Immune reconstitution syndrome associated with opportunistic mycoses

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Host immunity is essential in facilitating the eradication of infection. However, immunological recovery and an imbalance characterised by either suboptimum or excessive expression of immune responses can also be harmful to the host. Inflammatory responses triggered by rapid resolution of immunosuppression can lead to a series of localised and systemic reactions, termed immune reconstitution syndrome (IRS), that are often misconstrued as failure of specific antifungal therapy to eliminate the offending fungal pathogen. Recognition of IRS has become increasingly relevant in the context of our current use of potent immunosuppressive agents and immunostimulators that allow rapid manipulation of the immune system. Whereas the conceptual principles of IRS underscore the adverse effects of an overzealous and dysregulated immune response, they also support a role of immunotherapies to augment immunity if induction of endogenous responses is inadequate for the control of infection.

Introduction

In the era of improved antifungal drugs and the frequent use of immunomodulators with both immunostimulatory and immunosuppressive capabilities, clinicians have reached an important therapeutic crossroads. As Casadevall and Pirofski have elegantly outlined, the primary focus of a fungus–host interaction is a disease that arises from both the invading organism and host immunity. A major determinant of outcome after fungal infection is the characteristics of the host immune response, which may range from suboptimum or ineffective to excessive and inappropriate. Indeed, the host achieves the so-called Goldilocks paradigm, whereby the immune response is neither too much nor too little, and just right; however, this rarely happens efficiently except in normal hosts.

All too often in the treatment of fungal infections, therapeutic failure is identified as our inability to kill the invading yeasts or moulds. Our therapeutic focus has been on providing as rapid and as effective an immune reconstitution as possible in immunosuppressed patients with refractory fungal infections. However, what is not fully appreciated is that although host immunity is crucial in the eradication of infection, immunological recovery can also be detrimental and may contribute towards worsening disease expression. For instance, soon after the advent of potent antiretroviral therapy, successful immune restoration in HIV-infected patients became associated with an exuberant inflammatory response and worsening clinical manifestations of opportunistic infections. This entity, known as immune reconstitution syndrome (IRS), is also seen in other immunocompromised hosts, and even in immunocompetent individuals. IRS is best characterised as a collection of localised and systemic inflammatory reactions of varying degrees that have both beneficial and noxious features during an invasive mycosis.

However, the concept of IRS and its precise diagnosis in the context of opportunistic mycoses remains poorly characterised for health-care providers. Occurrence of IRS is almost always construed as failure of therapy or a relapse caused by inability to eliminate the fungus, often leading to unwarranted or inappropriate changes in specific antifungal treatment. Accordingly, its management is driven by sophisticated image studies and non-specific clinical signs and symptoms of inflammation rather than precise gauging of immunological recovery. Development of IRS also contributes to increased health-care costs and resource use. In this Review, we discuss the current state of knowledge about the pathophysiological basis, clinical characteristics, and approach to the management of opportunistic mycoses-associated IRS.

Pathophysiological basis of IRS

Host immune responses

The conceptual paradigm underlying IRS is clinically detectable immune reconstitution in an immunosuppressed host—for instance, enhanced immune responsiveness resulting from initiation of antiretroviral therapy in HIV-infected patients or from reduction of immunosuppression in transplant recipients. The available evidence suggests that reconstitution of pathogen-specific immunity and an inflammatory response triggered by resolving immunosuppression is the most likely basis for IRS (figure).

Cytokine-secreting effector T cells play a major part in mediating immune responses to self and foreign antigens. Activation by cognate ligand causes precursor CD4 lymphocytes (T-helper 0 [Th0] cells) to differentiate into cells with distinctive cytokine responses. Th1 cells, characterised by the production of interferon γ, elicit proinflammatory responses. Th2 cells produce anti-inflammatory and immunosuppressive cytokines (eg, interleukin 10). The cells that secrete transforming growth factor β are termed Th3 cells. These, in concert with Th2 cells, inhibit the development and function of Th1 cells. A normally functioning immune system is the result of fine balance between Th1 and Th2 or Th3 cells. An imbalance characterised by an inadequate or
Alemtuzumab, a CD52 antibody, provides prolonged initiation of antiretroviral therapy.21 Usual clinical occurrence of IRS within 2 months of stage of immunity, and this timing is consistent with the development of IRS has been proposed to occur at this antiretroviral therapy.18 Initial repopulation with CD45RO immune effector cells ensues after the start of HIV-RNA concentrations decline and recovery of (memory T cells) is followed 4–6 weeks later by an shift in T-cell receptor repertoire from Th2 to Th1.6,18–20

