Biomarkers of HIV Immune Reconstitution Inflammatory Syndrome

Shuli Bonham, MD[1,2], **David B Meya, MBChB, MMed**[3], **Paul R Bohjanen, MD, PhD**[1,2,4], and **David R Boulware, MD MPH DTM&H**[1,2]

[1]*Division of Infectious Disease & International Medicine, Department of Medicine, University of Minnesota, Minneapolis, MN* [2]*Center for Infectious Diseases and Microbiology Translational Research, University of Minnesota, Minneapolis, MN* [3]*Infectious Disease Institute, Makerere University, Kampala, Uganda* [4]*Department of Microbiology, University of Minnesota, Minneapolis, MN.*

Abstract

Dysregulation of the immune system drives HIV pathogenesis. As we develop new ways to treat HIV and AIDS, we encounter new clinical ramifications of our treatment on regulatory components of the immune system. HIV-associated Immune Reconstitution Inflammatory Syndrome (IRIS) occurs after initiation of anti-retroviral therapy (ART) with inappropriate and dysbalanced restoration of the immune system resulting in pathologic inflammatory reactions with significant morbidity. IRIS is most commonly associated with latent, occult, or past infections, including tuberculosis, *Cryptococcus neoformans*, and *Mycobacterium avium*-complex. We discuss common clinical presentations, new diagnostic modalities, current hypotheses of IRIS pathogenesis, and future directions of IRIS-related research, focusing on the identification of biomarkers that can be used to predict and diagnose IRIS.

Keywords

Introduction

HIV immune reconstitution inflammatory syndrome (IRIS) is a newly described clinical syndrome which occurs after initiation of antiretroviral therapy (ART), resulting in clinical deterioration due to pathological inflammatory reactions. Biomarkers to predict or diagnosis IRIS are limited, and the pathogenesis is poorly understood. As access to HIV treatment has expanded, there have been remarkable increases in survival and decreases in morbidity worldwide. Yet, new challenges have emerged, such as IRIS. IRIS events are often an exaggerated, inflammatory response to a known pathogen. The clinical symptoms may mimic that of an active infection or relapse of a prior infection, but microbiological cultures are often negative. IRIS events occur as the immune system recovers from an anergic state with the reconstitution of T-cell antigen-specific immunity. IRIS appears to be an antigen-specific

Corresponding Author Address: David R Boulware, MMC 250, 420 Delaware Street SE, Minneapolis, MN 55455 USA, Phone: +1.612.624.9996, Fax:+1.612.625.4410, Emails: scbonham@gmail.com; bohja001@umn.edu; boulw001@umn.edu; david.meya@gmail.com.

exaggerated inflammatory response in which the normal immune regulatory mechanisms that usually limit the degree of inflammation are lacking. Although IRIS is a clinical syndrome, studies directed at understanding the pathogenesis of IRIS have led to the identification of candidate biomarkers that could be used to diagnose IRIS and predict which patients are at risk.

Categorization

IRIS is often categorized by the associated pathogen and the clinical scenario. Pathogens commonly associated with IRIS include, *Mycobacterium tuberculosis, Cryptococcus neoformans, Mycobacterium avium*-complex, and cytomegalovirus (CMV). The most common clinical scenarios are: 1) unmasking IRIS and 2) paradoxical IRIS (Table 1). In either scenario, an essential feature is dysregulated restoration of antigen-specific immunity leading to a pathological, exaggerated inflammatory response. The primary difference between these clinical scenarios is whether the infection is newly identified after initiation of ART (unmasked) or was previously treated but clinical worsening developed after initiation of ART (paradoxical). In unmasking IRIS, the *typical* clinical course is often greatly accelerated with abrupt atypical manifestations in both disease severity and location.

At present, it is unclear if these two forms of IRIS occur through a general, universal mechanism or whether distinct pathogenic mechanisms are involved in the different clinical scenarios (unmasking vs. paradoxical). As well, it is unclear whether pathophysiology differs by pathogen. Current knowledge suggests there may indeed be distinct pathogenic mechanisms for different organisms, which may also be related to this distinction between paradoxical and unmasking IRIS. Thus, an emphasis has been made for IRIS researchers to use these categorizations when describing the epidemiology and investigating the pathogenesis. Examples of the common IRIS clinical scenarios are presented in Table 2 by pathogen. The terminology of 'IRIS', 'Immune Reconstitution Disease' or 'Immune restoration disease' (IRD), and 'Immune Reconstitution Syndrome' are often used interchangeably.

A major diagnostic challenge for clinicians is distinguishing IRIS from recurrence or relapse of an infection, as often the clinical presentation is similar. At present, IRIS remains a diagnosis of exclusion which necessitates eliminating the possibility of disease relapse, drug resistance, non-compliance, and alternative diagnoses. While IRIS is frequent within the first 12 weeks of ART, IRIS can occur at anytime as can alternative diagnoses.

Potential Biomarkers predictive of IRIS

The four principal factors associated with an increased risk of developing IRIS are: 1) low baseline CD4^{+} T-cell count, 2) excellent virologic response to ART, 3) increased antigen burden of an opportunistic infection (OI), 4) early initiation of ART after an OI. These factors have been identified in some retrospective case-control and cohort trials; however, these risk factors are by no means universal. For example, extrapolating from a case series on TB-IRIS may not be informative for defining the risk of hepatitis B IRIS. Of course, the statistical risk for a group does not perfectly predict the outcome for an individual patient, either.

