MINIREVIEWS

Immunological Features of Pneumocystis carinii
Infection in Humans

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INTRODUCTION

Pneumocystis carinii is an important opportunistic pulmonary pathogen in human immunodeficiency virus (HIV) patients and other immunocompromised hosts. Basic research on P. carinii has been hampered by the lack of a reliable in vitro culture system; nevertheless, through the use of molecular techniques and experimental models, progress has been made over the last 2 decades in our understanding of the immunological features of the infection. Advances have included identification of major P. carinii antigens as well as the roles of CD4 cells, CD8 cells, macrophages, cytokines, and antibodies in the host defenses against the organism (5, 8, 9, 21–23, 30, 37, 40, 49, 60, 68, 80, 110, 124). Immunodeficient and immunosuppressed mice and rats, which have been the principal animal models, have been valuable because they provide a ready supply of organisms and are amenable to experimental manipulation (4).

By contrast, the small quantity of human-derived P. carinii available for use has limited the types of immunological studies which have been performed with humans. Although it is likely that much of what has been learned from animal models can be applied to humans, several lines of evidence emphasize the need for immunological studies to actually be performed with humans. Over the past decade, there has been an increasing appreciation of the genetic diversity and host specificity of P. carinii (121). Studies using experimental animals and humans have shown that the immune response to P. carinii may have harmful as well as helpful effects on the host, but the underlying mechanisms are poorly understood (87, 100, 101, 124, 127). There are aspects of P. carinii infection in humans (e.g., the high frequency of recurrent episodes of pneumocystosis and adverse reactions to anti-P. carinii drugs among HIV patients) which cannot be adequately studied in animal models (131). This review summarizes the current state of our knowledge of the immunological features of P. carinii infection in humans, compares this information with data obtained from experimental animals, and suggests areas for future investigation.

P. CARINII ANTIGENS

P. carinii antigens have mainly been identified by immunoblotting studies using polyclonal or monoclonal antibodies. The group which has attracted most of the attention is a surf-
Based on its prominence and surface location, it is surprising that MSG was not detected as often as the 35- to 45-kDa antigen. One possible answer for this finding is that MSG undergoes antigenic variation. MSG gene expression is controlled by a single telomeric expression site termed the upstream conserved sequence (UCS), which is thought to permit only one MSG gene to be transcribed and hence only one isoform of the antigen to be expressed on the surface of \textit{P. carinii} (114). Antigenic variation (i.e., the introduction of a new surface MSG) results from changing the MSG gene linked to the UCS and most likely results from recombination. Studies with rodents have shown that different forms of MSG can be found within a population of \textit{P. carinii} organisms in the lung (3, 41, 72, 128). Different patterns of reactivity of monoclonal antibodies to MSG have been found with the BALF of patients with single episodes of pneumocystosis as well as with the BALF of patients with recurrent episodes of the disease (114).

Although detection of soluble \textit{P. carinii} antigens in the respiratory tract or serum by techniques such as immunoblotting, counter immunoelectrophoresis, or enzyme immunoassay (EIA) is theoretically attractive, these approaches have not been developed into clinically useful diagnostic tests. On the other hand, immunofluorescence has been shown to be a highly sensitive and specific method of detecting \textit{P. carinii} in BALF as well as induced sputum (6), and a number of commercial kits have been developed.

**HUMORAL IMMUNE RESPONSES TO \textit{P. carinii}**

Exposure to \textit{P. carinii} stimulates a serum antibody response in the host. Serologic studies of humans have been performed using complement fixation, indirect fluorescent antibody (IFA), EIA, and immunoblotting techniques. Analysis of the older (119) and more recent (18, 29, 43, 66, 67, 75–77, 91, 117) literature indicates that serology has helped establish that infection with \textit{P. carinii} is common but has otherwise been of limited value as a diagnostic or epidemiologic tool. \textit{P. carinii} antigens used in serologic studies have mainly consisted of whole or fractionated organisms or antigens (e.g., MSG) obtained from infected human or rodent lungs. Such crude preparations have been unable to consistently distinguish past from present infection or colonization from active disease.

Several reports have shown that most healthy people throughout the world have serum antibodies to \textit{P. carinii} and that exposure to the organism begins in early childhood (85, 91, 96, 117, 119, 130). Overall, the 35- to 45-kDa band is the most commonly recognized antigen. Geographic differences in the prevalence of antibodies to MSG and higher (>95 kDa) molecular weight antigens have been found, raising the question of exposure to antigenically different strains of the organism (117). Serologic studies have also suggested that the lower frequency of \textit{P. carinii} pneumonia in HIV patients in tropical and developing countries compared to that in HIV patients in industrialized nations is not due to a difference in exposure to the organism; rather, it is more likely due to the high prevalence of more virulent infections such as tuberculosis and to poor access to health care (104, 117).

