New Insights Into Transmission, Diagnosis, and Drug Treatment of Pneumocystis carinii Pneumonia

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CASE PRESENTATION
A 49-year-old Hispanic man was referred to the National Institutes of Health (NIH) for therapy of a human immunodeficiency virus (HIV)–related lymphoma. The patient was diagnosed with HIV infection in 1995 and was treated with various antiretroviral regimens with poor viral control. In January 2001, he was diagnosed to have Pneumocystis carinii pneumonia (PCP) and was treated initially with trimethoprim-sulfamethoxazole, but therapy was changed to intravenous pentamidine when the patient developed a diffuse rash.

In April 2001, the patient presented to an emergency department complaining of fever, shortness of breath, and cough that had persisted for 2 weeks. He was taking an antiretroviral regimen that consisted of lopinavir/ritonavir, abacavir, lamivudine, and didanosine. He was also taking itraconazole, atovaquone, aerosolized pentamidine, and clarithromycin, although adherence to his drug regimens was uncertain. He was empirically treated with a combination of augmentin and levofloxacin, but 2 weeks later reported that the shortness of breath and fever had not improved. A chest radiograph showed diffuse interstitial infiltrates and an anterior mediastinal mass. His white blood cell count was 4500/µL with 55% neutrophils. The CD4 T-lymphocyte count was 4/µL and Pneumocystis carinii has been recognized as a human pathogen for nearly 50 years. We present a case of P carinii infection that typifies clinical presentation in the era of the acquired immunodeficiency syndrome epidemic. The high incidence of P carinii pneumonia in persons infected with human immunodeficiency virus (HIV) has served to focus laboratory and clinical research efforts on better understanding the biology of the organism and on improving diagnosis, treatment, and prevention of this disease. Although inability to culture P carinii has hampered research efforts, molecular and immunologic approaches have led to the recognition that the organism represents a family of fungi with a very restricted host range and have allowed characterization of clinically relevant antigens and enzymes. Molecular epidemiologic studies have identified more than 50 strains of human-derived P carinii and have suggested that recently acquired infection, as opposed to reactivation of latent infection, may account for many cases of clinical disease. Diagnosis has been improved by the development of organism-specific monoclonal antibodies and, more recently, by polymerase chain reaction using multicopy gene targets, together with induced sputum or oral wash samples. Chemotherapeutic prophylaxis is very effective in preventing P carinii pneumonia; the combination of trimethoprim-sulfamethoxazole remains the first-line agent for both therapy and prophylaxis. Prophylaxis needs to be administered only during periods of high risk; in HIV-infected patients responding to effective antiretroviral therapies, prophylaxis no longer needs to be lifelong. Molecular studies have identified mutations in the target of sulfa drugs that appear to represent emerging resistance in P carinii. Resistance to atovaquone, a second-line agent, may also be developing.

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his viral load was 250,000 copies/µL. Room air PaO2 was 55 mm Hg. Biopsy of the anterior mediastinal mass revealed a large B-cell lymphoma. Bronchoalveolar lavage (BAL) failed to demonstrate any pathogen. Cytology was not performed.

The patient developed respiratory failure requiring intubation and mechanical ventilation. He was transferred to the NIH for therapy of his lymphoma, where he presented with a temperature of 39.3°C. Chest examination revealed diffuse rales. Arterial blood gases on 40% oxygen were: pH, 7.48; Po2, 95 mm Hg; and Pco2, 40 mm Hg. His white blood cell count was 2300/µL. His chest radiograph showed diffuse infiltrates that were interstitial and alveolar. Over the next 4 days, the patient began EPOCH (etoposide, prednisone, vincristine [Oncovin], cyclophosphamide, and doxorubicin [hydroxydaunorubicin]) chemotherapy for lymphoma, but his respiratory status worsened. A BAL was performed and many P carinii cysts and trophozoites were seen by direct fluorescent antibody (DFA) staining. By quantitative polymerase chain reaction (PCR), 18,640 copies of the P carinii–specific major surface glycoprotein (MSG) gene were detected per 20 µL of BAL fluid.

The patient was treated with intravenous pentamidine for 7 days. Because his condition was not improving, therapy was switched to intravenous trimethoprim-sulfamethoxazole. The DFA stain remained positive and by PCR, 61,260 copies/20 µL of the MSG gene were detected. The patient slowly improved, but after 9 days of trimethoprim-sulfamethoxazole the patient developed a rash and received an additional 14 days of intravenous pentamidine. At 3 weeks and 5 weeks after initiation of therapy, 1190 and 6 copies/20 µL of the MSG gene, respectively, were detected; DFA staining was positive at 3 weeks but negative at 5 weeks. Analysis of the P carinii dihydropteroate synthase (DHPS) gene, the target of sulfamethoxazole therapy, revealed a single Thr→Ala mutation at position 55, suggesting possible sulfa resistance. The patient was able to be weaned from mechanical ventilation after 6 weeks of ventilatory support and was discharged to the oncology service for additional therapy of his lymphoma.

