSL and MHC class I, while ICOSL-expressing CTLs remain in the connective tissue surrounding muscle fibers in DMD [7*]. These findings indicate distinctions in CTL activation in DMD compared with the IIMs that are not yet understood.

Immune cell involvement in dysferlinopathies

Mutations in the dysferlin gene cause the progressive muscular dystrophies Miyoshi myopathy and limb girdle muscular dystrophy 2B [14*]. Observations that link dysferlin with membrane repair following cell damage suggest that mechanical damage to muscle fibers followed by defects in the repair process underlie the pathologic progression of the disease [14*]. Although defects in membrane repair following damage may be a primary pathogenic mechanism in dysferlinopathy, the prominent inflammatory infiltrate in dysferlin-deficient muscle suggests that inflammation may play a significant but unexplored role in promoting muscle damage. Furthermore, dysferlin-deficient fibers express MHC class I on their surfaces [15–17], and complement activation precedes fiber necrosis, which implicates a nonspecific immune response in promoting fiber damage [18]. Macrophages, which are the most prevalent leukocyte population to invade dysferlin-deficient muscle, are found within or near injured fibers [18], which would be consistent with their role in either causing or responding to muscle fiber damage. However, this inflammatory infiltrate is distinct from either IIMs or dystrophinopathies; limb girdle muscular dystrophy 2B or Miyoshi myopathic muscle biopsies rarely contain CTLs, although CD4+ T-cell populations are greatly elevated [18]. Although dysferlin deficiency and dysferlin deficiency both involve muscle membrane damage that is associated with inflammation, there are scattered reports that are not yet well documented stating that treatment of patients with dysferlinopathy with
anti-inflammatory corticosteroids may decrease muscle strength [16,19]. If this is generally true for patients with dysferlinopathy, the observation would indicate important differences in inflammatory cell involvement compared with patients with DMD, in whom corticosteroids typically have beneficial effects.

**Do nonimmune factors contribute to idiopathic inflammatory myopathy pathogenesis?**

Our developing understanding of the role of the immune system in promoting the pathology of muscular dystrophies, in particular DMD and mdx dystrophy, suggests an interesting possibility: could nonimmune factors play a significant role in the pathogenesis of the IIMs? Although the pathologies of the major IIMs are certainly driven by autoimmunity, in each case the pathogenesis is unknown. Typically, autoantibodies expressed by patients with IIM recognize antigens that are expressed by tissues that do not experience an autoimmune pathology, illustrating the tissue specificity in the presentation of the antigens that is required for the autoimmune response [20••].

The question of what conditions are necessary for developing a muscle-specific autoimmune disease became even more intriguing with the recent discoveries of Casciola-Rosen et al. [20••]. In their analysis of the expression of the autoantigen Mi-2, against which 15–30% of patients with dermatomyositis express autoantibodies, they unexpectedly found that Mi-2 was expressed in muscle biopsies of healthy control participants. Similarly, other autoantigens that are present in dermatomyositis and polymyositis muscle, U1-70, HRS, and DNA-PKcs, were also present in healthy muscle. In each case, autoantigen expression was tremendously elevated after the clinical onset of the dermatomyositis or polymyositis. Further analysis showed that autoantigen expression was specifically elevated in muscle cells located in foci enriched in regenerating muscle fibers, proliferating myogenic cells, and inflammatory cells. These regions contained elevated concentrations of myogenic cells and myotubes that expressed markers of muscle development or regeneration, such as neural cell adhesion molecule and neonatal myosin heavy chain. These observations suggested the intriguing possibility that muscle regeneration was accompanied by elevation in autoantigen expression that eventually triggered an autoimmune response. In a scenario described by the investigators, muscle damage that induced muscle regeneration along with elevated MHC class I expression could provide the initial trigger for the ensuing autoimmune response. Interestingly, previous investigators have demonstrated that interferon-γ can induce expression of MHC class II on proliferative myoblasts but not on fully differentiated muscle fibers [21]. This potential dependence on stage of muscle cell differentiation on the capacity for antigen presentation may be sufficient to explain the induction of autoimmunity by regenerative muscle, but not healthy muscle.

**Immune-targeted interventions for the muscular dystrophies**

Despite the accumulating evidence of immune cell involvement in promoting dystrophinopathies, uncertainties remain concerning whether the functional benefits of corticosteroid treatments in DMD or mdx dystrophies are primarily attributable to immunosuppression. Recent studies in young mdx mice confirm that treatment with prednisolone significantly decreased the concentrations of inflammatory cells in dystrophin-deficient skeletal muscle [22]. In addition, the prednisolone-treated mdx mice experienced reduced expression of cellular adhesion molecules by leukocytes and endothelial cells, suggesting that prednisolone’s anti-inflammatory functions may be mediated by attenuating inflammatory cell extravasation into muscle. However, initiation of prednisolone therapy in mdx mice after the peak inflammatory phase of the disease did not have an effect on macrophage number, although gene array analysis showed induction of an anti-inflammatory expression profile [23]. For example, annexin A1, an expected mediator of glucocorticoid anti-inflammatory functions, was expressed at higher levels in prednisolone-treated animals, and expression of tumor necrosis factor (TNF) receptor was decreased [23]. Together, these findings show that prednisolone treatments in mdx dystrophy can have immunosuppressive effects if treatments are started early in the disease. This may be relevant to anecdotal reports that prednisolone treatments of DMD patients are more effective if they are begun at an early age. However, the findings do not eliminate the possibility that significant functional benefits may also derive from immunosuppressive functions of prednisolone. Unfortunately, the clinical efficacy of prednisone is accompanied by negative side effects. Although a recent clinical study showed that DMD patients and their families perceived the benefits of intermittent prednisolone therapy to outweigh the adverse side effects [24], development of specifically targeted immune interventions may offer greater promise for reducing the negative side effects associated with prednisolone treatments.

Functional blockade of TNF-α has been explored recently as a more specific therapeutic strategy for the treatment of dystrophinopathy, because TNF-α can promote inflammation, and its expression is increased in patients with DMD. However, whether perturbations of TNF-α expression or activity have a net beneficial effect on dystrophinopathy has not been demonstrated conclusively. Mdx mice treated with a soluble receptor fusion protein that binds TNF-α showed decreased mRNA for type I collagen and for the profibrotic cytokine transforming growth factor (TGF)-β1 in diaphragm muscles, which suggested that
blocking TNF-α may attenuate fibrosis and thereby improve ventilatory function [25]. However, treatment of mdx mice with an anti-human TNF-α antibody produced a less clearly beneficial outcome. Mdx mice receiving anti-human TNF-α showed delayed onset of pathology [26], but the treatment also induced a cyclic pattern of pathology that perhaps reflected the weekly dosage regimen.

Continuing studies of the mechanisms through which anti-inflammatory drugs can affect the severity of muscular dystrophies have shown that some of the observed treatment effects may occur through direct action on muscle cells. For example, treatment of young mdx mice with the immunosuppressant cyclosporin prevented exercise-induced loss of strength and fiber damage and decreased fibrosis, perhaps by attenuating TGF-β1 expression rather than by actions as an immunosuppressant [27]. Interestingly, the cyclosporin treatment also inhibited the typical, dystrophic fast-to-slow fiber type transition, likely via calcineurin inhibition. A similar protective effect on fiber type transition, also attributed to calcineurin inhibition, was observed following prednisolone treatment [23], although it is unknown whether functional benefits occurred.

Conversely, other experimental therapeutics intended to have beneficial effects by direct action on dystrophic muscle cells may be mediated in part through anti-inflammatory effects. For example, a recently completed pilot study of the effects of albuterol, a β2-agonist, on knee-extensor strength of patients with DMD showed beneficial effects on muscle strength that could reflect inhibition of calpain-mediated proteolysis of muscle proteins that can be signaled through binding β2 receptors [28]. However, albuterol can also inhibit T-cell and macrophage activation [29–31], suggesting that the protective effect of albuterol in DMD may be attributable in part to its immunosuppressive functions.

Chemokines and their receptors as potential targets for therapeutics in muscular dystrophies and idiopathic inflammatory myopathies

The prominent involvement of the immune system in promoting the pathologies of IIMs and dystrophin deficiencies indicates that more specifically targeted immune interventions could be designed to disrupt disease-specific components of pathologic immune responses. The growing body of information concerning the roles of specific chemokines in the recruitment and activation of leukocytes suggests that disruption of chemokine-mediated signaling could be valuable in treatment of IIMs and muscular dystrophies in which inflammation is a pathologic feature. The use of chemokine receptor antagonists has already yielded promising results in rheumatoid arthritis, multiple sclerosis, and HIV [32]. Recent findings have suggested several potential targets for disruption of chemokine-mediated pathology in IIMs or muscular dystrophies. Beta-chemokine receptors (CCR) expressed on vascular endothelial cells and monocytes provide potential targets for affecting inflammatory cell invasion into diseased muscle, and the presence of CCR1, 4, and 5 expressed by muscle fibers themselves suggests their involvement in promoting an inflammatory response [33,34*,35*].

Although chemokines and their receptors may present potentially valuable therapeutic targets in muscle disease that have an inflammatory component, the large number of chemokines that are up-regulated in IIMs and dystrophin deficiency (Table 1), and their redundancy of function may make the design of effective therapeutic interventions difficult. In addition, there are conflicting reports regarding CCR1 expression in the IIMs [34*,35*] that may be a result of inter-patient variability; this would indicate that a single therapeutic approach based on chemokine blockade may not be effective for all patients diagnosed with a common IIM. Furthermore, the identity of chemokines involved in promoting inflammation may differ between muscles and between stages of the pathology. For example, gene array and reverse transcription polymerase chain reaction analyses showed significant differences in the identity of chemokine ligands that were expressed at higher levels in the diaphragm or hind-limb muscles of mdx mice [33,36].

Although blocking chemokine-mediated signaling may provide a useful strategy for the treatment of IIMs and dystrophies in which there is inflammatory involvement, recent findings also show that chemokine-mediated signaling may also promote muscle regeneration. For example, ablation of CCR2 signaling in CCR2-null mice results in impaired muscle regeneration following injury [37**]. Similarly, patients with dermatomyositis who experienced clinical improvement following intravenous immunoglobulin G therapy showed increased expression of α-chemokine ligands CXCL9 and CXCL11, suggesting they may have a beneficial role [38]. Therapeutic interventions based on disruptions of chemokine-mediated signaling will require careful dissection of the specific roles played by chemokines and their receptors in promoting immune cell–mediated damage or repair of muscle.

Conclusion

Recent advancements of our understanding of the pathogenesis of muscular dystrophies and IIMs reveal that the events that initiate and promote the pathologies of these muscle diseases are complex and subtle and display shared features between inflammatory myopathies and non-inflammatory myopathies. Growing evidence shows a significant inflammatory cell involvement in promoting dystrophinopathies, and the extensive inflammatory cell involvement in dysferlinopathies suggests that similar
Table 1. Summary of chemokine and chemokine receptor expression and localization in idiopathic inflammatory myopathies and muscular dystrophy from data published during review period 2004–2005.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Chemokine</th>
<th>Chemokine receptor</th>
<th>Tissue assayed</th>
<th>Expression level compared with control</th>
<th>Expressing cells</th>
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<td>ND</td>
<td>[36]</td>
<td></td>
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<td></td>
<td>CXCL13</td>
<td>mdx diaph</td>
<td>Increased</td>
<td>ND</td>
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<tr>
<td></td>
<td>CXCL14</td>
<td>mdx hindlimb</td>
<td>Increased</td>
<td>ND</td>
<td>[36]</td>
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<tr>
<td></td>
<td>CCR1</td>
<td>mdx diaph, TA</td>
<td>Increased</td>
<td>Myotubes, monocytes</td>
<td>[33]</td>
<td></td>
</tr>
<tr>
<td></td>
<td>CCR2</td>
<td>mdx diaph, hindlimb</td>
<td>Increased</td>
<td>ND</td>
<td>[36]</td>
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<tr>
<td></td>
<td>CCR3</td>
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<td>Increased</td>
<td>ND</td>
<td>[33]</td>
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<tr>
<td></td>
<td>CCR4</td>
<td>Patient biopsy</td>
<td>ND</td>
<td>Regenerating myonuclei</td>
<td>[35]</td>
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</tbody>
</table>
immune cell involvement in the pathogenesis of these muscular dystrophies may also exist. Exciting new findings also indicate that early events that trigger the pathology of the best characterized IIMs may be nonimmunologic in at least some instances and result from a pathologic immune response to muscle regeneration following injury.

More specific and effective therapies for dystrophin-deficient muscular dystrophies and for IIMs are needed. Although corticosteroids and immunosuppressants such as cyclosporin can have beneficial effects, their lack of specificity limits their usefulness. Identification of specific inflammatory mediators that promote autoimmune or cytotoxic interactions between inflammatory cells and target muscle cells may allow the design of interventions that attenuate pathogenic mechanisms without disrupting regenerative processes.

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

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 768—769).

Table 1. Summary of chemokine and chemokine receptor expression and localization in idiopathic inflammatory myopathies and muscular dystrophy from data published during review period 2004—2005.

<table>
<thead>
<tr>
<th>Disease</th>
<th>Chemokine receptor</th>
<th>Tissue assayed</th>
<th>Expression level compared with control</th>
<th>Expressing cells</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>CCR5</td>
<td>mdx diaph, hindlimb</td>
<td>Increased</td>
<td>ND</td>
<td>[36]</td>
<td></td>
</tr>
<tr>
<td>CXCR4</td>
<td>mdx diaph, TA</td>
<td>Increased</td>
<td>ND</td>
<td>[33]</td>
<td></td>
</tr>
</tbody>
</table>

“Expression levels of chemokines and their receptors in pathological muscle are compared with control muscles. The specific cells shown to express select chemokines and chemokine receptors are indicated.

CD4, CD4+ T cell; CD8, CD8+ T cell; diaph, diaphragm; hindlimb, gastrocnemius and soleus; MAC, macrophage; MIP, macrophage inflammatory protein; ND, not determined; PARC, pulmonary and activation-regulated chemokine; PE, perivascular exudate; PI, mononuclear cells partially invading nonnecrotic muscle fibers; RANTES, regulated upon activation normal T-cell expressed and secreted; TA, tibialis anterior; VEC, vascular endothelial cell.

9 Dominov JA, Kravetz AJ, Ardelt M, et al. Muscle-specific BCL2 expression ameliorates muscle disease in laminin alpha2-deficient, but not in dystrophin-deficient, mice. Hum Mol Genet 2005; 14:1029—1040. This investigation uses a clever experimental strategy to test whether the stage of differentiation on dystrophic muscle in which the anti-apoptotic protein BCL2 is expressed trans-genically affects muscle pathology. Their findings show that laminin–2–deficient mice experience reduced pathology and increased lifespan when BCL2 is expressed in myoblasts or muscle fibers. However, mdx mice experienced no change in pathology with BCL2 transgene expression.


12 Greenwald RJ, Freeman GJ, Sharpe AH. The CD8+ T cell revisited. Annu Rev Immunol 2005; 23:515—548. This review is a comprehensive, up-to-date review of the CD8+ T cell family of costimulatory molecules.


Gosselin LE, Martinez DA. Impact of TNF-α blockade on TGF-β1 and type 1 collagen mRNA expression in dystrophic muscle. Muscle Nerve 2004; 30:244—246. This investigation provides clear evidence that blocking TNF-α interactions with its receptor can improve respiratory function in dystrophic mdx mice, perhaps by preventing fibrosis.


Cyclosporine A-treated mdx mice showed significant functional and histological improvements that may be mediated by direct effects of the drug on muscle tissue.

Fowler EG, Graves MC, Wetzel GT, Spencer MJ. Pilot trial of albuterol in Duchenne and Becker muscular dystrophy. Neurology 2004; 62:1006—1008. This report provides promising results from a pilot trial to demonstrate that the β2-agonist, albuterol, can increase knee-flexor strength in patients with DMD and Becker muscular dystrophy.


Ribeiro S, Horuk R. The clinical potential of chemokine receptor antagonists. Pharmacol Ther 2005; 107:44—58. This is a current and very comprehensive review of chemokines, their receptors, and recent progress using chemokine receptor antagonists in a variety of auto-inflammatory diseases.


Civatte M, Bartoli C, Schleinitz N, et al. Expression of the β chemokines CCL3, CCL4, CCL5 and their receptors in idiopathic inflammatory myopathies. Neuropathol Appl Neurobiol 2005; 31:70—79. Expression and localization of β chemokine receptors and their ligands is described in biopsies from patients with dermatomyositis, polymyositis, and IBM. Immunohistochemical data show that CCR1 and 5 can be expressed on muscle fibers.

De Paepe B, De Bleecker JL. B-chemokine receptor expression in idiopathic inflammatory myopathies. Muscle Nerve 2005; 31:621—627. Analysis of β-chemokine receptor expression and localization in biopsies from patients with IBM (dermatomyositis, polymyositis, IBM) was performed. CCR1 expression data conflict with those of Civatte et al. [34], which may be a result of inter-patient variability.


Warren GL, Hulderman T, Mishra D, et al. Chemokine receptor CCR2 involvement in skeletal muscle regeneration. FASEB J 2005; 19:413—415. In this investigation, CCR2-null mice were used to provide provocative new evidence that signaling through the CCR2 receptor is involved in muscle regeneration following injury. The results of this study have important implications with regard to the development of chemokine receptor—targeted therapeutics.

Purpose of review
Recent characterization of the expression and functioning of muscle-derived positive and negative regulators of the immune response will be highlighted in view of the concept that muscle cells can act as facultative antigen-presenting cells and should be considered as active participants rather than passive targets of immune reactions.

Recent findings
Although lacking detectable major histocompatibility complex expression under physiologic conditions, under pathologic conditions muscle cells can express a variety of immunologically important molecules. Advances were made in characterizing the expression and functioning of classical and nonclassical major histocompatibility complex, adhesion, and costimulatory molecules. Muscle-related expression of the B7-family member called the inducible costimulatory signal ligand was identified as an important costimulatory signal for muscle immune interactions. In contrast, inducible expression of B7-H1 (PD-L1) and the nonclassical major histocompatibility complex molecule human leukocyte antigen-G were identified as relevant immune-inhibitory pathways.

Summary
The recent identification of muscle-derived positive and negative signals has broad implications for understanding the active role of muscle in modulating muscle–immune interactions: these signals could modify the immune response against muscle fibers in cell-mediated injury in autoimmune muscle disorders or in various muscle infections. Furthermore, they could modulate the immune responses after protein-based or DNA-based vaccinations and influence muscle-directed antigen-specific and nonantigen-specific immune responses in either condition.

Keywords
coinhibitory B-7 family members, costimulation, human leukocyte antigen-G, muscle immunobiology, nonclassical major histocompatibility complex molecules

Abbreviations
APCs antigen-presenting cells
HLA human leukocyte antigen
IFN-γ interferon-γ
MHC major histocompatibility complex
tGF transforming growth factor
TNF-α tumor necrosis factor-α

Introduction
Many immune reactions occur in the skeletal muscle. These develop spontaneously during the course of autoimmune and infectious muscle diseases [1,2] or are deliberately induced by immunotherapeutic gene transfer into muscle [3]. Local immune reactions pose a serious problem after intramuscular injection of vectors for gene therapy. They also represent the major obstacle for the success of myoblast transfer therapy, a cell-mediated gene transfer method aimed at restoring normal protein expression in some hereditary muscular disorders [4]. Muscle is an immunologic microenvironment [5•]: because it is one of the few body compartments that lack major histocompatibility complex (MHC) expression under physiologic conditions, immune reactions triggered by or directed against muscle cells proceed along specific pathways. Recently, advances have been made in the characterization of positive and negative muscle-derived regulators for immune interactions, extending the view that muscle has important immunoregulatory capacities under certain conditions [5•].

Expression of classical and nonclassical major histocompatibility class I and class II molecules by muscle cells
Cultured human myoblasts constitutively express the classical human leukocyte antigen (HLA) class I antigens HLA-A, HLA-B, and HLA-C [6,7–12]. This level is increased by proinflammatory cytokines (interferon-γ [IFN-γ], tumor necrosis factor-α [TNF-α], interleukin-1α, interleukin-1β) and the chemokine MIP-1α, whereas transforming growth factor (TGF)-β reduces the basal levels of HLA-class I expression [13]. IFN-γ induces myoblasts and myotubes to express the HLA-class II antigen HLA-DR. HLA-DP and HLA-DQ are also inducible by IFN-γ, but the kinetics of induction and the levels of expression vary with the different HLA-class II molecules [6,9,10]. The expression of the different HLA-class II molecules seems to be developmentally regulated, in that more differentiated myotubes can be induced to express HLA-DR, but not DQ and DP on their surface [9]. In vivo, under physiologic
conditions neither MHC-class I nor MHC-class II antigens are detectable on normal mature muscle fibers, but MHC-class I antigens [14,15] are up-regulated in various inflammatory myopathies. It should be noted, however, that the expression of HLA-DR on the surface of muscle fibers in inflammatory lesions in inflammatory muscle disorders is inconsistent [14,16–18], but conceivably the level of HLA-class II expression in vivo may be so low as to escape detection by conventional immunostains. If muscle fibers do express HLA-DR in vivo, they could theoretically present not only viral or bacterial antigens but also muscle autoantigens or alloantigens to CD4 T cells.

In addition to the classical MHC class I and MHC class II molecules, myoblasts can be induced to express HLA-G [7]. HLA-G is a ‘nonclassical’ MHC-class I molecule (class Ib) structurally related to classical MHC-class Ia (HLA-A, HLA-B, HLA-C). HLA-G exhibits a highly restricted tissue distribution under physiologic conditions [19]. In contrast to MHC class Ia molecules, HLA-G is characterized by a limited polymorphism and the alternative transcription of spliced mRNAs that encode at least seven different isoforms, including membrane-bound HLA-G1, HLA-G2, HLA-G3, HLA-G4, and soluble HLA-G5, HLA-G6, HLA-G7 proteins. The immunobiological role of HLA-G is still not entirely clear. It is thought that HLA-G prevents maternal lymphocytes from attacking fetal tissue. Like classical HLA-class I molecules, HLA-G binds CD8 and antigenic peptides, therefore theoretically acting as a possible antigen-presenting molecule [20]. However, HLA-G has chiefly been identified as a molecule mediating immune tolerizing functions [21]. For example, HLA-G protects target cells from the cytotoxic activity of T lymphocytes and natural killer cells through direct or indirect interaction with several inhibitory receptors such as immunoglobulin-like transcript (ILT)-2, ILT-4, and the killer-inhibitory receptor KIR2DL4 (Fig. 1).

Costimulatory molecules
Cultured myoblasts do not express the classical costimulatory molecules B7.1 (CD80) and B7.2 (CD86) [22,23] and treatment of these cultures with IFN-γ and TNF-α has no effect on the expression of these molecules.

As the first functional co-stimulatory member of the B7-family on human muscle cells, inducible costimulator ligand (ICOSL, B7-H, B7-H2) was recently identified [24,25*]. ICOSL interacts with its receptor, ICOS, on activated T cells to costimulate CD4 and CD8 T-cell responses [24–26]. Although expression of ICOS-L on muscle fibers in vivo is low (or absent) under physiologic conditions, under inflammatory conditions (presence of TNF-α in vitro, muscle fibers of inflammatory myopathies in vivo) demonstrate markedly increased ICOSL expression [24,25*]. These findings corroborate the relevance of ICOSL-ICOS interactions as a major immunostimulatory pathway under inflammatory conditions [24,25*].

Perhaps one of the most interesting discoveries was the finding of the B7-homologue B7-H1 (PD-L1) in cultured human myoblasts as well as its presence in inflammatory myopathies [27]. Although absent under normal culture conditions, B7-H1 was inducible in the presence of IFN-γ. Interestingly, B7-H1 exerted strong immune-inhibitory
properties for CD4 as well as CD8 T cells in co-culture assays in that it reduced cytokine production and up-regulation of T-cell activation markers. Expression of B7-H1 on muscle fibers in vivo correlated with the presence of inflammatory cells [27]. Thus, B7-H1 could serve as a negative immune-regulatory principle exerted by muscle cells and induced upon inflammatory stimuli. In inflammatory myopathies this might partly protect muscle fibers from immune aggression. However, the observation of muscle-related expression of B7-H1 might have much broader implications for the immunobiology of muscle, because it could play an important role in many different immune reactions that occur in this tissue. As hypothesized, B7-H1 could protect muscle fibers from cell-mediated injury in autoimmune muscle disorders (polymyositis, dermatomyositis, or inclusion body myositis) or in various muscle infections. Furthermore, B7-H1 could modulate the immune responses after protein-based or DNA-based vaccinations and efficiently reduce muscle-directed antigen-specific and non-antigen-specific immune responses in either condition. These findings provide evidence for the strong capability of muscle to promote immunoprotective or immunosuppressive mechanisms and support a concept proposing that the expression of B7-H1 in the periphery may regulate local levels of immune inflammation [27,28*].

