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Pathological and Protective Immunity to *Pneumocystis* Infection

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Abstract

Pneumocystis jirovecii is a common opportunistic infection in the HIV-positive population and is re-emerging as a growing clinical concern in the HIV-negative immunosuppressed population. Newer targeted immunosuppressive therapies and the discovery of rare genetic mutations have furthered our understanding of the immunity required to clear *Pneumocystis* infection. The immune system can also mount a pathologic response against *Pneumocystis* following removal of immunosuppression and result in severe damage to the host lung. The current review will examine the most recent epidemiologic studies about the incidence of *Pneumocystis* in the HIV-positive and HIV-negative populations in the developing and developed world and will detail methods of diagnosis for *Pneumocystis* pneumonia. Finally, this review aims to summarize the known mediators of immunity to *Pneumocystis* and detail the pathologic immune response leading to *Pneumocystis*-related immune reconstitution inflammatory syndrome.

Keywords

Pneumocystis; Diagnosis; Immunity; Immune Reconstitution Syndrome; Therapy

Introduction

Pneumocystis was first described in the lungs histologically by Dr. Carlos Chagas in the early 1900's. Approximately forty years later during World War II, the first cases of a diffuse interstitial pneumonia caused by *Pneumocystis* were documented in malnourished infants in orphanages (1). *Pneumocystis* at that time was considered a rare infection observed in patients with genetic immunodeficiencies. Fast forwarding another forty years, the CDC released the first case report of *Pneumocystis* pneumonia in homosexual men in Los Angeles in 1981 (2). *Pneumocystis* was and remains one of the most common and most devastating opportunistic infections in the HIV/AIDS population. Currently, thirty years after the connection between *Pneumocystis* and HIV was elucidated, *Pneumocystis* is re-emerging onto the clinical scene in the HIV-negative population. The use of newer immunosuppressive agents and chemotherapeutics has left patients with autoimmune conditions, transplantation, and hematologic malignancies at-risk to developing

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Pneumocystis pneumonia. Given the clinical problem that *Pneumocystis* presents, we will discuss the epidemiology, clinical features, and diagnostic methods for *Pneumocystis* in the current review. We will also review the protective immunity responsible for eliminating *Pneumocystis* infection, as well as the pathologic immune response following reconstitution of the immune system.

Epidemiology

The epidemiology of *Pneumocystis* can be categorized into the HIV and non-HIV populations. As described above, *Pneumocystis* first emerged as a common opportunistic infection in the HIV/AIDS population. As a result, anti-*Pneumocystis* prophylaxis was recommended for any individual with low $CD4^+$ T cell counts (<200 cells/μL), which led to a reduction in the incidence of infection (3). This reduction was furthered by the implementation of combined anti-retroviral therapy (cART) regimens (3). Within three years of the use of cART, *Pneumocystis* incidence (as measured by infection rates per 1,000 person-years) decreased by approximately half (3). Although the incidence of *Pneumocystis* has been reduced, a study by Walzer *et al*. demonstrated that the mortality of *Pneumocystis* pneumonia has largely been unchanged by the implementation of antiretroviral therapy (4). Prior to cART, mortality rates of *Pneumocystis* pneumonia in HIV patients were 10.1%; following cART, mortality rates were modestly reduced to 9.7% (4).

Despite the use of cART and anti-*Pneumocystis* prophylaxis, *Pneumocystis* pneumonia remains the most common serious opportunistic infection in HIV patients in the United States (3, 5, 6). One study reported 322 cases of *Pneumocystis* pneumonia in 2,622 patients with AIDS-defining events (5). Unsurprisingly, most cases of *Pneumocystis* in the developed world are in patients unaware of their HIV-positive status and/or patients not receiving prophylaxis or antiretroviral therapy (6).