**DISCUSSION**

*Pneumocystis carinii* (FIGURE 1) is a fungus whose importance in the United States as a human pathogen increased dramatically following the onset of the acquired immunodeficiency syndrome (AIDS) epidemic 20 years ago. Since that time, advances in understanding the biology and in improving the diagnosis, treatment, and prevention of PCP have substantially reduced the morbidity and mortality associated with this organism.

**Biology and Epidemiology of P carinii**

**Historical Perspective.** *Pneumocystis carinii* was first identified in 1909 and 1910 in trypanosome-infected lungs of animals by Chagas1 and Carini,2 who thought that it was a form of trypanosome. In 1912, *Pneumocystis* was recognized as a new genus by Dela noe and Delanoe3 and was named in honor of Carini.

In the 1930s and 1940s, epidemics of interstitial plasma cell pneumonia were recognized in premature and malnourished infants in Europe. *Pneumocystis carinii* was identified as the cause of this pneumonia by Vanek and Jirovek4 in 1952. *Pneumocystis carinii* pneumonia was subsequently recognized with increasing frequency as a major cause of pneumonia and death in immunodeficient and immunosuppressed patients. The number of recognized cases exploded in the 1980s, as PCP became one of the hallmark manifestations of AIDS as well as a common complication of cancer chemotherapy and organ transplantation.5,6

**Incidence of PCP.** In the late 1960s and the early 1970s, there were fewer than 100 cases per year of PCP reported in the United States (FIGURE 2A).7 Following the onset of the AIDS epidemic in 1982, there was a marked increase in the incidence of cases reported to the Centers for Disease Control and Prevention that peaked in 1990 at about 20,000 cases per year. In the early 1990s, there was a decline in incidence that was largely due to the widespread use of PCP prophylaxis, which followed a report from a Public Health Service task force that recommended that prophylaxis should be universal for HIV-infected patients who met certain criteria, most importantly a CD4 cell count less than 200/µL.8

After 1995, there was a further decline due to the widespread use of highly active antiretroviral therapy (HAART), which is very effective in suppressing HIV, augmenting immune function, and protecting from opportunistic infections, as documented by the Adult and Adolescent Spectrum of Disease study, a prospective cohort study conducted by the Centers for Disease Control and Prevention (Figure 2B).9 Despite this dramatic decline in incidence, PCP remains the most common life-threatening opportunistic infection diagnosed in HIV-infected patients.

**Clinical Presentation**

*Pneumocystis carinii* causes clinically apparent pneumonia virtually exclusively in immunosuppressed patients. The clinical presentation is character-
ized by fever, shortness of breath, substernal tightness, and a nonproductive cough. Especially in HIV-infected patients, the symptoms can be relatively mild and slowly progressive, which may delay diagnosis, as occurred in this case. The chest radiograph characteristically demonstrates bilateral interstitial infiltrates, which progress to an alveolar pattern (Figure 3A), although in 10% or more of cases, it may be entirely normal. In the latter situation, a high-resolution (thin section) computed tomography scan of the chest will usually demonstrate a characteristic ground glass attenuation.

Diagnosis is dependent on detection of the organism in a clinical specimen by colorimetric or immunofluorescent stains (Figure 3B). Histopathologically, PCP shows a very characteristic intra-alveolar acellular eosinophilic exudate that can fill the alveoli (Figure 3C) and is primarily composed of large numbers of P carinii organisms. The combination of trimethoprim-sulfamethoxazole is the current first-line therapeutic agent; treatment should be continued for 14 to 21 days for most patients.

**Advances in Understanding the Biology of P carinii**

Substantial advances have been made over the past 2 decades in understanding the biology of P carinii. To highlight these advances, it is helpful to compare what was known about the organism in the pre-AIDS era in the 1970s with what is known today. In the 1970s, P carinii was thought by most experts to be a protozoan. Based largely on mo-
molecular studies, *P carinii* has been reclassified as a fungus.

In the 1970s, *P carinii* was thought to be a single strain with a broad host range. Molecular as well as immunologic studies have shown that in fact there are multiple strains of *P carinii*, each of which is restricted to infecting a single host species. Thus, human-derived *P carinii* cannot infect mice, rats, or ferrets, or even monkeys, whereas mouse-derived *P carinii* cannot infect rats or humans. These different strains are potentially unique species.