Baseline CD4 Profiles

IRIS occurs more frequently in patients who have advanced HIV/AIDS when they start ART and in those who are previously ART naïve [1,2]. When CD4^{+} cells are <200 cells/µL prior to ART initiation, patients are more likely to develop IRIS [1,3,4]. The degree of immunosuppression likely affects IRIS risk in two ways. First, the lower the CD4^{+} count is, the greater the risk of an OI. Secondly, progressive damage to the immune system disrupts the homeostatic regulatory mechanisms.

IRIS rarely occurs in persons starting ART with relatively high CD4$^+$ counts >350 cells/μL [5]. This observation has particular implications for populations in resource-limited regions where persons are more likely to have more advanced AIDS, more co-infection with OIs, and have lower CD4$^+$ counts when they initiate treatment. In resource-limited regions, the incidence of IRIS appears to be 20–25% [6,7,8].

The degree of absolute CD4$^+$ rise with ART is not a risk factor [2,7,9]. Earlier case definitions used CD4$^+$ increase after start of ART as a criterion [10]; however, IRIS can occur without an appreciable CD4$^+$ increase. Some cohort studies have reported that IRIS is associated with an increase in the CD4 percentage or the ratio of CD4 to CD8 cells. One reported risk factor with the highest positive predictive value, based on the available data, is an increase in the CD4 percentage of ≥12% in the first 30 days of ART.[11] This occurred in nearly half of patients with HIV-associated IRIS due to TB in France, but in none of patients without IRIS [11]. Similarly, a rapid increase in the CD4 to CD8 ratio of >0.33 in the first 30 days of ART in patients with TB co-infection appeared to be strongly correlated with IRIS [11,12].

Higher levels of CD8$^+$CD25+ cells prior to ART initiation may be a predictive biomarker for later IRIS events [13]. In a cohort study of 50 Spanish patients with <100 CD4 cells/μL, those developing IRIS (number and IRIS-type not specified) had 17% ±32% CD8$^+$CD25$^+$ cells versus those without IRIS who had 4%±6.8% (P=0.07) and rapid decreases to 3–4% within the first month of ART. This study illustrates the problematic nature of the existing data on IRIS pathogenesis; the available data are derived from relatively small cohorts with a wide inter-personal heterogeneity. Thus, while a biomarker may be seem to be predictive within a specific study population, the results may not be generalizable to individuals.

Virologic Response

The non-modifiable IRIS risk factors include being ART naïve and having an excellent response to ART. For example, patients with a > 2 log drop in HIV-1 RNA viral load at 90 days of ART are at higher risk for IRIS [2,14]. The virologic response may be more important as a risk factor for IRIS in heavily ART pretreated populations with pre-existing HIV virologic resistance where only those patients who have a good response to ART are at high risk for IRIS [2]. In ART naïve populations, the virologic response is usually good whether or not patients develop IRIS [7,8]. Overall, those lacking a virologic response are unlikely to achieve a meaningful immune recovery, and thus, are at lower risk of IRIS. In populations with extensive virologic resistance, ineffective ART regimens are more likely to place populations at risk for new AIDS events rather than IRIS events. Thus, in retrospective cohorts composed of both ART naive and treatment experienced populations, being ART naive is often a risk factor for IRIS.

Antigenic Burden

Because of the link between IRIS and prior OIs, patients who have OIs that were identified prior to HIV therapy may benefit from delayed initiation of ART until they complete a period of therapy for the OI. The decision about when to start ART should be made on a case by case basis, depending on the type of OI and the severity of the infection. The antigen burden of an OI may be a indicator of IRIS risk. In a retrospective cohort study of TB patients, those with disseminated TB or extra-pulmonary TB had greater incidence of IRIS compared to those with pulmonary infection only [15,16,17]. In patients with disseminated cryptococcal fungemia, rates of IRIS were 6-fold higher compared to those with cryptococcal infection but without cryptococcemia. Higher CSF cryptococcal antigen titers at initial meningitis diagnosis and persistence of positive cultures after treatment were both risk factors for later development of IRIS upon commencing ART [3,18].

Timing of Starting ART after an OI

After an OI, persons starting ART within 2 months of an OI appear to have anywhere from zero to up to a ten-fold increase in the risk of IRIS [14,19,20,21]. The recently completed ACTG A5164 trial prospectively randomized 282 persons with an OI to starting ART immediately (<14 days, median 12 days) vs. delayed (>28 days, median 45 days) [21]. In this cohort, the median CD4 count was 29 cells/μL. The cohort consisted principally of patients with *Pneumocystis jirovecii* pneumonia (PCP) (63%), and the study found earlier initiation of ART resulted in fewer deaths and fewer new AIDS events (14% immediate ART vs. 24% deferred ART; *P*=.035) [21]. In this ACTG study, rates of IRIS were low, but importantly, did not differ by timing of ART initiation (5.6% immediate vs. 8.5% deferred ART) [21].

