REVIEW ARTICLE

Pneumocystis Pneumonia

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Pneumocystis pneumonia (PcP) in humans is caused by *Pneumocystis jirovecii*, which has recently been reclassified as a fungus because its cell wall composition and nucleotide sequences are more similar to those of fungi. PcP occurs only in immunocompromised individuals such as those with AIDS. Despite the use of highly active antiretroviral therapy, PcP remains the leading opportunistic infection in AIDS patients. Based on nucleotide sequence variations in the internal transcribed spacer region of rRNA genes, more than 60 different types of *P. jirovecii* have been identified. Although type differences do not appear to correlate with the clinical characteristics of PcP, nucleotide sequence variations of the organism have been useful in epidemiologic studies. As a result, some recurrent infections are found to be due to re-infection with new types, and outbreaks due to the same types of *P. jirovecii* have been identified. Initial diagnosis of PcP is usually based on symptoms and chest radiography. A characteristic histopathologic feature is the presence of acellular eosinophilic exudates and organisms in the alveoli. Ultimate diagnosis of PcP is achieved by demonstration of the organism in induced sputum or bronchoalveolar lavage fluid by tinctorial staining or polymerase chain reaction (PCR). Among the many different PCR methods, the nested PCR that targets the large subunit mitochondrial rRNA gene is the most sensitive and specific. Combination of trimethoprim and sulfamethoxazole is the first choice of drugs for both treatment and prophylaxis of PcP. Other drugs that can be used include a combination of primaquine and clindamycin, pentamidine, atovaquone, and a combination of dapsone and trimethoprim. *Pneumocystis* organisms have the ability to inactivate the phagocytic activity of alveolar macrophages and to induce them to undergo apoptosis. This apoptosis is due to activation of caspase 9 by polyamines that are present in high levels in the lung and alveolar macrophages during PcP. [*J Formos Med Assoc* 2008;107(11):830–842]

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Pneumocystis Pneumonia in AIDS Patients

Pneumocystis is a major cause of pneumonia in immunocompromised individuals, including those with AIDS and those receiving immunosuppressive therapy. During the early stage of the AIDS epidemic, there were approximately 20,000 cases of *Pneumocystis* pneumonia (PcP) per year. The incidence of PcP started to decline when prophylaxis was recommended in 1989 for HIV-infected patients with a $CD4^+$ cell count less than 200/μL.[1,2] A further decline in the incidence of PcP occurred around 1996 when highly active anti-retroviral therapy (HAART) became widely used. At present, PcP occurs mainly as an AIDS-associated illness in patients who do not know that they are HIV-positive or who are not compliant with HAART

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therapy. However, PcP is still the most common opportunistic infection in AIDS patients.[3]

The Organism

Pneumocystis was first identified as a form of trypanosome in guinea pigs by Chagas[4] and in rats by Antonio Carinii.[5] In 1912, Delanoe and Delanoe[6] found that it was not a trypanosome and named it *Pneumocystis carinii* in honor of Carinii. Morphologically, *P. carinii* has the characteristics typical of protozoa and therefore was considered as a protozoan. *P. carinii* is now classified as a fungus because the composition and structure of its cell wall[7,8] and nucleotide sequences are more similar to those of fungi than to those of protozoa. The first molecular biological evidence suggesting that *P. carinii* is a fungus was described by Edman et al.[9] They determined the nucleotide sequence of the 16S rRNA gene of *P. carinii* and found that this sequence was more similar to that of fungi than to that of protozoa. Similar results were later reported by Stringer et al.[10] Additional molecular information with the same implication includes the finding that thymidylate synthase (TS) and dihydrofolate reductase (DHFR) of *P. carinii* are two distinct enzymes,[11,12] whereas those activities in protozoa are contained within a single bifunctional protein.[13] Results of studies on the *P. carinii* β-tubulin gene,[14,15] 5S rRNA gene,[16] mitochondrial rRNA gene and other mitochondrial protein genes[17] indicate a closer relationship to filamentous fungi than to yeasts. *Pneumocystis* is now classified as a member of the phylum Ascomycota. Although much evidence suggests that *Pneumocystis* is a fungus, it is not susceptible to antifungal drugs such as amphotericin B because of the lack of ergosterol, which is the major sterol and a hallmark of most fungi.