In the 1970s, the life cycle of *P carinii* was unknown, in large part because the organism could not be cultured. As a consequence, metabolism of the organism was also poorly understood. Currently, the life cycle remains unknown, since *P carinii* still cannot be consistently cultured, but there are some encouraging recent reports of success in this area. However, advances in understanding the metabolism have been made through molecular biological approaches. Target enzymes of some available therapeutic regimens have been cloned, sequenced, and characterized. These include the genes encoding dihydrofolate reductase (*DHPS*) and dihydrofolate reductase (*DHFR*), which are the targets of sulfamethoxazole and trimethoprim, respectively.

In the 1970s, protective host immune responses were ill-defined, although cell-mediated immunity was believed to be important. Studies in animals and humans have documented a critical role for CD4 cells in controlling *P carinii* infection, while CD8 cells appear to have a minor role at best, presumably because *P carinii* is exclusively an extracellular pathogen. Macrophages appear to be important effector cells and certain cytokines, such as tumor necrosis factor α, also appear to be important.

In the 1970s, the antigens of *P carinii* were essentially uncharacterized. Today a number of antigens have been identified and cloned, the most important of which is the MSG. This protein is encoded by a multicopy gene family with 100 or more genes per genome that presumably exists to allow *P carinii* to use antigenic variation as a means of avoiding host immune defenses.

**Epidemiology in Humans**

*Pneumocystis carinii* pneumonia has been diagnosed in patients throughout the world. The mode of transmission in humans is unknown, though the respiratory route is likely important; this route has been documented in animal studies to be the primary mode of transmission. The reservoir for human infection is unknown, but could include environmental sources, other humans, or animals, although the restricted host range argues strongly against the latter.

Serologic studies have shown that healthy humans have a high rate of prior infection with *P carinii* and that exposure often occurs by 2 years of age. There is no well-defined clinical syndrome associated with acute infection in the immunocompetent host; presumably patients are asymptomatic or experience mild, nonspecific symptoms. A few studies have demonstrated focal pneumonitis in association with *P carinii* in infants and there is an intriguing recent report suggesting association of mild PCP with sudden infant death syndrome, although whether this is causal or coincidental needs to be evaluated further. It has been widely held that latency is established following acute infection in healthy individuals and that latent infection can reactivate in the setting of immunosuppression.

Molecular typing of isolates has been recently used as a tool to address some of the epidemiologic questions. Typing with the internal transcribed spacer region of the ribosomal RNA (*rRNA*) gene has identified more than 50 unique isolates. Coinfection with multiple *P carinii* isolates has been demonstrated in 20% to 30% of PCP cases. Type variation has been demonstrated in some recurrent episodes, suggesting that recurrence may be the result of re-infection with a new type rather than reactivation of the type causing the previous episode. Type variation has also been associated with place of diagnosis but not with place of birth, again suggesting recently acquired rather than remotely acquired organisms are responsible for clinical disease.

**Diagnosis of PCP**

Over the past 2 decades, major improvements have been made in the laboratory diagnosis of PCP. Diagnosis in the 1960s and 1970s was primarily made using stains specific for the cyst form of the organism, such as Giemsa or toluidine-blue O. Experienced investigators used Giemsa stains to look for trophozoite forms of *P carinii* or clusters of cysts containing intracytic bodies. Because patients with PCP frequently raised little if any sputum and harbored low numbers of organisms, the specimen of choice was open lung biopsy. Expectorated sputum as well as bronchial washes obtained using rigid bronchoscopes were insensitive for *P carinii* detection.

In the early to mid-1970s, the introduction of flexible fiberoptic bronchoscopy led to transbronchial biopsy and BAL specimens. These specimens have sensitivities for *P carinii* detection that are comparable with open lung biopsies. Bronchoalveolar lavage alone was subsequently used effectively for diagnosing PCP. During the mid-1980s, sputum induction using nebulized saline was found to provide an even less invasively obtained specimen with a sensitivity ranging from 60% up to 95%, though in some centers it was lower.
In 1986, monoclonal antibodies were developed that reacted with human *P carinii*. Several of these antibodies proved to be both sensitive and specific for *P carinii*, permitting development of a fluorescent antibody stain that allowed detection of the organism within 2 hours. A stain with such specificity has greater accuracy so morphologically similar yeast like *Candida* will not be misidentified as *P carinii*. Moreover, a stain that detects both cysts and trophozoites, such as fluorescent stains using monoclonal antibody 2G2, has greater sensitivity than stains that detect cysts only, such as silver stains and other monoclonal antibody stains, because trophozoites greatly outnumber cysts.