There are caveats, however, to the broad generalization of this study to other populations. First, two-thirds of those with PCP (50% of the cohort) were receiving corticosteroids and this may have prevented the development of IRIS. While corticosteroids are part of standard PCP treatment regimens, there is evidence that steroids blunt over-all thymic recovery after ART initiation. Second, patients with TB were excluded from the study, and TB is the most common IRIS-associated infection. Finally, other OIs that have been associated with IRIS, such as cryptococcal meningitis, occurred infrequently, and therefore this study was statistically underpowered for detecting differences in IRIS rates. Thus, the optimal timing for starting ART in patients with recent OIs has not yet been determined for most clinical scenarios. The interplay between the antigen burden and antigen clearance with the recovery of antigen-specific immunity and recovery of T-cell subsets is a complex dynamic (Figure 1).

Other Potential Demographic Risk Factors

There are other postulated risk factors for IRIS which may include sex, age, or specific ART regimen. Sex is unlikely to be an independent risk factor, but some retrospective studies have reported men to have a 2–3 fold higher risk of IRIS [3,14,22], yet in other prospective cohorts, sex has no association with IRIS [7,9,17]. The current belief is that sex is not an independent risk factor for IRIS. Age has not been established as a consistent risk for IRIS [2], though some retrospective case-control studies have matched for age [23]. In a U.K. cohort, the median age of those experiencing IRIS was 33.7 years vs. 35.6 years without IRIS [24]. In a Texas cohort, average age at time of ART initiation did not differ at 38.5 years with IRIS vs. 38.9 years without IRIS [14]. In 169 U.S. patients with TB prospectively enrolled, there was no association between age and risk of TB-IRIS [17]. Lastly, protease inhibitors have been suggested as a risk factor for IRIS [2], yet other cohorts have not replicated this finding [14,17,21]. Once again, extrapolating across all IRIS conditions may be incorrect, and age and gender may be specific risk factors in specific IRIS scenarios with specific pathogens.

Cell Surface Biomarkers related to IRIS Pathogenesis

The clinical features of IRIS and its association with specific OIs suggests that IRIS is an antigen-driven process in which normal immune homeostasis is dysregulated. Inflammatory responses in IRIS are exaggerated and the mechanisms that normally limit inflammation seem to be broken. These features implicate particular components of the immune system in IRIS pathogenesis, namely those components which normally maintain homeostasis and modulate the function of the "effector" cells of the immune system [25]. Damage to these homeostatic control mechanisms may allow "overreaction" to certain antigen triggers, causing pathological inflammatory responses in IRIS patients. Figure 2 displays the major components of the immune system involved in immune regulation and effector functions [26]. Antigens, such as *M. tuberculosis*, activate dendritic cells and phagocytic macrophages, which seek out and consume foreign material. These cells then activate Th1 memory cells, promoting their differentiation into CD4+ Th1 effector cells.

Functional T helper (TH) cells are classified into subsets such as interferon-γ (IFN-γ) producing TH1 and interleukin (IL)-4 producing TH2 cells which have long been known to be important mediators of host defense. TH1 cells are crucial for clearance of intracellular pathogens, such as *M. tuberculosis*, while TH2 cells are crucial for clearance of extracellular organisms. Recently, the discovery of TH17 cells, a new lineage of helper T cells preferentially produce IL-17, IL-17F, and IL-22 as signature cytokines, appear to play a critical role in sustaining inflammatory responses [27,28]. A reciprocal relationship has been described between pro-inflammatory TH17 lineage and anti-inflammatory, regulatory T-cells [29].

Regulatory T-cells (T$_{REGs}$)

The maintenance of a homeostatic state is made possible by T$_{REGs}$. T$_{REGs}$ suppress the proliferation of effector $CD4^+$ and $CD8^+$ cells and suppress their cytokine production, thus limiting the immune response to microbial antigens. The differentiation of naïve helper T cells (Th0) into T$_{REGs}$ is via signaling by TGF-β, IL-7, and IL-10. Disruption of T$_{REG}$ signaling may upset the delicate balance between an appropriately controlled immune response to an infectious pathogen and the development of exaggerated inflammatory states which are harmful. A critically important cytokine in this balance is IL-6. In the presence of TGF-β, IL-6 preferentially induces TH17 cells, and in the absence of IL-6, T$_{REG}$ differentiation can occur [29,30].

Normally, there is an approximately 2:1 ratio between pro-inflammatory TH17 cells and the T$_{REGs}$. During immune reconstitution, various subsets may reconstitute at differing rates, and this ratio may become unbalanced. Altered or absent T$_{REGs}$ function may be an integral part of the cellular and cytokine dysregulation leading to IRIS. Such defects in modulatory function may occur because of alterations in T$_{REG}$ production, or changes in their functional capacity.