Taxonomy

Pneumocystis infects many different species of mammals, including human, monkey, horse, pig, rabbit, ferret, rat, mouse, etc. However, *Pneumocystis* is host specific.[18] *Pneumocystis* organisms from rats do not infect mice, and those from humans do not infect other animal species, and vice versa. Although *Pneumocystis* organisms from different host species are morphologically similar, analyses of nucleotide sequences of ribosomal RNA genes and other loci of *Pneumocystis* from different hosts indicate that they are distinct. To reflect this difference, a trinomial naming system was proposed in 1994.[19] In this system, human *P. carinii* was named *Pneumocystis carinii* f. sp. *hominis*, and rat *P. carinii* was called *Pneumocystis carinii* f. sp. *carinii*. Mouse *P. carinii* was referred to as *Pneumocystis carinii* f. sp. *muris*, and ferret *P. carinii* was named *Pneumocystis carinii* f. sp. *mustelae*. This naming system has proven to be very cumbersome to use; therefore, another naming system was proposed.[20] This new system first adopts the name *Pneumocystis jirovecii*, which was first proposed by Frenkel,[21] as the official name for human *Pneumocystis*. *P. carinii* now refers to the original rat *Pneumocystis*. Mouse *Pneumocystis* is now called *P. murina*, and ferret *Pneumocystis* is named *P. mustelae.*

Strain Variation

Both rat and human *Pneumocystis* have been found to have more than one type. Typing of *Pneumocystis* organisms is based on the number and size of chromosomes separated by pulsed-field gel electrophoresis (PFGE) or on nucleotide sequence variations of certain genetic loci. PFGE studies reveal that *Pneumocystis* from various host species has approximately 15 chromosomes ranging from 300 kb to 900 kb in size.[22,23] Conventional microbial typing methods such as biochemical reactions or antigen profiles are not possible because these methods require large number of organisms and *Pneumocystis* organisms are very difficult, if not impossible, to culture. Rat *Pneumocystis* can be cultured for very limited generations on feeder cells.[24–27] An axenic culture system has been reported,[28] but has not been widely used. Several types of rat *Pneumocystis* have been detected

by molecular karyotyping based on chromosome number and length polymorphism.[29,30] One of these types is named *P. wakefieldiae* (formerly *P. ratti*) in memory of Dr A. E. Wakefield. *P. wakefieldiae* differs from *P. carinii* in genomic sequence by 4–7%. *P. carinii* has 12 electrophoretic karyotypes, whereas *P. wakefieldiae* has only one karyotype.[31]

More than 60 different types of *P. jirovecii* have been discovered.[32] These types differ in the nucleotide sequences of the internal transcribed spacer (ITS) regions (ITS1 and ITS2) of nuclear rRNA genes.[33] Some of these types also have nucleotide sequence variations in the large subunit mitochondrial rRNA gene.[34–36] Although results of early studies suggested that the development of PcP was due to activation of latent infection,[37] several outbreaks have been described.[38–41] An increase in the incidence of PcP in non-AIDS patients who were in contact with AIDS patients was reported.[42] There are also reports of transmission of *Pneumocystis* from AIDS patients to transplant recipients.[43] The development of typing systems based on nucleotide sequence variations in the ITS regions and the large subunit of the mitochondrial rRNA gene has made it possible to conduct epidemiologic studies, and some recurrent infections were found to be due to re-infection with a new type.[44–46]

Approximately 30% of PcP cases are coinfected with multiple types of *Pneumocystis*.[42,43,47] The finding that type variation is associated with place of diagnosis but not with place of birth[48,49] suggests that *Pneumocystis* infection is acquired. Experimentally, normal mice can be infected when they are cohoused with *Pneumocystis*-infected mice. A report that 12 of 16 renal transplant recipients in the same hospital developed PcP and were infected by the same type (Ne) of *P. jirovecii* also supports this possibility.[41,50] Rabodonirina et al[50] found that five renal transplant recipients and one HIV-infected patient harbored the same type of *P. jirovecii* as the PcP patients they had been in contact with. Using multilocus sequencing typing, Schmoldt et al[40] found a very similar situation in 14 patients who received renal transplants at the same hospital and were infected with the same type of *P. jirovecii*. These observations suggest a patient-to-patient transmission route. Colonization of *Pneumocystis* in individuals with no signs of PcP has been recognized. There is speculation that children are reservoirs of *Pneumocystis* as 85% of children have antibodies against *Pneumocystis* by 20 months of age.[51] Based on PCR results on nasopharyngeal aspirates, 10% of healthy children and 15% of those with respiratory symptoms are considered to have colonization by *Pneumocystis*. In adults, 10–69% of nasopharyngeal aspirates from HIV-infected patients are positive for *Pneumocystis* PCR.[52–54] Most healthy people are *Pneumocystis* free,[55] but *Pneumocystis* can be detected in 7–19% of patients with respiratory illness.[56–58]