In addition to specimen type and stain, another significant factor affecting diagnosis is the patient being examined. In general, patients with AIDS who are not receiving *P carinii* prophylaxis have a higher organism burden than those receiving prophylaxis, and the diagnostic yield of BAL and induced sputum may be decreased in patients receiving aerosol pentamidine prophylaxis. Also, AIDS patients often have more organisms than other immunocompromised patients, such as cancer patients.

The algorithm used for many years at the NIH Clinical Center for diagnosis of PCP has been to obtain an immunofluorescent-stained smear of induced sputum, and if that smear is negative, to proceed to BAL. In order not to compromise patient care, the induced sputum results are available promptly, so that a BAL can be performed within 24 hours. Although lung biopsies were requested frequently in the past, these requests have decreased from an average of 20 to 30 submissions per year in the late 1980s to our current rate of about 5 per year, despite an enlarging population of highly immunocompromised patients. Moreover, in the past 5 years, none of these biopsies has been positive for *P carinii*. Experience with PCP diagnosis at NIH over the past 13 years is shown in Figure 4. Our data for AIDS patients are consistent with the national trend, with a decrease in both the number of specimen submissions and the percentage that were positive. Currently, PCP is diagnosed more often in non-HIV immunocompromised patients. The quantities of *P carinii* in these patients are generally low, making detection more difficult.

Fortunately, the introduction of molecular-based methods has provided detection assays that are more sensitive than traditional stains and even immunofluorescent stains. The first report using molecular amplification methods such as PCR for detection of *P carinii* was published by Wakefield et al in 1990. Since then, there have been many PCR methods described that use a variety of gene targets. Those with the highest sensitivity use either a multiplex PCR procedure requiring 2 amplification rounds or a nested PCR procedure requiring 2 amplification rounds. All studies using these assays have shown PCR to be more sensitive than stains for detection of *P carinii* in either BAL or induced sputum. This increased sensitivity may permit the use of PCR to detect *P carinii* in more easily obtained specimens such as oral washes or gargles. Increased sensitivity may also allow earlier detection of infection when there may be very few organisms that are difficult to find in stained smears.

Several recent studies using PCR to detect *P carinii* have found individuals who are positive by PCR but negative by stain. Although some of these patients have clinical disease, others do not develop PCP, suggesting either asymptomatic colonization or carriage. We can therefore expect to encounter occasional patients in whom a positive PCR may not represent active infection. As further epidemiological studies are undertaken, we should begin to understand carriage and transmission of this organism better, particularly in different populations of immunocompetent and immunocompromised hosts.

Currently at NIH, we are looking at *P carinii* PCR by 2 different assays, one using primers for the mitochondrial rRNA gene and the other using newly described primers for the major surface glycoprotein. Preliminary results with oral washes show a sensitiv-

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**Figure 4. National Institutes of Health Experience in the Diagnosis of Pneumocystis carinii Pneumonia (PCP), 1987-1999**

HIV indicates human immunodeficiency virus. Both the number of episodes of pulmonary symptoms evaluated for PCP (A) and the number of cases of PCP diagnosed (B) showed a decrease over time in patients with HIV infection (black circles) in contrast to patients without HIV (gray circles).

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ity of up to 80% when compared with concomitantly obtained BAL or induced sputum samples examined by direct immunofluorescent staining. By using a sensitive PCR assay, we can now look at the possibility of detecting PCP earlier than by traditional smears and determining if there are certain PCP-positive but smear-negative patients who will develop *P. carinii* infection later. By following up PCR-positive patients who are without disease, we can also look at whether carriage or colonization is transitory or long-term and determine carrier rates among different patient populations.

Our goal is to develop a new algorithm in which a noninvasive specimen such as an oral wash is screened for the presence of *P. carinii* by PCR. Although the significance of a positive PCR assay would need to be interpreted in conjunction with the patient's clinical findings and risk factors, a negative PCR would suggest a low likelihood of PCP and the need to look for other etiologies. Our group has developed a quantitative PCR assay that could help predict the likelihood of colonization vs disease and may also provide prognostic information, as suggested in the case presentation.55 For now, however, pending prospective validation of this approach, diagnosis should follow the traditional algorithm (FIGURE 5), namely to examine an induced sputum sample, ideally by direct immunofluorescence, and if this is negative to follow with BAL, reserving bronchoscopic or open lung biopsy for patients in whom the BAL is nondiagnostic.

**Prevention of PCP**

Prevention of PCP is a logical strategy to reduce the morbidity and mortality that *P. carinii* can produce in susceptible humans. There are 4 basic approaches that could be taken to prevent PCP. Because it is highly likely that human *P. carinii*, like murine, is spread by an aerosol route,26 reducing exposure of susceptible individuals would be a logical approach to prevention. However, *P. carinii* appears to be a ubiquitous organism, there are no data demonstrating transmission of *P. carinii* from an acutely infected host to a susceptible patient, and molecular epidemiologic studies do not suggest single source outbreaks.38 Thus, respiratory isolation would not be expected to prevent most cases of PCP.