In the developing world, *Pneumocystis* pneumonia is a common complicating factor in the HIV-positive population. *Pneumocystis* was detected in the bronchoalveolar lavage (BAL) fluid of 33% of HIV-infected patients presenting with a diffuse pneumonia in southern Africa (7). Furthering those findings, additional studies in Africa have shown that HIV-positive patients with symptoms (e.g. cough/dyspnea) of pneumonia are likely to have *Pneumocystis* infection; the incidence of *Pneumocystis* in such populations were found to be between 37.2% and 48.6% in South Africa and Kenya, respectively (8, 9). Asian countries, such as Thailand, India, and Malaysia, also have high incidences of *Pneumocystis* infection in the HIV-positive population with diagnosis rates between 12.2–25% (10–12). Developing countries in South America, such as Chile and Venezuela, also report high incidences of *Pneumocystis* in HIV-positive patients with respiratory symptoms (~37%) (13, 14). More alarming than any individual percentage, the above studies all further the point that *Pneumocystis* remains a global clinical concern for patients with HIV/AIDS where prophylaxis and/or cART use is limited for a variety of reasons.

Pneumocystis is also re-emerging in developed countries in the HIV-negative population. A study conducted in Sweden demonstrated that 75% of patients presenting to the hospital with *Pneumocystis* pneumonia were HIV-negative (15). Another study conducted in the

United Kingdom between 2000–2010 found that the number of hospital episodes of *Pneumocystis* pneumonia more than doubled during the study period, with transplant and hematologic malignancy patients representing the highest risk groups (16). In addition to malignancy and transplantation, several other conditions including autoimmune conditions and inherited immunodeficiencies have been implicated as emerging risk factors for *Pneumocystis* infection (Table 1) (5, 17–29).

Although there are several immunologic changes associated with each of the above conditions, the immunosuppressive therapy for each disease undoubtedly contributes to the risk of developing *Pneumocystis* pneumonia (Table 1). One such example is the use of Rituximab, a monoclonal antibody against the B-cell marker CD20, for the treatment of hematological malignancies such as diffuse large B-cell lymphoma. Martin-Garrido *et al*. found that approximately 30% of patients receiving Rituximab went on to develop *Pneumocystis* pneumonia over the course of the study period (30). Perhaps more troubling, acute respiratory failure was seen in 40% of patients with Rituximab-associated *Pneumocystis*, while mortality in these patients was as high as 30% (30). Although several other agents can increase a patient's risk for *Pneumocystis* (Table 1), this example of Rituximab highlights two important points. First, the use of these targeted immunosuppressive agents has lead to a greater understanding of the immune response required to protect against *Pneumocystis* (see Immunity against *Pneumocystis* section). Rituximab selectively targets B-cells and leaves the often-implicated $CD4^+$ T cells intact; however, these patients are exquisitely susceptible to *Pneumocystis* to the point where universal prophylaxis is being discussed. Second, much like the above study found with Rituximab, non-HIV cases of *Pneumocystis* tend to have increased morbidity (e.g. higher mechanical ventilation rates) and mortality than HIV-positive cases (31, 32). At this time, it remains unclear if the direct cause of the increased mortality is due to changes in the disease or differences in clinical management.

Clinical Features

Pneumocystis pneumonia in the HIV-positive population is generally characterized by a sub-acute onset of low-grade fever, nonproductive cough, and progressive dyspnea (33). Further nonspecific findings, such as tachypnea and tachycardia, can be found on physical exam, while the lung exam may range from normal to diffuse crackles upon auscultation (33). While the above characteristic presentation of *Pneumocystis* pneumonia is common in HIV-positive patients, HIV-negative patients can present much differently. Typically, the HIV-negative patients will have a more acute or fulminant presentation with substantial dyspnea, fever, and chills (17). Furthermore, HIV-negative patients have a wider alveolar-arterial oxygen gradient and are more likely to require mechanical ventilation (17).

Diagnosis

Radiologic methods are a useful first step in making a diagnosis of *Pneumocystis*, as most patients presenting with fever and dyspnea will receive a chest x-ray (CXR). The classic CXR of patients with *Pneumocystis* shows diffuse, bilateral interstitial and alveolar infiltrates (Figure 1A). Less common findings, such as pneumatoceles, lobar infiltrates, and

pneumothoraxes have been reported (17). Some patients presenting with *Pneumocystis* will have near normal or unimpressive radiographic findings, in which case higher resolution radiologic approaches are recommended, such as high-resolution chest CT (6, 17). Chest CT most commonly demonstrates ground glass opacity with relative peripheral sparing, although mosaic and diffuse patterns can be observed (34). Notably, the findings described above are not specific for *Pneumocystis* and a broad differential for opportunistic pneumonias (e.g. *Aspergillus*, *Mycobacterium avium-complex*) should be maintained (6).