Using a monoclonal antibody against BB-1 (named after the reacting antibody clone raised against B7.1), it was shown that muscle fibers of myositis patients express BB-1 reacting molecules [23,29]. Functional experiments on human myoblasts demonstrated that the BB-1 reacting molecule has putative costimulatory function. However, the BB-1 antigen is found inconsistently in a minority of muscle cell lines in vitro [23]. The issue is further complicated by the observation that the BB-1 monoclonal antibody obviously (cross) recognizes the CD74 antigen (invariant chain Ii) [31], which is also detectable in muscle cells. The importance of BB-1 in providing costimulation will only be answered after molecular identification and characterization of the BB-1 antigen in muscle cells. Cultured muscle cells as well as muscle fibers in inflammatory myopathies also express B7-H3 (Wintterle and Wiendl, unpublished results) a third identified member of B7-homologues [28,33]. Its significance for muscle-immune interactions is currently not known but available data thus far suggest an inhibitory role of B7-H3 for immune cells.

Unstimulated cultured human muscle cells express CD40, and these levels are increased synergistically by the proinflammatory cytokines TNF-α and IFN-γ. Ligation of CD40 with anti-CD40 antibodies results in increased expression of ICAM-1 on muscle cells [23,38]. Thus, the presence of these costimulatory molecules further emphasizes the important immunologic role of myoblasts and their role in muscle immune interactions (Fig. 2).

**Cytokines**

Human myoblasts in vitro constitutively express a variety of cytokines, such as interleukin-6 and TGF-β, which can be induced after cytokine stimulation. Other cytokines, such as interleukin-4, interleukin-10, and IFN-γ, have never been reported to be detectable in myoblasts, even under stimulatory conditions. Interleukin-1α, interleukin-1β,
and TNF-α are found in myoblasts after stimulation only. However, it has to be noted that the expression of these molecules in the literature is inconsistent, and technical differences may account for these discrepancies. In biopsy specimens, normal muscle cells do not reveal any cytokine expression pattern, as illustrated in most of the studies published so far. In inflammatory myopathies, somewhat conflicting data have been reported, most likely because of differences in the patient cohorts investigated as well as in the chosen technical approach. In general, not only invading lymphocytes and endothelial cells, but also muscle cells secrete pro-inflammatory cytokines, such as interleukin-1α, interleukin-1β, TNF-α, interleukin-6, interleukin-2, and IFN-γ, but not TGF-β. Expression of interleukin-1 was depicted in regenerative fibers as well as in areas of myosinolysis, proposing a role of this cytokine in myofibrillar protein breakdown as well as in regeneration in inflammatory myopathies. In spite of the inconsistencies in the reports so far, an emerging body of evidence suggests that muscle fibers actively create a local milieu for chronic inflammation by the secretion of pro-inflammatory cytokines. Notably, the expression of anti-inflammatory cytokines by muscle fibers has not been reported at present (reviewed in [39]).

Relevance of positive and negative regulators for muscle–immune interactions

Many molecules of the ‘immunological synapse’ are indeed inducible on myoblasts (Figs. 1 and 2). These results suggest that myoblasts have sufficient immunologic ‘potential’ to qualify them to act as facultative antigen-presenting cells. For example, they might (re)stimulate memory and effector T cells in muscle. Cultured myotubes and myoblasts express HLA class I molecules and are efficiently killed by cytotoxic T cells and natural killer cells [8,40,41]. Death of muscle fibers in vitro as well as in vivo is mediated by a form of necrosis rather than apoptosis, presumably because of the counterbalancing effect or protection by the anti-apoptotic molecules Bcl-2, hILP, and FLIP, which are up-regulated in inflammatory myopathies [42–44]. Fas is also expressed, but it does not mediate apoptosis [42,43].

Antigen presentation to CD4 T cells depends on the constitutive or induced expression of HLA class II on the presenting cell. HLA class II-positive human myoblasts can act as facultative local antigen-presenting cells in muscle by providing the signals necessary to trigger both antigen-specific lysis and T-cell proliferation [6,24,27]. In addition to presentation of exogenous antigens processed in the classical MHC class II–restricted pathway, human myoblasts seem to be capable of presenting endogenous antigen to MHC class II restricted CD4+ T cells [45,46]. It remains unclear, however, to what extent myoblasts can stimulate naïve antigen-inexperienced T cells. Professional antigen-presenting cells (APCs) can be found at low numbers within muscle tissue. Under inflammatory conditions, however, they are recruited to the muscle [34,47,48,49]. Numerous experimental systems have shown that presentation of antigens by nonprofessional APCs, which lack appropriate costimulatory molecules, is more likely to result in tolerance than stimulate T cells. In certain situations, however, nonprofessional APCs, such as B7-transfected fibroblasts, are able to induce an MHC class I and MHC class II restricted lymphoproliferative response [50]. This implies that the antigen presentation and costimulation do not need to be provided by the same cell, as long as it is provided in an appropriate local environment (transcostimulation).

The detection of HLA-G expression on skeletal muscle raises several possibilities regarding its potential pathophysiological role in inflammatory myopathies and for the immunobiology of muscle in general. Locally accumulating inflammatory cells produce a plethora of proinflammatory cytokines including TNF-α and IFN-γ. Because these cytokines are known to induce HLA expression in many cell types, it seems likely that the HLA-G expression noted in inflammatory myopathies at least partly results from stimulation by locally produced cytokines [7]. Functional experiments delineating the role of HLA-G for muscle–immune interactions demonstrated a strong inhibitory function of transmembranous as well as soluble HLA-G isoforms [41]. The inhibitory effects of HLA-G were directed virtually against all immune effector cells because both isoforms inhibited natural killer cell lysis and prevented direct alloreactive killing by CD8 as well as CD4 cells [41]. Thus, HLA-G seems capable of modulating antigen-specific as well as non-antigen-specific (cytotoxic) T-cell responses during primary and secondary immune responses against muscle.

Although on professional APC, HLA-G acts as a strong inhibitor of CD4 activation [51,52], in nonprofessional APC such as muscle, HLA-G expression might be a protective mechanism activated in response to immune-mediated damage of MHC-expressing target cells and provoked by inflammatory cytokines such as IFN-γ [53]. Interestingly, HLA-G does not seem to be the only inhibitory principle expressed by muscle cells. The B7-homologue B7-H1 on muscle fibers acts as an inducible protein directly inhibiting CD4 and CD8 T-cell activation in muscle–immune cell interactions [27]. B7-H1 thus could represent another inhibitory mechanism by which muscle counterbalances immune damage [27]. In addition to its implications for the immunopathogenesis of inflammatory myopathies, the observations on the potential role of muscle-derived inhibitory signals might be of interest for understanding and fine tuning of therapies that rely on muscle such as gene therapy, DNA vaccination, or myoblast transplantation. Myoblasts and myotubes themselves are potent inducers of primary and secondary
allogeneic immune responses, thus partly explaining the rapid graft destruction after myoblast transfer [4]. Muscle-derived immune inhibitory signals might enhance survival of muscle cells under allogeneic conditions. We therefore surmise that gene transfer of B7-H1 or HLA-G into myoblasts could constitute a possible future strategy for circumventing the problem of myoblast death after therapeutic injection in vivo.

**Conclusion**
Recent research has refueled the concept that muscle plays an active part in muscle–immune interactions: myoblasts can participate as facultative APCs both in MHC class I-dependent and MHC-class II-dependent immune reactions. They secrete a surprising number of soluble factors, including important cytokines and chemokines, and they can be stimulated to express several positive and negative immune regulatory molecules. Thus, myoblasts can form a functional immunologic synapse with T cells, at least in vitro. Although some of these molecules are less abundantly expressed in more differentiated myotubes and muscle fibers, it seems likely that even mature muscle fibers can actively participate in various immune reactions. Demonstrating the interaction of muscle-derived ICOSL with ICOS on activated T cells brought the long-awaited characterization of a muscle-related B7-molecule expressed on muscle that is required for costimulating muscle–immune interactions. The identification of muscle-derived inhibitory signals (the immunotolerogenic MHC molecule HLA-G and the co-inhibitory B7-family member B7-H1) has broad implications for the immunobiology of muscle, because they could play an important role in many different immune reactions that occur in this tissue. These signals could protect muscle fibers from cell-mediated injury in autoimmune muscle disorders or in various muscle infections. Furthermore, they could modulate the immune responses after protein-based or DNA-based vaccinations and efficiently reduce muscle-directed antigen-specific and non-antigen-specific immune responses in either condition.

**Acknowledgement**
Part of this work was adapted from Ref. [5*].

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

- of special interest
- of outstanding interest

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 769).

25 Schmidt J, Rakovecic G, Rau R, Dalakas MC. Upregulated inducible • co-stimulator (ICOS) and ICOS-ligand in inclusion body myositis muscle: significance for CD8+ T cell cytotoxicity. Brain. 2004; 127:1182—1190. Second paper identifying ICOS ligand/ICOS interactions as crucial costimulatory pathway for the (re)stimulation of putatively pathogenic T cells and inflammatory myopathies. Most important, elaborated multicolor immunohistochemistry demonstrates the relevance of ICOS ligand/ICOS interactions for certain CD8 T cells involved in driving the pathogenesis of inclusion body myositis.


44 Interesting study elucidating the presence and possible importance of monocyte/macrophage differentiation with regard to inflammatory myopathies.


47 LeMaoult J, Krawice-Radanne I, Dausset J, Carosella ED. HLA-G1-expressing monocytes and fibroblasts of skin and muscle-derived regulators of immune response. Wiendl et al. 719
Muscle regeneration through myostatin inhibition
Kathryn R. Wagner

Purpose of review
Myostatin is an endogenous, negative regulator of muscle growth. Selective inhibition of myostatin may have broad clinical utility by improving regeneration in diverse and burdensome muscle disorders. An understanding of this potential is relevant because inhibitors of myostatin have recently entered clinical trials.

Recent findings
This article reviews the structure and function of myostatin, the effect of inhibiting myostatin in models of disease, and potential therapeutic approaches to blocking myostatin pharmacologically. The possibility that a myostatin inhibitor will promote muscle regeneration in human disease, as seen in animal models, is suggested by the observation that loss of myostatin results in muscle hypertrophy in a human subject.

Summary
Multiple approaches to inhibiting myostatin are suggested by the recent elucidation of its signaling pathway. An inhibitor of myostatin may be the first drug specifically designed to enhance muscle growth and regeneration.

Keywords
muscle, myopathy, myostatin, regeneration

Introduction
Muscle harbors its own progenitor cells and has the potential to regenerate from an array of injuries including traumatic, inflammatory, degenerative, and wasting disorders. Clinicians who treat these disorders, however, are faced with the reality that lost muscle bulk and strength are frequently never completely regained. There are currently no approved medications specifically designed to improve and hasten muscle regeneration. One recent approach to this clinical problem has been to attempt to modulate myostatin, an endogenous, negative regulator of muscle growth. This article will briefly review the background science of this muscle-specific growth factor and discuss recent findings suggesting its potential as a therapeutic target for a wide range of muscle disorders.

A muscle-specific, transforming growth factor beta family member
Myostatin, originally named growth differentiation factor 8, was identified through its homology to other members of the transforming growth factor-β (TGF-β) superfamily [1]. It is expressed almost exclusively in developing and mature skeletal muscle. Genetically engineered mice lacking myostatin have a dramatic hypermuscular phenotype. Homozygous myostatin null mice are approximately 30% larger than their littermates, due entirely to diffusely increased muscle mass. Both axial and appendicular muscles are enlarged, weighing two to three times those of their wild-type counterparts. On histologic analysis, the mutant muscle appears normal with the exception of exhibiting both muscle fiber hypertrophy (increased fiber size) and hyperplasia (increased fiber number) [1]. Other animal models with altered myostatin function confirm the importance of myostatin in postnatal muscle growth and reveal that the degree of muscle fiber hypertrophy and/or hyperplasia is in part secondary to the timing and mode of myostatin inhibition during development and postnatal life [2†]. These characteristics of myostatin-deficient animals indicate that myostatin normally functions as a negative regulator of muscle growth.

Myostatin shares several structural features with other members of the TGF-β superfamily. It is synthesized as a prepro-protein by muscle. Myostatin is secreted and proteolytically processed yielding a propeptide and active C-terminal domain [3]. A disulfide-linked dimer of myostatin C-terminal domains circulates in the blood as a latent complex, inhibited by a noncovalent bond to its own propeptide [3,4]. In vitro, myostatin can be activated by dissociation of the propeptide after proteolytic cleavage by a metalloproteinase of the bone morphogenetic
protein (BMP)/tolloid family, and this is postulated to be an in vivo mechanism for activation [5]. The circulating inhibitory complex also contains FLRG (follicatin-related gene) and GASP-1 (growth differentiation factor associated protein-1) [6,7]. GASP-1 binds to the pro-region and has domains found in protease inhibitors, suggesting that it may act to inhibit the BMP/tolloid metalloproteinases that cleave the propeptide [7].

Most members of the TGF-β superfamily signal through heteromeric complexes of type I and type II serine/threonine kinase receptors, which in turn activate intracellular proteins, called Smads, that regulate expression of downstream genes. The activated C-terminal dimer of myostatin, dissociated from its inhibitory propeptide, initiates a similar signal transduction cascade [8•]. In crosslinking studies, myostatin binds to the ActRIIB receptor and ALK-4 and/or ALK-5 coreceptors [3,9]. Further support of ActRIIB as the in vivo receptor for myostatin came from the development of a phenotypically similar hypermuscular transgenic mouse line expressing a dominant negative form of the ActRIIB without the kinase domain [3]. Downstream from the heteromeric receptor, myostatin signaling leads to activation of Smad proteins. Smad2 and Smad3 are phosphorylated and then complex with Smad 4 [9–11]. The Smad complex translocates to the nucleus to regulate transcription. Myostatin modulates transcription of a variety of muscle-specific genes as well as Smad7 that acts as a negative feedback mechanism [12•]. In summary, myostatin appears to act through binding to activin type II receptors and type I co-receptors that in turn activate Smad proteins, inhibiting transcription of muscle-specific genes.

Inhibition of muscle progenitor cells

The development of skeletal muscle in vertebrates arises from embryonic and fetal myoblasts that by birth have differentiated into multinucleated, postmitotic myofibers. Postnatal muscle growth and regeneration are largely dependent on satellite cells that reside in a quiescent state between the sarcolemma and muscle fiber basal lamina. A variety of stimuli, such as injury to the myofiber, activate the satellite cells to proliferate and differentiate into mature myofibers. The potential of satellite cells to repopulate and regenerate muscle is quite remarkable, as seen in experimental myotonic injuries where after only 3 to 4 days after complete destruction of myofibers, myogenic progenitor cells have rapidly repaired the damaged muscle with nascent myofibers.

Several lines of evidence indicate that myostatin acts to keep muscle progenitor cells in a quiescent state. Myostatin-coated beads down-regulate expression of myogenic regulatory factors and lead to deficient limb muscle formation in developing chick embryos [13]. Recombinant myostatin inhibits the proliferation and differentiation of stable C2C12 myoblasts, primary bovine myoblasts, and mouse satellite cells in culture [14–18,19•]. Conversely, myoblasts and satellite cells cultured from myostatin null animals proliferate and differentiate more rapidly [18,19•]. A major function of myostatin therefore appears to be to maintain myoblasts and satellite cells in a quiescent state, and when myostatin levels are reduced, such as from injury to the myofiber, these muscle progenitor cells are released from growth arrest.

An intriguing question that has not been fully investigated is whether myostatin also plays a role in determining cell lineage. Animals lacking myostatin have been found to develop less connective tissue [20] and adipose tissue [1,21,22•] as well as increased muscle. In dystrophic animals, fibrosis and fatty replacement are decreased in the absence of myostatin [23]. These findings raise the question of whether a pluripotent progenitor cell having the ability to pursue alternate pathways of myogenesis, adipogenesis, or fibrogenesis is directed away from myogenesis in the presence of myostatin. Potentially in support of such a hypothesis, high concentrations of myostatin recombinant protein applied to multipotent mesenchymal cells C3H 10T(1/2) was recently reported to inhibit myogenesis with decreased expression of myogenic regulatory factors and stimulate adipogenesis with up-regulation of adipogenic markers C/EBPα and adiponectin [24•]. Others have seen only a very small induction of adipogenesis with myostatin compared with other TGF-β family members, such as BMP2 and BMP7, and this question of cell fate will require additional investigation [9].

Muscle regeneration in the absence of myostatin

If a major function of myostatin is inhibition of satellite cells, then in the absence of myostatin, one would anticipate enhanced muscle regenerate. This appears to be true from studies of acute and chronic muscle injury in mice. Muscle from myostatin null animals acutely injured with cardiotoxin express myogenic regulatory factors and regenerate large-diameter myofibers earlier than injured controls with normal myostatin [19•].

Chronic muscle injury is exhibited in the mdx mouse model of muscular dystrophy, where repeated cycles of muscle degeneration and regeneration result from the loss of dystrophin. Limb muscles are only moderately affected, exhibiting excellent regeneration. However, the diaphragm more closely parallels human disease with fibrosis and fatty replacement of muscle over time. Mdx mice genetically lacking myostatin are more muscular with greater functional strength than their mdx counterparts. Diaphragms of 9-month-old and 18-month-old animals show significantly decreased fibrosis in the absence of myostatin, with a virtual absence of fatty replacement [19•,23]. Mdx mice expressing the dominant negative ActRIIB
similarly showed increased muscle mass and decreased interfascicle space [25]. Blockade of myostatin using intraperitoneal injections of myostatin-neutralizing antibodies resulted in increased muscle mass, absolute force, and functional strength, indicating that postnatal inhibition of myostatin alone has measurable effects on muscle growth and regeneration in the mdx model [26].

Myostatin in humans

Myostatin is highly conserved across species with 100% amino acid identity in its active C-terminal domain among mouse, rat, pig, chicken, and human. The function of myostatin as a negative regulator of muscle growth is similarly conserved. ‘Double-muscled’ cattle known for their massive muscle bulk and low fat content have been found to be natural myostatin mutants [27–29]. We have recently described a loss-of-function mutation in the human gene for myostatin associated with gross muscle hypertrophy in a child, suggesting that myostatin also plays an important role in regulating muscle mass in humans [22*].

A German baby came to medical attention because of extensively developed musculature [22*]. Ultrasonography showed that the cross-sectional plane of the patient’s quadriceps muscle was 7.2 SD above the mean value for 10 age-matched and sex-matched controls (6.7 cm² vs 3.13 ± 0.49 cm²). The subcutaneous fat pad was 2.88 SD below the mean value for controls, and there was no difference in the diameter of the femoral bone between the patient and controls. A single base pair substitution at the splice donor site of the first intron was detected in both alleles of the patient and one allele of his mother but not in 200 controls. Expression of genomic wild-type and mutant constructs in vitro showed that approximately 70% of the mutant construct was misspliced with a 108-bp insertion from intron 1 that would predict a premature termination codon. No myostatin protein could be detected from this transcript in vitro or from the child’s serum. The discovery of this human mutation has provided strong evidence that myostatin plays an important role in regulating muscle mass in humans.

The human gene for myostatin resides on chromosome 2q [29,30]. The gene is composed of three exons and two introns and is normally processed to produce a 3.1-kb mRNA expressed in human skeletal muscle [30]. Additional mutations in the human myostatin gene have been sought in association with response to strength training and with strength in the elderly [31,32]. Six missense substitutions in conserved amino acid residues have been identified (A55T, R65H, K153R, E164K, P198A, and I225T) [31,32]. In addition, two polymorphisms within the 5’UTR have been identified [32]. The K153R variant appears to be a common polymorphism, and there are suggestions of association with muscle strength [31,32].

Studies aiming to determine myostatin levels in humans have been hampered in part by very low circulating levels of myostatin in humans, compared with rodents, and difficulties in producing specific antibodies to the myostatin protein, which is not only highly conserved but also highly homologous to another TGF-β family member, growth differentiation factor 11.

Therapeutic approaches

Because the function of myostatin is conserved in humans, there are great expectations that the enhancement of muscle regeneration from myostatin inhibition observed in animal models will apply to human disease. Numerous strategies can be used to develop therapeutic agents to decrease myostatin signaling, including decreasing the biosynthesis of myostatin, blocking myostatin extracellular activity, or inhibiting the intracellular signal transduction. The first therapeutic agents developed to inhibit myostatin signaling have been neutralizing antibodies. In preclinical studies, monoclonal antibodies were developed by immunizing myostatin null mice to recombinant myostatin, and these antibodies were screened by their ability to bind to and inhibit the signaling of myostatin [33]. Wyeth has subsequently developed a humanized, anti-myostatin antibody called MYO-029 and is sponsoring a multicenter phase I/II trial of this myostatin inhibitor in adult patients with muscular dystrophy (www.clinicaltrials.gov). If safe and effective at increasing strength in the setting of muscular dystrophy, MYO-029 or similar agent will hopefully be tested for its ability to stimulate muscle regeneration in a number of other clinical settings including inflammatory myopathies, cachexia, and sarcopenia.

In addition to neutralizing antibodies, there are a number of endogenous inhibitors of myostatin, including the myostatin propeptide, follistatin, FLRG, and GASP-1, which could potentially be modified for use as therapeutic agents. As described above, the myostatin propeptide forms a noncovalent bond with the active C-terminal domain, inhibiting signal transduction until the propeptide is proteolytically processed. Wild-type myostatin propeptide is unstable in vivo, but a propeptide modified so that it is not susceptible to the BMP/tolloid family of metalloproteinases has a dramatic effect on muscle mass when injected intraperitoneally [5]. Mice injected for 4 weeks with 10 mg/kg of the modified propeptide fused to immunoglobulin G (IgG)-Fc had an 18–27% increase in weight of each skeletal muscle examined versus 10–16% muscle weight increase for the JA-16 anti-myostatin antibody and no increase for wild-type propeptide fused to IgG-Fc [5]. When wild-type propeptide fused to IgG-Fc was injected at the same dose into mdx mice, however, significant increases in muscle weights as well as improved pathophysiology were reported [34]. The reason for these apparent contradictory results is not clear; however, the mdx study did involve treatment over a longer time period.
(3 months) and of dystrophic rather than normal muscle. A modified myostatin propeptide or protease inhibitor that specifically blocks the function of the putative metalloproteinase responsible for cleaving the endogenous myostatin propeptide are potential future therapeutic agents.

Follistatin is a secreted glycoprotein that inhibits the activity of a number of TGF-β family members. There is significant data that follistatin is an in vivo inhibitor of myostatin [3,35**]. Follistatin binds myostatin with high affinity, is co-expressed with myostatin in somites, and prevents myostatin-mediated inhibition of limb muscle development in chick embryos [35**]. However, the inhibitory effects of follistatin are clearly not specific to myostatin even in regard to muscle growth. Transgenic mice overexpressing follistatin in skeletal muscle have increased muscle weights of up to 327%, markedly in excess of those in the myostatin null mice, suggesting that follistatin is inhibiting other TGF-β family members in addition to myostatin during muscle growth [3]. If the lack of specificity of follistatin can be circumvented, follistatin may be a powerful target for therapeutic agents. In fact, deacetylase inhibitors, which include the popular drug valproate, up-regulate the expression of follistatin in myotubes and induce myoblast fusion into myotubes [36**]. Alternatively, FLRG and GASP-1, which bind to and inhibit circulating myostatin, may prove to be more specific inhibitors of myostatin for therapeutic use [6,7].

**Conclusion**

Since 1997, when the cloning and characterization of myostatin as a novel TGF-β family member was first published, rapid advances have been made to the point where an inhibitor of myostatin entered clinical trials in early 2005. This is in part due to the appeal of this muscle-specific growth factor that when inhibited produces such dramatic images of increased musculature and in part due to good collaborative efforts between academia and industry. As reviewed in this article, inhibition of myostatin is a promising therapy for muscle regeneration for a number of reasons including myostatin’s specificity for skeletal muscle, its extreme conservation across species and its direct effect on muscle progenitor cells. Although inhibition of myostatin with neutralizing antibodies is the first to enter clinical trials, it may not be the most specific or potent and there will doubtlessly be additional inhibitors to study in the future. Also, myostatin inhibitors may be the first therapies specifically designed for muscle regeneration, but they will not be the cure for most muscle diseases. Myostatin inhibition does not address the underlying pathophysiology of inflammatory myopathies, muscular dystrophies, or cachexia. Effective treatment of these disorders in the future will likely be combination therapies, underlining the importance of private and government funding of multiple strategies to combat muscle disease.

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**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**

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 769—770).


9. A comprehensive and authoritative review of the biosynthesis and signaling pathway of myostatin. Several sites along the pathway are potential targets for therapeutic agents.


Schuelke M, Wagner KR, Stolz LE, et al. Myostatin mutation associated with gross muscle hypertrophy in a child. N Engl J Med 2004; 350:2682—2688. This first characterization of a human myostatin mutation demonstrates that the function of myostatin is conserved. The implication is that the effects of myostatin inhibition, particularly in models of disease, may also be conserved from rodents to humans.


Role of major histocompatibility complex class I molecules in autoimmune myositis
Kanneboyina Nagaraju

Purpose of review
Recent work has continued to clarify the role of major histocompatibility complex class I in the pathogenesis of autoimmune myositis. In the past year, several new observations have been made in this area. This review describes these findings and discusses their relevance to the pathogenesis of autoimmune myositis.