Diagnosis of *Pneumocystis* Pneumonia

Patients with PcP usually develop fever, shortness of breath, substernal tightness, and nonproductive cough. Their chest radiographs exhibit patchy consolidation with bilateral interstitial infiltrates. Histopathologically, the alveoli of PcP patients are filled with acellular eosinophilic exudates and *Pneumocystis* organisms. Laboratory diagnosis of PcP is usually achieved by demonstrating the presence of *Pneumocystis* organisms in induced sputum or bronchoalveolar lavage (BAL) fluid. The success rate of using induced sputum for diagnosis of PcP ranges from 50–90%.[59] Since it is noninvasive, it is usually done first. BAL follows if induced sputum fails to yield satisfactory results.

Pneumocystis has two different morphological forms, trophozoites and cysts. Diff-Quik, a modified Wright's Giemsa stain, is commonly used to visualize trophozoites, and Grocott-Gomori methenamine silver (GMS) is used to stain cysts.[60–62] Calcofluor white (CW) stain, in which the active ingredient cellufluor nonspecifically binds to β-linked polysaccharides such as β-glucan, chitin and cellulose, is also commonly used. Immunofluorescence staining, such as MeriFluor (MF) Pneumocystis (Meridian Bioscience Inc., Cincinnati, OH, USA) using monoclonal antibodies, is also

commonly done.[63–65] A recent study comparing these four different staining methods on 310 respiratory specimens concludes that all of these methods have a specificity greater than 94% but their sensitivities range from 48.4% to 90.8%, with Diff-quick the least sensitive (48.4%), followed by CW (73.8%), GMS (76.9%), and MF (90.8%). Among these, only CW and GMS have positive and negative predictive values greater than 90%.[66]

Many different PCR methods for diagnosis of PcP have been reported; the targets of these methods include dihydropteroate synthase (DHPS), dihydrofolate reductase (DHFR), thymidylate synthase, β-tubulin, cdc2 gene, the ITS of the rRNA, 5S rRNA, 18S rRNA, major surface glycoprotein (MSG), mitochondrial large subunit rRNA (mtLSUrRNA), and mitochondrial small subunit rRNA.[67–80] A recent study comparing the efficiency of nine different PCR methods for diagnosis of PcP found that the mtLSUrRNA nested PCR is the most sensitive and specific method for detection of *P. jirovecii* in sputum, tracheal aspirates, BAL fluids, or lung biopsy tissues.[65] The primers for the first round of this nested PCR are pAZ102-E (GATGGCTGTTTCCAAGCCCA) and pAZ102-H (GTGTACGTTGCAAAGTACTC) that amplify a fragment of 346 bp, and those for the second round are pAZ102-X (GTGAAATACAAATCGGACTAGG) and pAZ102-Y TCACTTAATATTAATTGGGGAGC. The final PCR product is 260 bp.

Prophylaxis and Treatment

The first choice of drug for treatment of PcP is trimethoprim-sulfamethoxazole.[81,82] The second choice is a combination of primaquine and clindamycin for those who are allergic to sulfa. Pentamidine, atovaquone, and a combination of dapsone and trimethoprim are alternative choices. For prophylaxis, trimethoprim-sulfamethoxazole is also the first choice, followed by dapsone, aerosolized pentamidine, and atovaquone. Sulfamethoxazole and dapsone inhibit the folate biosynthesis enzyme DHPS, whereas trimethoprim inhibits DHFR. Sulfamethoxazole is a very widely used antibiotic, and bacteria resistant to this drug due to DHPS mutations are very common. Similar mutations on *Pneumocystis* DHPS have been discovered,[83–88] suggesting the occurrence of sulfamethoxazole-resistant *Pneumocystis* isolates. Evidence indicating that *Pneumocystis* DHPS mutation is due to exposure to sulfamethoxazole is the observation that the mutation at amino acid position 55 or 57 is found in *Pneumocystis* isolates in 62–100% of patients receiving prophylaxis and in only 11–47.5% of those from patients not receiving prophylaxis.[84,85,87–91] Mutations in the *Pneumocystis* DHFR gene have also been found and correlated with failure of prophylaxis.[90] Atovaquone is structurally similar to ubiquinone; it binds to cytochrome b. Mutations on *Pneumocystis* cytochrome b have also been detected.[92–94] Approximately 30% of *Pneumocystis* isolates from patients receiving atovaquone have the mutation, whereas only 6% of those from patients with no atovaquone exposure have the mutation.[93] This observation suggests that *Pneumocystis* cytochrome b mutation is inducible and may render *Pneumocystis* resistant to atovaquone.