One finding has been increased ratios of T$_{REG}$ to effector/memory cells in patients with IRIS [31]. Seddiki noted a significant numeric expansion of the $CD4^+$ $CD25^+$ $CD127^+$ $FoxP3^+$ subset of T$_{REGs}$ after ART initiation in patients who developed IRIS, compared with HIV-negative controls and HIV patients who did not develop IRIS. One would expect increased T$_{REG}$ numbers to correlate with increased anti-inflammatory activity. However, Seddiki also found that the actual functional ability of these T$_{REGs}$ to suppress inflammation appears to be diminished in TB IRIS patients. In the experiments, T$_{REGs}$ showed blunted ability to suppress the release of the pro-inflammatory cytokines IL-6, IL-4, TNF-α, and IFN-γ, resulting in increased expression [31]. On the other hand, levels of IL-10, an inhibitory cytokine released directly by T$_{REGs}$ in effector cell modulation, were lower in some IRIS patients, supporting the concept of unbalanced homeostasis leaning too far in favor of immune activation (Seddiki, personal communication). This result might not be generalizable to all forms of IRIS; in cryptococcal meningitis patients, we found no differences in IL-10 production in the serum from patients who did or did not develop IRIS. Though there may be greater numbers of T$_{REGs}$, these cells *may* produce less effective homeostatic regulation in persons with IRIS.

Cytokines as Biomarkers of Inflammation

All of these cellular interactions are mediated by signaling molecules, including the interleukins and other immunologic cytokines. Some cytokines are pro-inflammatory and others have anti-inflammatory / suppressive / homeostatic functions. Finding consistent patterns in cytokine activity during IRIS events will likely be the key to understanding the mechanisms by which some people to develop severe IRIS symptoms and others have none. A number of studies have found elevations in IL-6, one of the general pro-inflammatory cytokines, at IRIS events. In our prospective experience in Uganda, IL-6 levels in serum are 3-fold higher in IRIS patients than in comparable control patients without IRIS matched by $CD4^+$, OI-status, and time of ART. A similar retrospective observation of increased plasma

IL-6 *after* IRIS events was reported among persons with a history of a variety of IRIS events in Australia [33]. Many cells produce and modulate IL-6, though macrophages, in particular, are implicated in IL-6 hyperproduction associated with IRIS [32,33]. As well, in a case-control study of one person with nontuberculous mycobacterial IRIS manifesting as abdominal lymphadenopathy reported a temporal increase in IL-6 levels at time of IRIS event, returning toward levels of controls thereafter [56].

In our experience in Uganda, patients without a known active OI, who then develop IRIS after initiating ART, predominantly developed the unmasking form of IRIS manifest as dermatologic conditions, such as Herpes Zoster, Herpes simplex, and genital warts. The cytokines which were up-regulated at time of IRIS events in these IRIS patients were IFN-γ, TNF-α, IL-6, and MIP-1α. Similarly, among human herpesvirus-related IRIS, bio-available IL-6 was increased [34].

IRIS-associated Infections

As we develop more refined understanding of the cytokine/immune cell interactions occurring in particular infections, we may find common underlying mechanisms of IRIS pathogenesis. Because of the heterogeneity of IRIS-associated infections, however, most current studies examining the molecular underpinnings of IRIS have focused on specific infectious pathogens. Thus, we will organize our next discussion around the most common IRIS-associated microbes; *M. tuberculosis* and other mycobacteria, Cryptococcus, viral pathogens (including CMV, VZV and HSV), and Pneumocystis.

Biomarkers for Tuberculosis (TB) IRIS

Tuberculosis was one of the first identified pathogens to be associated with IRIS. As discussed above, it can occur either as a "paradoxical" IRIS event to previously treated TB or as "unmasking" of underlying latent or occult infection. With ART, TB-antigen-specific immunity reconstitutes; however the timing is variable and not consistent [35]. Those with TB-IRIS have increased numbers of TB-specific CD4+ T cells when measured by ELISpot interferon-release assay [36]. In TB-IRIS, Th1 cytokines appear to be up-regulated at the time of IRIS, with increases in interleukin (IL)-2, IL-12, and IFN-gamma as well as chemokines such as interferon-inducible protein 10 (IP-10/CXCL10) and monokine-induced by gamma interferon (MIG/CXCL9) [36]. Unpublished data also suggest C-reactive protein (CRP) is frequently elevated at time of TB-IRIS events [37]. Morlese et al also reported elevated CRP and IL-6 in a person with unmasking of abdominal TB [38].

Clinically, TB-associated IRIS most often occurs in the early phase after ART initiation, when memory T cells redistribute [39]. Th-1 cells are integral to the immune component of TB granuloma formation, and IL-6 may play a role in development of TB-associated IRIS responses, as well [40,41]. Other studies investigating ex-vivo stimulation of PBMCs report increased production of interferon-gamma (IFN-γ), human interferon-inducible protein-10 (IP-10), and monokine induced by IFN-γ (MIG) in patients with TB-associated IRIS events [42].

A different hypothesis by Shankar et al. postulates that patients may be protected by tolerance to the most antigenic component of TB, bacterial lipopolysaccharides. Having this tolerance, either because of long-term immune exposure or up-regulated T_{REG} function, may decrease the likelihood of developing IRIS. Having diminished T_{REG} function or a very high antigen burden may then predispose a patient to developing IRIS after they start ART [43].

Biomarkers for Mycobacterium avium-complex IRIS

In one case series, 30% of patients with *M. avium-complex* (MAC) subsequently developed IRIS upon ART initiation with a cumulative incidence rate of 15 episodes per 100 patient years [44]. The incidence of MAC in North America and Europe has decreased considerably in the ART era. In a Canadian case series from 1993 through 2004, the cumulative incidence of IRIS among those starting ART with CD4 counts <100 cells/µL or <50 cells/µL was 3.5% and 3.6% respectively [45]. MAC IRIS primarily presents as lymphadenitis, but fever of unknown origin (FUO), hepatosplenomegaly, abscess, or hypercalcemia have also been reported [14]. Both unmasking and paradoxical IRIS events are possible.