Recent findings
Recent studies have confirmed earlier observations of the up-regulation of major histocompatibility complex class I antigens in myositis. In particular, a recent study has strengthened the conclusion that major histocompatibility complex class I expression is highly specific to inflammatory myopathies and may be of diagnostic value. Two new studies have indicated that endoplasmic reticulum stress response pathway (the endoplasmic reticulum stress response [GRP78]) is highly activated in patients with myositis. One study using transgenic mice has further indicated that abnormal accumulation of major histocompatibility complex class I in the endoplasmic reticulum of muscle may be responsible for the initiation of this endoplasmic reticulum stress response. Furthermore, studies of normal muscle cells have shown that endoplasmic reticulum stress also plays an important role in skeletal muscle development. Investigations of autoantigen expression in myositis biopsies have revealed that regenerating muscle cells express high levels of autoantigens and major histocompatibility complex class I, indicating that these cells are the targets of cytotoxic T-cell attack and may participate in the initiation of a myositis-specific autoimmune response.

Summary
Defining the role of major histocompatibility complex class I in autoimmune myositis may be useful not only for diagnosis of this group of diseases but also for therapeutic opportunities for these difficult disorders.

Keywords
autoimmune response, endoplasmic reticulum stress response, major histocompatibility complex class I, myositis

Introduction
Autoimmune inflammatory myopathies (polymyositis, dermatomyositis, and related diseases) are a heterogeneous group of muscle disorders. The etiology and pathogenesis of these conditions remain unclear [1,2]. Some evidence exists that the muscle fiber injury present may be secondary to an autoimmune process [3–7]. For example, most patients with myositis possess autoantibodies to various antigens of protein translation (e.g., tRNA synthetases and signal recognition particles). The role of autoantibodies in the pathogenesis of myositis, however, is unknown.

The immunophenotypic profiles of the cells found at the various sites differ from one inflammatory myopathy to another [8]. In polymyositis, the endomysial inflammatory aggregate contains a high percentage of T cells, activated CD8+ T cells, very few natural killer cells, and no B cells. Immuno-electron microscopic studies have provided striking evidence for the invasion, replacement, and eventual destruction of nonnecrotic muscle fibers by T cells and macrophages [9,10]. Invasive cytotoxic T cells also express perforin granules, which are characteristically oriented toward the target muscle fiber, indicating that the muscle fiber injury may be partially mediated by perforin-dependent cytotoxic mechanisms [11,12]. In dermatomyositis, there is a high percentage of B cells and CD4+ T cells in the infiltrate. The loss of capillaries in dermatomyositis is attributed to complement-mediated damage.

On the basis of these data, it has been suggested that T lymphocytes (cytotoxic T cells) and humoral (antibodies and complement) effector pathways play a role in the pathogenesis of polymyositis and dermatomyositis, respectively. Several studies have shown that the degree of inflammation varies from patient to patient, and does not correlate consistently with the severity of the structural changes in the muscle fibers and clinical disease, suggesting a potential role for nonimmune mechanisms in the pathogenesis of myositis [8]. Thus, the exact contributions of immune and nonimmune-mediated pathways to muscle damage are still unknown. The developments that have occurred...
during the past year in these areas of research are discussed below.

**Major histocompatibility complex class I**
The major histocompatibility complex (MHC) genes are located on chromosomes 17 (in the case of H-2) and 6 (human leukocyte antigen [HLA]) in mice and humans, respectively. MHC class I molecules are composed of a transmembrane heavy-chain glycoprotein (H), a non-covalently associated soluble protein called β2-microglobulin, and a short peptide of 8–10 residues derived from endogenous proteins. These genes are constitutively expressed in most adult tissues, although the relative levels of class I expression in different tissues vary widely. Higher levels of constitutive expression are seen in tissues and cells of the immune system; in contrast, little or no expression is seen in the brain cortex, cerebellum, sympathetic ganglia, hypophysis, parathyroid gland, thyroid, exocrine pancreas, and skeletal muscle [13,14]. The MHC class I protein superfamily includes not only the classic MHC class I proteins (human HLA-A, HLA-B and HLA-C; mouse H-2K, H-2D and H-2L) that present intracellular antigens to cytotoxic CD8+ T lymphocytes, but also the nonclassic (human HLA-E, HLA-F and HLA-G; mouse Qa1, TL) and MHC class I related chain whose functions are distinct from antigen presentation. Class I-like proteins are often encoded by genes unlinked to the MHC and serve a variety of functions, including presentation of nonpeptide antigens, transport of immunoglobulin across the placenta, and control of iron metabolism [15].

The folding and assembly of class I MHC molecules occurs in the endoplasmic reticulum. The H chain and β2-microglobulin are cotranslationally translocated into the endoplasmic reticulum, where they fold, form intrachain disulfide bonds, and associate with each other. At this point, the complex is relatively thermolabile and is retained within the endoplasmic reticulum. Peptides are transported into the lumen of the endoplasmic reticulum by the transport-associated antigen processing; those with the appropriate sequence motif can bind to the H chain/β2-microglobulin complex. Once the complex has bound a peptide, the association between the H chain and β2-microglobulin is stabilized, and the trimeric complex is released from the endoplasmic reticulum and transported to the cell surface. Several endoplasmic reticulum chaperones (GRP78 [BiP], calnexin, calreticulin, endoplasmic reticulum p57, and Tapasin) associate with MHC class I molecules at different stages during maturation and peptide loading. When the peptide is bound to the MHC complex, all the chaperones dissociate from the MHC. The mature H chain/β2-microglobulin/peptide complex is then allowed to exit the endoplasmic reticulum to the cell surface. Thus, various chaperones have been shown to facilitate folding and assembly and are involved in retaining peptide-empty MHC class I molecules in the endoplasmic reticulum. Prolonged retention of misfolded and incompletely folded proteins in the endoplasmic reticulum leads to their degradation [16–18].

Endoplasmic reticulum-associated degradation is mainly performed by the 26S proteasomes, located in the cytosol. The process occurs in several steps: First, terminally misfolded or unassembled proteins are recognized by endoplasmic reticulum chaperones such as calnexin and BiP or by other factors such as specific mannose lectins. They are then retro-translocated through the Sec61 channel into the cytosol, deglycosylated (in the case of glycoproteins), and polyubiquitinated before proteosomal degradation. Until recently, the machinery involved in the extraction of misfolded proteins from the endoplasmic reticulum was poorly defined. Two independent reports [19,20] have highlighted the role of a mammalian equivalent of the yeast Der1 protein, Derlin-1, in the extraction of aberrantly folded proteins from the mammalian endoplasmic reticulum. It would be interesting to investigate the behavior of these molecules in MHC class I-associated disease conditions such as myositis.

**Major histocompatibility complex class I in autoimmune diseases**
The over-expression of MHC class I molecules is an early event in many autoimmune diseases, particularly in tissues such as muscle, pancreatic β cells, neuronal cells, and thyrocytes that show little or no constitutive expression [21–24]. The over-expression of these molecules can occur in the absence of an inflammatory infiltrate, suggesting that it may be independent of, and possibly precede, the effects of cytokines released from infiltrating mononuclear cells. Transgenic over-expression of MHC class I in these tissues has resulted in the destruction of the target tissue, leading to insulin-dependent diabetes, a shivering phenotype with severe demyelination of the central nervous system, and Graves’ disease in the absence of lymphocyte infiltration [25–27]. These data clearly demonstrate that MHC class I molecules by themselves can have a deleterious effect on some cell types that do not constitutively express these molecules.

**Expression of major histocompatibility complex class I antigens in skeletal muscle cells in vitro and in vivo**
Normal human skeletal myoblasts constitutively express low levels of HLA class I molecules under cell culture conditions [28,29]. Prolonged expression of MHC class I antigens is associated with an increase in the expression of MHC class I antigens on muscle cells. IFN-γ induces a stronger level of expression than do the other cytokines, and TGF-β reduces the basal levels of HLA class I expression [28]. Muscle fibers in normal individuals do not express detectable levels of MHC class I antigens [29,30];
however, several studies have shown that in individuals with autoimmune muscle diseases, the myofibers can indeed express MHC class I [24,31] with the staining observed in both the sarcolemma and sarcoplasm. The expression in some patients was restricted to a few clusters, whereas in others, almost every fiber was positively stained [32]. Generally, all of the invaded muscle fibers and some of the noninvaded fibers show MHC class I reactivity [33]. In contrast, MHC class I expression was not detected on the muscle fibers of patients with neurogenic atrophy, distal myopathy with rimmed vacuole formation, or acid maltase deficiency [34].

The potential diagnostic value of MHC class I staining in autoimmune myositis was recently examined in 61 patients with inflammatory myopathies, 69 patients with muscular dystrophies, and 20 healthy controls [35]. A diffuse pattern of staining was observed in all samples, showing positive staining of the sarcolemma. Expression of MHC class I was found in 67% of the muscle biopsies from patients with dermatomyositis, in 61% from those with polymyositis, and in 96% from those with inclusion body myositis. Expression was not detected in patients with congenital or metabolic myopathies or neurogenic disorders, or in the healthy controls. Expression of MHC class I was found in only 11% of the biopsies from patients with muscular dystrophy and in 4% of those with miscellaneous neuromuscular disorders. The sensitivity for diagnosing inflammatory myopathies using the detection of MHC class I expression on the sarcolemma was 78% (95% confidence interval [CI], 66–88%), with a specificity of 95% (91–98%). The sensitivity for tests conducted before starting immunosuppressive treatment was 89% (76–96%). The sensitivity was unaffected by the inclusion of all patients who had been on immunosuppressive treatment for less than 4 weeks before muscle biopsy (90% [79–97%]). A particularly interesting finding was the observation that six patients who fulfilled the established diagnostic criteria for idiopathic inflammatory myopathies had no inflammatory infiltrates in their muscle biopsies; however, all the biopsies stained positive for MHC class I antigens, suggesting a nonimmune role for class I MHC [35*]. The expression of a nonclassic MHC class I antigen, HLA-G, in muscle fibers in various inflammatory myopathies has been reported; the distribution of this antigen closely resembled that of classical HLA-A, B, and C antigens in muscle fibers, infiltrating mononuclear cells, and capillaries [36]. These results suggest that both classic and nonclassic MHC molecules may play a role in the pathogenesis of inflammatory myopathies.

**Role of major histocompatibility complex class I in pathogenesis**

The role of MHC class I molecules has been further evaluated in a recently developed mouse model in which syngenic mouse MHC class I (H-2Kb) antigens are conditionally up-regulated in skeletal muscle. The overexpression of MHC class I molecules in skeletal muscle leads to muscle fiber damage, with the clinical, biochemical, histologic, and immunologic features of human myositis [37]. The following observations in human patients with myositis and in the mouse model of myositis suggest that MHC class I molecules themselves may potentially mediate muscle fiber damage and dysfunction in the absence of lymphocytes:

1. Induction of MHC class I antigens in muscle fibers precedes the inflammatory cell infiltration in myositis [38].
2. MHC class I staining of human myositis biopsies reveals both cell surface staining and a sarcoplasmic reticulum pattern of internal reactivity [32,39,40], suggesting that some of the MHC class I molecules may be retained in the endoplasmic reticulum–Golgi network in these fibers.
3. Persistent MHC class I over-expression occurs in muscle fibers in the absence of any inflammatory infiltrate (as assessed by immunohistochemistry, histology, and magnetic resonance imaging) [41].
4. The MHC expression is seen early in the disease process, before any inflammatory infiltrate is present [42].
5. MHC class I transgenic mice show muscle weakness before mononuclear cell infiltration [37].
6. Gene transfer of MHC class I plasmids in vivo and in vitro attenuates muscle regeneration and differentiation [43].

Taken together, these observations indicate that the muscle fiber damage seen in myositis may be mediated not solely by immune attack (e.g. cytotoxic T lymphocytes and autoantibodies) but may also be mediated through nonimmunologic mechanisms such as the endoplasmic reticulum stress response. In a recent study, we have demonstrated that the endoplasmic reticulum stress response, the unfolded protein response (glucose-regulated protein 78 pathway), and the endoplasmic reticulum overload response (NF-κB pathway) are significantly activated in muscle tissue of human patients with myositis and in the mouse model [44**]. These pathways may play a significant role in the induction of self-sustaining disease and the loss of skeletal muscle mass in myositis. Future experiments should define the exact contribution of these pathways to the skeletal muscle damage and dysfunction associated with autoimmune myositis. Earlier, independent studies conducted in sporadic inclusion body myositis have also shown that endoplasmic reticulum chaperones (calnexin, calreticulin, GRP94, BiP/GRP78, and endoplasmic reticulum p72) are not only increased but also colocalized with amyloid-β in the inclusions, suggesting that unfolded protein response plays a role in the pathogenesis of other members of this group of diseases [45**]. Another
recent study [46\*] has shown that endoplasmic reticulum stress signaling, especially that mediated by activating transcription factor 6, plays a role in the induction of developmental apoptosis in muscle tissues through activation of caspase-12. The results of this study also demonstrated that induction of endoplasmic reticulum stress-responsive proteins (BiP and CCAAT/enhancer-binding protein (C/EBP)-homologous protein) occurs in both apoptotic and differentiating muscle cells. Regeneration of muscle cells is a prominent feature of inflammatory myopathies, and the endoplasmic reticulum stress response in diseased muscle is apparently a reflection of ongoing developmental and cell death processes.

**Role of major histocompatibility complex class I in autoimmune response**

Unique autoantibody specificities are strongly associated with distinct clinical phenotypes. For example, in myositis, muscle cells are the target, and the autoantibodies against aminoacyl-tRNA synthetases, the signal recognition particle, or the Mi-2 nuclear protein (ubiquitous antigens) that occur in a significant proportion of cases are highly specific for myositis. Recently, our group investigated the mechanisms underlying this striking association. We found that autoantigen expression (protein DNA-dependent protein kinase, histidyl-tRNA synthetase, and Mi-2) is significantly increased in myositis but not in normal muscle and is restricted to regenerating muscle cells. This pattern of expression in myositis muscle is strikingly reminiscent of MHC class I expression [47**]. It is likely that cytotoxic lymphocytes target MHC class I expressing regenerating muscle cells and initiate a feed-forward cycle of muscle injury that is focused in regions of muscle regeneration. The uniform susceptibility of myositis autoantigens to cleavage by granzyme B [48] and the marked enrichment of activated, oligoclonal CD8+ T cells in dermatomyositis, polymyositis, and juvenile dermatomyositis muscle [11,49] further strengthen these novel observations [47**].

**Possible mechanisms of muscle cell damage in myositis**

At this time, it is not clear what factors initiate MHC class I expression in myositis. A number of different events as diverse as denervation, cytokine or chemokine stimulation, and viral infection can lead to transient changes in the expression of MHC on the surface of muscle cells. Antecedent infection or muscle injury is not usually followed by sustained myositis, so one may speculate that only prolonged up-regulation of MHC class I expression, perhaps only in individuals with certain genetic backgrounds, provokes self-sustained inflammation. We propose that MHC class I molecules may mediate muscle fiber dysfunction and damage through immune/extrinsic and nonimmune/intrinsic mechanisms (Fig. 1).

**Figure 1. Immune and nonimmune pathways may contribute to the damage and dysfunction of major histocompatibility complex class I-expressing muscle fiber.**

The damage to major histocompatibility complex class I-expressing muscle fibers may occur through immune (cytotoxic T-cell (CTL)–mediated death [e.g. granzyme B], cytokine [e.g. tumor necrosis factor-\(\alpha\)], or anti-class I antibodies) or nonimmune (endoplasmic reticulum overload response [EOR], unfolded protein response, and/or growth factor depletion) pathways. These pathways are known to induce cell death via autophagy or apoptosis or necrosis or a combination of the three.

**Immune/extrinsic mechanisms**

The muscle cell damage may be mediated by CD8+ cytotoxic T lymphocytes, because MHC class I molecules are involved in the presentation of endogenous antigens to these cells. It might be expected that these cells could play an important role in provoking an autoimmune disease process. The overexpression of transgenic class I MHC may trigger anti-class I antibody synthesis, and the stimulation of MHC on muscle cells by these antibodies may impair muscle cell function and viability. Indeed, the ligation of MHC class I antigens by anti-MHC class I antibodies on lymphocytes has been shown to induce intracellular signaling events that lead to growth inhibition and apoptosis [50,51]. The tissue damage and secretion of proinflammatory cytokines into the milieu may activate macrophages, and these in turn may be involved in both damage and clearance of cellular debris.

**Nonimmune/intrinsic mechanisms**

MHC class I overexpression may interfere with the synthesis or transport of proteins that are critical for cell survival, or improper assembly and export of class I molecules caused by limitations in the available concentration of native \(\beta\)-2-microglobulin may result in stagnation and stress in the endoplasmic reticulum, which can impair cellular function. By displacing \(\beta\)-2-microglobulin, the over-expressed MHC class I \(\alpha\) chains may structurally associate with insulin receptors and interfere with insulin-mediated survival signals in the muscle cells [52]. Indeed, MHC molecules have been shown to interact noncovalently with
membrane receptors for insulin, glucagon and epidermal growth factor [33,34]. Therefore, the inappropriate expression of MHC molecules could interfere with key functions of other molecules and ultimately impair cell viability. These and other unknown mechanisms may be simultaneously operating both in the mouse model and in human inflammatory myopathies.

Conclusion
The role of MHC class I antigens in myositis pathogenesis is fascinating. Recent evidence affirms that these antigens play important roles in the initiation and perpetuation of the auto-inflammatory response in myosis, and future investigations may yield unexpected surprises.

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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
Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 770).

4 Miller FW, Waite KA, Biswas T, Plotz PH. The role of an autoantigen, histidyl-tRNA synthetase, in the induction and maintenance of autoimmunity. Proc Natl Acad Sci U S A 1990; 87:9933——9937.
36 Study suggests that detection of sarcolemmal MHC is a valid diagnostic test for inflammatory myopathies.
44 Nagaraju K, Casciola-Rosen L, Lundberg I, et al. Activation of the endoplasmic reticulum stress response in autoimmune myositis: potential role in muscle fiber damage and dysfunction. Arthritis Rheum 2005; 52:1824–1835. This study demonstrates that endoplasmic reticulum overload (NF-kB) and unfolded protein response pathways are highly activated in human myositis and in the MHC class I transgenic mouse model of myositis.
45 Vattemi G, Engel WK, McFerrin J, Askanas V. Endoplasmic reticulum stress and unfolded protein response in inclusion body myositis muscle. Am J Pathol 2004; 164:1–7. This study demonstrates that the endoplasmic reticulum chaperone (calnexin, calreticulin, GRP94, GRP78, and endoplasmic reticulum p72) expression is increased in sporadic inclusion body myositis muscle, and these chaperones are associated with amyloid-β precursor protein.
46 Nakanishi K, Sudo T, Morishima N. Endoplasmic reticulum stress signaling transmitted by ATF6 mediates apoptosis during muscle development. J Cell Biol 2005; 169:555–560. This study shows that endoplasmic reticulum stress is up-regulated during non-pathological conditions such as myoblast differentiation.
Cytotoxic T lymphocytes and autoimmunity
Patrick Blancoa,b,c, Jean-François Viallard, Jean-Luc Pellegrina and Jean-François Moreaub,c

Purpose of review
The possibility of the recognition by cytotoxic T lymphocytes of tissue autoantigens has been largely ignored in explaining organ-specific autoimmune diseases. Recent advances in the understanding of human leukocyte antigen class I-binding peptides motifs have led to the detection and the characterization of those autoreactive CD8+ cytotoxic T lymphocytes involved in organ-specific autoimmune diseases. The purpose of this review is to discuss recent studies that shed light on the implication of cytotoxic T lymphocytes in several autoimmune disorders as well as the mechanisms underlying their stimulation.

Recent findings
Significant progress has been made in the characterization of autoantigens targeted by cytotoxic T lymphocytes in several class I-restricted autoimmune diseases, including Behcet’s disease and ankylosing spondylitis, and their implication in systemic autoimmune disease such as systemic lupus erythematosus. Moreover, the signals involved in the activation of autoreactive cytotoxic T lymphocytes have been better characterized, particularly the molecular requirements of the antigen presentation at the surface of the dendritic cell system, mainly because of a better understanding of the Toll-like receptor—induced signals or the discovery of a defect in regulatory T cells.

Summary
New findings in the pathophysiology of cytotoxic T lymphocytes in autoimmunity and especially a better comprehension of their activation may give a new impetus for the development of targeted immunologic therapies in various autoimmune disorders.

Keywords
autoimmunity, cytotoxicity, lupus (systemic lupus erythematosus), T-lymphocytes

Introduction
Cytotoxic T lymphocytes (CTLs) are the guided missiles of the immune system because of their well known role in the destruction of targeted cells including virally infected cells as well as tumoral cells. The direct cytotoxic damage to the target cells could be the result of several mechanisms involving CD95 or the perforin/granzyme system as well as the production of effector cytokines such as TNF-α or IFN-γ [1]. This effector step follows the specific recognition by the T lymphocyte T-cell receptor of major histocompatibility complex class I molecules loaded with the relevant antigenic peptide, expressed at the surface of the target cells. By their lytic capacity, these cells may well represent key effectors in various autoimmune diseases. Delineating their precise roles and functions in autoimmunity therefore remains an important challenge that widens the number of the putative targets for a selective immuno-therapeutic approach [2].

The attractive hypothesis that CTLs can target tissue autoantigens directly and induce organ-specific autoimmune diseases is based on recent advances in the understanding of HLA class I-binding peptides motifs that have enabled detection, monitoring, and characterization of the autoreactive CD8+ cytotoxic T lymphocytes involved in organ-specific autoimmune diseases [3]. Not only CTLs are able to destroy target cells, but also they do so through qualitatively different mechanisms that may end up with specific apoptosis products favoring the breakdown of tolerance against nuclear components, a feature that has mainly been implicated in systemic autoimmune diseases [4].

On the basis of a recent review of the literature and results obtained by our group, we attempt to pinpoint the most recent observations documenting the direct implications of CTLs in various autoimmune diseases, including systemic autoimmune diseases such as systemic lupus erythematosus. In addition, we summarize recent findings that may explain why and/or how those CTLs are activated to lead to autoimmunity.

Cytotoxic T lymphocytes induce tissue damage
If the implication of CTLs in various autoimmune diseases including type I diabetes, thyroiditis, and polymyositis [5] is well known and well documented, recent reports have directly implicated CTLs in other autoimmune diseases, particularly in diseases characterized by a known genetic linkage with class I major
histocompatibility alleles. Yasuoka et al. [6] were first to report on Behcet’s disease characterized by an increase of autoreactive CTLs directed at a transmembrane peptide of the stress-inducible antigen major histocompatibility complex class I chain-related gene A (MIC-A) expressed on epithelium and endothelium and presented to autoreactive CTLs in the context of HLA-B51. In this setting, the role of MIC-A would be indirect, but nonetheless, MIC-A autoreactive CTLs could lead to an excessive and prolonged inflammatory response at a site of stress. Interestingly, another group found that the aqueous humor from Behcet’s uveitis (and none from other etiologies of uveitis) was characterized by the predominant presence of activated CD8+ T lymphocytes [7], and more generally, peripheral blood CD8+ T lymphocytes are found to exhibit an activated phenotype in patients with active Behcet’s [8].

Another situation in which a class I major histocompatibility complex allele is associated with disease is ankylosing spondylitis. HLA-B27 restricted peptides derived from type II collagen and type IV collagen were found to be stimulatory for CD8+ T cells in four of seven patients with ankylosing spondylitis. In this report, the investigators used an HLA-B27-binding and proteasome-cutting prediction program for the human cartilage proteins, selecting for potentially immunogenic nonamer peptides bound to the B27 allele. Although their results suggest that cartilage cellular autoimmunity might play an important role in joint-specific tissue damage in patients with ankylosing spondylitis, they did not perform any in-vitro studies to test the ability of CD8+ T cells to become cytotoxic against appropriate cell lines [9].

In multiple sclerosis, in which CTLs have long been suspected to be causative, CTLs have received a recent increase of interest as effectors of the pathologic immune reactions that damage central nervous system in an animal model as well as in humans [10]. CD8+ T-cell clones could be found in the brain, the blood, and the cerebral spinal fluid of different patients. Moreover, T-cell clones that infiltrate central nervous system could persist in the cerebral spinal fluid and blood for many years as clonal expansions. The antigen specificity is not known, but one can argue that central nervous system autoantigens including myelin antigens could be the target for those CTLs, particularly because in another study, patients with multiple sclerosis were found to be characterized by a high prevalence of autoreactive neuroantigen-specific CD8+ T cells [11]. In agreement with these data, we detected a high proportion of activated CD8+ T lymphocytes expressing perforin and granzyme B in the blood of a group of 61 patients with systemic lupus erythematosus (SLE). This T-cell subpopulation increase correlated with disease activity as assessed by systemic lupus erythematosus disease activity index, and those lymphocytes were functional. In an in-vitro nonspecific killing assay, large amounts of soluble nucleosomes as well as unique granzyme B autoantigenic fragments, previously described by Casciola-Rosen et al. [12,13], could be generated by the fresh cells isolated from a patient’s blood. In addition, neuropsychiatric SLE patients (NPSLE) with white matter lesions were found to have an increase of myelin autoreactive CD8+ T lymphocytes circulating. In none of these patients was NPSLE related to a thrombotic event in the context of an antiphospholipid syndrome, whereas the steroids or cyclophosphamid given as therapy resulted in the disappearance of those cells as well as the stabilization of the lesion (manuscript in preparation). In addition, in SLE nephritis biopsies, we found the CD8+ T lymphocytes to be the major infiltrating mononuclear cells located in the interstitium and in the peri-glomerular area (unpublished observation). Although those results could appear surprising on the basis of the dogma that autoantibodies and immune complexes are the key effectors in this type of disease, they could be balanced by the recent data obtained in Goodpasture’s disease, another classic antibody-mediated autoimmune disease. The kidney insults can be reproduced by the passive transfer of heterologous antibody from humans to new world monkeys, and plasmapheresis represents the standard treatment for this disease. Observational human studies and data gathered from experimental systems suggest a role for cell-mediated effector injury, however. The reality of T-cell involvement was brought by inducing the disease through the immunization of mice deficient for B cells and immunoglobulin with a3-a5(IV)NC1 [14]. Beyond the conclusion that effectors cells including CTLs are at work in experimental autoimmune Goodpasture’s disease, those results suggest that one should be cautious in delineating autoimmunity as antibody-mediated immune disease or cellular-mediated immune disease, because both may well coexist.