A second approach to prevention would be to enhance immunocompetence so that patients were not immunologically susceptible to PCP for extended periods. Management of transplant recipients, cancer patients, and patients with HIV seeks to minimize the period and severity of immune deficiency by minimizing the intensity of chemotherapy or by controlling HIV replication. Other strategies to enhance immunocompetence specifically against *P. carinii*, such as active or passive immunization, have not been evaluated in humans.

The most widely used and successful strategy for preventing PCP is to use specific chemoprophylaxis in patients during defined periods of susceptibility. This strategy requires that susceptible patients be identified and that a convenient, nontoxic, effective drug regimen be developed.

**TABLE 1** shows the estimated risk for PCP in selected patient populations.7,56-61 The risk for developing PCP can be suggested by the degree of cellular or humoral deficiency that the patient has by virtue of an underlying disease or an immunosuppressive therapy; however, laboratory measures of immune competence are imprecise measures of susceptibility. Thus, clinical studies are needed to identify patient populations at high risk, and to define the periods when patients are most susceptible.

For most patients, the period of susceptibility is discrete, based on the natural history of their underlying disease, the extent and duration of immunosuppression induced by their therapy, and the response to therapy. For patients with HIV infection, this period of susceptibility can be successfully assessed by measuring the peripheral CD4 cell count (ie, patients are most susceptible when their CD4 cell count is lower than 200/μL). For other patients, there are no laboratory markers that are predictive of susceptibility; observational studies have been necessary to define when the high-risk period begins and ends.62,63 While such studies have led to the routine use of PCP prophylaxis during high-risk periods for certain patients without HIV infection (including those listed in Table 1), for other populations the level and period of risk are more difficult to define.62,64-65 Further, changes in management of underlying diseases, which may increase the intensity of immunosuppression, require an ongoing reassessment of risk. The recognition that the period of susceptibility is discrete (ie, there is a beginning and end) is an
important concept, especially for patients with HIV infection in whom guidelines now recommend cessation of prophylaxis following an adequate response to HAART, as defined by a sustained CD4 T-lymphocyte count greater than 200/µL.66

For patients at high risk, what are the chemoprophylactic regimens that are preferred based on efficacy, toxicity, convenience, and cost? In 1975, Hughes et al66 demonstrated that daily trimethoprim-sulfamethoxazole was almost 100% effective for preventing PCP in heavily immunsuppressed children with cancer. That regimen is extremely well tolerated in most patient populations: rash, fever, transaminase elevation, interstitial nephritis, and crystalluria can occur, but these toxicities are manageable. Patients with HIV infection have an unusually high frequency of toxicity, although several strategies for gradual dose escalation and for using lower maintenance doses appear to enhance tolerability.67 A variety of dosing regimens has been assessed, including 1 single- or double-strength tablet twice daily or once daily, 1 double-strength tablet 3 times weekly, and 1 double-strength tablet twice daily 2 consecutive days per week. While sufficiently powered studies to accurately compare their efficacy and toxicity have not been done, all of these regimens appear to have a high degree of efficacy and safety.68 The lower dose regimens (ie, a single-strength tablet daily rather than a double-strength tablet daily) are probably better tolerated. Trimethoprim-sulfamethoxazole prophylaxis is quite inexpensive; the wholesale cost for a month of prophylaxis can be less than $10.

The use of trimethoprim-sulfamethoxazole is associated with adverse effects in addition to toxicity. The widespread use of trimethoprim-sulfamethoxazole for PCP prophylaxis has an effect on bacterial ecology, enhancing the prevalence of bacterial resistance to these agents among common enteric and respiratory flora.69 Such use can also affect the sensitivity of *P carinii* to sulfa drugs.

Table 1 lists some potential alternative regimens for patients who cannot tolerate or who fail the trimethoprim-sulfamethoxazole regimen. Most of these regimens have been assessed most completely in patients with HIV infection, but are used in other patient populations as well. Dapsone and dapsone-pyrimethamine appear to be the most effective alternatives, although a substantial number of patients who cannot tolerate a sulphonamide also cannot tolerate the structurally similar sulfone-based regimens. Aerosolized pentamidine is effective, but not as effective as trimethoprim-sulfamethoxazole. Aerosolized pentamidine also has the disadvantage that it is not well distributed to all lobes of the lungs, especially in patients with obstructive lung disease, and it may predispose to extrapulmonary *P carinii* infection, though such cases are rare (<1%) even in patients receiving aerosolized pentamidine.70 Aerosolized pentamidine is also more expensive to buy and administer than the oral regimens, but it can be given once monthly, which is an advantage in terms of convenience and adherence. Atovaquone is quite effective, but is also expensive.