Microbial antigen (fungi, HIV) Iatrogenic immunosuppression Potent antiretroviral therapy Appropriate antimicrobial therapy Withdrawal of immunosuppression Administration of immunostimulatory molecules Proinflammatory responses Immune reconstitution syndrome Immune reconstitution syndrome

excessive expression of either response can be detrimental to the infected host. During retroviral infections, opportunistic infection-associated IRS is usually observed after initiation of antiretroviral therapy in naive HIV-infected patients.4,6 HIV-RNA concentrations decline and recovery of immune effector cells ensues after the start of antiretroviral therapy.18 Initial repopulation with CD45RO (memory T cells) is followed 4–6 weeks later by an increase in CD45RA and CD62L (naive CD4 cells) and a shift in T-cell receptor repertoire from Th2 to Th1.5,19,20 Development of IRS has been proposed to occur at this stage of immunity, and this timing is consistent with the usual clinical occurrence of IRS within 2 months of initiation of antiretroviral therapy.21

Th1 cytokines are also the primary mediators of allograft rejection and are the main targets of immunosuppressive agents in transplant recipients.22,23 In patients receiving calcineurin inhibitors, the concentrations of interleukin 10 compared with Th1 cytokines were raised.24 Intragraft mRNA expression of interferon γ and interleukin 12 was also inhibited by tacrolimus.25 In fact, suppression of interferon γ was greater with tacrolimus than with interleukin-10 concentrations in the cerebrospinal fluid.32–34 Furthermore, higher interferon-γ concentrations in the cerebrospinal fluid correlated with an improved clinical response during treatment of CNS cryptococcosis.35 However, the receipt of antifungal therapy in a patient with Cryptococcus gattii meningitis was associated with a pronounced reversion of Th2 to Th1 response with an exacerbation of clinical inflammatory manifestations, although the yeast was ultimately eradicated from the site.9

In addition to antigen-specific responses, C. neoformans is capable of eliciting an innate T-lymphocyte response as a mitogen.9 Mitogen-activated T cells could potentially lead to potent proinflammatory responses and therefore IRS. Even specific antibody responses to the invading fungus may contribute to inflammatory imbalance during immune reconstitution. A phenomenon, similar to a prozone-like effect, whereby too much antibody enhances disease, has been described in cryptococcosis.36,37 In fact, in experimental cryptococcosis, antibody-mediated immunity may be protective, non-protective, or even harmful to the host, depending on its concentration relative to the inoculum of C. neoformans.38 For instance, concentrations of IgM and IgG antibodies specific to glucuronoxylomannan, a component of the cryptococcal polysaccharide capsule, were higher in transplant recipients who developed cryptococcosis than in those

Figure: Proposed pathological basis of IRS
Microbial antigen and receipt of immunosuppressive agents lead to anti-inflammatory responses. Receipt of appropriate antimicrobial therapy, reduction of immunosuppression, or direct use of immune-stimulatory cytokines such as interferon γ and granulocyte colony-stimulating factor or granulocyte-macrophage colony-stimulating factor promote an inflammatory response that might lead to IRS.

Pathogen-mediated immunosuppression
Offending pathogens may also contribute to a state of immunosuppression that is then potentially reversible on initiation of antifungal therapy. Experimental studies have shown that Cryptococcus neoformans has immunomodulatory characteristics and preferentially inhibits Th1 while inducing Th2 responses.11,29,30 The exact relevance of these data in vitro and in animals for human disease is incompletely discerned. Nevertheless, several reports have shown that patients with cryptococcal meningitis have defective production of interferon γ and tumour necrosis factor α,11 with or without high interleukin-10 concentrations in the cerebrospinal fluid.32–34 Furthermore, higher interferon-γ concentrations in the cerebrospinal fluid correlated with an improved clinical response during treatment of CNS cryptococcosis.35 However, the receipt of antifungal therapy in a patient with Cryptococcus gattii meningitis was associated with a pronounced reversion of Th2 to Th1 response with an exacerbation of clinical inflammatory manifestations, although the yeast was ultimately eradicated from the site.9

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who did not. The precise contribution of this immunoglobulin dysregulation to IRS remains to be elucidated, but it shows the potential ability of the fungus to directly drive an inappropriate immune response.