Once sufficient clues indicating that CTLs are involved in autoimmune diseases have been collected, the major next step would be to understand why those autoreactive T cells have been activated.

**Mechanisms of activation of cytotoxic T lymphocytes in autoimmune diseases**

Every healthy individual has autoreactive T cells; however, the vast majority will not develop an autoimmune disease, and the answer to this question may be environmental through sophisticated pathways. As an example, elegant studies led to the conclusion that signaling through TLRs in different autoimmune diseases, including type I diabetes and lupus, could trigger autoimmunity following several mechanisms (cross-presentation, enhancement of lytic function, overexpression of MHC class I molecules, and so forth) [15–17]. In a mouse model of type I diabetes, TLR3 stimulation (i.e. TLR3 and 7) triggered autoimmune disease, and IFN-α was the key cytokine by its
capacity to activate cellular immune response [18**]. Those results fit very well with the fact that viral infections shortly precede or coincide with the development of autoimmunity and that type I interferon may represent a valuable target for immunotherapeutic intervention in autoimmune diseases [19].

It is currently believed that dendritic cells, which are tolerogenic when they migrate and present self-antigens in a basal state, acquire the necessary properties to activate immune cells when they mature in response to infection or injury [20]. In a double-transgenic mouse model, however, in which peripheral tolerance was disrupted and animals developed lethal CD8+ T-cell–dependent autoimmune disease, the Langerhans cells, a subset of dendritic cells residing in the epidermis, were unique in their ability to present self-antigens and promote autoimmunity as they trafficked to lymph nodes at the basal state [21*]. Even though more work is needed to address the relevance of this series of findings in human diseases, it may open a new area of investigations on the real implications of those dendritic cells in various autoimmune diseases, including skin autoimmune disease.

**Decrease of regulation**

The first hint that tolerance could be a result of an active mechanism at an effector stage came from the earlier studies on allograft rejection. Later, Sakaguchi et al. [22] showed that the depletion of the CD4+CD25+ subset of cells was sufficient to cause the onset of systemic autoimmune diseases in mice, giving the formal proof of the existence of suppressor T cells. Furthermore, cotransferring these cells along with CD4+CD25+ T cells prevented the development of experimentally induced autoimmune diseases such as colitis, gastritis, type 1 diabetes, or thyroiditis. Since this pioneering work, it has been conceded that several types of regulatory T cells exist. The so-called natural CD4+CD25+ regulatory T cells are thought to arise during the normal process of T-cell maturation in the thymus and strongly suppress the proliferation of responder T cells on coculture. Other species of inducible regulatory T cells such as Tr1 or T helper type cells can develop from conventional CD4+ T cells that are exposed to specific stimulatory conditions including blocking of costimulatory molecules, deactivating cytokines [23,24]. As a result of the crucial role of those negative regulators on the effector functions of the immune system, their alterations, either quantitative or qualitative, have been directly implicated in the pathogenesis of several autoimmune disorders [25–30]. One of the first studies to describe such an alteration at work in a disease has been conducted in patients with multiple sclerosis and showed a significant reduction in effector functions of the regulatory T-cell population in patients with multiple sclerosis compared with healthy individuals. Through the use of different stimulatory conditions such as varied concentrations of plate-bound anti-CD3, the authors drew the conclusion that the defect found in the regulatory function of CD4+CD25+ T cells could affect only the autoreactive T cells from autoimmune patients, compared with T cells stimulated by microbial antigens from infected patients [27]. The story may even be more subtle, because it was reported in active rheumatoid arthritis. In this study, the authors did not find any reduction in regulatory T cell numbers in patient with active rheumatoid arthritis compared with the healthy individuals. The regulatory T cells were able to suppress the proliferation of effector T cells; however, they did not suppress the cytokine production from effector T cells including TNF-α and IFN-γ. Interestingly, they showed that this phenotype was partially reverted by anti-TNF therapy [28]. Obviously, the dissection of the molecular mechanisms underlying these observations may help decipher new therapeutical approaches in patients with RA by specifically targeting regulatory T cells, which is partially achieved by giving patients anti–TNF-α monoclonal antibody.

**Conclusion**

As we understand better the pathways underlying the cellular effector mechanisms operating in autoimmunity, we will get better handles to design targeted immunotherapeutic strategies to treat autoimmune diseases. Recent studies on CTLs and autoimmunity have helped us understand the autoantigens recognized by autoreactive CTLs, but also, and maybe more importantly, they have helped us understand why they were activated. In this regard, the interplay among CTLs, dendritic cells, and regulatory T cells is likely to be the main focus of interest for the next years.

**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

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 770).


This study shows that autoimmune diabetes in a mouse model is exacerbated by signaling through Toll-like receptors. These results emphasize the notion that a viral trigger could help set off autoimmune disease. In addition, they provide some clues of how this might happen at a molecular level.


This study shows that Langerhans cells do not have tolerogenic properties in the steady state. This is a new concept in terms of generation of autoimmunity, with the implication of unactivated DC in autoimmune responses.


OBITUARY

Joseph H. Korn, MD
1947–2005

Remembering Joe Korn
The international scleroderma community lost a leader and friend with the death of Joseph H. Korn, MD, at the age of 58. Joe died after a brief struggle with cancer. Joe’s influence on scleroderma research and on the lives of his colleagues, trainees, and patients has been enormous.

Career
Joe was born in a camp for displaced persons in Augsburg, Germany, to Polish Jewish parents who survived the Holocaust. The family arrived in New York in 1948 and started a new life. Joe adapted enthusiastically to his new homeland. An outstanding student, he graduated from the City College of New York magna cum laude in 1968 and then pursued his dream of becoming a doctor by enrolling in the College of Physicians and Surgeons of Columbia University. On receiving his MD, Joe headed south for internship and residency in internal medicine at the University of North Carolina. Another move followed when he became a Rheumatology Fellow at the Medical University of South Carolina in Charleston. The training program had been just established there under the directorship of the E. Carwile LeRoy. The meeting with Dr LeRoy was serendipitous, sparking Joe’s lifelong interest in scleroderma and connective tissue research. On completion of his fellowship, Joe secured an academic appointment at the University of Connecticut School of Medicine in Newington, Connecticut, where, except for a sabbatical at the Weizmann Institute for Science in Rehovot, Israel, he remained for 15 years, rising to full Professor and Chief of Rheumatology at the VA Medical Center. In 1993, Joe was named the Alan S. Cohen Professor of Medicine and Chief of Rheumatology at Boston University. Under his skilled leadership, the Rheumatology Section emerged as a leading center for scleroderma research. Joe recruited talented young physician-scientists, sparked their interest in scleroderma, and guided their development as independent investigators. They now carry forward the research he started.

Joe was a man of enormous gifts and a passion for life. He took pride and pleasure in his family, friends, profession, and work. He was a caring physician, an inspiring teacher, a curious scientist, an energetic organizer, an engaging colleague, and a dependable friend; and more, throughout his life he taught and modeled common sense, fairness, compassion, and integrity. Yet, despite his impressive professional and personal accomplishments, and numerous titles and accolades, Joe was a modest and unpretentious man.

Influence on scleroderma
Joe Korn’s effect on scleroderma research is virtually unmatched. During a career spanning 3 decades, Joe published over 100 original papers, reviews, and book chapters related to the pathogenesis and treatment of scleroderma, and he was a guiding force behind the creation and successful evolution of the International Scleroderma Workshops.

Throughout his professional life, Joe’s research explored the interface between inflammation and connective tissue in health and in scleroderma. He pioneered the study of immunity and inflammation in the context of fibroblast biology. In a series of early papers initiated in Carwile LeRoy’s laboratory, he showed that inflammatory prostaglandins suppressed fibroblast growth and collagen synthesis. These studies took advantage of then-innovative experimental techniques for propagating skin fibroblasts in...
culture. Joe further pursued the link between immune cells and fibroblasts by showing, with Terry Piela-Smith and Barry Gruber, the importance and mechanisms of fibroblast interactions with T cells and mast cells. Starting in the 1980s, Joe described and characterized the phenomenon of fibroblast heterogeneity. In collaboration with Steven Goldring and Ante Jelaska, he showed that individual fibroblasts were markedly divergent in prostaglandin synthesis, collagen production, and sensitivity to apoptosis. In subsequent studies focusing on scleroderma fibroblasts, with Carol Black, David Abraham, and Keith Elkon, Joe demonstrated intrinsic abnormalities in extracellular matrix synthesis, apoptosis, and gene expression that contribute to the pathogenesis of fibrosis.

Well grounded in science and always exploring new opportunities, Joe was among the first to recognize the potential of the genomics revolution to accelerate scleroderma research. In ambitious studies pursued with David Strehlow and Humphrey Gardner, Joe pioneered the use of transcriptional profiling to illuminate the mysteries of the scleroderma fibroblast. In another line of investigation, working with Robert Lafyatis, Joe pursued the molecular basis of scleroderma in animal models, focusing on the Tsk1 mouse and the roles of fibrillin and fibulin in fibrosis. In the 1990s, together with Robert Simms, Peter Merkel, and other colleagues at Boston University, Joe initiated studies focusing on the evaluation, outcome, and management of scleroderma. As a member of the Scleroderma Clinical Trials Consortium, Joe played a major role in the design, performance, and interpretation of the results of clinical trials of iloprost, relaxin, cyclophosphamide, bosentan, and anti-transforming growth factor-β antibody. Some of these interventions have since been incorporated into the therapeutic armamentarium and are now improving the lives of patients with scleroderma. Joe’s was a consistently productive research career marked by thematic consistency, eagerness to explore novel concepts and apply innovative methodologies, commitment to high quality science, and a wonderful ability to collaborate.

In addition to this significant body of scleroderma research, Joe contributed mightily to the field as an enthusiastic teacher and engaging speaker. He presented seminars, updates, grand rounds, and review courses all over the world, infusing his lectures with humor, erudition, and deep understanding. Joe was a superb spokesman for scleroderma, articulating the research challenges and opportunities to his colleagues, to study sections, and to lay audiences. He played a huge role in changing public perceptions about scleroderma as a mysterious and untreatable disease and in prodding drug companies to embrace scleroderma as a potential therapeutic target.

Together with Professor Black, now President of the Royal College of Physicians, Joe was the founder of the International Scleroderma Workshops, linking scleroderma researchers in the United States and Europe. From its modest beginning with 25 interested colleagues gathered in a Chicago hotel, the workshops evolved to include over 150 international participants. The workshops, held every other year at locations alternating between England and the United States, have become recognized for introducing the latest research advances to the scleroderma research community, engaging with those outside the field, and promoting innovative thinking. From these workshops have come many collaborative research projects and many long-lasting friendships connecting colleagues across the world. Joe’s personal warmth and people skills were never more in evidence than at these forums. Carol and Joe, as co-organizers, never had a cross word between them; they enjoyed working together, providing mutual support and friendship that lasted over 20 years. Two days before Joe died, the Council of the Royal College of Physicians made him a Fellow in acknowledgment of his international endeavors on behalf of patients with scleroderma. Sadly, Joe never came to know of this singular honor, the highest that the College can bestow.

Joe Korn was a giant in the field. He leaves behind a memorable personal and scientific legacy, and the determination of those who were privileged to work with him to fulfill his vision of finding the pathologic bases of scleroderma, so that effective treatments be developed for what Osler described as ‘that most terrible of human diseases.’

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New developments in scleroderma interstitial lung disease
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Purpose of review
To review the recent medical literature pertaining to interstitial lung disease found in association with systemic sclerosis remains a major contributor to morbidity and mortality. Significant progress is being made in terms of understanding the pathogenesis, the best approaches to clinical evaluation, and various options for therapy of systemic sclerosis patients whose disease course is complicated by interstitial lung disease.

Recent findings
Recent studies highlight the importance of microvascular disease, autoimmunity, and fibroblast differentiation/activation in the pathogenesis of systemic sclerosis-interstitial lung disease, particularly in the early phase of disease. It appears as if the balance between various pro-fibrotic/pro-inflammatory and anti-fibrotic/anti-inflammatory mediators may be central to interstitial lung disease pathogenesis, which presents potential opportunities for therapeutic intervention. The clinical approach to staging of disease activity remains controversial. High resolution computed tomography scans, bronchoalveolar lavage and various serum markers (e.g., surfactant protein D and KL-6) each may provide useful information about the degree of activity of the systemic sclerosis-interstitial lung disease. Currently, treatment recommendations are limited by a scarcity of well designed clinical trials, but the recently completed Scleroderma Lung Study is a model for future studies and is providing useful information about this important complication of systemic sclerosis.

Summary
Basic and clinical studies of systemic sclerosis patients with interstitial lung disease are yielding promising data that ultimately will be translated into more effective diagnostic and therapeutic strategies.

Keywords
interstitial lung disease, pulmonary fibrosis, scleroderma, systemic sclerosis

Introduction
Clinically significant interstitial lung disease (ILD) is observed in approximately 40% of patients with systemic sclerosis (scleroderma, SSc) and is a leading cause of morbidity and mortality. To summarize the recent literature on scleroderma interstitial lung disease, we searched the MEDLINE database from January 1, 2004 through May 31, 2005, using the following free-text search words: scleroderma, systemic sclerosis, interstitial lung disease, pulmonary fibrosis, pulmonary, or lung. The search was limited to the English language.

Pathogenesis
The pathogenesis of SSc-ILD is complex and involves fundamental abnormalities in fibroblasts, endothelial cells, and cells of the immune system. These alterations often result in severe and progressive fibrosis, widespread vascular and microvascular disease, and humoral and cellular immunologic abnormalities, which include the production of autoantibodies, cytokines, chemokines and growth factors.

Histopathology
In a study [11] of postmortem SSc lung tissue, two major types of cellular abnormalities were seen in the early active stages of SSc lung fibrosis; one was the induction of a large number of smooth muscle α-actin-positive myofibroblasts in the interstitium and the other was excessive formation of irregularly shaped alveolar capillaries (neovascularity) accompanied by an increase in the number of microvascular endothelial cells. However, as fibrosis progresses to end-stage lung disease, the population of
myofibroblasts and capillary endothelial cells declines. These temporally progressive abnormalities suggest that myofibroblast proliferation and vascular abnormalities may provide key information relevant to the etiology and/or pathogenesis of SSc-ILD (Fig. 1).

**Autoantibodies**

Antinuclear antibodies (ANA) are detected in more than 90% of patients suggesting that systemic autoimmunity is a central feature of SSc [2]. Anti-topoisomerase I antibody levels correlate closely with disease activity and severity and are associated with ILD [3]. Anti-topoisomerase I has been shown [4•] to display antifibroblast antibody activity by reacting with determinants at the fibroblast cell surface. Antifibroblast antibodies (AFA) were characterized in sera from 99 patients with SSc and 30 age- and sex-matched healthy controls. Patients with SSc had significantly higher IgG AFA levels compared with healthy controls \( (P < 0.05) \). High titer AFA was associated with increased frequency of pulmonary involvement (restriction \( P < 0.005 \); diffusion for carbon monoxide [DLCO] <60% predicted \( P < 0.02 \)). Patients who were AFA-positive at diagnosis had an increased risk of death (odds ratio 3.47, 95% confidence interval [CI] 1.35–8.9). There was a striking correlation between the fibroblast-binding activity of these AFAs and the presence of anti-topoisomerase I in SSc sera \( (P < 0.0001) \). The AFA activity was directly mediated by anti-topoisomerase I autoantibodies that were capable of recognizing and binding to a fibroblast surface antigen.

Other autoantibodies that have been recently examined in SSc include anti-matrix metalloproteinases (MMPs) and rheumatoid factor. Connective tissue homeostasis is a balance between the synthesis and degradation of the extracellular matrix (ECM). ECM degradation is regulated mainly by MMPs. In serum samples of 58 patients with SSc [5], IgG anti MMP-3 was significantly elevated compared with normal controls \( (P < 0.0001) \) and correlated with fibrosis of the skin \( (P \leq 0.001) \), lung \( (P \leq 0.05) \) and renal vasculature \( (P \leq 0.05) \). IgG anti MMP-3 antibodies inhibited MMP-3 activity, suggesting that anti-MMP-3 autoantibodies may contribute to the development of fibrosis by inhibiting MMP-3 activity and reducing the turnover of ECM.

In a study [6] measuring rheumatoid factor (RF) isotypes in 79 serum samples from patients with SSc, the levels of IgM-RF, IgG-RF, and IgA-RF were significantly higher in patients with SSc than in healthy controls. The prevalence of ILD was significantly greater in patients with elevated IgA-RF levels than in those with normal levels (56% versus 26%, \( P < 0.05 \)). In addition, the prevalence of an abnormal vital capacity (<80% predicted) was also significantly greater in patients with elevated IgA-RF than those with normal levels (50% vs. 11%, \( P < 0.05 \)).

**Cytokines and chemokines**

There is a growing body of evidence to suggest that perturbed immunoregulation is involved in the pathogenesis of SSc, and dysregulation in production of soluble factors may play a central role. Among these factors, chemokines and cytokines are important mediators in modulating leukocyte-endothelial interactions [7•, 8–10]. Serum samples, activated peripheral blood mononuclear cells and
T cell lines obtained from 54 SSc patients and 20 healthy donors were examined for representative Th-1 type cytokines (IFN-γ and IL-18), Th-2 type soluble factors (CD30s and IL-4), regulatory (IL-10 and transforming growth factor-β1), inflammatory cytokines (IL-6 and TNF-α), and C-C chemokines (macrophage derived chemokine, monocyte chemotactic protein [MCP-1], macrophage inflammatory protein-1α and regulated on activation, normal T-cell expressed and secreted [RANTES]) [7*]. Multiple linear regression analysis revealed a significant correlation between ILD (forced vital capacity [FVC] < 70% predicted or abnormalities on high resolution computed tomography [HRCT]) and serum IL-6 (P = 0.042) and IL-10 from T cell lines (P = 0.04), and an inverse relation to MCP-1 from T cell lines (P = 0.032). Other authors [11] have shown evidence for an IL-4-mediated phenotype change in alveolar macrophages from patients with SSc-ILD resulting in sustained inflammation and Th2 immune response.

The clinical and histopathologic differences between SSc-ILD and idiopathic pulmonary fibrosis (IPF) may be due, in part, to the presence of different bronchoalveolar lavage fluid (BALF) cytokine profiles. Meloni et al. [12] found that the BALF cytokine profile in SSc-ILD reflects a more favorable balance between fibrotic (MCP-1) and anti-fibrotic or anti-inflammatory factors (IL-12 and IL-10) than that observed in IPF. SSc-ILD patients had increased levels of IL-10 and IL-12; MCP-1 was significantly raised in IPF patients, but only slightly increased in SSc-ILD. Moreover, MCP-1 levels in BALF correlated significantly with BALF eosinophil counts, which are thought to be a poor prognostic factor in SSc-ILD.

Accumulating evidence suggests that the fractalkine (FKN)-CX3CL1 interaction may play a role in inflammation and vascular injury in SSc. In a study of 67 Japanese patients [13*], FKN expression was increased in the vascular endothelial cells of lesional skin and lung tissues. CX3CR1 expression was enhanced in peripheral CD4+ T cells, CD8+ T cells, and CD14+ monocytes/macrophages; the number of mononuclear cells expressing CX3CR1 was increased in the affected skin and lung tissues. Reflecting the overexpression of FKN in the skin and lung, serum soluble FKN was also increased. Furthermore, soluble FKN levels were significantly associated with the severity of ILD.

**Microvascular injury**

The presence of thrombin, endothelin-1, β thromboglobulin, and platelet factor 4 suggest the presence of microvascular injury in SSc-ILD. Thrombin differentiates normal lung fibroblasts to a myofibroblast phenotype via protease-activated receptor (PAR-1) and a protein kinase C (PKC)-ε pathway [14,15]. In a study of lung tissue from scleroderma patients [16*] PAR-1 expression was found to be increased and associated with inflammatory fibroproliferative foci. The authors also provided compelling evidence that thrombin is an important regulator of cell survival and DNA synthesis and that these two processes are regulated via two distinct PKC isoforms: resistance to apoptosis by thrombin-induced myofibroblasts is regulated by PKC-ε, whereas activation of PKC-α is followed by activation of MAPK and cyclin D1, leading to enhanced proliferative capacity of myofibroblasts. These results lend additional support for thrombin-induced signaling in the emergence, proliferation, and persistence of the myofibroblast phenotype critical for the development and progression of pulmonary fibrosis, thus making thrombin and its receptor potentially attractive therapeutic targets in scleroderma.

Endothelin-1 (ET-1) is a mediator of fibrosis and is overexpressed in plasma as well as dermal fibroblasts cultured from SSc patients [17]. ET-1 is mitogenic for a number of cell types, can modify ECM metabolism, promote contractile ability of normal dermal fibroblasts, and participates in wound healing and scar formation [18*]. Similar to thrombin [15,16*], ET-1 is able to induce myofibroblast formation [18*] These authors demonstrated that lesional SSc pulmonary fibroblasts have increased expression of ET-1 protein and have substantially higher levels of ET-1 surface binding. They further demonstrated that ET-1 directly contributed to lung fibrosis by enhancing contractile ability of the SSc fibroblast through an Akt/Pi3-kinase-dependent pathway, thereby promoting the formation of scar tissue. The authors conclude that there may be potential therapeutic advantage in using Pi3-kinase inhibitors or endothelin antagonists to prevent the pathologic fibrosis observed in SSc-ILD. A trial (BUILD2) of the dual endothelin receptor antagonist, bosentan, in SSc-ILD is currently underway. This is a 12-month study to determine if treatment with Tracleer (bosentan) will improve exercise tolerance, as measured by the 6-minute walk test, in diffuse or limited cutaneous SSc-ILD patients.

B thromboglobulin (BTG) and platelet factor 4 (PF4) are platelet specific α granule proteins released from activated platelets and are considered markers of platelet activation. Activated platelets may play a role in inflammation through release of chemotactic factors and production of proinflammatory eicosanoids. BTG was detected in BALF from 11 of 37 patients with SSc (29.7%), and all patients in whom BTG was detected had features of SSc-ILD [19*]. PF4 was detected in eight of the 37 SSc patients (21.6%). The BTG: PF4 ratio was greater than 2, indicating in vivo release. Patients with detectable BTG or PF4 had significantly shorter disease duration than SSc-ILD patients without detectable platelet activation markers, suggesting that platelet activation occurs in the early phase of disease. This is consistent with the timing of vascular...
abnormalities on histopathology [1] and suggests a possible role of antiplatelet drugs in the prevention of SSc-ILD.

**Adhesion molecules**

Leukocyte recruitment from the circulation to sites of inflammation is a multi-step process regulated by multiple cell surface adhesion molecules. The soluble forms of various adhesion molecules are significantly elevated in sera of SSc patients [20,21]. The selectins primarily mediate tethering and rolling of leukocytes. P-selectin glycoprotein ligand-1 (sPSGL-1) is a high affinity ligand for P-selectin found on most leukocytes [22,23]. Serum sPSGL-1 concentrations from 65 patients with SSc were examined using ELISA, including 177 sera from 35 patients in a longitudinal analysis [24]. sPSGL-1 was raised in SSc patients compared with healthy controls ($P < 0.05$). Raised sPSGL-1 concentrations were observed in 42% of patients with SSc. SSc patients with raised sPSGL-1 concentrations less often had pulmonary fibrosis ($P < 0.05$) and decreased vital capacity ($P < 0.05$) than those with normal sPSGL-1 levels. sPSGL-1 concentrations were positively correlated with vital capacity ($P < 0.005$) and DLCO ($P < 0.01$). In the longitudinal study, patients without pulmonary fibrosis had consistently increased sPSGL-1 concentrations early in disease, while those with ILD had decreased sPSGL-1 throughout the follow-up period. Measurement of sPSGL-1 in patients with SSc may offer an important approach to the evaluation of pulmonary involvement. Furthermore, since a raised serum sPSGL-1 is associated with a lower frequency and severity of pulmonary fibrosis in SSc, sPSGL-1 may be protective against the development of pulmonary fibrosis and as such would be a possible therapeutic target.

