Immune reconstitution inflammatory syndrome associated with pulmonary pathogens

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ABSTRACT Immune reconstitution inflammatory syndrome (IRIS) is an exaggerated immune response to a variety of pathogens in response to antiretroviral therapy-mediated recovery of the immune system in HIV-infected patients. Although IRIS can occur in many organs, pulmonary IRIS, associated with opportunistic infections such as *Mycobacterium tuberculosis* and *Pneumocystis jirovecii*, is particularly associated with high morbidity and mortality. The pathology of IRIS is associated with a variety of innate and adaptive immune factors, including $CD4^+$ T-cells, $CD8^+$ T-cells, γδ T-cells, natural killer cells, macrophages, the complement system and surfactant proteins, Toll-like receptors and pro-inflammatory cytokines and chemokines. Although there are numerous reports about the immune factors involved in IRIS, the mechanisms involved in the development of pulmonary IRIS are poorly understood. Here, we propose that studies using gene-deficient murine and nonhuman primate models will help to identify the specific molecular targets associated with the development of IRIS. An improved understanding of the mechanisms involved in the pathology of pulmonary IRIS will help to identify potential biomarkers and therapeutic targets in this syndrome.

Introduction

A variety of opportunistic pathogens including viruses (human herpesvirus-8 and cytomegalovirus), fungi (*Cryptococcus*, *Pneumocystis* and *Histoplasma*) and bacteria (*Mycobacterium*) [1, 2] are associated with the development of immune reconstitution inflammatory syndrome (IRIS) after initiation of antiretroviral therapy (ART). IRIS presents as one of two forms, characterised by the timing of IRIS diagnosis in relation to the start of ART [3]. Paradoxical IRIS refers to worsening of symptoms secondary to a known opportunistic infection in the setting of the initiation of ART and treatment of the opportunistic infection. The second form is "unmasked" or ART-associated IRIS, in which infection with an opportunistic

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pathogen is undiagnosed until the initiation of ART [3]. Pulmonary IRIS has been described in *Mycobacterium tuberculosis*, *M. avium* complex, *Pneumocystis jirovecii*, *Cryptococcus neoformans* and Kaposi's sarcoma and can present with a spectrum of clinical manifestations, including pneumonitis, pulmonary infiltrates, lymphadenopathy, cough and dyspnoea [3, 4]. Low $CD4^+$ cell counts, high antigen load from an opportunistic infection and a short interval between the treatment of an opportunistic infection and the start of ART are risk factors involved in the development of IRIS [5, 6]. Several studies of pulmonary IRIS have suggested that the adaptive immune response, especially an antigen-specific T-cell response to opportunistic infection, is crucial for the development of IRIS [7–9]. However, recent studies have shown that dysregulation of the innate immune system due to opportunistic infection is also a key factor in the subsequent development of IRIS [10, 11]. Although many reports demonstrate a variety of immune factors involved in IRIS, the complexity of the disease process and variation in clinical presentations limit the understanding of the pathogenesis of disease. Thus, more research is necessary to understand the molecular mechanisms associated with the development of IRIS. IRIS has been modelled using T-cell deficient mice or severe combined immunodeficiency (SCID) mice infected with pulmonary pathogens including *M. tuberculosis*, *P. jirovecii* and *C. neoformans*. More studies using these models will help to overcome the limitations in understanding the mechanism involved in IRIS. In this review, we discuss epidemiology, immunopathology, animal models, diagnosis, treatment, prevention strategies and future research of pulmonary infections associated with IRIS.