Surveys of immunocompromised hosts have revealed high rates of seropositivity to \textit{P. carinii} among patients who have experienced a documented episode of pneumocystosis as well as among those who have not (119). Of greater interest have been studies of patients monitored over time. HIV patients demonstrate on immunoblot a variety of antibody responses to single or recurrent episodes of \textit{P. carinii} pneumonia: loss of antibodies prior to the episode, no change in antibodies, and development of active IgG and/or IgM antibody responses following recovery from pneumocystosis (91). The development of active serologic responses has been less evident by the IFA and EIA techniques (20, 29, 76, 78). In fact, one IFA study found that HIV patients were less able to mount a humoral antibody response to the organism than other immunocompromised hosts (29).

The occurrence of pneumocystosis in patients with preexisting serum antibodies to \textit{P. carinii} led to the belief that humoral immunity has little role in host defenses against the organism. However, the importance of humoral immunity is supported by cases of \textit{P. carinii} pneumonia in patients and animals with B-cell defects, the therapeutic value of administration of anti-serum against the organism, and protection against pneumocystosis in actively immunized, T-cell-depleted mice (5, 30, 35, 36, 40, 48, 80, 101). A determination of which antibodies produced against the organism are functionally important awaits delineation of the protective B-cell epitopes. One role for antibodies might be to serve as opsonins (82, 88).

The development of serum antibodies to \textit{P. carinii} after recovery from an episode of pneumocystosis illustrates the ability of HIV patients and other immunocompromised hosts to mount an immune response to the organism even at an advanced stage of their disease. These antibody responses are also of interest in light of recent evidence which has shown that recurrent episodes of pneumocystosis occur by two different mechanisms (114, 121): (i) the episode represents infection with a new \textit{P. carinii} isolate, and thus antibodies may be produced in response to new antigenic determinants, and (ii) the episode represents relapse of an existing infection, and thus antibodies may be formed in response to antigenic variation.

Reports of outbreaks of clusters of \textit{P. carinii} pneumonia have stimulated interest in factors (e.g., exposure to asymptomatic carriers) that influence transmission of infection (114, 121). Some studies have shown a higher frequency or level of serum antibodies among hospital personnel who cared for \textit{P. carinii}-infected patients than among personnel who did not (43, 66, 103, 112). However, other studies have failed to confirm these findings (67, 75).

Analysis of local immune responses to \textit{P. carinii} has mainly been performed with BALF by the IFA technique (15, 63, 98). Some reports have found that HIV patients can mount a local antibody response to the organism (98), whereas other studies have reported that this antibody response is impaired (63).

**CELLULAR IMMUNE RESPONSES TO \textit{P. carinii}**

The importance of impaired cellular immunity in predisposing to the development of pneumocystosis in humans has been based on consideration of the immune defects in the underlying disease rather than on a specific relationship to the organism. These disorders include HIV infection, primary immunodeficiency diseases (especially severe combined immunodeficiency disease), prematurity, protein malnutrition, and the effects of immunosuppressive agents (particularly corticosteroids) used in the treatment of cancer, organ transplantation, collagen-vascular disorders, and other conditions (131). Analysis of lymphocyte subsets has shown that the risk of \textit{P. carinii} pneumonia in HIV patients is inversely related to the number of circulating CD4 cells (95): this has led to the recommendation for \textit{P. carinii} prophylaxis for CD4 counts of ≤200/mm^3. Limited evidence suggests that other immunocompromised hosts with low CD4 counts are also at increased risk of pneumocystosis (55, 113, 132). Thus, the human data support the results studies with animal models which have shown (i) that \textit{P. carinii} pneumonia occurs spontaneously in scid/scid and athymic (nude) mice, (ii) that \textit{P. carinii} pneumonia can be
induced in normal mice and rats by corticosteroid administration and protein-malnutrition, and (iii) that CD4 cells play a central role in the host defenses against the organism (shown by cell depletion and reconstitution experiments or by the use of knockout mice) (4, 46, 47, 49, 100, 110, 124). The interaction of CD4 cells with B cells and other cells via the CD40-CD40L pathway is also important to clearance of \textit{P. carinii} from the lungs (137). Although CD8 cells have been shown to participate in host resistance to the organism in experimental models (8, 124, 127), studies with humans have not yet been performed.

CD4 cells may also affect other clinical aspects of pneumocystosis and its management in HIV patients. The number of CD4 cells in peripheral blood and/or BALF has been shown to be related to disease survival and to the risk of developing adverse reactions to anti-\textit{P. carinii} drugs such as trimethoprim-sulfamethoxazole (1, 19, 62).