Diagnosis is primarily based on histopathology. Once again, the most serious differential diagnosis is treatment failure due to the development of drug resistance [46]. To differentiate IRIS from resistance, obtaining tissue for AFB staining, culture, and resistance testing is essential. IRIS lymphadenitis is marked by copious granulomatous inflammation with a paucity of organisms, at times no organisms may be seen, and indentifying the inciting pathogen can be difficult. Mycobacterial antigens persist long after culture sterility. Among HIV-seronegative persons treated for TB, 25% have PCR-positive sputum after two and four months of anti-mycobacterial therapy [47]. PCR probes may help delineate the inciting pathogen, but PCR cannot distinguish IRIS from persistent infection.

In a small U.K. case series of unmasking MAC-related IRIS presenting at an average of 11.5 months, Imami et al found that many patients lacked antigen-specific responses [48]. Similar unpublished findings have been reported by Elliot and colleagues in Australia in that restoration of antigen-specific responses can vary by person as well as by different antigens of a pathogen. For example, persons may selectively respond to the *M. tuberculosis antigens* of ESAT-6 or CFP-10 or PPD during immune recovery. The response by peripheral blood mononuclear cells (PBMCs) to antigen when measured from peripheral blood during an IRIS event can be either overwhelming or undetectable (unpublished). Sampling the compartment where the relevant inflammation is occurring, such as the lungs or lymph nodes, is not always possible to determine the composition of the antigen specific response. Imami et al reported patients with MAC unmasking, who had failed standard therapy, responded to adjuctive therapy with IL-2 and GM-CSF. The combination of IL-2 and GM-CSF supplementation was also associated with increased proliferative responses and increased IL-2 production [48].

Biomarkers for Cryptococcal Meningitis (CM)

There have been three retrospective and one prospective cohort studies of cryptococcal meningitis IRIS conducted among patients in France, Uganda, Houston, and Thailand [3,9, 18,49]. Among the 255 patients cumulatively described, the incidence of IRIS is near 30%. The common triad for CM-IRIS is: 1) treated CM, 2) recently started HAART, and 3) new aseptic meningitis [9]. The presentation of the aseptic meningitis of IRIS is clinically indistinguishable from cryptococcal treatment failure and/or relapse. In IRIS, a culture-negative meningitis occurs which appears to be triggered by the presence of residual antigen in the CSF. The spectrum of cryptococcal disease in IRIS is not always restricted to the meninges and can also manifest as atypical disseminated disease, such as cutaneous lesions, lymphadenopathy, mediastinitis, pneumonitis with ARDS, and CNS cryptococcomas [9,18, 50,51,52]. In these cases, a paucity of organisms is usually recovered but copious granulomatous inflammation exists.

At time of a CM-IRIS event, a CSF WBC pleocytosis and elevated protein are often present, but these levels can overlap with what is observed in CM relapse. Of persons with CM-IRIS, 80% will have a CSF WBC cell count <100 cells/µL [18]. The CSF glucose level is usually in the low normal range. In general, an increased intracranial pressure (ICP) of >20 cm H_2O is

very common in patients with CM. An elevated ICP occurs in 50% of individuals and pressures >35 cm H_2O occur in 25% of individuals with CM in the U.S [53]. In resource limited regions, the ICP has been reported to be higher, with a median CSF opening pressure of 33 cm H_2O and with 81% having elevated pressure ($\geq$20 cm) in patients with CM [9]. In cases of IRIS, the ICP is equally or even more elevated than with culture positive cryptococcal meningitis. Greater than 75% of patients with CM IRIS will have a CSF opening pressure >30 cm H_2O, and the average is near 45 cm H_2O at the time of IRIS events [9,18].

Obtaining a culture is critical to differentiate treatment failure or relapse from IRIS [54]. In cases of IRIS, the CSF cryptococcal antigen (CRAG) titer usually has decreased dramatically following initiation of ART and the initial anti-cryptococcal therapy [18]. The average level of the CSF CRAG titer in patients with culture positive cryptococcal meningitis is 1:2048. In CM-IRIS with negative CSF cultures, the CSF CRAG titers average 1:128 [18]. In patients with IRIS, 75% will have a CSF CRAG titer $\leq$ 1:256, whereas in culture positive meningitis 75% of CRAG titers are $\geq$1:256 [18].

In a Ugandan cohort [9], we identified heterogeneous cytokine profiles in patients with CM-IRIS marked by increases in IL-2, IL-6, IL-9, IL-17, and TNF-α in the serum of individual patients. Notably, interferon-γ, IP-10, and MIG in serum were not increased at time of IRIS events compared to those without IRIS. Additionally, at time of IRIS events, preliminary data suggest serum CRP levels are often, but not always, markedly elevated (mean 170 ± 100 mg/ L). At time of initial CM diagnosis, CSF cytokine profiles are similar and are not predictive of future IRIS events. Based on published studies, predictive biomarkers of CM IRIS have not been identified.