It is also important to interpret radiologic findings in the context of the underlying immunosuppression. HIV-negative patients with *Pneumocystis* tend to have a greater extent of ground-glass opacity on chest CT (34). Moreover, HIV-negative *Pneumocystis* patients are more likely to have lung consolidations, perhaps reflecting a more robust host immune response (34).

Although radiologic methods, coupled with the appropriate clinical context, can highly suggest a diagnosis of *Pneumocystis*, the gold standard of diagnosis remains harvesting organism from bronchoalveolar lavage (BAL) fluid. Several stains can be utilized to identify *Pneumocystis* microscopically: Gomori-methenamine silver stain (GMS) (Figure 1B), Wright-Giemsa, toluidine blue O, or Calcofluor white (33). Monoclonal antibodies conjugated to a fluorescent marker are also available to stain *Pneumocystis*. In fact, these conjugated monoclonal antibodies have greater sensitivity and specificity for detecting *Pneumocystis* than most non-immunofluorescent stains (33). In addition to BAL sampling, induced sputum samples can also be analyzed for *Pneumocystis* by the above stains, with monoclonal antibodies again having the highest sensitivity and specificity (18). Importantly, HIV-negative patients with suspected *Pneumocystis* infection may have a negative induced sputum sample, as HIV-negative patients tend to have a lower organism burden than their HIV-positive counterparts (18). In HIV-negative patients, BAL is recommended (18).

Several molecular techniques have been tested as means to diagnose *Pneumocystis* from BAL or sputum samples. One such method developed in the 1990's was the use of a single-round polymerase chain reaction (PCR) to amplify the mitochondrial small subunit rRNA of *Pneumocystis* (35, 36). Since that time, the use of nested-PCR (two round PCR) has been used on several gene targets, such as dihydropteroate synthase (*DHPS*), dihydrofolate reductase (*DHFR*), major surface glycoprotein (*MSG*), and loci within the region coding for rRNAs (36). While using the nested approach increases sensitivity to nearly 100%, this often comes at the cost of decreasing specificity due to the ability to detect colonized individuals who may not have active *Pneumocystis* pneumonia (36).

Newer techniques include the use of quantitative real-time PCR (qPCR) on many of the same targets described above. In addition to reduced turnaround times, qPCRs provide semi-quantitative data to discriminate the cases of colonized individuals from the truly infected individuals. As such, qPCRs for *Pneumocystis* tend to have high sensitivities, along with increased specificities when compared to nested techniques (36). One study that illustrates this point was conducted by Flori *et al*. and examined the diagnostic value of a qPCR test on *MSG* (37). In this study, which examined both HIV-positive and HIV-negative patients, the *MSG* qPCR test had a sensitivity of 100% and specificity of 98.6% (37). While this indicates

such diagnostic tests can be optimized to have value in the clinical setting, implementing a qPCR test requires validating the gradations of *Pneumocystis* PCR product to distinguish colonization from infection in the heterogeneous population of the immunocompromised (36). At this time, this challenge still persists in the field and limits the clinical use of such tests.

Two serum markers, β-1,3-glucan and KL-6, have been evaluated as diagnostics for *Pneumocystis*. β-1,3-glucan is a component of the fungal wall, particularly of the ascus (see Microbiology of *Pneumocystis* below) and can enter the serum upon active infection. One study demonstrated that serum β-1,3-glucan above 100 pg/mL had a sensitivity of 100% and a specificity of 96.4% for diagnosing *Pneumocystis* pneumonia using a retrospective analysis (38). However, specificity for serum β-1,3-glucan testing is difficult to establish, as this test does not discriminate between fungal species. As such, serum β-1,3-glucan is often used as an adjunct to clinical suspicion and other diagnostic tests to confirm a *Pneumocystis* infection rather than a stand alone diagnostic. Similarly, KL-6, a glycoprotein expressed on pneumocytes, can enter the serum in the setting of infectious lung disease. One study has shown that KL-6 levels are elevated in HIV-positive cases of *Pneumocystis*, but the generalizability of serum KL-6 to the HIV-negative population has yet to be demonstrated (39).