Clinical presentation and epidemiology of pulmonary IRIS

The incidence of pulmonary IRIS is difficult to measure, given the wide range of possible disease presentations, local incidence of opportunistic infection and variations in local diagnostic capacity. In a retrospective analysis of patients in USA initiating ART after experiencing an opportunistic infection, IRIS was observed within 1 year in 16% of patients with tuberculosis (TB), 10% of patients with *M. avium* complex infection, 29% of patients with Kaposi's sarcoma and 4% of patients with *P. jirovecii* infection [12]. An international meta-analysis showed that TB-associated IRIS occurred in 15.7% of patients who were treated for both HIV and TB [13]. Risk of developing IRIS increased with shorter duration between initiation of TB treatment and ART, or with low $CD4^+$ T-cell count at ART initiation [13]. Clinical manifestations of TB-IRIS include weight loss, fever, lymphadenopathy and worsening of pulmonary symptoms, including development of abscesses, respiratory failure, acute respiratory distress syndrome and death. Morbidity and mortality secondary to TB-IRIS may be difficult to assess accurately, given the broad differential for clinical deterioration while on therapy, particularly in resource-limited environments. A *post mortem* analysis of a cohort of South African patients showed that IRIS secondary to mycobacterial infection was associated with the majority of early deaths occurring within 3 months of initiating ART [14]. World Health Organization guidelines recommend early ART initiation in patients with TB and very low $CD4^+$ T-cell counts, as there are increased risks associated with delaying ART, such as death from another opportunistic infection, compared to complications from IRIS. Given these newer guidelines, with increasing numbers of patients starting ART with lower $CD4^+$ T-cell counts, the global incidence of IRIS may rise [15].

IRIS associated with *P. jirovecii* infection presents as a worsening of existing *Pneumocystis* pneumonia (PCP) or unmasking of a previously asymptomatic infection. While many patients exhibit an inflammatory response upon initiation of antibiotics directed against *Pneumocystis* tempered by treatment with steroids, patients may also have worsening of pulmonary disease after initiation of ART, with symptoms of fever, dyspnoea, worsening hypoxia and increased inflammatory cells on bronchoscopy [16]. An analysis of published case reports of PCP shows that the IRIS syndrome tends to present early, at a median 15 days after initiation of ART [17].

IRIS associated with *M. avium* complex infection usually presents as either focal or diffuse lymphadenitis, occurring typically within 3 months of initiation of ART, and often with suppuration. *M. avium* complex related IRIS rarely presents as focal pulmonary disease [18].

Cryptococcosis manifests frequently as a multiorgan disease process, affecting the brain, lungs and skin; and while cryptococcal IRIS usually involves meningeal disease, pulmonary cryptococcal IRIS may present as a pneumonitis, or as cavitary or nodular lesions [3]. In some instances of *Cryptococcus*-associated IRIS, relapse of infection is increased after treatment for suspected IRIS given use of steroids for treatment.

Kaposi's sarcoma-associated IRIS (KS-IRIS) manifests as progression of lesions in the context of initiation of ART, and is noted to have high morbidity and mortality, especially in the setting of visceral disease, which frequently affects the lungs and gastrointestinal tract [19]. One US cohort exhibited an incidence of 16% within 1 year of starting ART [12]. Increased susceptibility to KS-IRIS correlates with a rapid rate of $CD4^+$ T-cell increase during ART, as well as peripheral oedema consistent with advanced-stage Kaposi's sarcoma [20].

Studies of the incidence of IRIS in children are limited. In a multicentre study in Uganda, IRIS was observed in 38% of paediatric patients in the first 6 months of ART therapy, predominantly in patients co-infected with TB or bacterial pneumonia [21].