Zheng et al have found increased macrophage phagocytosis of *C. neoformans* in response to increased IFN-γ production by $CD4^+$ T cells. The $CD4^+$ cells themselves had direct cytotoxicity against *C. neoformans* through secretion of granulysin [55]. GM-CSF also plays a critical role in the phagocytosis of Cryptococcus by macrophages [56,57,58], and may play an important role in the rate of antigen clearance. In HIV patients, this process of antigen clearance appears to be impaired, but could potentially improve after initiation of ART and play a role in the association between IRIS and more rapid expansion of $CD4^+$ cells.

Biomarkers for Viral-related IRIS

The understanding of viral related IRIS is very limited at present. In part, this comes from the poor understanding of the immunology and pathogenesis of many viral related infections. One particular limitation is in understanding the basis between asymptomatic viral shedding and symptomatic disease; specifically, whether the manifestations of 'disease' are caused by a viral cytopathic affect or the activation of an immune response.

Hepatitis B virus (HBV) or hepatitis C virus co-infection with HIV are common conditions worldwide, especially in Asia or among illicit drug use populations. Differentiating between what is hepatotoxicity due to ART versus an IRIS event is difficult. At present, there are no reliable biomarkers to distinguish the two, and hepatitis-associated IRIS is probably under-recognized. Moderate to severe hepatitis flares after starting ART are fairly common, affecting 10–12% of patients who start ART; however, most of these flares are self-resolving [59,60]. In the setting of IRIS, HBV viral loads are decreased from pre-ART baseline with the restoration of $CD4^+$ HBV-specific immunity [61], causing an elevation of liver enzymes that has the same clinical appearance as hepatotoxicity.

Kaposi sarcoma (KS) is an AIDS defining condition that typically improves with immune reconstitution; however clinical flares and worsening can occur shortly after starting ART [62]. The timing of onset of KS IRIS appears to be similar to other forms of IRIS, and usually

occurs within the first 2–3 months of ART. In patients with KS IRIS, cutaneous KS lesions may initially expand or become painful; visceral KS-associated IRIS may be fatal. The diagnosis of KS IRIS is clinical, and specific biomarkers have not been identified.

Although numerous viral infections are associated with IRIS, we presently do not know if these diverse clinical diseases are due to common pathophysiological mechanisms, but we hypothesize that dysregulated immune homeostasis plays a significant role.

Summary

HIV patients starting on anti-retroviral therapy have profound changes in the function of components of their immune system as immune recovery occurs. Our previous understanding of immune recovery treated the immune system and its cellular and protein components as a single unit, which gradually improved as HIV diminished. We now find that 'immune reconstitution' happens at different rates for different subcomponents of the immune system, and reconstituted immune function does not always mimic normal immune function. IRIS develops when the immune response is exaggerated due to lack of homeostatic regulation. Elucidating the subtle patterns within specific cell populations in the recovering immune system and the intrinsic immune modulatory pathways will give us further insight the pathogenesis of this clinically important phenomenon. In the future, a better understanding of IRIS pathogenesis will lead to development of new biomarkers to identify those patients at greatest risk and to develop specific treatments to return the immune system to homeostasis following initiation of HIV therapy.

Future Perspectives

The importance of biomarkers in the future of medicine cannot be overemphasized. From the early 19th century, various laboratory-based tests have been unveiled with a positive impact on the practice of medicine. We envision the use of biomarkers in predicting who is at risk of developing IRIS. However, until then, the next few years will require dedicated scientists working to better define the pathogenesis of IRIS.

Over the next decade, increasing numbers of patients will require ART, especially in Sub-Saharan Africa. For example, in Uganda, there are 1.5 million people living with HIV. But only about 70,000 currently receive ART. With the increasing roll out of ART and consolidation of established ART programs in Africa, IRIS has emerged as an issue that cannot be ignored.

The early development of solutions to the problem of IRIS will depend on the establishment of collaborative research networks responsible for the convergence of research ideas. In order to be successful, multi-disciplinary research teams will need to combine investigators skilled in clinical, epidemiological, and basic science research to define the pathophysiology of IRIS and the clinical management of IRIS.

One limitation of IRIS research has been the paucity of data from prospective cohorts. Most of the IRIS data to date have been generated from retrospective cohorts using a variety of non-conforming case definitions. A major task is to define IRIS in a generalizable way that can be used by all researchers in the field. The International Network for the Study of HIV-associated IRIS (INSHI), composed of researchers from Australia, Europe, South Africa, Uganda, and the United States will produce IRIS definitions and likely play a significant role in research on the pathogenesis, treatment, and prevention of IRIS. Such networks will also lead to the development of biomarkers that can be used to diagnose IRIS and will lead to better definitions in the future. As various IRIS- related events are studied, one of the questions to be answered will be whether the pathophysiology is pathogen-specific or if general pathways of immune

regulation are involved in all forms of IRIS through a common mechanism. Thus, biomarkers for diagnosis and prediction of IRIS may be general to all forms of IRIS or may be pathogen-specific.

A major ongoing dilemma is the adequate characterization and categorization of populations and IRIS conditions being studied. Differentiating unmasking from paradoxical IRIS events is critical to correctly studying the epidemiology and pathophysiology of IRIS. Extrapolating across pathogens is likely unwise in comparing pathophysiology of IRIS in the near term.