Perivascular infiltrates of inflammatory cells consisting mainly of CD4$^+$ T cells are another hallmark of SSc. In a study [25] that investigated the distribution of the skin and mucosal committed CD4$^+$ T lymphocytes subsets, the authors found a significant increase of both $\alpha_4\beta_1$ and $\alpha_4\beta_7$-expressing CD4$^+$ T cells and a reduced number of CLA$^+$ CD4$^+$ cells in the peripheral blood of SSc patients, providing indirect proof of autoreactive T cell proliferation. The authors found that the increase of $\alpha_4\beta_1$ CD4$^+$ T cells was related to the presence of ILD, while the increase in $\alpha_4\beta_7$-expressing CD4$^+$ T cells was associated with esophageal dysmotility; patients with renal involvement had a lower number of CLA$^+$ CD4$^+$ cells.

**Diagnosis**

Most experts rely on a combination of pulmonary function testing, high resolution chest computed tomography and bronchoalveolar lavage for diagnosing scleroderma interstitial lung disease.

**Chest computed tomography**

Until recently, it was believed that SSc-ILD was indistinguishable from idiopathic pulmonary fibrosis. However, in a study [26$^{**}$] comparing HRCT characteristics in patients with SSc-ILD to patients with usual interstitial pneumonia (UIP, IPF) and idiopathic nonspecific interstitial pneumonia (NSIP), the ILD in patients with SSc was less extensive, less coarse ($P < 0.001$) and characterized by a greater proportion of ground-glass opacification ($P < 0.001$) than in UIP. The HRCT characteristics in SSc closely resembled those in idiopathic NSIP (Fig. 2). This is consistent with several recent pathologic series that have shown NSIP to be the more prevalent histologic pattern in SSc [27–29]. Interestingly, nearly one-third of the SSc-ILD patients [26$^{**}$] had coarse fibrosis and a lower proportion of ground-glass opacification suggestive of IPF. However, the same overlap was apparent when HRCT features were compared between the idiopathic UIP and NSIP subgroups, suggesting that microcystic disease (honeycombing) occurs in a subset of patients with NSIP.

**Bronchoalveolar lavage**

Although early identification and treatment of alveolitis may prevent deterioration of lung function, the best approach for diagnosing active alveolitis remains controversial.

![Figure 2. Computed tomography (CT) scans obtained in two patients with systemic sclerosis demonstrate the range of CT appearances.](image)
In 1990, BAL was first used to evaluate for the presence of inflammation in the alveoli and its association with lung function changes over time in patients with SSc-ILD [30]. Ground-glass opacification on HRCT may also be used to diagnose alveolitis. However, ground-glass opacification may also be seen with pulmonary edema, resolving inflammation and/or infection and may be confused with mosaic perfusion patterns seen in patients with pulmonary vascular disease. Importantly, abnormalities on BAL may precede HRCT abnormalities in a majority of patients [31]. In a study [32-34] of 15 SSc-ILD patients that underwent both HRCT and BAL of the middle lobe or lingula and a lower lung segment, the utility of HRCT in comparison to BAL was investigated. Bronchoalveolar lavage of the middle lobe or lingula was found to underestimate the presence of active alveolitis. While ground-glass opacification on HRCT accurately predicted alveolitis in the middle lung fields ($P < 0.0001$), agreement was poor for the lower lobes ($P = 0.24$). The correlation between fibrosis on HRCT and the presence of alveolitis on BAL was significant for the lower lobes ($P = 0.04$), but not the middle lung fields ($P = 0.31$). The addition of a lower-lobe BAL to the one performed in the right middle lobe or lingula increased the number of patients classified as having inflammatory alveolitis by 23%. Furthermore, 20% of patients (n = 3) had an unsuspected infection. Since HRCT does not detect all sites of inflammation and does not identify infectious etiologies, the authors concluded that, in addition to HRCT, BAL with lavage, differential cell counting, and culture from at least 2 segments of lung should be performed for diagnosing SSc alveolitis. It is important to note, that BAL results often varied by up to several percentage points, suggesting that there can be considerable variability in reporting of the leukocyte subsets in a BAL sample even with specially trained personnel. Unfortunately, reliable techniques and trained personnel for interpreting BAL differential cell counts are not widely available in the community setting. Therefore, caution is warranted in making treatment decisions solely on BAL findings.

**Nailfold capillaroscopy**

In addition to collagen deposition and fibrosis, systemic sclerosis is characterized by widespread microvascular abnormalities. Nailfold capillaroscopy is a noninvasive method to evaluate vascular dysfunction. A majority of patients with SSC have capillary dilatation associated with avascular areas and loss of normal capillary organization [33]. The clinical features associated with capillaroscopic alterations and the value of nailfold capillaroscopy in predicting the presence and activity of pulmonary disease in SSC were defined in 91 patients with pulmonary disease diagnosed by ground-glass opacities on HRCT [34-36]. Total skin score, duration of Raynaud’s phenomenon, presence of digital pitting scars or finger amputations, pulmonary ground-glass opacities on HRCT, and age ≥50 years were independently associated with the mean avascular score. Although the mean avascular score tended to correlate with the extent of ILD in the entire patient sample ($P = 0.083$), the association between ground-glass opacities and higher avascular scores was stronger in patients with disease duration ≤5 years ($P = 0.04, n = 27$). Among these patients, ground-glass opacities were present in 14 of 19 patients with severe nailfold alterations (dilated capillaries and areas of avascularity), but were absent in all patients with mild or no nailfold alterations ($P < 0.001$). In this subgroup, the sensitivity of severe nailfold capillaroscopic alterations for ground-glass opacities was 100% (95% CI: 74.7 to 100) with a specificity of 61.5% (95% CI: 32.3 to 84.9) Receiver operating characteristic curves confirmed the ability of nailfold capillaroscopy to discriminate between patients with and without ground-glass opacities among those with disease duration ≤5 years (area = 0.83, 95% CI: 0.63–0.94). This study is consistent with a prior study [35], which observed that patients with inflammatory BAL tended to have a greater prevalence of abnormalities on nailfold capillaroscopy than patients with a non-inflammatory BAL. Interestingly, the study by Bredemeier et al. [35] did not show an association between the mean avascular score with abnormalities on pulmonary function testing and with the presence of honeycombing; this suggests that abnormalities in nailfold capillaroscopy may be an early finding. Therefore, nailfold capillaroscopy may be useful for the selection of patients in need of more extensive evaluation and aggressive therapeutic interventions.

**Surfactant protein D and KL-6**

There is marked hyperplasia of type II epithelial cells in patients with ILD, which may contribute to the pathogenesis of lung fibrosis by the secretion of fibrogenic cytokines, e.g., TGF-β, and growth factors, e.g., CTGF. Products of proliferating type II alveolar epithelial cells could, therefore, function as ‘lung-specific’ markers of ILD.

KL-6 is a mucin-like glycoprotein that is strongly expressed on alveolar type II epithelial cells and has been shown to be associated with the presence of and severity of ILD [36,37]. KL-6 was measured in 39 serum samples [38-39] from 12 children with the diffuse cutaneous form of juvenile SSC and 20 healthy controls. Elevated levels of KL-6 were found in 1 of 12 samples obtained from patients without ILD (8.3%) and in 24 of 27 samples obtained from patients with ILD at time of sampling (88.9%). Increased serum levels of KL-6 were associated with the presence and extent of ILD on HRCT, and inversely correlated with FVC ($P < 0.001$), DLCO ($P = 0.012$), and response to therapy, which is consistent with findings in adults with SSc-ILD [37].

Surfactant proteins are also produced and secreted by alveolar type II epithelial cells. Surfactant proteins A and D
are elevated in patients with SSc-ILD [39] and correlate with disease activity and KL-6 levels and inversely with pulmonary function [40**]. Serum levels of surfactant protein-D (SP-D) and KL-6 were measured in 42 Japanese SSc patients, including a retrospective longitudinal study of 83 samples from 6 patients [40**]. SP-D was a more sensitive marker for ILD than KL-6 (91% versus 39%), and KL-6 showed higher specificity than SP-D (88% versus 100%). In the longitudinal study, SP-D levels were more responsive to change and tended to reflect ILD activity more so than KL-6. The measurement of serum KL-6 and surfactant protein D may be useful noninvasive markers of ILD in patients with SSc, and the combined use of these two serum markers would be more helpful in diagnosing and monitoring SSc-ILD activity than use of either marker alone.

**Pulmonary hypertension**

Systemic sclerosis, when complicated by pulmonary hypertension, has a very poor prognosis [42]. Doppler echocardiography has become a useful screening tool for pulmonary hypertension. In a study of 227 patients with early SSc, [43**] referred from hospitals throughout Sweden during 1992–2001, echocardiograms were obtained at initial visit and at 1 year; patients on immunosuppressive medication were investigated annually, diffuse cutaneous patients were examined every 2 years and those with limited cutaneous SSc every 3 years. An increased tricuspid gradient, indicating possible pulmonary hypertension, was common and found in 44.9% at the first assessment point, and in 61.2% of patients at follow-up. Notably, an increased tricuspid gradient was found in 40.7% of patients with disease duration of 2 years or less. A higher tricuspid gradient was associated with longer disease duration, age, the presence of anti-topoisomerase 1 antibodies, ILD, ground glass opacification on HRCT and treatment with cyclophosphamide. There was no relation between tricuspid gradient and gender, the extent of skin involvement, the presence of anti-centromere antibodies and fibrosis on HRCT. At the first assessment point, the tricuspid gradient in patients with ILD was 26.8 ± 9.7 mm Hg, and it increased 2.0 ± 5.7 mm Hg/y; the tricuspid gradient in patients without ILD was 23.5 ± 8.6 mm Hg (P = 0.031) and increased by 1.0 ± 3.5 mm Hg/y. There was also a significant inverse correlation between DLCO and tricuspid gradient at the first assessment point (P < 0.01). The increased tricuspid gradient in patients with ILD may be secondary to coexistent pulmonary arteriopathy, hypoxic pulmonary vasoconstriction, or microvascular capillary dropout. Alternatively, it is important to note that left heart disease was not measured in this study; systolic and diastolic dysfunction are known to occur frequently in SSc as a result of fibrosis of the ventricles [44].

**Treatment**

The results of the Scleroderma Lung Study (SLS), a parallel-group, double-blind, multicenter, randomized controlled trial of the safety and efficacy of oral cyclophosphamide versus placebo for the treatment of alveolitis in early SSc, are eagerly awaited. This study was initiated in response to multiple uncontrolled case series that reported clinical (dyspnea, survival), [45,46] spirometric [46–50] gas transfer (DLCO) [45,50–52] and radiographic improvement (HRCT) [46,49,53] in SSc-ILD patients treated with oral or intravenous cyclophosphamide. Although we await the publication of the final results of the SLS, this large study has already provided us several lessons.

Greater severity of dyspnea in patients with SSc-ILD is associated with lower health-related quality of life, greater functional disability, poorer patient global assessment of disease, and greater decreases in physiologic measures of lung function (FVC and DLCO). In patients with active alveolitis associated with SSc, values for Mahler’s Baseline Dyspnea Index (BDI) and the Visual Analogue Scale (VAS) for breathing (both measures of dyspnea) were associated with performance on both the physical and mental component summary measures of the Short Form 36 (SF-36) and, to a lesser degree, inversely with DLCO. The SF-36 was able to discriminate among SSc-ILD patients with more severe and less severe breathlessness, complementing the BDI and VAS and, therefore, should be included as an outcome measure in intervention trials in patients with SSc-ILD [54*].

**Possible future therapies**

In a retrospective study [55*], the records of 11 patients with SSc-ILD treated with azathioprine and low-dose prednisone were reviewed. Three patients received less than 6 months of therapy secondary to adverse effects. The remaining eight patients received at least 12 months of treatment and the results suggested an improvement in the percent predicted FVC from a baseline value of 54.25 ± 3.53 to 63.38 ± 6.15 after 12 months (P = 0.101). Overall, five patients improved and three patients remained stable. The mean dyspnea score improved from baseline in all 8 patients (P = 0.011). Importantly, there was no significant deterioration in the symptoms or FVC in all the patients who received treatment for 12 months. Furthermore, azathioprine was effective in three patients who relapsed after previous cyclophosphamide therapy. Since all patients had deteriorating pulmonary function observed prior to the initiation of azathioprine, the stabilization of FVC together with improvement in symptoms is clinically significant.

Curcumin is a major component of the spice turmeric. Turmeric has been used in Chinese and Indian herbal
medicine to treat a wide range of conditions, although the efficacy of tumeric in these folk applications has not been proven. Practitioners of alternative medicine recommend curcumin as a treatment or autoimmune disease. Curcumin has been reported to act as an anti-inflammatory agent, to inhibit tumor promotion, tumor cell proliferation, and metastasis, to be a powerful antioxidant, and to be an effective topical microbicide [56]. Curcumin has also been reported to protect rats against lung fibrosis induced by bleomycin, by cyclophosphamide and by whole-body irradiation [57–59]. Recently, curcumin was shown to induce apoptosis in scleroderma lung fibroblasts (SLF), but not in normal lung fibroblasts (NFL). This effect appears to be due to aberrant PKCε signaling in SLF and due to the failure of SLF to up-regulate the expression of phase 2 detoxification enzymes in response to curcumin [56]. Curcumin is already being used in clinical trials for other diseases and may be an attractive treatment for scleroderma lung disease.

Survival

In a meta-analysis [60**] of 1645 incident cases from SSc cohorts recruited from seven medical centers in the United States, Europe, and Japan, 578 deaths occurred over 11,521 person-years of follow-up. After adjusting for age, sex, and year of enrollment, the presence of anti-topoisomerase I antibodies (hazard ratio [HR] = 1.3; 95% confidence interval [CI]: 1.0 to 1.6), as well as renal (HR = 1.9; 95% CI: 1.4 to 2.5), cardiac (HR = 2.8; 95% CI: 2.1 to 3.8), and pulmonary (HR = 1.6; 95% CI: 1.3 to 2.2) involvement, increased mortality risk. Furthermore, renal, cardiac, and pulmonary involvement tended to occur together (P < 0.001). Pulmonary involvement was defined as bibasilar pulmonary fibrosis (bilateral reticular linear or reticulonodular densities, most pronounced in the lung bases, on standard chest radiograph), crackles (‘Velcro’ rales), forced vital capacity <70% of predicted normal plus forced expiratory volume in 1 second >70% of the FVC.

Conclusions

SSc-ILD is a very significant complication and important cause of morbidity and mortality among systemic sclerosis patients. Recent studies have highlighted the importance of microvascular changes, autoimmunity and fibroblast activation that together culminate in interstitial fibrosis and pulmonary dysfunction. As progress is made toward understanding the basis of vascular dysfunction, autoimmunity and fibroblast activation in SSc-ILD, potential therapeutic targets will emerge. Until then, it would appear as if immunosuppressive therapy may be the best means of stabilizing pulmonary function. Careful clinical assessment and sound clinical judgment are essential for the evaluation and management of SSc patient with dyspnea or other features suggestive of ILD.

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

Additional references related to this topic can also be found in the Current World Literature section in this issue (pp. 772–773).


7. Scala E, Pallotta S, Frezzolini A, et al. Cytokine and chemokine levels in sys- temic sclerosis; relationship with cutaneous and internal organ involvement. Clin Exp Immunol 2004; 138:540–546. This study of 54 consecutive patients with SSc identifies quantitative and qualita- tive changes in cytokine and chemokine production compared to healthy sub- jects. Cytokine and chemokine profiles vary correlated with cutaneous and internal organ involvement. There was a significant direct correlation between pul- monary fibrosis and serum IL-6 and IL-10 from T cell lines, and an inverse relationship to MCP-1 from T cell lines.


This study suggests that the cytokine profile of BALF in SSc-ILD may reflect a more favorable balance between fibrotic (MCP-1) and anti-fibrotic (IL-12) or anti-inflammatory (IL-10) factors as compared to patients with UIP. These data should be interpreted with caution given the small numbers of patients and the wide range in BALF cytokine concentrations.

13. Hasegawa M, Sato S, Echigo T, et al. Up regulated expression of fractal- line/CXCR1 in patients with systemic sclerosis. Ann Rheum Dis 2005; 64: 21–28. In this study, patients with SSc-ILD have increased expression of fractaline and CXCR1 compared to normal controls. Furthermore soluble FKN levels were significantly associated with the involvement and severity of pulmonary fibrosis.


16 Bogatkevich GS, Gusto E, Oates JC, et al. Distinct PKC isoforms mediate cell survival and DNA synthesis in thrombin-induced myofibroblasts. Am J Physiol Lung Cell Mol Physiol 2004; 288:L190–L201. This article provides compelling evidence that thrombin stimulates unrestricted proliferation and cell survival and initiates tissue repair in scleroderma lung fibroblasts, which may have significant relevance to the pathogenesis of lung fibrosis.

17 Abraham DJ, Vancheeswaran R, Dashwood MR, et al. A4b1+ and A4b7+ T cell numbers in bronchoalveolar lavage fluid of patients with systemic sclerosis: association with lower frequency of pulmonary fibrosis. Ann Rheum Dis 2004; 63:184–189. This study shows that abnormalities on nailfold capillaroscopy may be early findings in SSc-ILD, and nailfold capillaroscopy is a highly sensitive test in predicting patients with ground-glass opacity on HRCT with less than 5 years of disease duration.


35 Bogatkevich GS, Gusto E, Oates JC, et al. Distinct PKC isoforms mediate cell survival and DNA synthesis in thrombin-induced myofibroblasts. Am J Physiol Lung Cell Mol Physiol 2004; 288:L190–L201. This article provides compelling evidence that thrombin stimulates unrestricted proliferation and cell survival and initiates tissue repair in scleroderma lung fibroblasts, which may have significant relevance to the pathogenesis of lung fibrosis.


SSc-ILD patients with greater severity of dyspnea have lower health-related quality of life, greater functional disability, poorer patient global assessment of their disease and greater decreases in physiologic measures of lung function (FVC and DLCO). In this report from the SLS the patient self-assessed measures of disease were noted to be stronger correlates of dyspnea than were physiologic measures.


This is the first case series of patients with SSc-associated ILD treated with azathioprine. The results suggest that azathioprine may have a role in stabilizing lung function and improving symptoms in SSc, although this needs confirmation by a randomized trial.


This is the first case series of patients with SSc-associated ILD treated with azathioprine. The results suggest that azathioprine may have a role in stabilizing lung function and improving symptoms in SSc, although this needs confirmation by a randomized trial.


This is the largest case series to date that looks at predictors of mortality in SSc. The authors found that SSc confers a high mortality risk, but there is considerable heterogeneity across settings. Internal organ involvement and anti-topoisomerase I antibodies are important determinants of mortality.
B lymphocytes and systemic sclerosis
Manabu Fujimotoa and Shinichi Satob

Purpose of review
Systemic sclerosis is characterized by fibrosis and autoimmunity. Systemic sclerosis displays a variety of abnormal immune activations, including the production of disease-specific autoantibodies, although the pathogenic relation between systemic autoimmunity and the clinical manifestations of systemic sclerosis remains unknown. Recent studies have rediscovered that B cells play critical roles in systemic autoimmunity and disease expression through various functions more than autoantibody production, such as antigen presentation and cytokine production. This review focuses on recent advances in understanding the B cell’s role in systemic sclerosis.

Recent findings
Patients with systemic sclerosis have altered B-cell homeostasis characterized by expanded naive B cells and diminished memory B cells. Although memory B cells are decreased in number, they are chronically activated, possibly because of CD19 over-expression in B cells from patients with systemic sclerosis. CD19 over-expression can be genetically explained in part by a polymorphism of CD19 promoter region. Similarly, B cells from a tight-skin mouse, a genetic model of systemic sclerosis, show augmented CD19 signaling and chronic hyper-reactivity. CD19 hyper-phosphorylation in tight-skin B cells is caused by impaired function of CD22, a negative response regulator expressed on B cells. Classic roles of autoantibody secretion may also be important in systemic sclerosis because autoantibodies to matrix metalloproteinases can be pathogenic in vivo.

Summary
B cells may have more pathogenic roles in systemic sclerosis than had been appreciated. Further studies are required to clarify the precise molecular basis that links B cells and fibrosis. Collectively, B cells and B-cell-specific response regulators such as CD19/CD22 appear to be potential therapeutic targets of the disease.

Keywords
CD19/CD22 autoimmune loop, memory B cells, polymorphism, systemic autoimmunity, tight-skin mouse

Abbreviations
BCR B-cell antigen receptor
dcSSc diffuse cutaneous systemic sclerosis
MMP matrix metalloproteinase
SHP-1 Src homology 2 domain-containing tyrosine phosphatase 1
TSK tight skin

Introduction
Systemic sclerosis is a connective tissue disease characterized by collagen accumulation, vascular injury, and immune activation. Immune activation is characterized by autoantibody production, lymphocyte activation, and production of various cytokines [1**]. More than 90% of patients possess antinuclear antibody, a central feature of immune activation of the disease. The specificities of autoantibodies closely correlate with the clinical manifestations. For example, anti-DNA topoisomerase I antibody and anti-RNA polymerase antibody are associated with diffuse cutaneous systemic sclerosis (dcSSc), while ant Centromere antibody is found in limited form. In particular, anti-topoisomerase I antibody levels reflect disease activity and severity of dcSSc [2] and correlate with the extent of fibrosis in skin, lung, and renal blood vessels. Furthermore, the decreased levels of anti-topoisomerase I antibody parallel improvement of skin fibrosis during follow-up, while their increased levels are associated with new onset or worsening of organ involvement. Consistently, it is reported that 20% of anti-topoisomerase I antibody-positive patients with systemic sclerosis lost anti-topoisomerase I antibody during the disease course and had a favorable outcome [3]. Also, we have experienced a patient with dcSSc who showed a marked improvement of skin sclerosis after the occurrence of anti-centromere antibody [4]. Collectively, these data suggest that autoantibodies are closely linked to the pathogenesis of systemic sclerosis.

Nonetheless, these autoantibodies are not likely to have a direct pathogenic role in systemic sclerosis, because most systemic sclerosis-related autoantigens such as topoisomerase I, centromere, and RNA polymerases are intracellular components of mitosis-related functions. Autoantibodies are usually not internalized into the cell. Even if they can penetrate viable cells, the inhibition of these autoantigen functions is unlikely to induce fibrosis. A recent study has shown that anti-topoisomerase I antibody can directly bind to the cell surface of fibroblasts [5*], although the pathologic relevance remains unclear. Therefore, at minimum, the presence of autoantibodies
demonstrates that abnormal B-cell activation exists in systemic sclerosis.

Recent assessments of the role of B cells in the immune system have indicated that B cells have various fundamental roles in regulating immune responses than had previously been appreciated [1**,6]. These functions include antigen presentation, cytokine production, lymphoid organogenesis, differentiation of T cells, and influence on dendritic cell function. Therefore, not only autoantibody production but also abnormalities of other B-cell functions could lead to the induction or development of autoimmune disorders. Patients with systemic sclerosis exhibit polyclonal B-cell hyperactivity and hyper-γ-globulinemia in addition to autoantibody production. Interestingly, a gene expression analysis using DNA microarrays has revealed up-regulation of B-cell-related genes in sclerotic skin lesions of systemic sclerosis [7]. These observations collectively point to the presence of intrinsic B-cell abnormalities in systemic sclerosis and suggest that B cells may have pathogenic roles other than autoantibody production.

**Intrinsic B-cell abnormalities in patients with systemic sclerosis**

B-cell homeostasis is tightly regulated in the immune system. CD19+ blood B cells are grouped into naive B cells negative for CD27, memory B cells that express medium levels of CD27, and plasmablasts/early plasma cells expressing high levels of CD27. In systemic sclerosis, peripheral B-cell homeostasis is disturbed [8**]. Total blood - cell number is increased in patients with systemic sclerosis. Among the B-cell subsets, the number of naive B cells is increased, while those of memory B cells and plasmablasts/early plasma cells are markedly decreased (Fig. 1). Memory B cells from patients with systemic sclerosis are chronically activated in vivo because they have up-regulated the cell surface density of CD80 and CD86. Furthermore, CD95 expression, which is increased on B-cell activation, is also higher on systemic sclerosis memory B cells. Consistent with this, memory B cells from patients with systemic sclerosis are more sensitive to spontaneous apoptosis. This enhanced spontaneous apoptosis of systemic sclerosis memory B cells may account for the diminished number in the blood. Furthermore, the continuous loss of memory B cells and plasmablasts/early plasma cells may result in the increased production of naive B cells, leading to augmented CD95-mediated apoptosis that may result in their diminished number in the blood. The continuous loss of memory B cells and plasmablasts/early plasma cells may increase naive B-cell production in bone marrow to maintain the B-cell homeostasis in systemic sclerosis (feedback).