**Epidemiological characteristics and risk factors**

**HIV infection**

Although IRS has been reported with *Histoplasma capsulatum*, *Pneumocystis jirovecii*, and aspergillus infections, the syndrome is best characterised in context of *C neoformans* infection. IRS was reported in 30–33% of HIV-infected patients with *C neoformans* after the initiation of antiretroviral therapy. Lower CD4-cell count and higher HIV-RNA concentrations at the onset of infection correlated with a higher risk of IRS. Patients with IRS also had a greater reduction in HIV-RNA concentration within 90 days of antiretroviral therapy initiation, and had a greater fungal burden at the onset of infection. Furthermore, initiation of antiretroviral therapy within 30–60 days of the treatment of fungal infection has been associated with a higher risk of IRS.

**Transplantation**

IRS was observed in 5% of the solid-organ transplant recipients, a median of 5–5 weeks after the start of antifungal therapy. Transplant recipients with IRS were more likely to have received a potent immunosuppressive regimen than those without IRS. After reduction of immunosuppressive therapy, a relative increase in Th1 response may have been greater in patients receiving more potent immunosuppression and therefore a higher risk for the occurrence of IRS.

**Pregnancy**

An immunosuppressive status characterised by anti-inflammatory responses is crucial for maintaining pregnancy. This is largely established by the expression of interleukin 10 and transforming growth factor β by maternal tissue and simultaneous inhibition of Th1 cytokines. Rapid reversal of this immunological repertoire in the postpartum period can lead to severe complications caused by IRS with mycoses. For instance, the advent of specific therapy, a woman who tolerated chronic cryptococcal meningitis for more than 15 years deteriorated after her second pregnancy and died. Another woman developed IRS with induction of a vigorous Th1 response after delivery of a baby and treatment for cryptococcal meningitis.

**Neutropenia**

In neutropenic patients with pulmonary aspergillosis, the risk of life-threatening pulmonary complications substantially increased when the neutrophil counts rose rapidly from severe neutropenia to greater than 4500 per µL within 5 days of receiving colony-stimulating growth factor compared with patients with a more gradual recovery from neutropenia. There are many reports of respiratory failure or worsening radiographs in patients with invasive pulmonary aspergillosis as rapid neutrophil recovery occurs. In fact, despite the widespread use of colony-stimulating factors and their ability to reduce the duration of neutropenia, a definitive benefit of these immunomodulators in the treatment of invasive aspergillosis has not yet been shown, a finding that may be related to imprecise control of inflammatory responses.

**Other risk factors**

A genetic predisposition to IRS has been suggested in patients with IRS associated with cytomegalovirus and mycobacterial infections. Therefore, robust studies are needed to validate and define potential genetic susceptibility to IRS during mycoses. Furthermore, specific characteristics of the fungus or strain variations might have a contributory role in IRS. For example, *C gattii* seems to cause a substantial chronic inflammatory reaction at the site of infection that is consistently more prominent than with *C neoformans*. This may be because of intrinsic differences in the characteristics of the yeast species or simply because *C gattii* preferentially infects more immunocompetent individuals.

**Clinical manifestations**

*C neoformans*-associated IRS typically presents as lymphadenitis, enhancing CNS lesions, or skin or soft-tissue lesions. Increased intracranial pressure in association with aseptic meningitis and inflammatory lesions within the spinal cord have also been reported. A hallmark of IRS lesions is granulomatous changes of which Th1 cells are the key initial mediators. Granulomas are strongly associated with containment and resolution of infection. In a renal-transplant recipient, administration of interferon γ was associated with the appearance of large granulomas at the site of cryptococcal cellulitis that heralded the resolution of an otherwise refractory infection. Granulomas in IRS, particularly in the lymph nodes, may undergo necrosis and manifest as suppurative lesions. Cryptococci may be visualised histopathologically; however, cultures have been consistently negative for the organism.