Genetic profiles

An active area of current research focuses on the genetic variations associated with IRIS. Patients with CMV-related IRIS have been found to have increased frequency of HLA-B44 haplotypes [63]. Specific regulatory cytokine gene polymorphisms have been reported to be protective against herpes virus-associated IRIS (IL12B-3'UTR) and against mycobacterial-associated IRIS (TNFα-308*2 and IL6-174*G) [64]. These alleles play a role in decreasing cytokine production. IL-6, IL-12, and TNF-α polymorphisms have been implicated in other studies in development of mycobacterial-associated IRIS. In the future, we may be able to exploit these types of genetic markers to determine genetic susceptibility to IRIS prior to initiating anti-retroviral therapy.

Genomic Biomarkers of Gene Expression

Our research group has used microarray technology to investigate the genome-wide differences in gene expression among HIV-infected persons with and without IRIS events in two prospective cohorts: those with AIDS but without an active OI and those with recent cryptococcal meningitis when starting ART in Sub-Saharan Africa. Our preliminary data suggest that expression of numerous genes involved in immune activation and anti-viral responses can be used as diagnostic and predictive biomarkers of IRIS. Thus, this research direction shows great promise.

Although traditional epidemiologic and HIV parameters have had limited capability to predict IRIS on an individual level, biomarkers of immune activation and anti-viral responses may predict the development of IRIS before the clinical syndrome becomes evident. The early identification of patients who are at high risk of developing IRIS will allow better strategies for prevention and treatment of IRIS.

The discovery of biomarkers can be expensive, tedious and time consuming. There are however, newer technologies that will become widely available in the future. These include Luminex technology which can be used for cytokine profiling as well as microarrays used to profile gene expression at the mRNA level. The use of gene sequencing and newer technology to identify genomic polymorphisms on a genome-wide scale will also play a role in identifying specific genetic polymorphisms that may be useful to identify patients at risk for IRIS. These powerful methods will become increasingly available and utilized in the next 5– 10 years and will likely revolutionize the discovery of biomarkers.

Executive Summary

HIV Immune Reconstitution Inflammatory Syndrome (IRIS) is a common complication of antiretroviral therapy

- IRIS typically occurs in persons with advanced HIV/AIDS with CD4$^+$ counts <200 cells/µL.

- Incidence of IRIS is 10–15% among persons with AIDS in North America & Europe. IRIS is more frequent in resource-limited regions (20–25%).

- Persons with recent opportunistic infections (OIs) are a greater risk of IRIS. Rates of IRIS following tuberculosis (TB) or cryptococcal meningitis are near 33%.

Two major clinical scenarios of IRIS occur

- Paradoxical IRIS is the symptomatic relapse of a previously treated infection. Inflammation is driven by dysregulated, immunologic mechanisms despite microbiologic treatment success.

- Unmasking IRIS is the accelerated presentation of a new opportunistic infection which was occult or latent when starting HIV therapy. Enhanced screening may detect this OI prior to starting HIV therapy.

Pathophysiology of IRIS is poorly understood but hypothesized to be due to a dysregulated, pathologic immune response

- Conditions for IRIS require: antigen, restoration of antigen-specific immunity, dysregulated immune response which is hypothesized to be functional imbalance in pro-inflammatory (Th1, Th17) versus anti-inflammatory (T_{REG}) T-cell populations.

- Cytokine profiles of IRIS events are heterogeneous based on the inciting pathogen (e.g. viral, fungal, mycobacterial, etc) with marked individual variation.

- Pro-inflammatory cytokines are generated at time of mycobacterial and fungal IRIS events, such as IL-6, IL-17, IFN-γ, and TNF-α.

- C-reactive protein is frequently elevated at time of mycobacterial and fungal-associated IRIS events.

Genetic and genomic studies are ongoing to elucidate the pathophysiology of IRIS

- Genetic polymorphisms of IL-6, IL-12, and TNF-α have been implicated as possible factors in risk and protection from IRIS.

- Differences in gene expression exist prior to beginning HIV therapy which may predict the risk of subsequent IRIS. The genes differentially expressed involve immune activation and anti-viral responses.

- Ongoing prospective cohort studies are validating these biomarkers.

IRIS Research is rapidly developing with ongoing prospective cohort studies

- In the next 5–10 years, a much greater understanding of IRIS should develop.

- Ongoing international cohort studies are investigating the pathophysiology of IRIS through evaluation of: cell surface markers of peripheral blood mononuclear cells (PBMCs), cytokine profiles, genetic DNA polymorphisms, and RNA gene expression.

- Future prospective cohort studies and clinical trials will be needed to validate prevention and treatment strategies.

Abbreviations

ART, anti-retroviral therapy; CM, Cryptococcal meningitis; CMV, cytomegalovirus; IRIS, Immune reconstitution syndrome; OI, opportunistic infection; TB, Tuberculosis; T_{REG}, regulatory T-cells.