If the period of patient susceptibility has been defined and effective prophylactic regimens are available, why

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<th>Table 1. Risk of <em>Pneumocystis carinii</em> Pneumonia (PCP) According to Underlying Disease*</th>
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<td>Disease</td>
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<tr>
<td>Myeloma</td>
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<td>Hodgkin disease</td>
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<td>Acute myelocytic leukemia</td>
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<tr>
<td>Acute lymphocytic leukemia</td>
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<td>4 drug</td>
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<td>4 drug + radiation therapy</td>
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<tr>
<td>Lymphoma</td>
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<tr>
<td>PROMACE-MOPP</td>
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<tr>
<td>PROMACE-cytaBOM</td>
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<tr>
<td>Allogeneic marrow transplant</td>
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<td>Kidney transplant</td>
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<tr>
<td>Heart/liver/lung transplant</td>
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<tr>
<td>HTLV-1 associated lymphoma</td>
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<td>HIV infection</td>
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*Based on references 7, 56-61. PROMACE-MOPP indicates prednisone, methotrexate, doxorubicin (Adriamycin), cyclophosphamide, etoposide, and vincristine (Oncovin); PROMACE-cytaBOM, prednisone, doxorubicin (Adriamycin), cyclophosphamide, etoposide, mustargen, vincristine (Oncovin), methotrexate, leucovorin; HTLV-1, human T-lymphotropic virus 1; and HIV, human immunodeficiency virus.

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<th>Table 2. Regimens for Chemoprophylaxis of <em>Pneumocystis carinii</em> Pneumonia*</th>
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<td>Drug</td>
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<td>First choice</td>
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<tr>
<td>Alternatives</td>
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<tr>
<td>Dapsone</td>
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<tr>
<td>Dapsone with pyrimethamine</td>
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<tr>
<td>Leucovorin</td>
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<tr>
<td>Dapsone with pyrimethamine</td>
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<tr>
<td>Leucovorin</td>
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<tr>
<td>Aerosolized pentamidine</td>
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<td>Atovaquone</td>
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*DS indicates double strength (sulfamethoxazole, 800 mg with trimethoprim, 160 mg); SS, single strength (sulfamethoxazole, 400 mg with trimethoprim, 80 mg).
do patients still develop PCP today? Many cases of PCP occur in patients who were not taking prophylaxis because (1) they were unaware of their susceptibility (eg, patients with HIV infection and low CD4 T-lymphocyte counts who are unaware of their HIV infection); (2) they did not adhere to prescribed chemoprophylaxis; or (3) their health care provider did not prescribe the appropriate regimen. A few patients develop PCP when they were not expected to be susceptible (eg, a solid organ transplant recipient with no episode of organ rejection who develops PCP more than 6-12 months post-transplant, or an HIV-infected patient who develops PCP when his or her CD4 T-lymphocyte count is greater than 200/µL). Some patients also fail an appropriate regimen, regardless of how adherent they are, because no regimen is 100% effective. How often these failures occur because of sulfonamide resistance remains to be determined.

Chemoprophylaxis of PCP has been one of the major successes in the field of infectious diseases in the past 25 years. Careful attention to using the well-established regimens during periods of immunologic susceptibility and proper education of patients about the importance of these regimens are essential to minimize the impact of PCP on susceptible patient populations.

Sulfa Drug-Resistant P carinii

Sulfa drugs have been the most important component of therapy for PCP since the 1970s. The most important sulfa-containing drug is a combination of 2 antifolates: sulfamethoxazole and trimethoprim. Animal models suggest that trimethoprim may be inactive against P carinii. Thus, while it is difficult to assess the contribution of trimethoprim to therapeutic efficacy in humans, trimethoprim-sulfamethoxazole may functionally be sulfa monotherapy. Dapsone, a sulfone, is an important second-line agent. Both dapsone and sulfamethoxazole act by inhibiting the folate biosynthetic enzyme DHPS, which catalyzes the condensation of para-aminobenzoic acid (pABA) and 6-hydroxymethyl-7,8-dihydropterin pyrophosphate.

Sulfa and sulfone resistance has been well characterized in a variety of bacterial pathogens including Streptococcus pneumoniae, Neisseria meningitidis, Escherichia coli, Mycobacterium leprae, and in malaria. Sulfa resistance in these organisms results from point mutations in the DHPS gene. In malaria, mutations tend to accumulate over time and impart greater degrees of drug resistance.