**CD19 expression on B cells from patients with systemic sclerosis**

Judging from flow-cytometric analyses of blood from patients with systemic sclerosis, surface CD19 density in systemic sclerosis B cells are significantly higher by ~20% than those of healthy individuals [9]. CD21, a complement receptor associated with CD19 on the B-cell surface, also shows higher expression in patients with systemic sclerosis, while CD20 and CD40 expressions are not altered [9]. CD19 over-expression is also increased on memory B cells, leading to augmented CD95-mediated apoptosis that may result in their diminished number in the blood. The continuous loss of memory B cells and plasmablasts/early plasma cells may increase naive B-cell production in bone marrow to maintain the B-cell homeostasis in systemic sclerosis (feedback).

CD19 is a 95 000 molecular weight (Mr) glycoprotein member of the immunoglobulin superfamily expressed from early pre-B cells until plasma cell differentiation [10**]. CD19 expression is tightly regulated during the B-cell activation process, suggesting that intrinsic CD19 expression levels may determine a genetic predisposition to autoimmunity. CD19 has an extracellular region containing two C2-type immunoglobulin-like domains and a cytoplasmic region of ~240 amino acids with nine conserved tyrosine residues [11]. Lyn, a Src-family protein tyrosine kinase member, is the dominant kinase that phosphorylates CD19 on various stimuli. Once tyrosyl-phosphorylated, CD19 serves as a membrane-bound

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**Figure 1. Disturbed peripheral B cell homeostasis and intrinsic B cell functional abnormalities in patients with systemic sclerosis.**

![Diagram showing disturbed peripheral B cell homeostasis and intrinsic B cell functional abnormalities in patients with systemic sclerosis.](Image)
adaptor protein for Src homology 2-containing signaling molecules such as Lyn, Vav, and phosphatidylinositol 3-kinase, which further mediate downstream activation cascades. In-vivo CD19 function has been clarified using CD19-deficient mice and CD19-transgenic mice, which over-express CD19 by 200%. CD19-deficient B cells are hyporesponsive to transmembrane stimuli, while B cells from CD19-transgenic mice show augmented responses. CD19-transgenic mice spontaneously produce high titers of autoantibodies including anti-DNA, anti-histone, and anti-topoisomerase I antibodies. Thus, over-expression of CD19 can lead to autoimmunity.

It was unclear how CD19 expression is up-regulated in systemic sclerosis B cells. A recent study has revealed that CD19 polymorphisms are associated with genetic susceptibility to systemic sclerosis [12]. A single nucleotide polymorphism (SNP) in the upstream region of CD19, -499G > T, is associated with the susceptibility to systemic sclerosis. Association is particularly evident in limited cutaneous SSc (lcSSc) with anti-centromere antibody. CD19 expression levels in peripheral blood B cells are significantly elevated by 18–19% in both naive and memory B cells from the patients with systemic sclerosis carrying the −499T allele compared with those without the −499T allele. This accounts, at least in part, for the enhanced CD19 expression in patients with systemic sclerosis.

While it is certain that CD19 expression levels are higher on B cells from patients with systemic sclerosis, the increase is rather small (~20%), and it remains unknown whether this small increase can affect the immune system. Another line of CD19-transgenic mice over-expressing CD19 by only 20–30% of wild-type mice, which is an equivalent level to human patients with systemic sclerosis, produces significantly elevated levels of various autoantibodies including systemic sclerosis-specific anti-topoisomerase I antibody as well as anti-DNA, anti-histone, and anti-topoisomerase I antibodies. Thus, over-expression of CD19 can lead to autoimmunity.

**B cells and tight-skin mice**
The tight-skin (TSK) mouse is a genetic model for human systemic sclerosis and was originally identified as a spontaneous mutation that results in increased accumulation of extracellular matrix proteins. A tandem duplication within the fibrillin 1 gene is considered to cause the TSK phenotype [14]. Fibrillin 1 is a major structural protein of a widely distributed class of connective tissue microfibrils. Homozygous mutation results in embryo lethality, while heterozygous (TSK+) mice survive but develop cutaneous hyperplasia, pulmonary emphysema, and cardiac hypertrophy. Although the phenotype of TSK/+ mice is not identical to human systemic sclerosis, TSK/+ mice produce autoantibodies against systemic sclerosis–specific target autoantigens including topoisomerase I, fibrillin 1, and RNA polymerase I, indicating that TSK/+ mice represent skin fibrosis and systemic autoimmunity observed in human systemic sclerosis.

The phenotype of TSK/+ mice appears regulated not by fibrillin 1 mutation alone but by multiple factors. For example, transgenic mice expressing a mutated fibrillin 1 gene develop cutaneous hyperplasia but not pulmonary emphysema and myocardial hypertrophy [15]. CD4 deficiency in TSK/+ mice results in decreased cutaneous hyperplasia but does not affect lung emphysema or anti–topoisomerase I antibody levels [16]. Also, disrupting one or both interleukin-4 alleles allows survival of 29% and 47%, respectively, of homozygous TSK/TSK mice [17]. They do not exhibit cutaneous hyperplasia but develop pulmonary emphysema. These data suggest that abnormal immune functions contribute to the phenotype of TSK mice. Consistent with this, polymorphisms of the transforming growth factor (TGF)-β1 promoter in TSK mice have been reported [18].

B cells from TSK/+ mice also have an abnormal phenotype [13,19]. As in human systemic sclerosis, TSK/+ B cells are chronically activated, characterized by reduced cell surface IgM level as well as up-regulated major histocompatibility complex (MHC) class II and CD23 density. Unlike human systemic sclerosis, CD19 expression is not altered in B cells from TSK/+ mice. Remarkably, however, CD19 tyrosine phosphorylation is constitutively augmented [19], suggesting that the CD19 signaling pathway is intrinsically activated in TSK/+ B cells.

On B-cell antigen receptor (BCR) cross-linking, TSK/+ B cells exhibit exaggerated calcium responses and augmented activation of extracellular signal-regulated kinase [13]. BCR-induced CD19 phosphorylation is also augmented compared with wild-type B cells. Among many signaling molecules assessed, CD22 phosphorylation was specifically impaired in TSK/+ B cells. CD22 is a B-cell-specific cell surface molecule that has immuno-receptor tyrosine-based inhibitory motifs in the cytoplasmic domain. Immuno-receptor tyrosine-based inhibitory motifs on CD22 can recruit a potent tyrosine phosphatase, Src homology 2 domain–containing tyrosine phosphatase 1 (SHP-1), and thus CD22 serves as an inhibitory signaling molecule. Decreased tyrosine phosphorylation of CD22 is consistent with CD19 hyper-phosphorylation in TSK/+ B cells, because CD19 is considered a major target of CD22− regulation via dephosphorylation by SHP-1 [20]. Furthermore, when TSK/+ mice deficient in CD22 expression were compared with CD22-deficient mice without TSK mutation, [Ca2+]i response and extracellular...
signal-regulated kinase activation in CD22-deficient TSK/+ B cells were identical to those in B cells from CD22-deficient mice without TSK mutation [13••]. This suggests that disruption of inhibitory signal provided by CD22 is the dominant mechanism of hyper-activated TSK/+ B cells.

Remarkably, CD19 deficiency in TSK/+ mice results in ~40% reduction of skin thickness [19]. Therefore, B cells contribute to skin fibrosis in TSK/+ mice through a CD19-dependent pathway. TSK/+ mice exhibit hyper-γ-globulinemia and elevated autoantibody levels including anti–topoisomerase I antibody, both of which are also eliminated by CD19 deficiency. Reciprocally, anti-topoisomerase I antibody levels are significantly augmented in TSK/+ mice carrying the CD19 transgene [13••]. Nevertheless, skin thickness does not increase in TSK/+ mice over-expressing CD19 or TSK/+ mice with CD22 deficiency [13••]. Therefore, while silencing B cell hyper-activation can reduce skin fibrosis, exaggerating B cell hyper-activation does not lead to further skin fibrosis. Also, these results confirm that anti-topoisomerase I antibody does not have a major pathogenic role. The molecular mechanism by which silencing B-cell hyper-activation by CD19 pathway can influence skin fibrosis also remains unsolved. One possibility is cytokine production. TSK/+ B cells stimulated with anti-IgM antibody plus anti-CD40 antibody produce higher amounts of interleukin-6 compared with wild-type B cells [19]. CD19 loss inhibits interleukin-6 production by TSK/+ B cells. Therefore, CD19 may regulate skin fibrosis by controlling the production of cytokines such as interleukin-6.

**Figure 2. CD19/CD22 autoimmune loop.**


**CD19/CD22 autoimmune loop**

B-cell fate is determined by signals through BCR and other costimulatory cell surface molecules. CD19 and CD22 represent specialized costimulatory molecules that also function as response regulators to modulate the intensity, duration, and quality of constitutive and BCR-induced signals. Importantly, these response regulators do not merely regulate BCR signals independently, but have their own regulatory network. For example, CD19 expression positively regulates CD22 phosphorylation by maintaining Lyn activation, while CD22/SHP-1 negative feedback targets CD19 phosphorylation [11]. Therefore, while CD19 and CD22 regulate BCR signaling, they establish a regulatory loop to modulate each other’s function. This CD19/CD22 loop is dysregulated in B cells from TSK/+ mice, as described.

The components of the CD19/CD22 loop appear closely linked to autoimmune disorders [10••] (Fig. 2). Disrupting their expression/function in mice results in autoimmune manifestation. Lyn-deficient mice and transgenic mice with a hyper-activated form of Lyn both result in lupus-like disease [21•]. Transgenic mice that over-express CD19 by ~3-fold lose tolerance and generate autoantibodies spontaneously [10••]. Mice lacking CD22 have chronically activated B cells with various spontaneous autoantibody production including anti-cardiolipin antibody and anti-myeloperoxidase antibody [22]. Motheaten viable (mev/mev) mice with SHP-1 mutations produce elevated levels of spontaneous autoantibodies, including anti-topoisomerase I antibody, hyper-γ-globulinemia, and tissue deposition of immune complexes [23]. Therefore, this
Raynaud phenomenon, scleroderma, overlap syndromes and other fibrosing syndromes

Human patients with systemic sclerosis have increased CD19 expression on B cells, whereas TSK/+ B cells have normal CD19 expression but augmented CD19 signaling. CD19 over-expression in patients with systemic sclerosis may be in part determined genetically by a polymorphism of CD19 promoter region. Increased CD19 expression/signaling may lead to autoantibody production through a breakdown of B-cell peripheral tolerance. B cells from human systemic sclerosis and TSK/+ mice are chronically activated in vivo, possibly because of enhanced CD19 signaling. Chronic B-cell activation results in the development of skin fibrosis through continuous production of cytokines, such as interleukin-6. Thus, systemic autoimmunity could be linked to skin fibrosis through chronic B-cell activation by enhanced CD19 signaling. This could also explain why anti-topoisomerase I antibody levels correlate closely with disease activity and severity of systemic sclerosis despite the lack of pathogenic role. It is also possible that systemic autoimmunity is linked to fibrosis through the production of possibly pathogenic autoantibody such as anti-MMP antibody or yet unidentified autoantibodies. Collectively, B cells or B-cell-specific response regulators CD19/CD22 are potential therapeutic targets in systemic sclerosis.

**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

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 773).


Recent studies have clarified that B-cell homeostasis is disturbed in systemic autoimmune diseases such as systemic lupus erythematosus and Sjögren’s syndrome. This study has revealed that systemic sclerosis B cells display a distinct pattern of altered B-cell subsets.

**Link between fibrosis and B-cell autoimmunity**

How can fibrosis be related to B-cell hyper-activity in systemic sclerosis? Evidence exists that fibrosis itself drives autoimmunity. Patients with systemic sclerosis exhibit autoantibodies to extracellular matrix regulation proteins, such as fibrillin 1 [24], matrix metalloproteinases (MMPs) [25,26], and heat-shock protein 47 [27], suggesting that they serve as immunogens. Autoantibodies to MMP-1 and MMP-3 are detected in ~50% of patients with systemic sclerosis [25,26]. MMPs are zinc-dependent endopeptidases that can digest extracellular matrix components. Fibrosis is caused by an abnormal accumulation of extracellular matrix components, which is regulated by both synthesis and degradation. Anti-MMP antibody is unique because it can possibly act as a pathogenic autoantibody in systemic sclerosis, inhibiting MMP enzymatic activity and reducing the turnover of the extracellular matrix. Consistent with this finding, anti-MMP-1 antibody levels in patients with systemic sclerosis correlate with the extent of fibrosis in the skin and lung. Thus, an up-regulated fibrotic process may drive autoimmunity, and in return, B-cell autoimmunity can contribute to the development of fibrosis by autoantibody as well.


Purpose of review
New insights in the pathophysiology and molecular mechanisms implicated in cutaneous vasomotor response to cooling are emerging from recent literature. These advances are introducing significant changes in the management of Raynaud’s phenomenon. In this review, we outline how these new findings are leading to novel methods of assessment and new opportunities for specific targeted therapy.

Recent findings
New potential targets for treatment of Raynaud’s phenomenon derive from experimental observations. Increased protein tyrosine kinase activity and tyrosine phosphorylation have been described in vascular smooth muscle cells in response to cooling and are linked to excessive α2-adrenergic response. Activation of Rho/Rho kinase pathway is triggered by increase of reactive oxygen species and up-regulates α2c-adrenergic receptors on the surface of vascular smooth muscle cells, thus determining an excessive vasoconstrictive response to cooling. This observation generated pilot trials testing rho-kinase inhibitors and α2c-adrenergic receptors antagonists in vasospastic conditions with encouraging results. Therapies already in use for pulmonary hypertension are also showing an effect in Raynaud’s phenomenon. Studies evaluating anti-endothelin-1 (bosentan), phosphodiesterases inhibitors (sildenafil), and prostanoids (given for critical ischemia) in the treatment of Raynaud’s phenomenon all determined improvement of symptoms and/or digital ischemic lesions. Novel techniques for better visualization and quantification of cutaneous microvascular defects are under development. The hope is that these new tools will allow earlier discrimination between primary and secondary Raynaud’s phenomenon as well as a better way to predict outcome and response to therapy.

Summary
Remarkable progress towards a rational approach to the management and treatment of Raynaud’s phenomenon is emerging.

Keywords
α2-adrenergic receptor, Raynaud’s, scleroderma, vasospasm

Introduction
Raynaud’s phenomenon represents a relatively common complaint in clinical practice, particularly among patients with rheumatic diseases [1]. It is characterized by a reversible vasospasm of arteries and arterioles within acral body segments precipitated primarily by cold or emotional stress that determines typical skin color changes. The patients with the primary form of Raynaud’s phenomenon (PRP) usually have long-standing history (i.e. since adolescence) of recurrent attacks, mild to moderate symptoms, and lack of complications or tissue damage from ischemia [2]. Conversely, when Raynaud’s phenomenon develops in the context of a connective tissue disorder, particularly systemic sclerosis or mixed connective tissue disorder, it may be responsible for significant morbidity, with digital ischemic manifestations, frequent digital ulcers and, on occasion, digital amputation [3].

Interestingly, despite the stereotypical and reproducible nature of the clinical features in Raynaud’s phenomenon, response to treatment remains poorly predictable and subject to considerable variability among different patients. Many suggested pharmacological treatments exist, but no gold standard therapy exists. For this reason, in the past decade, increasing emphasis has been placed on defining better the key pathogenetic mechanisms in Raynaud’s phenomenon, resulting in a new impulse for more targeted therapies [4]. This, together with technological advances in studying microvascular abnormalities involved in Raynaud’s phenomenon, is opening a new season in the management of this disorder. In this review, we identify the most recent breakthroughs in terms of understanding the pathophysiology, measuring the disease severity or outcome, and treating Raynaud’s phenomenon.

Pathogenesis of Raynaud’s phenomenon
Sir Thomas Lewis predicted from clinical observations that Raynaud’s phenomenon was caused by a ‘local defect’ in the digital and cutaneous arteries and was not secondary to central nervous system malfunction [5]. We now
appreciate that the biology of the blood vessels and regulation of local blood flow is complex. Herrick [6] published an excellent and comprehensive review of the pathogenesis of Raynaud’s phenomenon. The vascular endothelium, smooth muscle cells, and nerve terminals form an integrated unit in which specific interactions and soluble mediators released in the microenvironment contribute together to determine the final balance between vasodilatation and vasoconstriction. These interactions are influenced by a variety of factors including the level of physical activity, the ambient temperature, the individual’s emotional state, and direct traumatic or inflammatory insults to the vessels. Vasomotor control mechanisms can be subdivided into those that are intrinsic to the vessel wall or those that are extrinsic to the vessel (Table 1).

**Endothelial dysfunction**
The endothelial cells actively regulate vascular tone by secreting both vasodilative (NO, prostacyclin, prostaglandin, and leukotrienes) and vasoconstrictive (endothelin, angiotensin II, and thromboxane A2) mediators. Perturbation of endothelial cells homeostasis driven by a disturbed state (inflammation, cytokine activation, trauma/vibration) can lead to a significant imbalance in the profile of mediators secreted by the endothelium. Vasoconstriction may thus result when there is imbalance towards release of active vasoconstrictive mediators even without underlying permanent structural vascular damage. White et al. [7] studied the effect of fluid vibratory forces on the endothelium in the attempt to simulate the arm-hand vibration syndrome. This is a condition induced by chronic trauma from the use of vibration devices and is characterized as a secondary form of Raynaud’s phenomenon (SRP). Vibration exposes the endothelium not only to mechanical deformation but also to rapid changes in fluid shear stress. These mechanical forces are linked to other vascular disorders including atherosclerosis and intimal hyperplasia [8,9]. Rapid low-volume fluid oscillations applied to human umbilical vein endothelial cell were transduced into a biochemical signal with activation of extracellular signal-regulated kinase (ERK 1/2) pathway and increased production of endothelin-1, a potent vasoconstrictor. This in-vitro study demonstrates that external forces can alter endothelial functions and, as a consequence of endothelin release, can then enhance vascular reactivity.

We now realize that structural changes in the vessel such as intimal fibrosis as well as intrinsic endothelial defects can play an important role in Raynaud’s phenomenon. In patients with systemic sclerosis (scleroderma), aberrant vascular remodeling and insufficient repair mechanisms (abnormal angiogenesis, inadequate vasculogenesis, and defect in hypoxia response) are thought to determine the vascular disease and the clinical consequences of severe vasospastic attacks, critical digital ischemia, and digital losses [10]. This year, new investigations have contributed to our understanding of the molecular basis and link existing between cooling and vasoconstriction in peripheral arterial vessels. These studies offer an interesting insight about the interplay between molecular abnormalities in vessel smooth muscle cells (intrinsic) and the extrinsic control (neuroregulation and/or soluble mediators) of vascular tone. It is likely that vessel smooth muscle contractile properties vary in different arterial beds, explaining why some skin blood vessels have a unique response to cold stimuli and why Raynaud’s phenomenon has a selective distribution.

**Protein tyrosine kinase activity**
The two studies by Furspan et al. [11,12] provide evidence that the increased contractile response to α2-adrenergic agonists and cooling observed in patients with Raynaud’s phenomenon compared with healthy controls is associated with increased protein tyrosine kinase (PTK) activity and tyrosine phosphorylation. These abnormalities are described in arteries from patients with PRP and SRP, providing a theoretical unifying explanation for the cold-induced vascular reactivity. These investigators show that exacerbated contraction of human arterioles from patients with PRP and SRP in response to cooling (31°C) with or without α2-adrenergic agonists (UK14,304, serotonin, and angiotensin II) was associated with

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**Table 1. Pathogenesis of Raynaud’s phenomenon.**

<table>
<thead>
<tr>
<th>Intrinsic</th>
<th>Functional (locally produced mediators)</th>
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<tbody>
<tr>
<td>Structural</td>
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<tr>
<td>Inflammatory activation and damage</td>
<td>Decreased vasodilators</td>
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<tr>
<td>Endothelial cell apoptosis</td>
<td>Nitric Oxide, prostacyclin and leukotrienes</td>
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<tr>
<td>Intimal fibrosis</td>
<td>Increased vasoconstrictors</td>
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<tr>
<td>Smooth muscle hypertrophy</td>
<td>Endothelin, angiotensin II and thromboxane A2</td>
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<tr>
<td>Extrinsic</td>
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<tr>
<td>Neuroregulation</td>
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<tr>
<td>Sympathetic nervous system (vasoconstriction)</td>
<td>Soluble mediators</td>
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<tr>
<td>Noradrenephrine (α2-adrenoceptors)</td>
<td>Hormones (estrogens)</td>
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<tr>
<td>Neuropeptide Y (cotransmitter)</td>
<td>Platelet activation (thromboxane, platelet derived growth factor)</td>
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<tr>
<td>Parasympathetic nervous system (vasodilator)</td>
<td>White blood cell activation and oxidative stress</td>
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<tr>
<td>Vasoactive intestinal peptide (VIP)</td>
<td>(reactive oxygen species-ROS)</td>
</tr>
<tr>
<td>Sensory afferent fibers</td>
<td>Others</td>
</tr>
<tr>
<td>Calcitonin gene-related peptide (CGRP), substance P, neurokinin A</td>
<td>Smoking (ROS, viscosity, impaired fibrinolysis)</td>
</tr>
</tbody>
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increased intracellular tyrosine phosphorylation measured by fluorescence intensity and was reversed by genistein (a PTK inhibitor). They also demonstrate that arterioles from patients with Raynaud’s phenomenon lack vasodilator response to acetylcholine compared with controls. Interestingly, PTK activity has been linked to endothelial-dependent relaxation of vessels in response to different stimuli (i.e. platelet-derived growth factor). It is possible that patients with Raynaud’s phenomenon, in particular those with scleroderma, lack this counterbalancing response because of loss of endothelial integrity and/or function. In contrast, Flavahan et al. [13], using a similar ex-vivo system, found a normal response to acetylcholine and argued that endothelial dysfunction was a late manifestation of scleroderma vascular disease. The identity of the ultimate target protein for tyrosine phosphorylation is still unknown, and it is possible that PTK activity may increase secondary to abnormal levels of other mediators (i.e. platelet-derived growth factor, transforming growth factor-β, other cytokines, or reactive oxygen species) but while confirmation of this work is needed, the potential use of PTK inhibitor to treat Raynaud’s phenomenon is intriguing.

**Alpha-2c-adrenergic receptors and Rho/Rho kinase signaling**
Smooth muscle from cutaneous vessels is predominately innervated via the α2-adrenergic receptors. Three classes of the α2-adrenergic receptors (α2a, α2b, and α2c) exist, each with a unique distribution and function [14]. Chotani et al. [15] demonstrated that α2c-adrenergic receptors play a prominent role in vasoconstriction of cutaneous arteries after moderate cooling. Under normal conditions (37°C), α2c-adrenoceptors are silently stored within the Golgi apparatus and translocate to the cell surface after cold exposure, contributing to the adrenergic constrictive response. This year, these same investigators brought new evidence on how this process is regulated. Bailey et al. [16**] showed that cooling induces activation of Rho/Rho kinase signaling pathway, and this prompts translocation of α2c-adrenergic receptor from Golgi complex to the plasma membrane, together with augmented sensitivity to Ca++ of contractile proteins. Rho-kinase inhibitors (fasudil, Y-27632, and H-1152) were able to reverse constriction induced by α2-adrenergic agonists at cooling temperatures (28°C) but not at 37°C. They demonstrate that Rho-kinase inhibition with fasudil actually prevented what appears to be the normal translocation of α2c-adrenergic receptors to the cell surface, thus blunting the effect of the applied agonist. In an editorial commentary, Teixeira et al. [17] note that this investigation highlights the importance of Rho-kinase in trafficking molecules through microtubule depolymerization inside cells like vascular smooth muscle. In another study, Bailey et al. [18**] provide evidence that the initial trigger for Rho/Rho kinase signaling is provided by a rapid increase of reactive oxygen species (ROS) in smooth muscle cells following cold exposure (28°C). This is a relevant observation, because Raynaud’s vasospastic attacks may initiate a vicious cycle of ischemia-reperfusion with further production of ROS and activation of Rho/Rho kinase pathway via repeated episodes of vasospasm. To date, however, the use of antioxidants shows variable success in the treatment of Raynaud’s phenomenon. In a pilot study, Denton et al. [19] found that probucol reduced the frequency and severity of Raynaud’s attacks, while Mavrikakis et al. [20] did not show benefit with the antioxidant ascorbic acid on controlling Raynaud’s phenomenon. New investigations on Rho/Rho kinase inhibitors and antioxidants in the treatment of Raynaud’s phenomenon and other related vascular conditions remain of great interest. For example, new studies suggest benefit from Rho/Rho kinase inhibitors and statins (potent antioxidants) in treating pulmonary hypertension [21,22].

Chotani et al [23*], using human dermal arteriolar vascular smooth muscle cells (VSMs) in culture, demonstrated that serum (10% fetal bovine serum) stimulates a cyclooxygenase-2, cAMP, and Rap 1-dependent increase in α2c-adrenergic receptor expression on VSMs. This finding suggests that in-vivo vascular injury or other pathologic conditions could expose VSMs to serum factors that can increase the expression of cold sensitive α2c-adrenergic receptors, thus contributing to the severity of Raynaud’s phenomenon.