Immune Responses

Both cellular and humoral immune systems are important in defense against *Pneumocystis* infection. The $CD4^+$ T lymphocyte is critical as most patients with PcP have a very low $CD4^+$ T-cell count.[95–98] Furthermore, mice depleted of $CD4^+$ T lymphocytes by anti-$CD4^+$ antibodies develop PcP when they are inoculated with *Pneumocystis* organisms. The $CD8^+$ T lymphocyte may be less important than the $CD4^+$ T lymphocyte because $CD8^+$ T-lymphocyte-depleted animals can still clear a *Pneumocystis* infection.[99] However, $CD8^+$ T lymphocytes do play a role in host defense against the infection as depletion of both $CD8^+$ and $CD4^+$ T lymphocytes in animals results in a more severe PcP than those with depletion of only $CD4^+$ T lymphocytes.

The role of the humoral immune system in the host response to PcP is suggested by the

observations that SCID mice require B lymphocytes to clear *Pneumocystis* organisms,[100] and that agammaglobulinemia patients develop PcP despite the presence of an intact cellular immune system.[98,101,102] In addition, intraperitoneal injection of sera from mice recovered from PcP confers protection against PcP to $CD4^+$ lymphocyte-depleted mice.[103] Subcutaneous injection of antibodies against *Pneumocystis* organisms also confers partial protection against PcP in rats.[32] However, passive immunity alone is not sufficient because mouse pups born from PcP dams do not clear the transtracheally inoculated *Pneumocystis* organisms until they are of an age to mount a full immune response;[104] however, these mice have increased levels of IgG in their lungs and sera and clear the organisms sooner than those born to naïve dams.[105]

Active immunization is possible using soluble *Pneumocystis* proteins[106] or viable organisms[107] as antigens. Although immunization with the major surface glycoprotein (MSG, also called glycoprotein A) from *P. murina* induces an antibody response comparable to that of whole organisms, it is not protective in T-cell depleted mice.[108] Similar results have been observed in rats; both native MSG and a recombinant fragment of MSG confer only partial immunity in immunosuppressed rats, reducing organism load but not preventing infection.[109] An antigen referred to as A12, which is homologous to *P. carinii* Kex1, shows promise as a vaccine candidate. Mice immunized with the A12-thioredoxin fusion developed a good antibody response. When these mice were $CD4^+$ T-cell depleted and were then challenged with *Pneumocystis*, 70% were resistant to infection and were completely free of the organism.[110] The role of B lymphocytes in defense against *Pneumocystis* infection is shown by the observation that B lymphocyte-deficient mice are naturally susceptible to *Pneumocystis* infection.[111] B lymphocytes appear to play a vital role in the generation of $CD4^+$ memory T cells in response to *Pneumocystis* infection as $CD4^+$ T-cells from normal mice mount immune responses and help the clearance of *Pneumocystis* organisms in SCID mice, but those from B cell-deficient mice fail to control the infection.[112]

The Major Surface Glycoprotein

The trophozoites of *Pneumocystis* are coated with MSG, which is encoded by multiple genes.[113] MSG is the most abundant protein of *Pneumocystis* organisms with a molecular weight of approximately 120 kD. Similar to the variable surface glycoprotein of trypanosomes, *Pneumocystis* MSG also undergoes antigenic variation; this may be the reason why MSG fails to confer complete immunity against *Pneumocystis* infection on the host. It has been estimated that *Pneumocystis* contains approximately 100 different MSG genes located on different chromosomes;[114,115] however, only one MSG gene is expressed at any one time,[116–118] and only the MSG gene located at the MSG expression site is expressed. The MSG expression site is located close to the telomere of a chromosome and contains an upstream conserved sequence (UCS). This sequence is present at the 5′ end of all MSG mRNA,[119] but the mature MSG protein does not contain the UCS peptide.[120] Translocation of a MSG gene to the expression site is thought to occur by homologous recombination at the conserved recombination junction element (CRJE), which is located between the expression site and the MSG gene that is linked to the expression site.[119] The CRJE sequence contains a dibasic sequence that can be cleaved by a kexin-like (or subtilisin-like) protease[121–125] to remove the UCS sequence. MSG is thought to mediate attachment of the organism to type I alveolar epithelial cells and has been shown to bind to host factors such as surfactant proteins, vitronectin, and fibronectin[126] via a glycophosphatidylinositol anchor located at the carboxyl terminus.[127] MSG is very heavily mannosylated.[128–130] Recombinant MSG fragments have been used to study the serologic response against *Pneumocystis* infection, and sera from greater than 60% of HIV-infected patients with PcP reacted with the carboxyl terminal fragment of MSG, referred to as MsgC.[131] The cell wall of *Pneumocystis* cysts is rich in β-1,3-glucan,[7,132] and the gene encoding the catalytic subunit of β-1,3-glucan synthase has been identified.[133]