Microbiology of Pneumocystis

One of the unique features shared by all the *Pneumocystis* species is the multiphasic life cycle that occurs within the alveolar space of the host. The ascus (cyst) form of *Pneumocystis* is circular or ovoid in shape and is approximately 4–7 μm in diameter (1, 40). The ascus form has a distinctive thick outer wall made of β-1,3-D-glucan, while within the ascus, eight ascospores mature (1, 40, 41). Following maturation, the ascospores will leave the ascus through a small pore and become the troph life form (40, 42). The troph life form appears to be the more metabolically active and replicative form of *Pneumocystis*. Trophs range in size from 2–8 μm and are more irregular in shape. Trophs are thought to replicate in both an asexual and sexual manner. Although most fungal species replicate asexually through a process known as “budding,” trophs are thought to propagate via binary fission (43). Two trophs can also conjugate via the use of pheromone receptors and replicate sexually by fusing. Following fusion, the two previous trophs are now a single diploid early sporocyte, which divides using meiosis. This meiotic process is then followed mitosis, generating the eight ascospores (44). During the division processes, the wall of the sporocyte thickens and hardens and returns the life cycle to the ascus stage.

One study by Cushion *et al*. examined the life cycle *in vivo*; in particular, they examined the effects of β-1,3-D-glucan synthase inhibitors on the ascus and troph population within the lung (41). Mice treated with anidulafungin had a decrease burden of asci in the lung, while the level of trophs remained the same. More importantly, mice depleted of asci were no longer able to aerially transmit infection to immunodeficient mice, implicating the ascus as the infectious form. A second study examined the *Pneumocystis* life cycle *in vitro*, despite the fact that a continuous axenic culture method for *Pneumocystis* has yet to be discovered (45). In this study, Martinez *et al*. showed that asci were capable of producing new trophs

while the reverse (trophs becoming asci) did not occur. These results demonstrated that information regarding the *Pneumocystis* life cycle could be gleamed from *in vitro* studies, although the viability in culture is undoubtedly a confounding variable. Recently, a novel mechanism to grow *Pneumocystis jirovecii* has been reported using differentiated pseudostratified CuFi-8 cells, although the utility of such a culture system for propagating infection and/or directed therapy selection has yet to be determined (46). Further studies on the *Pneumocystis* life cycle would be greatly enhanced by any sustainable *Pneumocystis* culture method.

In addition to elucidating the life cycle of *Pneumocystis*, the genetic makeup of *Pneumocystis* has also been heavily studied. In fact, even though *Pneumocystis* was first described in the early 1900's as a protozoan, it would not be until the end of the century that *Pneumocystis* was correctly classified as a fungus (1, 47, 48). Early studies demonstrated homology between *Pneumocystis* and fungi, such as *Saccharomyces cerevesiae*, using alignments of mitochondrial and ribosomal gene sequences. Furthermore, several studies demonstrated that *Pneumocystis* is a unique genus that encompasses several host-specific species, including *Pneumocystis jirovecii*, the human pathogen (49–51). Despite these early genetic studies, it was only recently that the genome of *Pneumocystis* had been sequenced (52).

Immunity of Pneumocystis Infection

Much of what we have learned of the immune response to *Pneumocystis* has been gleaned from acquired and congenital immunodeficiencies leading to susceptibility and many of these human conditions have also been successfully replicated in animal models. High dose corticosteroid treatment remains a risk factor and steroid induce immunosuppression has been a widely used tool to induce infection in rodents (53, 54). Prior to the epidemic of the acquired immunodeficiency syndrome (AIDS), *Pneumocystis* infection in humans was associated with significant malnutrition or myelosuppressive chemotherapy for acute leukemia (55). When the epidemic of *Pneumocystis* infection was observed in AIDS, it was realized that the prevalence of *Pneumocystis* inversely correlated with the peripheral blood CD4+ T-cell lymphocyte count (56). This was recapitulated in a murine model where CD4+ T-cell depletion resulted in Pneumocystis pneumonia whereas CD4+ T-cell replete mice cleared the infection (57). Although CD4+ T-cells are essential the specific T-cell subsets required remain unclear. Experimental *Pneumocystis* infection induces Th1, Th2, and Th17 responses in mice. Mice deficient in Th17 immunity have delayed clearance of the pathogen but ultimately clear in the infection (58). Analogous to these findings, patients with STAT3 mutations that have reduced Th17 cells to *Candida albicans* (59) rarely develop clinical *Pneumocystis* infection (60). IL-21 is a cytokine produced by T-follicular helper cells in the germinal center of secondary lymphoid tissues (61) as well as Th17 cells. IL-21 appears to potentially play a key role in susceptibility to *Pneumocystis* as evidenced by a recent patient with an IL-21 receptor mutation who subsequently developed clinical *Pneumocystis* pneumonia (62).