Since P carinii has been widely exposed to sulfa, it is reasonable to expect that it might be developing sulfa resistance. However, drug resistance in P carinii cannot be confirmed by classic methods because it is not possible to culture patient isolates and determine their in vitro drug sensitivities. Thus, the only way to detect resistance at present is by an indirect method: looking for mutations in the P carinii DHPS gene. In 1997, the P carinii DHPS gene was sequenced from 6 patient isolates and specific genetic polymorphisms were found. All of the polymorphisms were nonsynonymous (ie, all resulted in changes in the encoded amino acids). This suggested that there was evolutionary selective pressure to induce mutations, possibly as a result of drug exposure.

Based on homology to the E coli enzyme whose 3-dimensional structure has been solved, 2 of the mutations are at the enzyme active site (Figure 6). Threonine 55 and Arg56 are involved in binding to the pterin substrate; Arg56 is also involved in binding to pABA and to sulfa. Mutant strains usually replace the Thr at 55 with an Ala, which lacks the hydroxyl group involved in binding. The Pro at 57 is also replaced with Ser, which may alter the position of the critical Arg56. Interestingly, mutations at the same 2 sites have recently been shown to cause sulfa resistance in M leprae.

Mutations at positions 55 (as seen in the case presentation) and 57 have now been seen in many other studies. Table 3 is a summary of 6 studies in which the frequencies of mutations in sulfa-exposed and nonexposed patients were compared. The studies range in size from 20 to 152 patients. DHPS mutations occurred in 62% to 100% of patients receiving prophylaxis compared with 11% to 47.5% of patients not receiving prophylaxis. In every study, there were significantly more mutations in the sulfa-exposed group than in the nonexposed group.

One other important piece of information was added by Ma et al. Their study was the first to look for mutations in DHFR, the target of trimethoprim. Even though they found many isolates with DHPS mutations, no DHFR mutations were found. This study is consistent with previous animal studies suggesting that trimethoprim was inactive; it also supports the hypothesis that trimethoprim-sulfamethoxazole may function as sulfa monotherapy.

As one would expect, there is widespread geographical variation in the prevalence of these mutations. In general, they are more common on the West Coast than the East Coast, with the lowest frequency in Denver and Indianapolis.
Three studies have looked at the incidence of mutations over time.84-86 Mutations were absent or infrequent before 1993 and then became quite common.84,85 In a Danish study, the prevalence appeared to decline again after 1997, possibly because sulfa drug use was declining.86

**Clinical Significance of DHPS Mutations**

The occurrence of PCP in patients receiving sulfa prophylaxis suggests that the DHPS mutations confer, at minimum, a low-level resistance that can overcome the inhibitory effects of the low doses of sulfa administered during prophylaxis. What is less clear is how the mutations affect response to therapeutic doses of trimethoprim-sulfamethoxazole. In the Danish study, patients with mutant DHPS were significantly less likely to survive for 3 months than were patients with wild-type P carinii infections.86 However, in a recent study, there was no effect on survival, though patients who failed sulfa therapy were found to be significantly more likely to be infected with mutant strains than with wild-type strains.84 In another recently completed study, no association was seen between mutations and either response to therapy or survival.37 Thus, it appears that the 55 and 57 mutations in the P carinii DHPS gene have, at most, a small effect on response to therapy. But what will happen when a strain with additional mutations arises? If and when that occurs, it could mean a much higher level of resistance and loss of the most effective drug for treating PCP.

**Table 3. Association of Mutation Frequency in the DHPS Gene of Pneumocystis carinii With Prior Sulfa (Sulfamethoxazole or Dapsone) Prophylaxis**

<table>
<thead>
<tr>
<th>Source, y</th>
<th>Prior Sulfa Prophylaxis</th>
<th>No Prior Sulfa Prophylaxis</th>
<th>P Value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Kazanjian et al,85 1998</td>
<td>5/7 (71)</td>
<td>2/13 (15)</td>
<td>.02</td>
</tr>
<tr>
<td>Helweg-Larsen et al,86 1999</td>
<td>18/29 (62)</td>
<td>13/123 (11)</td>
<td>&lt;.001</td>
</tr>
<tr>
<td>Ma et al,87 1999</td>
<td>11/13 (85)</td>
<td>3/19 (16)</td>
<td>.01</td>
</tr>
<tr>
<td>Santos et al,87 1999</td>
<td>5/5 (100)</td>
<td>3/12 (25)</td>
<td>.01</td>
</tr>
<tr>
<td>Kazanjian et al,85 2000</td>
<td>28/37 (76)</td>
<td>14/60 (23)</td>
<td>.001</td>
</tr>
<tr>
<td>Huang et al,87 2000</td>
<td>57/71 (80)</td>
<td>19/40 (47.5)</td>
<td>.001</td>
</tr>
</tbody>
</table>

*DHPS indicates dihydropteroate synthase.