**Estrogen**
While there is impressive evidence for an intrinsic defect of vascular control in Raynaud’s phenomenon, significant data support also the influence by extrinsic factors on the vascular reactivity. Skin blood flow is further regulated by nonadrenergic mechanisms of vasoconstriction that may be altered by environmental temperatures [24]. Epidemiological studies suggest that estrogen use is associated with Raynaud’s phenomenon, but the biologic evidence demonstrates that estrogens may act as vasodilators [25]. This year, the role of estrogen and neuropeptides on the extrinsic regulation of vascular tone was comprehensively reviewed by Generini et al. [26*]. Arterial vessels relaxation is linked to the ability of 17-β estradiol to increase NO synthase expression and to induce calcium-dependent NO production [27]. Other mechanisms for dilator responses to estrogens have also been described, such as estrogen receptor-dependent (PI3K/Akt-mediated) up-regulation of cytochrome P450 activity [28]. Subsequent studies addressing endothelial function and vasomotor changes in patients with Raynaud’s phenomenon showed that acute and chronic estrogen administration has some positive effect on flow-mediated dilation of the brachial artery [29,30]. No data were provided, however, about effects on distal circulation or about variation of the clinical manifestation such as the symptoms, number, and duration of Raynaud’s phenomenon attacks. Given the controversy
about estrogen and vascular disease and the lack of definite studies about their effect on digital and cutaneous circulation, it is premature to recommend the use or not of these agents in patients with Raynaud’s phenomenon.

Neuropeptides
Neuropeptides can stimulate vasoconstriction or vasodilatation via endothelial dependent or independent pathways. Dysregulation of neuro-endothelial control of vascular tone has been suggested as another mechanism altering extrinsic control of vascular reactivity [26]. Evidence suggests a deficit of calcitonin-related peptide (CRP)–releasing nerves and an increased of neuropeptide Y in the skin of patients with Raynaud’s phenomenon [31]. This year, Stephens et al. [32] confirmed that the sympathetic cotransmitter neuropeptide Y can induce a reflex cutaneous vasoconstrictor response after mild cooling (31.7°C), and this effect is independent of norepinephrine. These studies were performed on human participants after total body cooling and measured skin blood flow by laser Doppler flowmetry following locally injected antagonist of neuropeptide Y-Y1 receptors. This observation broadens our understanding of how the sympathetic nervous system can potentially regulate vascular tone and suggests a possible alternative to pharmacological modulation of sympathetic vasoconstriction.

’Systeamic’ Raynaud’s phenomenon
A continued area of interest is the question whether the same vascular defects proposed to be present in the skin and digital vessels of patients with scleroderma exist in other organ systems. Can similar defects cause cold-induced systemic vasospasm or Raynaud’s of the circulation of internal organs? Evidence for cold-induced vasospasm of the arteries to the heart and kidney has been reported [33]. This year, Mukerjee et al. [34*] investigated whether pulmonary artery vasospasm occurred in scleroderma patients following a cold challenge of either hand immersion into cold water or the direct injection of cold water into the right atrium. In 21 patients, they could not demonstrate a significant change in hemodynamics of the pulmonary circulation by either cold provocation, thus confirming similar work by Wise et al. [35] and Shuck et al. [36]. While vasospasm of the vascular of internal organs likely is pathologic in scleroderma, environmental cold may not stimulate this vasoconstriction. This finding emphasizes that the critical regulatory mechanism and/or mediators of each organ’s circulation likely differ from each other.

New targeted therapies in Raynaud’s phenomenon
The growing understanding of the basic pathologic mechanisms shared by PRP and SRP and the elucidation of functional and structural differences in the vascular districts affected by Raynaud’s phenomenon are opening new avenues in the approach and treatment of this condition. New investigations emerged in the past year introducing more targeted and specific therapies directed against key mediators of digital vessels vasomotor control. These advances are discussed in this section (Fig. 1).

Figure 1. Vascular endothelium, smooth muscle cells, and nerve terminals form an integrated unit where specific interactions and soluble compounds released in the microenvironment participate to the regulation of vascular tone.
Autonomic nervous system: α2c-adrenoreceptors blockade
The sympathetic nervous system mediates a tonic vasoconstrictive effect on the vascular wall and plays a critical role in cutaneous thermoregulation. Nevertheless, the sympatholytic approach to treat Raynaud’s phenomenon has been quite disappointing in the past. Use of nonselective postganglionic sympathetic nerve blockers (reserpine, guanethidine, and phenoxybenzamine) has not proven to be successful for long-term therapy. Prazosin, a α1-adrenergic receptor antagonist, was more effective than placebo, but the response was modest, and side effects were common [37]. A more selective α-adrenergic blockade has been recently explored by Wise et al. [38] in 13 patients with Raynaud’s phenomenon secondary to scleroderma. In a randomized, double-blind, placebo-controlled trial, the investigators measured the effect of the investigational drug OPC-28326, an antagonist of α2c-adrenergic receptor, on skin temperature and digital blood flow following an acute cold challenge. The time for digital skin temperature to rewarm after cold challenge was significantly shorter following ingestion of 40 mg OPC-28326 compared with placebo. The digital blood flow showed improvement but was not statistically significant. Wigley and Czerwiec [39] reported the results of a multi-center, blinded, randomized, placebo-controlled study of OPC-28326 in a 2-week winter ambulatory study of 209 patients with Raynaud’s phenomenon. Interestingly, a reduction in the frequency of Raynaud’s attacks was found in the scleroderma-associated Raynaud’s phenomenon subgroup but was not effective clinically in the PRP subgroup. These studies support the importance of the α2c-adrenergic receptor in regulating cold-induced vascular responses, but broader clinical trials are necessary to confirm the usefulness of OPC-28326 or similar agents in the treatment of patients with Raynaud’s phenomenon.

Rho-kinase inhibition
As previously discussed, growing evidences indicate that Rho-kinases, the immediate downstream targets of the small guanosine triphosphate–binding protein Rho, are implicated in cold-induced vasoconstriction by inducing translocation of α2c-adrenoreceptors to the surface of blood vessel smooth muscle cells [16]. Furthermore, overactivity of Rho-kinases has also been observed in cerebral ischemia, coronary vasospasm, hypertension, vascular inflammation, and atherosclerosis [40]. Inokuchi et al. [41] recently demonstrated that fasudil, a Rho-kinase inhibitor, is effective in suppressing coronary artery spasm in patients with vasospastic angina. Tanaka et al. [42] used effectively fasudil in patients with symptomatic vasospasm after subarachnoid hemorrhage. Finally, Fukumoto et al. [21] observed a significant vasodilator effect of fasudil in patients with severe pulmonary hypertension. Rho-kinases, therefore, may represent a relevant therapeutic target both for the acute and long-term endothelial dysfunction in Raynaud’s phenomenon. Clinical trials need to be performed in this area.

Calcium channel blockers
Calcium channel blockers (CCBs) remain the most widely used class of drugs to treat Raynaud’s phenomenon and its complications (i.e. digital ischemia or ulcerations). A meta-analysis of 18 randomized, placebo-controlled, and double-blinded trials by Thompson and Pope [43] evaluated the efficacy of CCBs compared with placebo in patients with PRP. An average decrease of 2.8–5.0 attacks over a 1-week period and a 33% reduction in the severity was observed. These findings are similar to those of a previous meta-analysis by the same author on CCBs treatment for Raynaud’s phenomenon secondary to scleroderma (decrease of four attacks over 1 week and 35% severity reduction) [44]. It is important to emphasize that in clinical practice, the effective dose of CCB may be higher than used in the research trials, and the response is quite variable from patient to patient. Therefore, a standardized trial may not reflect the true overall benefit achievable by these agents, that still represent the most practical and valuable treatment for Raynaud’s phenomenon.

The endothelium plays a critical homeostatic role by integrating signals between the vessel lumen and the vascular wall. Under physiologic conditions, it participates in the regulation of vascular tone, cell trafficking, and blood fluidity by elaborating a variety of factors, such as prostacyclin, nitric oxide, endothelin, and adhesion molecules. In the pathologic condition, however, the endothelium can develop an altered phenotype and imbalanced secretory profile, favoring vasoconstriction, inflammation, and thrombosis. Significant interest is growing around these endothelial-derived molecules as possible targets in the treatment of Raynaud’s phenomenon, either by blocking the vasoconstrictive mediators or by supplementing the vasodilative ones.

Prostaglandins
Prostacyclin is a potent vasodilator that has also shown an anti-proliferative effect on smooth muscle cells and inhibition of platelet aggregation. Iloprost, a prostacyclin analogue, is used to treat severe pulmonary hypertension and critical digital ischemia in patients with Raynaud’s phenomenon [45]. Interestingly, trials designed to assess efficacy on ischemic outcomes in both adult and pediatric populations reported a significant improvement on number and duration of vasospastic attacks after intravenous administration of iloprost [46,47]. In some cases, Raynaud’s phenomenon completely subsided during the follow-up period. A randomized, observer-blind, controlled study by Marasini et al.
hypertension [48] compared use of iloprost and alprostadil in a group of 21 women (18 with systemic sclerosis) affected by severe Raynaud’s phenomenon. Although the number of patients studied was small, iloprost and alprostadil were both effective in improving Raynaud’s phenomenon symptoms assessed by visual analogue scale; however, only alprostadil reached statistical significance. The modified Rodnan skin score decreased with iloprost but not alprostadil. This possible ‘disease-modifying’ property of prostaglandins is also reported in another trial on the basis of periodically repeated intravenous iloprost infusions [49]. Intravenous epoprostenol and alprostadil have also been reported as being helpful for severe digital ischemia and Raynaud’s phenomenon symptoms in scleroderma [50,51]. This year, inhaled iloprost is being introduced into the United States for the treatment of pulmonary hypertension [52].

**Endothelin receptor antagonists**

The endothelins contribute to complex homeostatic functions in a variety of organs, working in a paracrine and autocrine fashion [53]. In the blood vessels, the endothelin system exerts a basal vasoconstrictive effect and is involved in the pathogenesis of different vascular disorders such as arterial hypertension, atherosclerosis, and heart failure. Endothelin-1 (ET-1) is the most potent endogenous vasoconstrictor yet described and mediates vasoconstriction and cell proliferation through activation of specific endothelin(A) and endothelin(B) receptors on VSMCs.

The nonselective endothelin(A/B) receptor antagonist bosentan has been used for treatment of severe pulmonary hypertension, showing improvement of survival together with exercise endurance, hemodynamics, and functional class [54,55]. In the recent randomized placebo-controlled study on prevention of ischemic digital ulcers in scleroderma (RAPIDS-1) trial for prevention and treatment of digital ulcers in patients with scleroderma (n = 122), bosentan decreased in a 16-week period the mean number of new ulcers by 48% compared with placebo [56]. No benefit was found with bosentan on healing of existing lesions. Scores for Raynaud’s severity, calculated by patient’s assessment, were similar in the bosentan and placebo groups. These data suggest that the clinical benefit of anti-endothelin therapy may extend beyond the vasodilatory effect, given the proliferative and profibrotic effect of endothelin-1. In contrast with the RAPIDS-1 study experience, a significant improvement of Raynaud’s phenomenon and associated symptoms has been anecdotally reported after institution of bosentan therapy in four patients (three with pulmonary hypertension) [57]. New studies are now investigating the role of bosentan in healing new digital ulcers in scleroderma. While inhibition of endothelin clearly improves the pulmonary circulation, more insight into the role of these agents within the peripheral circulation and in treating Raynaud’s phenomenon is needed.

**Phosphodiesterases inhibitors**

Intracellular responses to prostacyclin and NO are mediated by the cyclic nucleotides cAMP and cGMP, respectively. Phosphodiesterases are a complex group of enzymatic molecules that contribute to the tight regulation of intracellular cyclic nucleotides levels by their degradation. Eleven families and more then 60 isoforms of phosphodiesterases have been described to date [58]. The therapeutic efficacy of selective phosphodiesterase inhibition is already appreciated in conditions such as erectile dysfunction and pulmonary hypertension. In the very recent Sildenafil versus Endothelin Receptor Antagonist for Pulmonary Hypertension (SERAPH) trial, the addition to conventional treatment of sildenafil, a phosphodiesterase 5 inhibitor, in patients with pulmonary hypertension (WHO functional class III) showed reduction of the right ventricular mass and improvement of cardiac function and exercise capacity [59]. Successful use of sildenafil in reducing severity and numbers of attacks in 13 patients with SRP (with or without digital ischemia) is described in two case series [60,61]. Moreover, tadalafil, a new phosphodiesterase 5 inhibitor, was effective in one patient with SRP failing to respond to sildenafil [62]. Cilostazol (100 mg orally twice a day) is the only phosphodiesterase inhibitor (phosphodiesterase 3) studied in a randomized, double-blind, placebo-controlled trial for the treatment of Raynaud’s phenomenon [63]. Results were modest and limited to an increase in flow-mediated dilation of the brachial artery in patients with PRP and SRP. No significant changes to the microvascular flow or symptoms were observed. Although these studies are quite limited, they provide rationale for further investigations into the use of phosphodiesterases alone or in combination with another agent (e.g. CCB) in the treatment of Raynaud’s phenomenon.

**Circulating factors**

Serotonin is a potent circulating vasoconstrictor released from platelets whose role in Raynaud’s phenomenon is not clearly defined. Nevertheless, in recent years, several reports have described the usefulness of the selective serotonin reuptake inhibitors (SSRI) in the treatment of Raynaud’s phenomenon [64–66]. The number of treated patients is extremely small, and worsening of Raynaud’s phenomenon symptoms is also described with use of SSRIs. Well designed placebo-controlled trials are now required to assess the true role of these drugs in the management of Raynaud’s phenomenon.

**Miscellaneous**

Other treatments normally applied to different conditions are now considered for Raynaud’s phenomenon because of...
their potential vasodilative effect. Sycha et al. [67*] observed subjective improvement of Raynaud’s phenomenon symptoms (visual analogue scale) and objective increase of cutaneous blood flow (laser Doppler) in two patients with Raynaud’s phenomenon (one PRP and one SRP) after intra-digital injections of botulinum toxin A. They based this trial on the observation that botulinum toxin A reduced the amplitude of arterial isometric contractions in response to repeated electrical stimulation of sympathetic axons [68].

Sibell et al. [69] presented an interested case report and proposed the use of cervical spinal cord stimulation to treat severe Raynaud’s phenomenon associated with refractory digital ischemia. The use of spinal cord stimulation in peripheral vascular disease is controversial, with no good controlled studies in Raynaud’s phenomenon. In addition, sympathetic nerve blocks and surgical digital sympathectomy also lack controlled trials or long-term follow-up. The spinal cord stimulation needs to be explored further as a possible alternative before the method can be recommended as treatment for severe Raynaud’s phenomenon.

Hirschl et al. [70] studied in a placebo-controlled, double-blind intervention the efficacy of low-level laser therapy in 48 patients with PRP by irradiating their fingers and dorsum of the hands for 30 to 40 minutes in five sessions over 3 weeks. A significant reduction in frequency and severity (82%) of Raynaud’s attack was detected in the participants receiving laser therapy compared with sham treatment. Similar beneficial effect has been described by in 47 patients with PRP or SRP by al-Awami et al. [71]. The mechanism of action of low-level laser therapy remains speculative, and the clinical importance of the degree of improvement measured is not clear from current published studies.

Measuring disease and outcome in Raynaud’s phenomenon

Both PRP and SRP are characterized by episodic vasospastic events that can be quantified (numbers and severity) and characterized (extension and distribution of involvement, complications). However, these measurements are based on the clinical assessment by the physician and on the observation/report by the patient. They do not reflect with precision the degree of vascular involvement or, in the case of PRP, reveal the presence of underlying conditions or predict the possible future progression to a secondary process such as a connective tissue disease. For this reason, there is an effort to design and develop more objective tools that provide visualization and quantification of the micro-vascular blood flow and measure abnormalities at baseline or in response to stimuli triggering vasospasm.

Laser Doppler applications

Laser Doppler flowmetry is used to measure blood flow in the skin of the extremities and is based on a single probe applied to a very small skin area (i.e. fingertips).

Laser Doppler imaging

Recently, Murray et al. [72*] reviewed laser Doppler imaging (LDI). This technique measures blood flow through Doppler scanning of larger skin areas (e.g. palmar surface of hands), thus avoiding the site-to-site variability and poor reproducibility of laser Doppler flowmetry. Since the development of the LDI more then 10 years ago, small cross-sectional studies have demonstrated its ability to measure microcirculatory blood flow and to discriminate between different forms of Raynaud’s phenomenon [73,74]. The use of LDI remains investigational.

Ziegler et al. [75] applied laser Doppler anemometry to determine microvascular defect in vibration white finger syndrome (VWF) and PRP. This technique incorporates a laser detector into a nail-fold capillary microscopy examination and measures the nutritional blood flow as well as the blood cells’ velocity in the digital skin capillaries. In this study, patients with VWF showed a marked reduction of postocclusive-induced vasodilation compared with PRP, suggesting the presence of underlying structural vessel wall defects (i.e. endothelium-dependent vasodilatation) in VWF.

Another application in the use of laser Doppler is laser Doppler skin perfusion pressure (LOSPP). Digital skin perfusion pressure is measured by coupling manometry and laser Doppler-mediated blood flow detection during inflation/deflation of a pneumatic cuff wrapped around the palmar aspect of the third finger middle phalanx. A significant drop in the perfusion pressure after cold exposure was detected in patients with PRP and SRP compared with controls [76].

Limb scintigraphy

Finally, a study by Sarikaya et al. [77] evaluated 99mTc sestamibi limb scintigraphy as a tool to measure skin perfusion in patients with PRP. As expected, cold exposure determined a statistically significant decrease of tracer uptake in the fingers of patients with Raynaud’s phenomenon compared with controls. Interestingly, the test was repeated after 2 months of treatment with a CCB (Nifedipine 30 mg daily), showing blood flow improvement in all study patients. Correlation between 99mTc sestamibi scintigraphy measurements of microcirculatory blood flow and Raynaud’s symptoms score was not formally investigated.

Conclusion

The clinical assessment of Raynaud’s phenomenon and the approach to its treatment are now being integrated by significant advances elucidating the pathways leading to vasomotor instability in the digital and cutaneous
circulation. New therapies based on the identification and targeting of molecules implicated in the pathogenesis of this disease are emerging, and encouraging preliminary results are reported. Ideally, further insight into the macro-vascular and micro-vascular abnormalities characterizing cold hyper-reactivity in PRP and SRP will be gathered. This, together with the identification of new bio-markers and the development of more sophisticated diagnostic techniques, will prompt early detection of underlying disorders and help to predict clinical outcome and response to 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

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 773).


5. Lewis T. Experiments relating to the peripheral mechanisms involved in spasmatic arrest of the circulation in the fingers, a variety of Raynaud's disease. Heart 1929; 18:7–101.


This is a highly recommended comprehensive and current review of pathogenesis in Raynaud's phenomenon.


This is the first report describing transduction of vibrational energy into a biochemical signal inside endothelial cells. Fluid vibratory forces applied to the endothelium induce extracellular signal-regulated kinase (ERK 1/2) phosphorylation and endothelin-1 release.


This article suggests that PTK activity and intracellular VSM phosphorylation are implicated in the increased contractile response to cold and adrenergic agonists in patients with primary Raynaud's phenomenon.


The first study to show that cooling induced activation of Rho/Rho kinase signaling pathway induces translocation to the plasma membrane of VSMs of alpha2c-adrenoceptors stored in the Golgi complex and augments sensitivity to Ca++ of contractile proteins. These effects and cold-induced vasospasm are clearly reversed by Rho-kinase inhibitors supporting the potential use of these molecules to treat Raynaud's phenomenon.


This extremely important study confirms that the initial trigger for Rho/Rho kinase signaling is provided by a rapid increase of ROS in smooth muscle cells after cold exposure. ROS inhibitors were able to abolish cold-induced increase in alpha2c-adrenoceptor constrictor activity. These results prompt reconsideration of anti-oxidant treatment strategies in Raynaud's phenomenon.


Findings in this study suggest that exposure to serum induces expression of alpha2c-adrenoceptor receptors on VSMs. Similarly enhanced vasoreactive responses to cooling can result in vivo from traumatic or inflammatory events exposing VSMs to serum.


Comprehensive review of the extrinsic regulation of vascular tone by neuropepti-des and estrogen. This article also discusses the controversial role of estrogen in regulation of vascular tone.


This study highlights the role of non-adrenergic molecules in mediating cold-induced vasoconstriction.


Marasini B, Massarotti M, Bottasso B, et al. Comparison between iloprost and alprostadil in the treatment of Raynaud’s phenomenon. Scand J Rheumatol 2004; 33:253—256. This article supports the notion that the prostaglandin PGE1 is as useful as the prostacyclin PGI2 in treating severe Raynaud’s phenomenon.


Introduction
Several lines of evidence suggest a genetic basis for the susceptibility to systemic sclerosis or scleroderma. Systemic sclerosis occurs significantly more frequently within families with systemic sclerosis than in the general population. A positive family history of systemic sclerosis confers the strongest relative risk for disease, although the absolute risk for each family member remains quite low (<1%) [1]. The concordance for the clinical phenotype is similar between monozygotic and dizygotic twins, ~5%, but the concordance rate for antinuclear antibodies was significantly higher in monozygotic twins (95%) compared with dizygotic twins (60%) [2]. Finally, microarray and quantitative real-time PCR studies of cultured dermal fibroblasts revealed that clinically discordant monozygotic twins are in fact concordant at the molecular level for increased expression of transcripts considered markers of the fibrotic phenotype (collagen type I α2 (COL1A2), SPARC, connective tissue growth factor (CTGF), TIMP1, and others). The concordance rate for monozygotic twins was 46% compared with 0% for dizygotic twins for this molecular phenotype (Dr Carol Feghali, Personal communication). These data provide compelling evidence for a genetic contribution to two of the most prominent features of systemic sclerosis, autoimmunity and fibroblast activation.

Gene polymorphisms
A Medline search revealed only a handful published studies on candidate genes relevant to systemic sclerosis pathogenesis in the review period (Table 1).

Immunologic factors
Genes affecting immune regulation and inflammation are natural candidates for investigation of associations with systemic sclerosis.

Tumor necrosis factor
Tumor necrosis factor (TNF)-α is a key proinflammatory cytokine that has recently gained importance in rheumatoid arthritis, psoriatic arthritis, and ankylosing spondylitis as an effective therapeutic target. TNF-α is involved in the pathogenesis of vascular occlusion and connective tissue remodeling and may have anti-fibrotic activities. TNF-α blocks transforming growth factor (TGF)-β-induced genes and signaling pathways in systemic sclerosis dermal fibroblasts and decreases production of type I collagen and tissue inhibitor of metalloproteinases 1 in systemic sclerosis fibroblasts [3].
Sato et al. [4**] investigated the frequency of five TNF-α promoter single nucleotide polymorphisms (SNPs) in 214 unrelated Caucasian patients with scleroderma and 354 controls. An increase in the frequency of the C allele at position −1031 was observed in patients with systemic sclerosis compared with controls (46.3% compared with 35.5%; \( P_{\text{corr}} = 0.05 \)). Stratification of patients into nonoverlapping autoantibody subsets showed a striking association between anticientromere antibodies and the C allele at position −1031 (\( P_{\text{corr}} = 0.00005 \)) and the A allele at position −863 (\( P_{\text{corr}} = 0.00002 \)). Using haplotype mapping, the observed associations with TNF-α promoter SNP was actually stronger than the human leucocyte antigen (HLA) class II [5,6] association with anticientromere antibodies (i.e. \( \chi^2 = 27.7 \) for TNF-863A compared with \( \chi^2 = 6.0 \) for HLA-DRB1*01), suggesting that the linkage disequilibrium between the TNF-α and HLA class II could account for latter’s association with anticientromere antibodies.

Tolusso et al. [7] investigated TNF-α (position −238 and +489) and TNF-RII (position +196) polymorphisms in 114 Italian patients with systemic sclerosis and 170 healthy blood donors. Weak associations were found with A alleles at positions −238 and +489 with systemic sclerosis (\( P = 0.03; \) OR = 2.4 \([0.95–6.44]\)) and \( P = 0.044; \) OR = 1.46 \([0.95–2.24]\), respectively). A subgroup analysis showed that the overall association was mainly a result of the diffuse systemic sclerosis. These associations did not withstand correction for multiple comparisons.

**Monocyte chemotactic protein 1**

Infiltration of mononuclear cells in the dermis and increased collagen synthesis are characteristic histologic features of systemic sclerosis. Monocyte chemotactic protein 1 (MCP-1) is one of the most potent chemokines for monocytes and macrophages and can stimulate type I collagen gene expression in fibroblasts [8,9]. Moreover, there is evidence for MCP-1 dysregulation in systemic sclerosis fibroblasts [9,10].

**Interleukin-1α**

Previous studies have shown that that unlike normal fibroblasts, systemic sclerosis dermal fibroblasts express interleukin-1α mRNA and protein constitutively. Studies of IL-1α SNPs report conflicting results. Kawaguchi et al. [12] reported an association of systemic sclerosis with the IL-1α-889 C allele (\( P < 0.0001 \)) in a Japanese population.

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### Table 1. Investigated single nucleotide polymorphisms in the review period (January 2004–May 2005).