B-cells also play a key role in susceptibility to infection. B-cell deficient mice are susceptible to infection (63), as well as patients with hyper IgM syndrome due to either

mutations in CD40 or CD40 ligand (64, 65). Consistent with these findings, both CD40−/− or CD40L−/− mice are also susceptible to *Pneumocystis*. In addition to obvious effects on antibody production, the increased susceptibility of these patients may also be due to the fact that B-cells can function as critical antigen presenting cells during the infection (63). Antigen presentation by B-cells may also explain the fact that patients with mutations that affect antibody production such as common-variable or X-linked agammaglobulinemia can, but rarely, develop clinical *Pneumocystis* pneumonia (66). One caveat to the low incidence of *Pneumocystis* pneumonia in these patients, however, is that intravenous immunoglobulin is typically given prophylactically, which may mask some of the susceptibility to *Pneumocystis.* Despite the ambiguity associated with genetic immunodeficiencies, the use of anti-CD20 monoclonal antibodies in humans has emerged as a strong risk factor for *Pneumocystis* pneumonia, which further implicates the B-cell as an important cell type for the normal host defense against *Pneumocystis* (30). Thus, the B-cell appears to be critical and the dual functions of antigen presentation and antibody production are likely important.

Further evidence suggests antibodies can provide protection against *Pneumocystis*. It has been demonstrated that antibodies can provide protective immunity by passive transfer of serum elicited by immunization (67) or of monoclonal antibodies that recognize surface epitopes on the organism (68) to immunodeficient mice. Thus, although the role of humoral immunity in conferring susceptibility in humans remains unclear, antibodies could still be exploited for prevention or therapy.

It is thought that ultimately macrophages are the key effector cells that actually clear the infection. Indeed, macrophage depletion increases organism burden in the lung (69). *Pneumocystis* has also been shown to induce apoptosis of lung macrophages and this could be a major host evasion strategy of the organisms (70). Non-opsonic phagocytosis and killing of the organism requires the c-type lectin receptor Clec7a (71). This pathway can be bypassed if the organism is opsonized with IgG (71). Complement may also play a role in control of *Pneumocystis* (72). GM-CSF treatment of macrophages also increases their fungicidal activity (73). Recently, it has been demonstrated that macrophages that have an alternative activation program have greater fungicidal activity. Thus, given that CD4+ T-cell depletion is sufficient to confer susceptibility, CD4+ T-cells must be required for T-cell dependent antibody production as well as the recruitment of fungicidal macrophages.

Immune Reconstitution Inflammatory Syndrome

Immune Reconstitution Inflammatory Syndrome (IRIS) is a clinical phenomenon that occurs within the context of an opportunistic infection acquired during an immunosuppressive state. Upon treatment to reconstitute the immune system or address the underlying immunosuppressive condition, the immune response to an opportunistic infection can actually become pathologic. Typically, IRIS is observed in the context of HIV/AIDS following treatment with combination antiretroviral therapy (cART) and can present as worsening dyspnea, fever, and cough. Depending on the treatment status of the opportunistic infection, there are two different classifications of IRIS (74, 75). First, unmasking IRIS is characterized by a smoldering, undetected opportunistic infection acquired during the immunosuppressive state that is untreated upon the start of cART. After initiating treatment,

an exaggerated immune response to the active infection can cause damage to host tissues while failing to clear the infection. The second form of IRIS, paradoxical IRIS, occurs despite the fact that adequate treatment for the opportunistic infection has already been received. The immune system, however, can still target residual non-self antigens and an overzealous response can again cause damage to the host.