Functional studies of cellular immune function have mainly focused on the proliferative and cytokine responses of peripheral blood mononuclear cells to \textit{P. carinii} antigen preparations similar to those used in serologic studies (31, 45, 50, 52, 77, 125). Most healthy adults exhibit a vigorous proliferative immune response, a finding which is consistent with the high frequency of serum antibodies to the organism in this population. HIV patients exhibit a decline in their proliferative response with progression of the disease and a decrease in the number of CD4 cells; a similar decrease occurs in the TH1-like cytokine response (gamma interferon [IFN-\(\gamma\)]) but not the TH2-like response (interleukin-4 [IL-4]) (45, 125). HIV patients who have recovered from an episode of pneumocystosis have higher proliferative and IL-4 responses (but not IFN-\(\gamma\) responses) than HIV patients at a similar stage of their disease who never had pneumocystosis; this result occurred despite the fact that the \textit{P. carinii} patients had lower mean CD4 counts (60 versus 121/mm\(^3\)). Thus, people infected with HIV who have experienced \textit{P. carinii} pneumonia retain a sufficient number of memory CD4 cells to recognize the organism; however, there is a shift in their response from a TH1 to a TH2-like pattern as HIV advances.

Alveolar macrophages represent the principal host effector cell against \textit{P. carinii} (60, 69, 136). The organism can activate macrophages in the absence of T cells; however, activated macrophages require the presence of CD4 cells to control \textit{P. carinii} infection in animal models (9, 47). Studies with humans have shown that macrophages ingest, degrade, and kill \textit{P. carinii}, releasing proinflammatory cytokines such as tumor necrosis factor alpha (TNF-\(\alpha\)) and IL-1, eicosanoids, and reactive oxidants (32, 61, 64, 86, 123). MSG plays a role in this interaction (11). A few studies have examined the effects of HIV on the interaction of macrophages with \textit{P. carinii} (25, 56, 59). The data suggest that HIV impairs the mannose receptor-mediated binding and phagocytosis of the organism and alters the cytokine response. Nitric oxide is released by macrophages, but it does not appear to play a major role in host defenses against the organism (109, 111). By contrast, TNF-\(\alpha\) and IL-1 are very important in host resistance to \textit{P. carinii}, particularly early in the infection (22, 23, 69). IFN-\(\gamma\) is not crucial to the resolution of pneumocystosis, but it influences the host inflammatory response (34). A recent study showed that deletion of IFN-\(\gamma\) or TNF-\(\alpha\) receptor genes resulted in no problems in clearing \textit{P. carinii} infection, whereas deletion of receptor genes for both cytokines resulted in severe disease (102). Although a variety of other cytokines have been produced in response to \textit{P. carinii}, their roles in the host defenses against the organism are poorly understood.

Of the other types of cells, one report has shown that \textit{P. carinii} activates NK cells in conjunction with macrophages as described above (136). A role for NK cells in host resistance to \textit{P. carinii} has been suggested by the occurrence of pneumocystosis in people with HIV infection or other immunodeficiencies who had low numbers of NK cells or impaired NK cell function (16, 17, 28, 44). A study of neutrophils has shown that neutrophils from \textit{P. carinii}-infected patients exhibited impaired respiratory burst compared with neutrophils of healthy controls (65).

**DUAL EFFECTS OF THE HOST IMMUNE RESPONSE**

Studies with animal models and humans have shown that the immune response to \textit{P. carinii} can have harmful as well as helpful effects on the host. \textit{scid/scid} mice display production of proinflammatory cytokines in the lungs only late in the course of the disease (138); in corticosteroid-treated rats, levels of these cytokines are increased in lungs but not in peripheral blood (92). The adoptive transfer of immune splenocytes to \textit{scid/scid} mice or corticosteroid-treated rats with pneumocystosis results in an inflammatory response with the production of multiple cytokines and clearance of the infection (22, 124, 138). However, the adoptive transfer of purified CD4 cells to these same animals produces an early hyperinflammatory response with high mortality; animals that survive then clear the organism from the lungs (100, 101, 124). The adverse effects of this immune or inflammatory response can be prevented by the addition of hyperimmune serum or CD8 cells to the CD4 cells (100, 101, 127). It seems likely that the events described are cytokine mediated, but the specific cytokines involved and the underlying mechanisms remain to be elucidated.