Renal-transplant recipients with cryptococcosis may experience allograft loss temporally related to the onset of IRS. In one study, the overall probability of allograft survival after *C neoformans* infection was significantly lower in patients who developed IRS than in those who did not (p=0.0004). The Th1 inflammatory response at the onset of IRS might have contributed to graft loss by upregulation of tissue rejection. IRS in histoplasmosis usually presents as focal or pronounced lymphadenitis that causes obstructive or compressive lesions. Inflammatory CNS masses, soft-tissue or osteoarticular lesions, and uveitis have also been reported. Unlike histiocytic infiltrates, typically
observed at the onset of infection, IRS-associated lymphadenitis was characterised by well-formed epitheloid and giant-cell granulomas. Up to 5% of the cases of *P. jirovecii* pneumonia have been shown to develop recurrent symptomatic disease 5–17 days after the start of antiretroviral therapy or discontinuation of adjunctive corticosteroids for the initial episode of pneumocystosis. Patients with recurrence were more symptomatic and often developed acute respiratory failure. Additionally, well before the recognition of *P. jirovecii* in HIV-infected patients, 73% of the episodes of pneumocystosis in children with acute lymphocytic leukaemia occurred during remission (ie, recovery from neutropenia).

Pulmonary infiltrates in patients with aspergillosis often show worsening during neutrophil recovery before improvement ensues on appropriate therapy. The most common presentations of IRS in patients with aspergillus infections were recurrent clinical symptoms, and worsening pulmonary lesions radiographically. Although it is difficult to ascertain whether these cases represented IRS with cell-mediated recovery or simply improvement in the numbers of inflammatory cells, histological findings in some patients can show granulomas consistent with IRS.

A potentially helpful surrogate laboratory clue to IRS is hypercalcaemia after initiation of appropriate antimicrobial therapy. Hypercalcaemia is a recognised response to granulomatous disorders, with endogenous overproduction of 1,25-dihydroxyvitamin D by activated macrophages being the proposed mechanism.

### Management

The optimum management of IRS is dependent on the awareness by health-care providers of its existence. Recognition that IRS is a manifestation of a poorly-controlled inflammatory response rather than direct treatment failure of antifungal agents to eradicate or kill the fungus is crucial in avoiding unnecessary modifications in therapy. Currently, no readily available markers exist that can reliably establish the diagnosis of IRS. However, on the basis of published data, the criteria outlined (panel) might be thought as representing IRS, and provide some reference in the discussion or study of this entity.

In therapy-naive HIV-infected patients with opportunistic infections, consideration should be given to deferring the start of antiretroviral therapy for 4–10 weeks until the infection seems to be microbiologically controlled. This approach has been proposed for the treatment of *Mycobacterium tuberculosis* and *C. neoformans* in these patients. Similar rationale can also be applied to the management of immunosuppression in transplant recipients with these infections. Withdrawal of immunosuppression in transplant recipients with opportunistic infections is a common practice and is intuitively logical. However, concurrent withdrawal of immunosuppression and initiation of antifungal therapy has been shown to predispose not only to IRS, but also allograft loss. Thus it is plausible that spacing or separating the reduction in post-transplant immunosuppression and initiation of antifungal therapy is a more prudent approach to the management of transplant recipients with cryptococcosis.

We note that antifungal agents per se may have immunomodulatory effects. For example, amphoterin B deoxycholate promotes the transcription of pro-inflammatory cytokines. The potential for induction of such responses is lower with the lipid formulations of amphoterin B. Modification of Toll-like receptor signalling with liposomal amphoterin B was shown to induce an antifungal effect while attenuating the inflammatory cytotoxicity to the host. Echinocandins, by modulating the amount of fungal β-glucan (that elicits proinflammatory responses), might also have indirect immunomodulatory effects. The clinical relevance of these observations has yet to be fully discerned. IRS has also been reported in patients with opportunistic mycoses treated with the azoles, even though these drugs have little or no proinflammatory activity.