<table>
<thead>
<tr>
<th>Patient, n</th>
<th>Control, n</th>
<th>Summary</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>TNF promoter 214 354</td>
<td>−1031 C allele and −863 A allele are strongly associated with ACA</td>
<td>[4**]</td>
<td></td>
</tr>
<tr>
<td>TNFα 114 170</td>
<td>−238 and +489 A alleles are weakly associated with SSC</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>TNF-RII 114 170</td>
<td>TNF-RII + 196 polymorphism is not associated with SSC</td>
<td>[7]</td>
<td></td>
</tr>
<tr>
<td>MCP-1 promoter 18 139</td>
<td>−2518 G/G genotype is associated with SSC</td>
<td>[11*]</td>
<td></td>
</tr>
<tr>
<td>IL-1α 46 150</td>
<td>−889 T allele is associated with SSC</td>
<td>[13]</td>
<td></td>
</tr>
<tr>
<td>CTLA-4 137 156</td>
<td>CTLA-4 polymorphisms were not associated with SSC</td>
<td>[15]</td>
<td></td>
</tr>
<tr>
<td>KIR 102 100</td>
<td>Combination of KIR2DS2+ and KIR2DL2− is associated with SSC</td>
<td>[16]</td>
<td></td>
</tr>
<tr>
<td>CD19 134 96</td>
<td>−499T allele was associated with SSC</td>
<td>[18*]</td>
<td></td>
</tr>
<tr>
<td>eNOS 77 49</td>
<td>eNOS G894T is not associated with SSC</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>eNOS 164 184</td>
<td>eNOS G894T is not associated with SSC</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td>NADPH oxidase 77 49</td>
<td>NADPH oxidase C242T is not associated with SSC</td>
<td>[22]</td>
<td></td>
</tr>
<tr>
<td>ACE 164 184</td>
<td>ACE ID is not associated with SSC</td>
<td>[23]</td>
<td></td>
</tr>
<tr>
<td>GST 51 61</td>
<td>GSTM1*B is associated with SSC</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td>MnSOD 51 61</td>
<td>MnSOD (alanine or valine polymorphisms are not associated with SSC</td>
<td>[26]</td>
<td></td>
</tr>
<tr>
<td>SPARC 121 200</td>
<td>SPARC polymorphisms are not associated with SSC</td>
<td>[30*]</td>
<td></td>
</tr>
<tr>
<td>NAT2 39 100</td>
<td>NAT2 polymorphisms are not associated with SSC</td>
<td>[27]</td>
<td></td>
</tr>
</tbody>
</table>

*Mantel–Haenzel analysis for the entire population.

ACA, anticientromere antibody; ACE, angiotensin-converting enzyme; CTLA, cytotoxic T-lymphocyte–associated antigen; eNOS, endothelial nitric oxide synthase; GST, glutathione S-transferase; IL, interleukin; KIR, killer cell immunoglobulin-like receptor; MCP, monocyte chemotactic protein; MnSOD, manganese superoxide dismutase; NADPH, nicotinamide adenine dinucleotide phosphate oxidase; NAT2, arylamine N-acetyltransferase 2; SPARC, secreted protein, acidic and rich in cysteine; SNP, single-nucleotide polymorphism; TNF, tumor necrosis factor.
In contrast, the same polymorphism was investigated in 46 Slovak patients with systemic sclerosis and 150 healthy controls. Hutyrova et al. [13] reported instead that the T allele was significantly associated with systemic sclerosis (63% compared with 42%; \( P = 0.01; \) OR = 2.3 [1.2–4.6]).

**Cytotoxic T-lymphocyte-associated antigen 4**

Cytotoxic T-lymphocyte-associated antigen 4 is a T-cell surface glycoprotein that negatively regulates T-cell function. The reports of genetic associations with this gene are also conflicting. Takeuchi et al. [14] did not find any significant association of systemic sclerosis and SNPs at positions +49 and −308 in the Japanese population. In contrast, Hudson et al. [15] studied this SNP plus three additional ones at positions −318, −1661, and −1722 in 100 Caucasian and 37 African American patients with systemic sclerosis, and 122 white and 34 African American controls. They report an association of heterozygosity with systemic sclerosis (\( P = 0.003 \)) and a negative association with homozygosity for the A allele (\( P = 0.007 \)) at position +49 in African Americans. However, the genotype distribution deviated significantly from Hardy–Weinberg equilibrium in the African American patients (\( P = 0.0004 \)).

**Killer cell immunoglobulin-like receptors**

Killer cell immunoglobulin-like receptors (KIRs) modulate T-cell activation. Momot et al. [16] investigated nine KIR genes in 102 German Caucasian patients with systemic sclerosis and 100 race-matched controls. Presence or absence of any single KIR subtype was not significantly associated with systemic sclerosis after correction for multiple comparisons. Twelve patients with systemic sclerosis, however, compared with only two controls, had KIR phenotypes characterized by presence of the activating KIR2DS2 and the absence of the corresponding inactivating KIR2DL2 (\( P = 0.005 \)). The authors suggest that the combination of KIR2DS2+ and KIR2DL2− is associated with systemic sclerosis.

**CD19 receptor**

CD19 is a B-cell-specific transduction molecule that defines signaling thresholds critical for humoral responses and autoimmunity. The CD19 is over-expressed by peripheral B cells from patients with systemic sclerosis [17]. Tsuchiya et al. [18] investigated a promoter SNP at position −499(G/T) and a GT repeat in 3′-UTR of the CD19 gene in 134 Japanese patients with systemic sclerosis and 96 controls. CD19 expression levels in peripheral blood memory and naive B cells were also examined by flow cytometry and correlated with the CD19 promoter SNP genotype in 40 systemic sclerosis cases. They found that the frequency of T allele (\( P = 0.003; \) OR = 2.18 [1.31–3.86]) was significantly increased in patients with systemic sclerosis and also correlated with higher CD19 expression levels.

**Vascular and (anti)oxidative factors**

Endothelial cell dysfunction and oxidative stress have been implicated in the pathogenesis of systemic sclerosis.

**Endothelial nitric oxide and nicotinamide adenine dinucleotide phosphate oxidase**

Endothelial cells constitutively express endothelial nitric oxide, which regulates the production of nitric oxide. Inducible nitric oxide synthase (NOS) is produced during inflammatory states, which leads to cellular injury by increased production of free oxygen radicals. Immunohistochemical studies of systemic sclerosis skin biopsies have shown that eNOS expression was inversely related to the grade of skin disease, whereas inducible NOS staining was increased with higher levels of skin involvement [19]. Conflicting data have been reported on the association of the eNOS G894T polymorphisms with systemic sclerosis in two Italian populations [20,21]. Recently, two additional studies of this polymorphism in patients with systemic sclerosis have been reported. Allanore et al. [22] genotyped 77 French Caucasian patients and 49 matched controls, and our group investigated this polymorphism in 164 patients with systemic sclerosis (76 white, 28 African American, 53 Hispanic) and 184 healthy controls of similar ethnic composition [23]. Both studies showed no association with the eNOS G894T polymorphism and systemic sclerosis.

Oxidative radical generation may play a role in pathogenesis of systemic sclerosis. NADPH oxidases are the largest producers of superoxide anion [24]. Allanore et al. [22] also studied the frequency of the p22phox NADPH oxidase subunit (C242T) polymorphism and found no significant association with systemic sclerosis.

**Angiotensin-converting enzyme**

Angiotensin-converting enzyme (ACE) catalyzes the conversion of angiotensin I into the vasoactive and aldosterone-stimulating peptide angiotensin II and inactivates bradykinin, a vasodilator. The ACE I/D polymorphism accounts for some of the observed variance of the serum ACE level in general population, with the ACE D allele correlating with higher levels. A previously study of 73 Italian patients with systemic sclerosis and 112 race-matched controls reported an association of ACE D allele with systemic sclerosis [20]. In contrast, we found no association with systemic sclerosis and the ACE D allele in our cohort [23].

**Glutathione S-transferase isoenzymes and manganese superoxide dismutase**

Glutathione S-transferases (GSTs) are ubiquitous multifunctional enzymes that protect the cells against the oxidative stress by conjugating them to glutathione. Manganese superoxide dismutase is another protective enzyme that limits the effect of oxidative stress on mitochondria.
by scavenging superoxide anions produced from the electron transport system. Null alleles for GSTM1 and/or GSTT1 were not associated with systemic sclerosis in a multi-ethnic cohort of 152 patients with systemic sclerosis [25]. Tikly et al. [26] investigated various subtypes of GSTs (GSTM1*A, GSTM1*B, GSTT1*I, GSTP1*A, GSTP1*B, GSTP1*C, GSTP1*D) as well as alanine or valine polymorphism in the manganese superoxide dismutase gene on 51 South African patients with systemic sclerosis and 61 ethnically matched controls. The authors reported a negative association of the GSTM1*B subtype with systemic sclerosis (Pcorr < 0.05; OR = 0.19; CI, 0.04–0.77). A subgroup analysis revealed no association with specific clinical features after correction for multiple comparisons.

Arylamine N-acetyltransferase 2

Arylamine N-acetyltransferase 2 (NAT2) plays an important role in the metabolism of a large and diverse number of aromatic amines. Such agents have been implicated as environmental triggers for systemic sclerosis. Skretkowicz et al. [27] investigated NAT2 SNPs in 39 Polish Caucasian patients with systemic sclerosis and 100 ethnically matched controls. Using isoniazid as a model drug, a strong correlation between NAT2 acetylation phenotype and NAT2 genotype was found. The frequency of the acetylator status and the NAT2 genotypes did not differ between patients with systemic sclerosis and controls, however.

Regulation of the extracellular matrix

Increased synthesis and deposition of extracellular matrix protein is a cardinal feature of systemic sclerosis.

Secreted protein, acidic and rich in cysteine

Secreted protein, acidic and rich in cysteine (SPARC) or osteonectin is an important regulator of extracellular matrix metabolism and has been found in a variety of tissues undergoing wound healing and remodeling. SPARC mRNA expression is increased in fibroblasts cultured from the affected skin of patients with systemic sclerosis [28]. Association studies with SPARC also show conflicting results. Three SPARC SNPs in the 3′-untranslated region were studied previously, and homozygosity for the C allele at position +998 was found to be associated with systemic sclerosis in a multi-ethnic population. In addition, the C-allele correlated with increased transcript half-life in skin fibroblasts [29]. In contrast, Lagan et al. [30] recently studied the same three SNPs and five additional SPARC SNPs (two in the promoter region, one in exon 3, and two in the 3′-untranslated region) in 121 United Kingdom Caucasian patients with systemic sclerosis and 200 controls. No significant differences in genotype, allele, or haplotype frequency were observed between patients with systemic sclerosis and controls or within systemic sclerosis subgroups (with or without fibrosing alveolitis).

Microarrays

Expression profiling using microarrays is a powerful technology that allows the assessment of the abundance of thousands of transcripts simultaneously. This is an attractive prospect in complex diseases such as systemic sclerosis because it allows the investigator to examine interactions of multiple gene products along biologic pathways, or it may reveal novel processes heretofore unrecognized. This model-free approach has been used to fingerprint pathologic processes, subset diseases at the molecular level, and predict disease outcome [31,32]. Significantly smaller sample sizes are needed to detect disease-specific-expression profiles with microarrays [33] because the gene expression profile in a given cell type is the result of the combined effects all the genetic polymorphisms of the donor [34], and the data from array studies have a higher dimension than the usual single-gene association studies.

Whitfield et al. [35] investigated gene expression pattern in skin biopsies of four patients with systemic sclerosis with diffuse disease and four normal controls. With this small sample size, the authors reported consistent differences in gene expression profiles between systemic sclerosis skin biopsies and skin biopsies of unaffected individuals. In agreement with previous observations that nonlesional skin in systemic sclerosis is already abnormal [36], this report found that clinically lesional and nonlesional skin areas of patients with systemic sclerosis showed nearly indistinguishable expression patterns. Genes characteristically expressed in endothelial cells, B lymphocytes, and fibroblasts showed differential expression between scleroderma and normal biopsies. The gene expression profile from cell culture fibroblasts of patients with scleroderma (n = 6), morphea (n = 4), and normal controls (n = 3) was also examined, but no obvious differences could be detected in the patterns of gene expression between these types of fibroblasts.

In contrast, we studied early passage nonlesional dermal fibroblasts from 21 diffuse patients with systemic sclerosis and 18 healthy controls and found that <5% of genes out of ~8500 fibroblast genes were differentially expressed (using rigorous statistical criteria to control false discoveries) [37**]. Despite the small proportion of differentially expressed genes, they affect a wide variety of biologic processes, including those involved with extracellular matrix formation, fibrillogenesis, angiogenesis, and complement activation (http://www.uth.tmc.edu/scleroderma). Some genes that were most discriminating for systemic sclerosis fibroblasts were the basement membrane nonfibrillar collagen genes, collagen type VII α1 (COL7A1), and XVIIα1 (COL17A1) or endostatin, as well as decay accelerating factor.

The fact that scleroderma fibroblast phenotype is eventually lost in vitro has been well described. This may be
a result of the loss during experimental manipulation of cofactors present in vivo. These array data suggest that the study of tissue samples such as intact skin biopsies or whole blood rather than cultured cells will capture a greater diversity and complexity of gene expression because the biologic state of the cells in the tissue samples is likely to be closer to that in vivo (so long as measures are taken to stabilize the RNA). Candidate genes or pathways identified through arrays can be explored as potential biomarkers, used for molecular phenotyping of systemic sclerosis, or targeted for future genetic association studies.

**Conclusion**

Investigation into the genetic contribution to systemic sclerosis in the general population is complicated by the low prevalence of disease and the lack of multiplex families. Genetic association studies have the potential to circumvent these obstacles but unfortunately are plagued with low reproducibility. For example, of the five candidate genes discussed in this review, none of initial associations were replicated in independent cohorts. What are the reasons for such poor reproducibility?

Population stratification has been put forth as an explanation for spurious associations. Stratification occurs when disease frequency varies across subpopulations, resulting in selection bias in the affected group for those subpopulations. It can also arise from recent population admixture when the frequency of the disease allele differs among the founding parent populations. With stratification, the \( \chi^2 \) test of independence between the disease and candidate gene (or allele) will be inflated, leading to a false positive. However, advances in population genetics have resulted in the development of methodologies to account for stratification post hoc. By genotyping a series of unlinked SNPs that are neutral with respect to disease susceptibility (also termed null SNPs), it is possible to derive a correction factor to adjust the critical value of the significance tests for the candidate gene. This approach is termed **genomic control** [38,39], and because of its simplicity, it is being used increasingly to correct for population stratification. With recent refinements, it has been estimated that at least 30 null SNPs will be sufficient to perform the genomic control adjustment [40]. As costs for multiplex, high-throughput genotyping continue to fall, it should be possible to incorporate such a panel of SNPs routinely into candidate gene studies in a given cohort as a one time cost. On the other hand, for genome-wide SNP studies, this will not add a significant cost because it is highly likely that the vast majority of SNPs typed in this sort of investigation will be neutral in terms of disease susceptibility.

The success of genetic association studies also depends on discovering close linkage disequilibrium between the disease gene, marker, or haplotype and the clinical phenotype. It can therefore be readily appreciated that a precise definition of the phenotype becomes critical. Current genetic association studies are handicapped by the heterogeneous presentation and course of systemic sclerosis. To mitigate these effects, genetic association studies could be carried out in more narrowly defined subtypes of systemic sclerosis. What parameters are to be used to define disease subsets is still a matter of debate, although it is likely that simple partitioning into diffuse or limited systemic sclerosis will not be adequate. Our gene expression data show that dermal fibroblasts from patients can be readily subgrouped (http://www.uth.tmc.edu/scleroderma/Cluster/Cluster%20analysis.html) despite being explanted from a group of donors that are clinically homogenous (i.e. all had diffuse systemic sclerosis).

A final consideration is that genetic variations associated with late-onset diseases such as systemic sclerosis usually allow transmission of genes to the next generation before the clinical onset of disease. Thus, unlike Mendelian diseases, there will be considerably more genetic noise because of involvement of multiple loci or risk alleles (epistasis). Allelic and locus heterogeneity are examples of this complexity. Allelic heterogeneity occurs when there are various alleles in a locus responsible for increased disease risk (e.g. multiple allelic variants of breast cancer 1 (BRCA1) associated with breast and ovarian cancer). Locus heterogeneity occurs when alleles at different loci are responsible for increased disease risk (e.g. factor V Leiden and prothrombin G20210A mutations associated with venous thrombosis). The total genetic effect might be partitioned among multiple loci, so the genotype–phenotype correlation conferred by any single risk allele is likely to be small (OR \( \leq 1.5 \)). On the other hand, if a given risk allele confers a large genetic effect, the allele frequency will be low because of negative selection pressure. For these reasons, candidate gene association studies in systemic sclerosis may suffer from insufficient statistical power, or the candidate locus itself may have low prior probability for disease association. For the former, the most straightforward solution will be to increase sample size. The use of SNP haplotypes rather than single SNPs would also increase power. Recently, it has become apparent that SNPs on the chromosomes are inherited in blocks. These haploblocks may contain a large number of SNPs, but a few haplotype-tagged SNPs are enough to uniquely identify all the haplotypes in a block (http://www.hapmap.org/index.html.en). This dramatically reduces the number of SNPs that will need to be genotyped for a given candidate region, although it should be pointed out that tagged SNPs may vary from African study participants compared with European Caucaian study participants because of the different patterns of linkage disequilibrium. Multiplex SNP assays such as SNPlEx (Applied Biosystems, Foster City, CA) or the Gene Chip Mapping 100K Set (Affymetrix, Santa Clara, CA) are promising developments for whole-gene or whole-genome
association studies. Genotyping a large number of SNPs will result in greater resolution to detect subtle genetic differences between populations [41], although in this setting, measures need to be undertaken to control the proportion of false discoveries [42]. At minimum, a suitable threshold level of significance needs to be specified to minimize the chances of false positives. For genome-wide SNP association studies, Risch and Merikangas [43] proposed a significance level of $10^{-8}$ to account for all SNPs in the human genome. Such stringent levels will be very difficult to achieve for any SNP or gene, leading some to seek more deterministic alternatives. According to Neale and Sham [44], for the ~30,000 expressed genes in the human genome, the threshold genome-wide significance level ($P$) can be estimated by $P = 1 - (1 - \alpha)^m$, where $m$ is the number of contributory genes in the genome and $\alpha$ is the level of significance at the level of the gene. Thus, a given gene needs to be significant at level of $1.7 \times 10^{-6}$ to achieve a genome-wide significance of $P = 0.05$. Similarly, Van den Oord and Sullivan [45] argued that highly stringent significance levels sacrifice power and that the goal instead should be to obtain an acceptable ratio of true and false discoveries. Using their formula for controlling false discovery rates (specifying a false discovery rate of 5%), we calculate a threshold significance levels of $7 \times 10^{-6}$ for a five-disease gene model and $1.4 \times 10^{-5}$ for a 10-disease gene model, assuming 30,000 expressed genes. Furthermore, if we then assume that the allele frequency of the putative disease allele was similar to that of the PTNP22 R620W SNP reported for rheumatoid arthritis and systemic lupus erythematosus [46, 47] (which incidentally confers an OR = 1.37 for disease), we can now estimate sample size required for association studies. Using Power for Association With Errors (http://linkage.rockefeller.edu/joanne/pave/), a minimum sample size of 1200 and 1500 patients, and an equivalent number of controls will have 80% power to detect an association at $\alpha = 10^{-5}$ and $10^{-6}$, respectively. Sample sizes of this magnitude from a single ethnic group in systemic sclerosis, especially if a second replication cohort or subset analysis is planned, will be possible only through multi-center collaborations or systemic sclerosis consortia.

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

Additional references related to this topic can also be found in the Current World Literature section in this issue (p. 772).


This study demonstrates the strong association of TNF promoter −1031 C allele with anticentromere antibodies in a United Kingdom Caucasian population. This association might be able to explain the previously reported links between anti-centromere antibody production and class II MHC alleles.


Genetics of scleroderma

Assassi and Tan


A large study of nonlesional dermal fibroblasts from patients with systemic sclerosis and controls. Only a small fraction of genes were found to be differentially expressed, but they affect a wide variety of biologic processes.


Myositis and myopathies

Interstitial lung disease and myositis

Related review: Interstitial lung disease in polymyositis and dermatomyositis (pp. 701–706)


Raynaud phenomenon, scleroderma, overlap syndromes and other fibrosing syndromes

Lung disease in systemic sclerosis

B cells in systemic sclerosis

Genetics

Understanding, assessing and treating Raynaud's phenomenon

Microchimerism in systemic sclerosis: an update

Novel therapies

Raynaud and vascular
Muscle-derived positive and negative regulators of the immune response

Muscle-derived positive and negative regulators of the immune response

Myostatin and muscle mass control

Related review: Muscle regeneration through myostatin inhibition (pp. 720–724)


Clinical advances in myositis


Miscellaneous


Muscle satellite cells and self-renewal: implications for myositis


Cytoxic T lymphocytes and autoimmunity

Review (pp. 731–734)


Sun DF, Zheng ZL, Tummala P, et al.: Chronic
Short KR, Nygren J, Bigelow ML, et al.: Effect of
SelvaOCallaghan A, Martinez Costa X, Solans
Schedel J, Butz B, Volk M, et al.: Nonerosive
Saunders MJ: Creatine phosphokinase and
772
63:2191–2192.

Primary respiratory failure in inclusion
focal myositis in a patient with signet ring
rashes. Rheumatol Int 2005,
( Jo-1 antibodies), myositis, hyperglycemia,
exercise tolerance test in patients with
reliability of an aerobic and an anaerobic
dermatomyositis: comment on the article by

lavage and thoracic high-resolution
computed tomography results in dyspnic
patients with systemic sclerosis. Arthritis

with systemic sclerosis - Comparison with idiopathic pulmonary fibrosis and nonspecific
interstitial pneumonia. Radiology 2004,
232:560–567. [67].

with systemic sclerosis. Comparison with idiopathic pulmonary fibrosis and nonspecific

Dheda K, Laloo UG, Casisi B, et al.: Experience with azathioprine in systemic sclerosis

a group of patients with dyspnea and
alveolitis entering the scleroderma lung
group by fractakin/OCXCR1 in patients
with systemic sclerosis. Ann Rheum Dis

binding of anti-DNA topoisomerase I autoantibodies to the cell surface of
fibroblasts in patients with systemic sclerosis. Arthritis Rheum 2004,
50:3265–3274. [04].

Hesselstrand R, Ekman R, Eskilsson J, et al.: Screening for pulmonary hypertension in
systemic sclerosis - The longitudinal
development of tricuspid gradient in 227

Joannidis VM, Vlachoyiannopoulos PG, Hadicok AB, et al.: Mortality in systemic sclerosis - 
An international meta-analysis of individual patient data. Am J Med 2005,
118:2–10. [63].

Khan D, Clements PJ, Furst DE, et al.: Correlation of the degree of dyspnea with
health-related quality of life, functional
abilities, and differing capacity for carbon
monoxide in patients with systemic sclerosis and active alveolitis - Results from the
scleroderma lung study. Arthritis Rheum 2005,
52:992–1000. [54].

Kowal-Bielecka O, Kowal K, Lewszuk A, et al.: B Thromboglobulin and platelet factor 4 in
bronchoalveolar lavage fluid of patients with systemic sclerosis. Ann Rheum Dis

in bronchoalveolar lavage fluid of patients with systemic sclerosis. Ann Rheum Dis

Ludwicz Bradly A, Bogatchevich G, Silver RM: 
Thrombin-mediated cellular events in
pulmonary fibrosis associated with systemic sclerosis (Scleroderma). Clin Exp Rheumatol

Mironi F, Caporali F, Macone Bianco A, et al.: Cytokine profile of bronchoalveolar
lavage in systemic sclerosis with interstitial lung disease - Comparison with usual
interstitial pneumonia. Ann Rheum Dis 2004,
63:892–894. [12].

Raynaud phenomenon, scleroderma, overlap syndromes and other fibrosing syndromes

Lung disease in systemic sclerosis

Related review: New developments in
scleroderma interstitial lung disease (pp. 737–745)

cyclophosphamide therapy for Systemic
Sclerosis. A single-center experience and
review of the literature with pooled analysis
lung function test results. Clin Exp
Rheumatol 2004, 22:733–742. [1078].

alteration in scleroderma lung fibrosis. Clin
Exp Rheumatol 2004, 22:733–742. [01].

alteration in scleroderma lung fibrosis. Clin
Exp Rheumatol 2005, 22:733–742. [01].

mixed conective tissue disease (MCTD).

Bogatchevich GS, Gusto E, Oates JC, et al.: Distinct PKC isofoms mediate cell survival and
DNA synthesis in thrombin-induced
myofibroblasts. Am J Physiol Lung Cell Mol
Physiol 2004, 288:L190–L201. [06].

Bredemeer M, Xavier RM, Capobianco KG, et al.: 
Nailfold capillary microscopy can suggest pulmonary disease activity in systemic sclerosis. 

patients affected by scleroderma treated with 
cyclosporin and pulse methylprednisolone. 

lavage and thoracic high-resolution
computed tomography results in dyspnic
patients with systemic sclerosis. Arthritis
Raynaud phenomenon, scleroderma, overlap syndromes and other fibrosing syndromes

B cells in systemic sclerosis


Understanding, assessing and treating Raynaud’s phenomenon

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