IRIS is defined clinically by the temporal relationship between cART initiation and a subsequent inflammatory condition associated with a positive response to cART (75). IRIS remains a fairly common condition, as approximately 16% of HIV-positive patients will develop IRIS following initiation of cART (76). A number of different opportunistic infections (and even some non-infectious conditions) can result in IRIS, including *Mycobacterium tuberculosis*, *Cryptococcus*, herpes infections, and *Pneumocystis* (74). Despite remaining one of the most common opportunistic infections in HIV, retrospective studies demonstrate that *Pneumocystis* only accounts for approximately 2.7–4% of IRIS cases (77, 78). One prospective study examined the incidence and causative agent of IRIS in 282 patients (79). Interestingly, 63% of the patients enrolled in the study were diagnosed with *Pneumocystis* pneumonia prior to the initiation of cART; out of the 177 patients with *Pneumocystis*, 13 (7%) developed IRIS (79). However, the true incidence rate of *Pneumocystis*-related IRIS and IRIS in general are difficult to calculate for a number of reasons. First, there are several different functional definitions of IRIS used in research, making the diagnosis of IRIS variable across studies. Second, most patients with *Pneumocystis*-related IRIS present two months after initiation of cART (with some patients presenting closer to a year after therapy), thereby requiring that the patients are extensively followed (77, 79).

Clinical studies, both retrospective and prospective, have elucidated certain risk factors for the development of IRIS. Several studies have demonstrated that individuals with lower $CD4^+$ T cell counts upon cART implementation are more likely to develop IRIS (74, 75, 77–79). Similarly, patients with increased HIV viral loads at the start of therapy are more susceptible to IRIS development (78, 79). Following cART, individuals who experience IRIS tend to have a more rapid decline in HIV viral load, followed by a sharp increase in $CD4^+$ T cell counts (74, 75). The above risk factors are highlighted in a case series following three patients, each of whom presented with low $CD4^+$ counts and high HIV viral loads, and subsequently developed a life-threatening *Pneumocystis*-related IRIS requiring mechanical ventilation (80). Interestingly, this case series suggested that early use of cART may increase the likelihood of severe *Pneumocystis*-related IRIS, calling into question the timing of cART implementation. However, one of the largest studies to date found an increase in overall and *Pneumocystis*-related mortality associated with delayed cART (81).

The pathophysiology of *Pneumocystis*-related IRIS has been further studied in mouse models of the disease. One of the first studies of *Pneumocystis*-related IRIS demonstrated that reconstitution of a SCID mouse infected with *Pneumocystis* resulted in increased expression of pro-inflammatory cytokines such as IL-1, IL-3, IL-6, TNF-α, TNF-β, and IFN-γ (82). *Pneumocystis*-related IRIS appears to be a T cell mediated phenomenon, as *Pneumocystis*-infected SCID mice receiving $CD4^+$ T cells alone develop severe lung pathology (83). Further studies using an anti-CD3 antibody targeting T cells abrogated the

inflammation associated with IRIS (84). $CD8^+$ T cells have also been implicated in IRIS, as $CD8^+$ cells have been shown to modulate the $CD4^+$ response, while sensitized $CD8^+$ cells may result in pathology independent of $CD4^+$ cells (85, 86). Another subset of T cells, T regulatory cells, has been shown to dampen the lung inflammation secondary to a robust anti-*Pneumocystis* $CD4^+$ T cell response (87). Local expression of IL-10, a hallmark cytokine of T regulatory cells, ameliorated the inflammation of *Pneumocystis*-related IRIS, while selective depletion of T regulatory cells worsened the course of the disease (88, 89). Reconstitution also appears to modulate lung mechanics, particularly surfactant regulation. Wright *et al*. demonstrated that reconstitution increased the protein:phospholipid ratios and minimum surface tension of bronchoalveolar lavage fluid when compared to wild-type mice (90). A more recent study further characterized the changes in the lung, as reconstituted animals had impaired surfactant biophysical function and decreased amounts of surfactant protein B and surfactant phospholipid (91). In addition, the *S*-nitrosylated form of surfactant protein D (SP-D) was increased; *S*-nitrosylated SP-D exists mostly as a monomer and has pro-inflammatory functions, such as increased cellular recruitment (91). While the above studies focus on the host's immune response in IRIS, the properties of *Pneumocystis* leading to the development of IRIS have also been explored. Mice infected with asci (cysts) and trophs of *Pneumocystis* have greater immunopathology than mice infected with trophs alone, as measured by increased cellularity and pro-inflammatory cytokine profile (92). These results were corroborated by the finding that treatment of *Pneumocystis*-related IRIS with an ascus-targeting dectin:Fc fusion protein limited hypoxemia in reconstituted mice (93).