Immunopathology of pulmonary IRIS

Several components of the immune system, including T-cells, macrophages, natural killer (NK) cells, pro-inflammatory cytokines and chemokines are involved in the pathology of IRIS (figure 1). In particular, increases in antigen-specific $CD4^+$ T-cells were observed in IRIS patients regardless of the opportunistic infection involved [7, 8]. Furthermore, increased numbers of $CD8^+$ T-cells were observed in IRIS patients when compared to non-IRIS controls [22]. Recent studies have shown the involvement of the memory T-cell population in the pathogenesis of IRIS. Thus, increased frequencies of $CD4^+$ cells expressing programmed cell death protein-1, Ki67, high levels of human leukocyte antigen D-related and effector memory population markers were observed in patients developing IRIS when compared to non-IRIS controls [8]. Pro-inflammatory cytokines and chemokines are other important factors in the exaggerated inflammatory response. Accordingly, elevated serum interleukin (IL)-6, C-X-C motif chemokine ligand (CXCL)-8, tumour necrosis factor (TNF)-α and interferon (IFN)-γ were observed in patients during the development of IRIS [23, 24]. Moreover, recent studies have shown that innate immune factors such as macrophages, monocytes, γδ T-cells, NK cells and the complement system also play a major role in IRIS associated with opportunistic infection [11, 24–27].

Immunopathology of IRIS associated with TB

Immune responses in patients with TB-IRIS demonstrate an important role for T-cell-mediated inflammatory responses [7, 8]. Several studies have shown the role of T-helper (Th) type 1 cells and relevant cytokines IFN-γ and TNF-α in the pathology associated with TB-IRIS. Accordingly, higher frequencies of IFN-γ^+ T-cells specific to *M. tuberculosis* antigens such as early secretory antigenic target-6, α-crystallin 1, α-crystallin 2 and purified protein derivative were observed in TB-IRIS patients than non-IRIS patients [25]. However, in some cohorts, antigen-specific IFN-γ responses were similar in both TB-IRIS patients and non-IRIS controls [28]. Recent studies have shown an increase in effector memory $CD4^+$ T-cell frequencies in TB-IRIS patients when compared to non-IRIS controls [29]. Moreover, elevated levels of the soluble IL-2 receptor sCD25 suggest a role for $CD4^+$ T-cells in the pathogenesis of TB-IRIS [30]. Th17 cells produce the cytokines IL-17, IL-22 and IL-21 following stimulation with polarising cytokines IL-23, IL-1β and IL-6. Many studies have shown an elevated level of IL-6, a critical driver of Th17 cells, in TB-IRIS [11, 31, 32]. A retrospective analysis of a randomised trial reported the elevation of Th17 cytokines in IRIS patients, suggesting a role for Th17 cells [23]. T regulatory (Treg) cells are known to be involved in maintaining immune system balance by suppressing the pathological effect mediated by Th1 responses. While one study demonstrated the presence of $CD4^+$ $FoxP3^+$ cells in TB-IRIS patients, their role in the development of TB-IRIS is not clear [25].

Innate immune cells such as macrophages and monocytes are implicated in the pathogenesis of IRIS [10, 33]. Accordingly, an elevated $CD14^+CD16^-$ monocyte population and an increase in the pro-inflammatory cytokines IL-6, TNF-α and C- reactive protein (CRP) were observed in TB-IRIS patients prior to the initiation of ART [32]. Furthermore, microarray analysis of monocytes from TB-IRIS patients showed dysregulation in pathways related to pattern recognition receptors, peroxisome proliferator-activated receptors, IL-6 and IL-10 during pre-ART [33]. Studies have shown the involvement of the complement system [10], as well as signalling *via* Toll-like receptors and triggering receptors expressed on myeloid cells-1 [34] in the development of TB-IRIS. Moreover, one study has demonstrated that $CD68^+$ macrophages were the predominant inflammatory cell population identified by immunohistochemistry in lung tissue from TB-IRIS patients [35]. Inflammatory cytokines and chemokines induced by macrophages and dendritic cells have also been shown to be involved in TB-IRIS. Accordingly, increases in IL-6, IL-1β, CXCL8, IL-10, IL-18 and CC chemokine ligand (CCL)2 were observed during pre-ART in HIV patients who subsequently developed TB-IRIS [29, 31, 36]. Additionally, IL-6 [11] and IL-18 [36] have been identified as potential biomarkers of TB-IRIS. In addition to macrophages and monocytes, γδ T-cells and NK cells are implicated in TB-IRIS. Studies have shown elevated frequencies of Kir-γδ T-cells [37] and $CD69^+$ NK cells [38] in TB-IRIS patients during pre-ART, suggesting that these cells may play a role in IRIS-associated pathology. Furthermore, one study demonstrated increased levels of CD107a, marker of NK cell degranulation in TB-IRIS patients during pre-ART [39]. These studies suggest a requirement for both innate and adaptive immunity in the pathogenesis of IRIS (figure 1).