Contributions of the inflammatory response to lung damage in HIV patients with \textit{P. carinii} pneumonia have been suggested by reports which have related increased levels of IL-8 (a potent neutrophil chemoattractant and activator) and neutrophils in BALF to more severe disease and worse prognosis (10, 13, 14, 27, 70, 73, 81, 129). Changes in the levels of TNF-\(\alpha\), IL-1, eicosanoids, and other cytokines and inflammatory mediators have also been observed in these and other studies; however, their relationship to lung injury is unclear (53, 61, 86, 92–94). Some studies have shown that HIV and non-HIV patients with pneumocystosis have elevated levels of proinflammatory cytokines in BALF but increased levels of anti-inflammatory cytokines (e.g., soluble TNF receptors or IL-1 receptor antagonist) in peripheral blood (92–94). HIV patients with pneumocystosis also frequently experience a deterioration in respiratory status soon after receiving anti-\textit{P. carinii} drugs; this can be prevented or reversed by the administration of corticosteroids (87). Although the beneficial effects have been assumed to be due to their anti-inflammatory properties, the effects of these drugs on the levels of cytokines and other inflammatory mediators in BALF have so far been inconsistent (15, 53, 86, 92–94). The principal effect of corticosteroids has been suppression of cytokine production by whole-blood cultures (12, 92–94).

Another possible mechanism for the action of corticosteroids is their effect on surfactant. Studies with animal models and humans have shown that pneumocystosis is characterized by a fall in the level of surfactant phospholipids and a rise in the level of surfactant proteins A and D and that these changes contribute to lung injury (51, 89, 108, 122). These changes are at least partly due to suppression of phospholipid mediated by MSG (74, 99). Since corticosteroids improve surfactant phospholipid secretion, it is possible that this is responsible for their beneficial effects on lung function in HIV patients treated with anti-\textit{P. carinii} drugs.
CONCLUSIONS AND FUTURE DIRECTIONS

It should be apparent from this review that one of the most pressing needs in immunological studies of *P. carinii* infection is a plentiful supply of purified, well-characterized antigens. MSG, which has been the most studied antigen, is a good starting point. Several human *P. carinii* MSG genes have been characterized and their fusion proteins have been shown to react with serum antibodies (33). A recent study showed that a highly conserved, immunodominant, recombinant MSG fragment was reactive with all 49 human serum specimens tested by immunoblotting (84); this frequency is considerably higher than the seroprevalence (30 to 40%) of antibodies to native MSG reported in other studies (78, 91). Epitope mapping of rat *P. carinii* MSG, which has already begun (72a), should be helpful in directing efforts toward finding which portions of MSG are best for serological studies and which contain protective B- and T-cell epitopes. Studies will also need to determine whether a single MSG or combination of MSGs will be needed to examine the full repertoire of host immune responses.

The 35- to 45-kDa antigen is another potential candidate, but it has not been biochemically purified and its gene has not been isolated; the rat recombinant p55 antigen, which is recognized by human serum antibodies (116, 118), might be explored. Additional antigens recognized by experimental animals following exposure to *P. carinii* or recovery from pneumocystosis might also be considered (38, 135).

A better understanding of *P. carinii* antigens might lead to immunological approaches to therapy and prophylaxis. Current anti-*P. carinii* drugs in clinical use are not lethal for the organism and are only effective as long as they are being given; their efficacy also tends to decrease as host immune function declines. On the other hand, the new potent antiretroviral agents have led to a decline in pneumocystosis and other opportunistic infections, preserved host immune function, and raised questions about whether drugs to prevent opportunistic infections are still needed. Answers to these questions will be helped by a better understanding of organism-specific immunity.

Support of immunotherapy for pneumocystosis comes from experimental studies demonstrating the value of hyperimmune serum, adoptive transfer of lymphocytes, and cytokine (IFN-γ and granulocyte-macrophage colony-stimulating factor) administration (5, 7, 40, 49, 101, 107, 124, 127). Active immunization with *P. carinii* and MSG has shown promising results in some (39, 48, 126) but not all (42, 54) immunodeficient and immunosuppressed animal models. Since most healthy people encounter *P. carinii* early in life, immunization of the general population makes little sense. However, active immunization of high-risk individuals who still have most of their immune function (e.g., HIV patients with >500 CD4 cells/mm² or newly diagnosed organ transplant or cancer patients) might prevent, delay, or decrease the severity of pneumocystosis (126). Immunization studies are currently in their early stages, and there is no consensus among investigators about which antigen preparation or experimental model offers the best potential for application to humans. HIV patients respond serologically to a variety of *P. carinii* antigens when recovering from pneumocystosis (91), but it is unknown if any of these moieties have protective value. In the opinion of this author, studies will have their greatest applicability to humans if they include an analysis of both humoral and cellular immunity.

Before immunologic approaches to therapy and prophylaxis in humans can be contemplated, there must be a thorough understanding of how the immune response is protective and how it can be deleterious to the host. Studies are needed to determine what aspects of the immune response in animal models are applicable to humans and what aspects are unique to humans. This information will also be helpful in designing more specific alternatives to corticosteroids in preventing the clinical deterioration that occurs with the initiation of treatment with anti-*P. carinii* drugs.

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