While the definitive mechanism of IRIS remains elusive, a model of *Pneumocystis*-related IRIS can be proposed based off of the above murine models and clinical findings (Figure 2). Prior to cART, a high *Pneumocystis* burden is observed in the lung due to the lack of functional $CD4^+$ T cells capable of controlling the infection. Following cART (+/− treatment with antifungals), *Pneumocystis* antigen, likely derived from asci, remains prevalent in the lung while the high-affinity memory T cell compartment shows increased proliferation early in reconstitution (as reviewed in (74, 75)). Furthermore, the early expansion of memory T cells is facilitated by the lack of T regulatory cells, as these cells show a slower response to cART (as reviewed in (74, 75)). Due to the abundance of antigen and the lack of cellular regulators, T cells in the lung undergo antigen induced cellular death, again limiting the number of functional T cells in the lung (as reviewed in (74, 75)). Reconstitution in the context of *Pneumocystis* also alters the surfactant properties of the lung and leads to an increase in cellular recruitment and pro-inflammatory cytokines such as IL-1, IL-6, TNF-α, and IFN-γ. The recruited macrophages and neutrophils, coupled with T cell dysregulation, ultimately result in a pro-inflammatory state and damage to the host tissues. Importantly, the above model needs further validation in *Pneumocystis*-specific IRIS, as it is evident that the nature of the underlying opportunistic infection can alter the host response.

Treatment

The first line therapy for active *Pneumocystis* infection and for *Pneumocystis* prophylaxis is trimethoprim-sulfamethoxazole (TMP-SMX). However, TMP-SMX can be associated with several side effects (e.g. rash, cytopenia) and is not recommended for patients with sulfa

allergy (94–96). Interestingly, HIV-infected patients appear to be more likely to develop adverse side effects to sulfa drugs, further limiting the efficacy of TMP-SMX in the population at-risk for *Pneumocystis* infection (97). Several other treatments are indicated as second line therapies (e.g. pentamidine and dapsone) but such regimens tend to have much higher treatment failure rates (94, 95).

Treatment for *Pneumocystis*-related IRIS typically consists of eradicating the underlying infection with an anti-*Pneumocystis* agent described above, such as TMP-SMX (98–100). Identifying the cause of immunosuppression and providing adequate care and therapy for the underlying condition is also crucial for treating IRIS. As described above, early initiation of cART in the setting of an HIV-positive patient is thought to reduce overall mortality (81). Because some cases can become life threatening, glucocorticoid therapy can be used to reduce the inflammatory environment in the lung and supportive care and airway maintenance should be provided when needed (99, 100).

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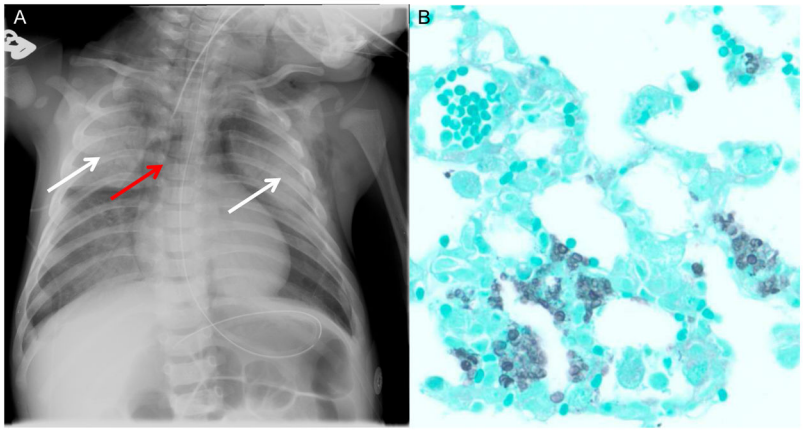

Figure 1.

Radiographic and microbiologic diagnosis of *Pneumocystis*. A. Chest radiograph of child with X-linked severe combined immunodeficiency showing bilateral ground glass infiltrates (white arrows) and air bronchograms consistent with *Pneumocystis* pneumonia. Note that this patient also has a pneumomediastinum (red arrow) with air dissecting into the soft tissue of the neck and an absent thymic shadow in the mediastinum consistent with athymia. B. Gomori-methenamine silver stain (GMS) on *Pneumocystis* infected mouse lung, showing lung architecture (green) with *Pneumocystis* organisms (black) filling the alveolar spaces.