Matrix metalloproteinases (MMPs) are a group of proteases involved in the degradation of various components of the extracellular matrix and which play a pathological role in inflammation, cancer and arthritis. It has been shown that MMP-mediated collagen degradation is a possible mechanism for TB-associated lung damage [40, 41]. Accordingly, one study has shown increased expression of MMP-1, -3, -7 and -10 in peripheral blood mononuclear cells from TB-IRIS patients [42]. Another study has

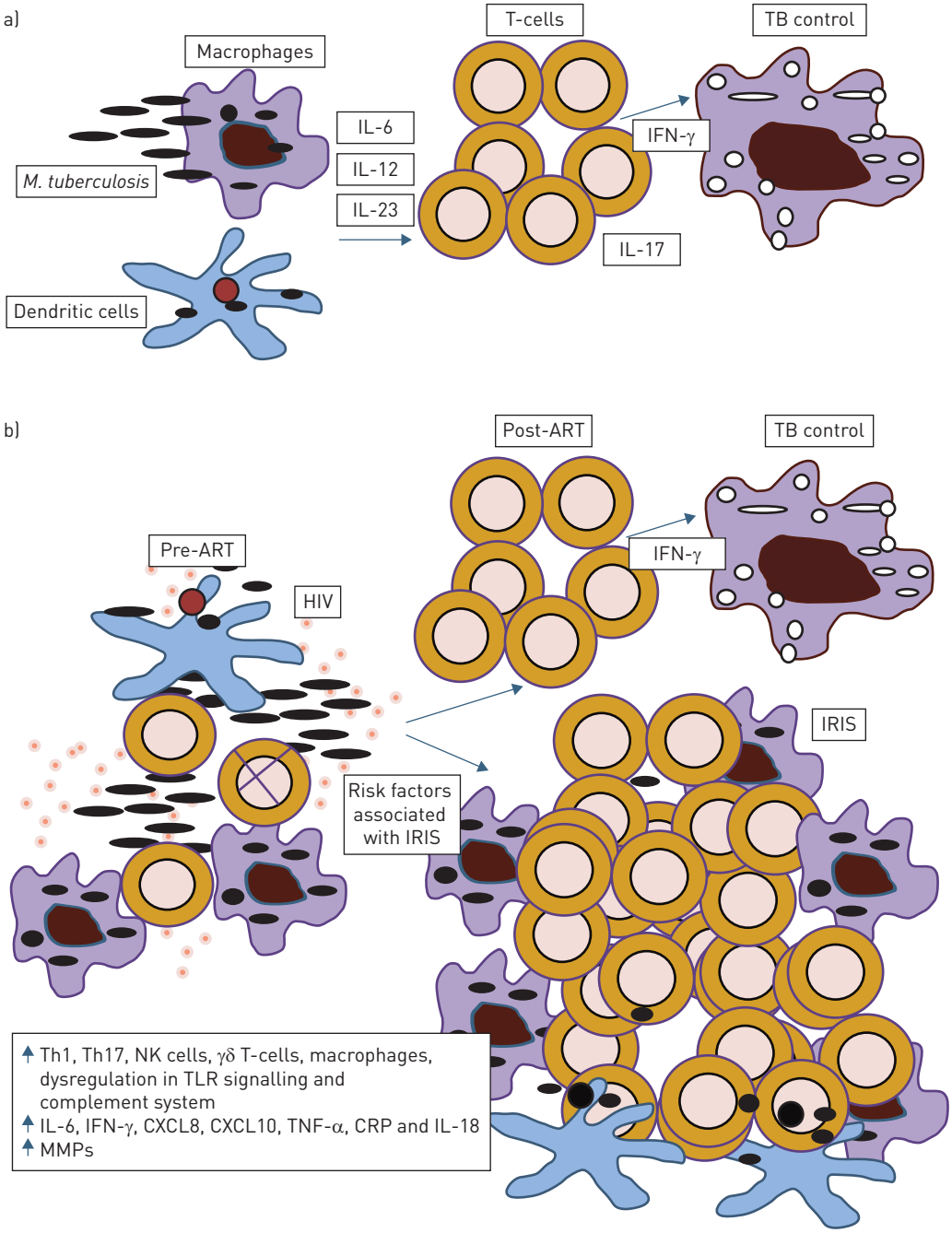

FIGURE 1 Immune reconstitution inflammatory syndrome (IRIS) associated with *Mycobacterium tuberculosis* (TB) infection. a) In healthy individuals in response to *M. tuberculosis* infection, macrophages and dendritic cells induce polarising cytokines including interleukin (IL)-6, IL-12 and IL-23. These cytokines polarise T-cells which in turn activate macrophages to kill *M. tuberculosis*. b) In HIV-infected patients there is no *M. tuberculosis* control because of lack of effective T-cell responses. IRIS develops in HIV-infected patients with pre-antiretroviral therapy (ART) risk factors, such as low $CD4^+$ cells, high HIV viral load, high *M. tuberculosis* antigen load and a short interval between *M. tuberculosis* treatment and ART. A variety of innate and adaptive immune cells, pro-inflammatory cytokines, chemokines and matrix metalloproteinases (MMPs) are involved in the exaggerated inflammatory response seen in IRIS. IFN: interferon; Th: T-helper cell; NK: natural killer; TLR: Toll-like receptor; CXCL: C-X-C motif chemokine ligand; TNF: tumour necrosis factor; CRP: C-reactive protein.