**Table 4. Regimens for Treatment of Pneumocystis carinii Pneumonia**

<table>
<thead>
<tr>
<th>Drug</th>
<th>Route</th>
<th>Dose</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>First choice</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>By mouth</td>
<td>2 DS every 8 hours</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>Trimethoprim 5 mg/kg plus sulfamethoxazole 25 mg/kg every 8 hours</td>
</tr>
<tr>
<td><strong>Alternatives</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Trimethoprim plus dapsone</td>
<td>By mouth</td>
<td>320 mg every 8 hours</td>
</tr>
<tr>
<td></td>
<td>Intravenous</td>
<td>100 mg daily</td>
</tr>
<tr>
<td>Atovaquone</td>
<td>By mouth</td>
<td>750 mg twice daily</td>
</tr>
<tr>
<td>Clindamycin plus primaquine</td>
<td>By mouth, intravenous</td>
<td>300-450 mg every 6 hours</td>
</tr>
<tr>
<td>Pentamidine</td>
<td>Intravenous</td>
<td>4 mg/kg daily</td>
</tr>
<tr>
<td>Trimetrexate plus leucovorin</td>
<td>Intravenous</td>
<td>45 mg/m² daily</td>
</tr>
<tr>
<td></td>
<td>By mouth, intravenous</td>
<td>20 mg/m² every 6 hours</td>
</tr>
<tr>
<td><strong>Adjunctive therapy</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Prednisone (if room air Pao₂ &lt;70 mm Hg within 72 hours of initiating therapy)</td>
<td>By mouth, intravenous</td>
<td>40 mg every 12 hours for 5 days, then 40 mg daily for 5 days, then 20 mg daily for 11 days</td>
</tr>
</tbody>
</table>

*DS indicates double strength (sulfamethoxazole, 800 mg with trimethoprim, 160 mg); for patients weighing <60 kg or >100 kg, adjust dose to equivalent of trimethoprim, 5 mg/kg every 8 hours.

**Atovaquone Resistance**

Pneumocystis carinii may also be developing resistance to atovaquone. Atovaquone is a second-line therapeutic and prophylactic agent for PCP and is also used against malaria. When used as monotherapy for malaria, almost all patients develop resistance.88 To slow the development of drug resistance, atovaquone is now used for malaria only in combination with another antimalarial drug, proguanil.

The mechanism of action of atovaquone is well known. Atovaquone mimics ubiquinone and binds to cytochrome b.89 There is a good molecular explanation for why resistance to atovaquone develops quickly. Cytochrome b is encoded on the mitochondrial genome where spontaneous mutation rates are 10-fold higher than in the nucleus.89 Biophysicals have been mapping mutations in cytochrome b for many years. Many electron transport inhibitors resemble atovaquone. Mutations conferring resistance to these have been mapped to the Qo box in bacteria, fungi, and protozoa.91

The P carinii cytochrome b genes from more than 70 patient isolates were recently sequenced.92,93 A number of mutations were seen in both of the peptides involved in the site. There were 7 different mutations and only 2 were found in more than 1 patient. Five of 15 patients with atovaquone exposure had P carinii cytochrome b mutations, while mutations were seen in only 3 of 45 matched controls with no atovaquone exposure (P = .02). Thus, mutations in the P carinii cytochrome b are significantly more common in patients exposed to atovaquone. The multiplicity of mutations suggests that atovaquone resistance develops de novo in each patient.

**Current Recommendations**

Despite the detection of these mutations, there is no compelling evidence to date that suggests a change is warranted in the approach to the treatment or prevention of PCP. Prophylaxis should continue to be administered as summarized in Table 2. Drugs of
choice for treatment of active PCP are summarized in Table 4. Since at present detection of DHPS mutations remains an experimental procedure not broadly available and many patients with DHPS mutations are treated with and do respond to trimethoprim-sulfamethoxazole, this drug remains the first-line agent for therapy as well as for prophylaxis. Folinic acid (leucovorin) should not be coadministered because it does not reduce toxicity and may be associated with an increase in failure of therapy and death.94 Patients intolerant of trimethoprim-sulfamethoxazole can be treated with one of the alternatives noted in Table 4. Adjunctive prednisone should be administered within 72 hours of starting therapy to patients with an arterial oxygen tension of less than 70 mm Hg.95

CONCLUSION

Despite the inability to culture P carinii, major advances have been made in management of PCP over the past 20 years, much of it based on advances in understanding the basic biology of the organism. Ongoing studies of this perplexing organism, including a project to sequence the genome,96 should continue to provide clinically relevant insights that will facilitate the care of patients with this potentially lethal infection.

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REFERENCES