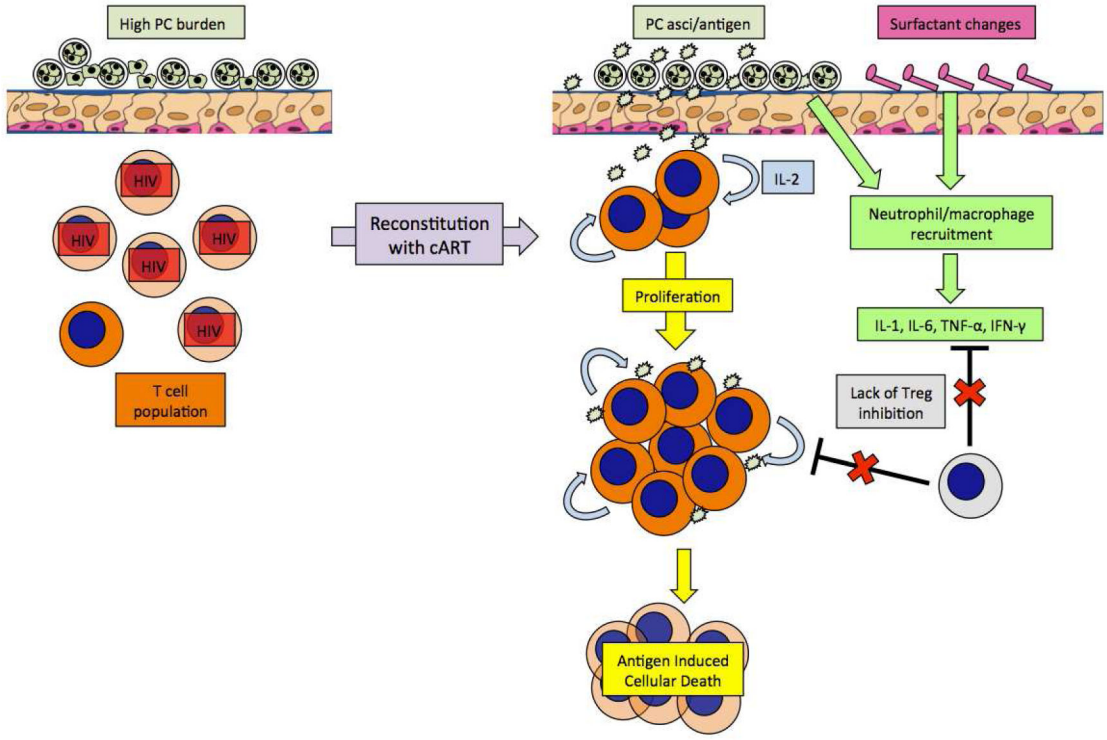

Figure 2.

Model of Immune Reconstitution Inflammatory Syndrome. In a patient with less than 200 cells/uL of $CD4^+$ T cells, *Pneumocystis* (PC) can propagate in the lungs and produce high PC burdens. Following reconstitution, high levels of PC asci or PC antigen can persist in the lungs and lead to activation of T cells through IL-2, which can induce apoptosis in the absence of regulatory T cell (Treg) inhibition. The lack of Tregs also allows for increased inflammatory cell recruitment due to PC and resultant surfactant changes, leading to systemic release of pro-inflammatory cytokines.

Table 1

Summary of conditions and procedures, along with the therapeutic agents used to treat those conditions, which have been implicated in increasing the patient's risk for developing *Pneumocystis* pneumonia (5, 17–29).

Conditions/procedures associated with *Pneumocystis* infection	**Therapeutic agents associated with *Pneumocystis* infection**
HIV	Corticosteroids
Hematologic malignancy	Alkylating agents (e.g. cyclophosphamide)
Solid tumors	Antimetabolite chemotherapeutics (e.g. methotrexate)
Hematopoietic stem cell transplantation	TNF inhibitors (e.g. Etanercept)
Solid organ transplantation	Azathioprine
Rheumatoid arthritis	Alemtuzumab
Severe combined immunodeficiency	Rituximab
Hyper-IgM syndrome	Sirolimus/Tacrolimus
Wegener's granulomatosis	Cyclosporine
Inflammatory Bowel Disease	
Collagen vascular disorders	