shown that increases in MMP-8 levels following ART were associated with impaired lung function in TB-IRIS [43]. These studies suggest that in addition to the innate and adaptive immune system response, MMPs also play a major role in the pathology caused by TB-IRIS.

IRIS-associated *Mycobacterium avium* complex infection has been modelled using T-cell antigen receptor (TCR)-$\alpha^{-/-}$ mice with *M. avium* infection [9]. In this model, TCR-$\alpha^{-/-}$ mice were infected with *M. avium*

for 2–3 months followed by reconstitution of $CD4^+$ T-cells from both spleen and lymph nodes [44]. It was demonstrated in this model that an antigen-specific $CD4^+$ T-cell response was crucial for the development of IRIS [9]. Additionally, increased levels of IL-6 and CRP were observed during the immune reconstitution process [11]. Moreover, this model demonstrated that the induction of CRP is IL-6 dependent. However, the induction of IL-6 is not dependent on IFN-γ production from T-cells transferred during experimental IRIS-associated *M. avium* infection. Furthermore, it has been shown that combined neutralisation of IFN-γ and IL-6 increased survival and pathology in this model [11].

Immunopathology of IRIS associated with fungal infections

Despite decreases in the incidence of PCP following the introduction of anti-*Pneumocystis* prophylaxis and ART, some people develop paradoxical IRIS following antiretroviral therapy [12, 13]. One case series demonstrated that decreased $CD4^+$ T-cell count and high HIV viral burden during pre-ART are associated with the development of PCP-IRIS following ART [45]. Another study has shown increased levels of CD4 and CD8 cells following ART in an HIV patient due to PCP-IRIS [16].