Effects of *Pneumocystis* Infection on Alveolar Macrophages

In animal experiments, transtracheally inoculated *Pneumocystis* organisms are not cleared in alveolar macrophage-depleted rats,[134] indicating that alveolar macrophages play an important role in the clearance of *Pneumocystis* organisms. In addition, administration of granulocyte/macrophage-colony stimulating factor (GM-CSF), which activates alveolar macrophages during *Pneumocystis* infection,[135] decreases the severity of PcP.[136] These results demonstrate the importance of the alveolar macrophage in the response to PcP. Alveolar macrophages interact with *Pneumocystis* organisms through various types of receptors, including the Fc-γ receptor,[112] complement receptor,[137] scavenger receptor,[138] mannose receptor,[139] and dectin-1, which recognizes β-glucan.[140–142] Recently, Toll-like receptor 2 (TLR2) was shown to also interact with *Pneumocystis* organisms.[143,144] TLR2 is a pattern recognition receptor for a large variety of ligands such as peptidoglycan, lipoprotein, lipopeptide, and zymosan.[145] Activated TLR2 transduces signals through a myeloid differentiation factor 88 (MyD88)-dependent pathway, inducing nuclear translocation of the transcription factor nuclear factor-kappa B (NF-κB). NF-κB activation is crucial for the production of proinflammatory cytokines and chemokines that initiate the host inflammatory response. In TLR2 knockout (TLR2-/-) mice with PcP, pulmonary inflammation was found to be much milder than that in normal mice with PcP.[144] However, these mice had much higher organism loads and died at least 1 week sooner than wild type mice with PcP. This observation indicates that inflammation is a normal host defense against PcP and cannot be completely inactivated.

Inflammation is due to recruitment of immune cells into infected tissue. These cells may include lymphocytes, neutrophils, monocytes and macrophages. They serve as the effector cells for the clearance of microorganisms. However, excessive inflammation may cause tissue damage. In fact, inflammation is considered to be the major cause of lung damage in PcP. Wright et al[146] found that immune-mediated inflammation directly impaired pulmonary function during PcP. A recent study showed that sensitized $CD8^+$ T cells failed to clear organisms but accelerated the onset of lung injury during PcP.[147] $CD8^+$ cells mediating lung damage during PcP have been shown to be dependent on MHC class I expression on non-bone marrow derived cells such as epithelial and endothelial cells, and the continued presence of *Pneumocystis* organisms.[148] However, the mechanisms by which $CD8^+$ T cells cause lung damage during PcP are still unclear. Surprisingly, neutrophils have been shown to play no role in lung damage during PcP, although an elevated number of neutrophils was correlated with poor outcome of PcP.[147]

Alveolar macrophages can produce interleukin (IL)-8, which attracts neutrophils and lymphocytes by chemotaxis as well as IL-1β, tumor necrosis factor (TNF)-α, IL-6, and GM-CSF, which participate in granulomatous lung inflammation.[136,139,149–152] Alveolar macrophages process and present antigens to naive T lymphocytes, activating them to become helper or cytotoxic T-cells. Alveolar macrophages can be activated by *Pneumocystis* MSG or the whole organism to release inflammatory mediators such as TNF-α and eicosanoid metabolites prostaglandin E2 and leukotriene B4.[153–155] This activation is enhanced by vitronectin or fibronectin, which accumulates in the lung during PcP.[156] Many investigators have investigated clearance of *Pneumocystis* organisms by alveolar macrophages. When alveolar macrophages from normal animals are separated from *Pneumocystis* organisms by a semipermeable membrane, they kill the organisms when activated by IFN-γ, probably through release of TNF-α.[157] The production and release of TNF-α from alveolar macrophages are increased in response to *Pneumocystis* infection in animals as well as in monocyte and macrophage cultures stimulated by *Pneumocystis* organisms.[158] The roles of IFN-γ and TNF-α in macrophage function have been investigated using IFN-γ and/or TNF-α and TNF-β receptor knockout mice. Both TNF (TNF-α and/or TNF-β) and IFN-γ were found to be important in the clearance of *Pneumocystis* organisms.[152,159,160]

Deletion of genes encoding TNF receptors, TNF-αR and TNF-βR, or IFN-γ did not render an animal susceptible to PcP, but deletion of TNF-αR, TNF-βR, and IFN-γ genes facilitated development of PcP.