There is no proven therapy for IRS. However, empirical treatment of symptomatic IRS in case reports or case series has been attempted using anti-inflammatory agents. Existing data support the use of prednisone at 80 mg per day and tapered over 3 weeks in the management of severe *P. jirovecii* pneumonia.

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**Panel: Suggested diagnostic criteria for IRS associated with opportunistic mycoses**

- **All three criteria must be present for a positive diagnosis of IRS**
  - New appearance or worsening of any of the following: clinical or radiographical manifestations consistent with an inflammatory process, such as contrast-enhancing lesions on neuroimaging studies (computed tomography or magnetic resonance imaging); cerebrospinal fluid pleocytosis (ie, >5 white blood cells per μL); increased intracranial pressure (ie, opening pressure ≥20 mm H₂O), with or without hydrocephalus; histopathology showing granulomatous lesions; unexplained hypercalcaemia.
  - Symptoms occurring during receipt of appropriate antifungal therapy† that cannot be explained by a newly acquired infection.
  - Negative results of cultures, or stable or reduced biomarkers for the initial fungal pathogen during the diagnostic work-up for the inflammatory process.

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*IRS is a collection of localised and systemic reactions of varying degrees with both positive and negative features, rather than a monolithic, rigidly definable entity.
†An attempt should be made to exclude intrinsic and de novo drug resistance, and suboptimum drug concentrations, particularly in case of the newer azoles.
A similar approach with longer tapering doses of corticosteroids has been used for the management of cryptococcosis-associated IRS. On the basis of these preliminary data and until definitive studies are done, the use of tapering doses of corticosteroids over 6–8 weeks is a reasonable option in T-cell reconstitution-associated IRS that presents with severe organ dysfunction, other life-threatening conditions, or CNS manifestations. However, careful consideration and selection of the patients with cryptococcal meningitis who are to receive corticosteroids is important because glucocorticoid therapy has been associated with a poor outcome in a retrospective study. We note, however, that in this study the use of corticosteroids was not based on clinical criteria and may simply have been a marker for patients with an extremely poor prognosis.

Although an overzealous or heightened inflammatory response may be life-threatening, the inflammatory reaction in IRS is ultimately beneficial in eradicating the infection. Induction of the endogenous immunological responses may be suboptimum for infection control, and augmentation of immune responses might be necessary for cure in severely immunosuppressed hosts. Indeed, a role for immunomodulatory therapies targeted towards neutralisation of suppressive cytokines, enhancement of Th1 responses with interferon γ, and transfer of adoptive cellular immunotherapy has been supported. Addition of recombinant interferon γ to antifungal therapy for cryptococcal meningitis in HIV-infected patients was associated with a trend towards improved clinical and mycological successes. On the one hand, the use of recombinant interferon γ for cryptococcal meningitis will probably not be routine since it may potentially precipitate IRS. On the other hand, the use of recombinant interferon γ might be ideal in combination with antifungal drugs in patients in whom cerebrospinal fluid is difficult to sterilise with standard therapies. Although interferon γ has been successfully used in transplant recipients, another concern with its use is the potential to exacerbate rejection.

Conclusions
IRS illustrates the complex host–parasite interactions in the evolution of opportunistic mycoses. IRS in fungal infections has been existent for years. However, the use in current medical practice of potent immunomodulators with their ability to rapidly alter immunological status has heightened its relevance. There remain major gaps in our knowledge about virtually all aspects of this entity, but IRS exists and we must deal with it. Characterisation of clinical variables predisposing to IRS, unravelling its immunological basis, and identifying markers that may be diagnostically helpful merit future studies. The treatment of IRS remains empirical, with little precision for agent, dose, or duration. Immunomodulatory therapies are potentially promising and necessary as adjuncts in the management of fungal infections, but balance in the modulation of the immune response that is not too little, not too much, but just right will be essential.

Conflicts of interest
NS has served on the advisory board of Schering Plough, has received grant support from Schering Plough, Enzon, and Astellas, and is on the speakers bureau for Pfizer. JRP has received support from and is a consultant for Schering Plough, Enzon, Merck, Pfizer, and Astellas.

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References