IRIS associated *P. murina* was first modelled in $Rag1^{-/-}$ and SCID mice [46, 47]. These immunodeficient mice were infected with *P. murina* for 2 weeks followed by reconstitution with whole splenocytes or $CD4^+$ T-cells [46, 47]. Using this model, it has been demonstrated that $CD4^+$ T-cells contribute the pathological immune response in PCP-IRIS. However, $CD8^+$ T-cells are the major inflammatory mediators in the absence of $CD4^+$ cells in PCP-IRIS [48]. The degree of lung injury in this model is driven by the fungal burden as well as the number of transferred cells. T-cells are known to be involved in suppressing the autoimmune response as well as hyperinflammation mediated through $CD4^+$ T-cells. Accordingly, it has been shown that depletion of Treg cells exacerbates IRIS in this model [47]. Lung injury associated with PCP-IRIS manifests with hypoxaemia, elevated lactate dehydrogenase and total protein in bronchoalveolar lavage fluid, weight loss and mortality [46, 47, 49]. A recent study using $Rag1^{-/-}$IFN-$\gamma R^{-/-}$ or $Rag1^{-/-}$IL-$4R^{-/-}$ mouse models of IRIS demonstrated that the activation of M1 or M2 macrophages is not required for *Pneumocystis* clearance [50]. Furthermore, this study has shown that IFN-γ is a critical mediator in the exacerbated inflammatory response by decreasing the $CD8^+Foxp3^+$ regulatory T-cell subset in PCP-IRIS [50]. Dectin-1 is involved in PCP-IRIS dependent lung injury, as antagonising dectin-1 signalling blocked the development of fungal IRIS [51]. B-cells are critical for effective $CD4^+$ T-cell response to control *Pneumocystis* infection [49, 52]. However, there are no current data supporting a role for B-cells in regulating the pathology associated with PCP-IRIS [49].

Pulmonary surfactants are lipoproteins that maintain surface tension in the lungs and protect the lungs from injury. Studies have shown that pathology in PCP is associated with decreased levels of surfactant protein (SP)-B and increased levels of SP-D [53, 54]. Recent studies have demonstrated that the immune reconstitution in IRIS is associated with the dysregulation of surfactant compounds in the lungs, increased pro-inflammatory cell recruitment [54] and exaggerated inflammation [55].

As in TB-IRIS, increased levels of IFN-γ, TNF-α, IL-6, CXCL8, granulocyte colony-stimulating factor and granulocyte-macrophage colony-stimulating factor before the start of ART were observed in cryptococcal IRIS patients [56]. Accordingly, these cytokines and chemokines were proposed to be potential biomarkers of cryptococcal IRIS [56]. The upregulation of chemokines CXCL10, CCL2 and CCL3 suggest a role for both T-cells and monocytes, reinforcing the interaction of innate and adaptive immunity in the pathogenesis of IRIS [57].

IRIS associated with *Cryptococcus* was modelled in the mouse successfully, in which $RAG^{-/-}$ mice were infected with *C. neoformans* and transferred with $CD4^+$ T-cells after 4 weeks. The data show the development of multiorgan immune reconstitution disease and systemic increases in the pro-inflammatory cytokines IFN-γ, IL-6 and TNF-α [58].

Diagnosis of IRIS

There is no single diagnostic test currently available for IRIS. Therefore, information regarding the opportunistic infection involved, diagnosis, treatment and response to treatment of opportunistic infection before the start of ART is crucial to the diagnosis of "paradoxical" IRIS. Diagnosis is further complicated in patients with "unmasking" IRIS, as it is difficult to prove both the previous existence of a hidden opportunistic infection and that the observed increase in inflammation is due to immune recovery. Thus, French *et al.* [59] recommended two major and three minor criteria for the diagnosis of IRIS. They defined these two major criteria as identification of atypical presentation of opportunistic infection and decrease in HIV RNA expression by >1 $\log_{10}$ after ART [59]. The three minor criteria are defined as an increase in $CD4^+$ cell count, increase in immunological response to opportunistic infection and spontaneous recovery of clinical symptoms [59]. In addition, some studies focus on the relationship between the decrease in viral load and increase in $CD4^+$ cell count after ART in the diagnosis of IRIS [6, 60]. However, the confirmatory diagnosis is