Although alveolar macrophages from normal hosts bind, phagocytose and degrade *Pneumocystis* organisms,[134,161,162] alveolar macrophages from *Pneumocystis*-infected hosts are defective in phagocytosis. Fleury et al[163] reported that *Pneumocystis* organisms were rarely seen intracellularly in alveolar macrophages from patients with PcP. Using a *P. murina*-infected SCID mouse model, Chen et al[164] demonstrated that phagocytosis of *Pneumocystis* organisms was not common. Phagocytosis of *Pneumocystis* organisms by macrophages is reduced in HIV patients with PcP, and macrophage mannose receptor expression is decreased in these patients.[165] There was also a report that *Pneumocystis* organisms cause alveolar macrophages to shed mannose receptors.[166] The mechanisms by which *Pneumocystis* disables alveolar macrophages are not clear. However, the transcription factor GATA-2 is downregulated in alveolar macrophages during *P. carinii* infection.[167] When GATA-2 expression is knocked down in normal alveolar macrophages, their phagocytic activity is greatly decreased.[168] These observations suggest that GATA-2 regulates phagocytic activity of alveolar macrophages and that *Pneumocystis* suppresses GATA-2 expression in alveolar macrophages, rendering them defective in phagocytosis.

The number of alveolar macrophages in patients with PcP is reduced.[163,169–172] Young et al[171] found that the numbers of both leukocytes and alveolar macrophages were low in the lungs of PcP patients. This is unusual because alveolar macrophages are usually increased in other types of infections in immunocompromised patients.[163] Furthermore, *Pneumocystis* MSG is chemotactic for monocytes;[173] it should have attracted monocytes from the blood to the lung and increased alveolar macrophage number. A more quantitative study was performed by Fleury et al.[163] They determined that alveolar macrophages comprised 69.5% of the total cell population in BAL fluids from immunocompromised patients without PcP but only 38.8% of the total cell population in BAL fluids from patients with PcP. A similar observation was reported by Sadaghdar et al[172] that alveolar macrophages account for 64% of total cells in BAL fluids from AIDS patients without PcP and only 45% in BAL fluids from AIDS patients with PcP. The reduction in the numbers of alveolar macrophages was found in both HIV-positive and HIV-negative patients with PcP but was not observed in either HIV-positive or HIV-negative patients without PcP.

With a rat PcP model, the number of alveolar macrophages was found to be reduced by approximately 60% within 6 days after the establishment of *Pneumocystis* infection.[174] Decreasing the organism burden by treatment with trimethoprim-sulfamethoxazole or cessation of immunosuppression of rats with PcP returned the alveolar macrophage number to normal levels,[168] suggesting that *Pneumocystis* plays a major role in the modulation of alveolar macrophage number. *Pneumocystis* organisms are known to produce polyamines, such as spermidine, spermine, N-acetylspermine, and N-acetylspermidine,[175] and the levels of spermine, N-acetylspermine, and N-acetylspermidine are greatly increased in both the alveoli and alveolar macrophages.[176] When normal alveolar macrophages are incubated with polyamines, they undergo apoptosis. These results indicate that *Pneumocystis* decreases alveolar macrophage number by producing polyamines to cause alveolar macrophage to undergo apoptosis. It is now known that H_2O_2, produced when the polyamines are metabolized, damages the mitochondria, leading to activation of caspase-9 and the cascade of apoptosis. Interestingly, suppressing the activity of this activated caspase-9 greatly decreases the severity of PcP,[177] suggesting that caspase-9 can be a target for treatment of PcP. Because alveolar macrophages are the first line of defense against *Pneumocystis* infection, it is conceivable that *Pneumocystis* must overcome this defense to survive. It appears that disabling the phagocytic activity and decreasing the number of alveolar macrophages are the means by which *Pneumocystis* ensures its survival in the host.

Therefore, preventing *Pneumocystis* from progressing to such actions is a viable approach for treatment of PcP. This possibility is being investigated.

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