often complicated due to factors such as drug resistance, drug interactions, treatment failure of the opportunistic infection and other possible opportunistic infections [61]. In TB-IRIS, the increase in drug resistance and emergence of extensively drug-resistant strains of TB in endemic areas add additional levels of complexity to diagnosis [62]. Although there are several studies that identify biomarkers for early diagnosis of IRIS, all of these factors must be considered for proper diagnosis and treatment.

Treatment of IRIS

Corticosteroids and nonsteroidal anti-inflammatory drugs (NSAIDs) may be helpful in the management of IRIS. In a study of patients newly initiated on ART with paradoxical TB-IRIS, patients received prednisone over 1 month in conjunction with antimicrobial therapy [63]. Addition of prednisone reduced TB-IRIS symptoms, number of days hospitalised, need for procedures and inflammatory markers [63]. In cases of pulmonary cryptococcal IRIS, corticosteroid treatment may be considered if acute respiratory distress syndrome is present in the context of IRIS [64]. The antifungal regimen should be intensified in patients experiencing complications of cryptococcal IRIS. In addition to corticosteroids and NSAIDs, medications such as thalidomide [65], hydroxychloroquine [66] and TNF-α inhibitors [67] are under investigation as possible anti-inflammatory agents in the treatment of IRIS. In all cases of IRIS, regardless of the opportunistic infections involved, ART should be continued unless there is risk of permanent sequelae from continuation or the patient's life is in danger [1].

Prevention strategies

The development of IRIS is based on the presence of residual antigens from opportunistic infection during recovery. Therefore, the diagnosis and treatment of opportunistic infection before the start of ART is important for the prevention of IRIS. Screening all patients for possible opportunistic infections before starting ART is recommended in TB-endemic areas [68]. Studies show that using this approach in South African cohorts substantially reduced the unmasking of TB before the start of ART [68, 69]. The timing of the start of the ART is crucial in preventing paradoxical IRIS. According to a trial that evaluated starting antiretroviral therapy at three points in TB (SAPiT), ART initiation in TB–HIV patients should be decided based on their CD4 cell counts [70]. If the CD4 counts are <50 cells·mm^{-3}, ART can be started immediately after the initiation of TB treatment. If the CD4^{+} cell counts are $\geqslant$50 cells·mm^{-3}, ART can be delayed [70]. In cases of cryptococcal-HIV co-infection, the timing of start of ART is crucial in controlling IRIS. Longley *et al.* [2] recommended starting ART 3 weeks after amphotericin B and 4 weeks after fluconazole treatment. In cases of PCP-HIV co-infection, it is recommended that ART be started within 2 weeks of treatment of opportunistic infection, based on a study that evaluated early ART and deferred ART [71].

Conclusions and future directions

Multiple pathogens cause IRIS, which can manifest systemically or localise to the lung. The pathogenesis of IRIS is dependent on multiple factors including CD4^{+} T-cells, pro-inflammatory cytokines, macrophages and other innate immune cells. More research is needed regarding the molecular mechanisms of IRIS to decipher crucial pathways in this aberrant immunological response which has severe consequences for the host. Although there are numerous reports defining biomarkers of IRIS, variations in clinical presentations and complexity of the disease process limit understanding of the mechanisms involved in immunopathogenesis. Furthermore, an understanding of the interplay between the innate and adaptive immune systems is crucial in identifying the mechanisms involved in the pathogenesis of IRIS. IRIS has been modelled using T-cell-deficient mice infected with pathogens implicated in pulmonary IRIS. Use of gene-deficient mouse models and nonhuman primate models will help to illuminate the gaps left by clinical studies, and will help to identify potential drug targets in preventing and treating pulmonary IRIS.

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