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IRIS Pathogenesis:

Interplay of Antigen Clearance, Immune Recovery of Antigen Specific-immunity, and Homeostatic T-regulatory Function
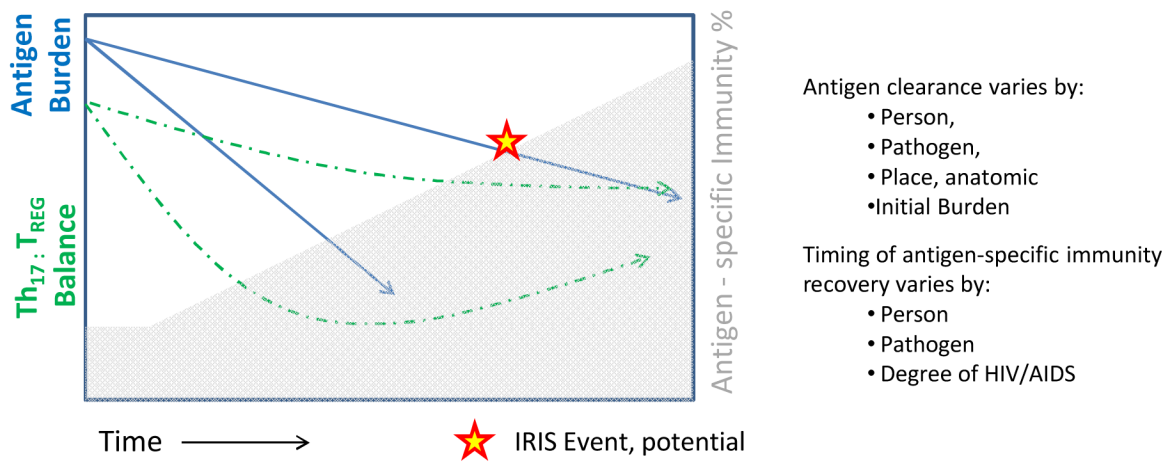

Figure 1.
The conditions for IRIS are a complex interplay between host and pathogen. The recovery of the host's immune system is critical to IRIS pathogenesis. With ART, persons gradually reconstitute their antigen-specific immunity to a pathogen (Shaded in Gray). Yet, the timing of reconstitution of T-cell subsets is variable. During reconstitution, the normal 2:1 balance between the pro-inflammatory (Th-17 subset) and the anti-inflammatory (T-regulatory subset) of the immune system (Dashed Line) may become unbalanced leading to the possibility of dysregulated responses.

Secondly, following an infection, antigen clearance is variable (Solid Line). Pathogens with rapid antigen clearance are less likely to generate an IRIS event, such as pneumococcal pneumonia. However, pathogens, such as TB and cryptococcus, in which antigens persist for months are at higher risk of developing IRIS (~33%). Overall, with HIV therapy, the immune system's reversal from an anergic state in the presence of abundant antigen and in the absence of normal homeostatic regulatory mechanisms create the potential for an IRIS event.
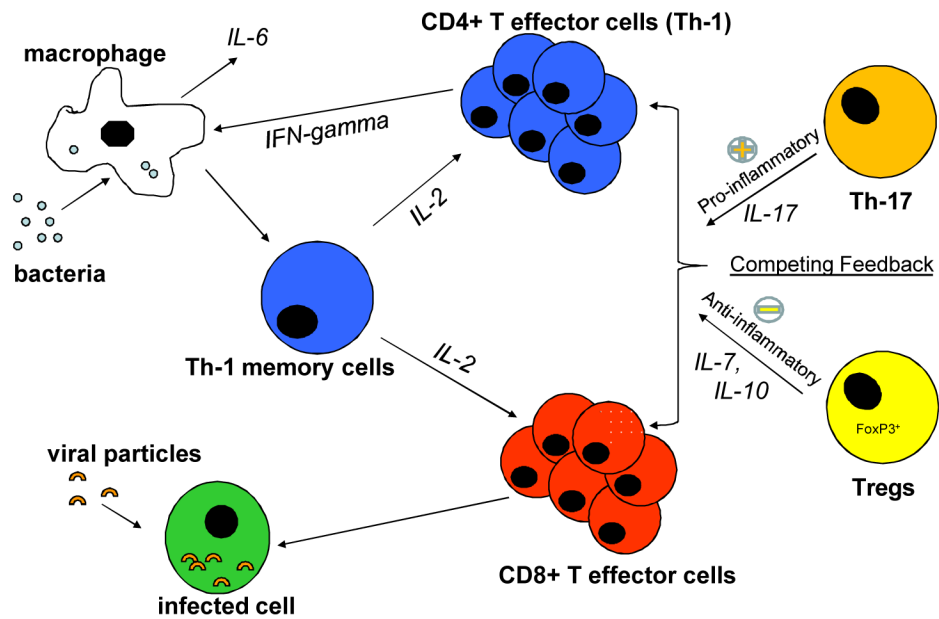
Figure 2.
Immune cells normally interact in a state of homeostasis mediated by T regulatory cells (T_{REGs}). Alteration in the balance between these components of the immune system and in their functionality may lead to IRIS. Adapted from Kestens et al.[26].

Table 1

Common Pathologic IRIS Scenarios

"Unmasking" IRIS
- Occult, subclinical opportunistic infection
- Unmasked by ART
- Infectious pathogens present

"Paradoxical" IRIS
- Clinical recrudescence of a successfully treated infection
- Symptomatic relapse despite microbiologic treatment success.
- Antigen driven immune activation
- Sterile cultures, typically [*]

[*] Cultures are often negative; however, this depends on the nature of the infection and the timing of the event. Though cultures are negative, histopathology stains, antigen, or DNA based testing is frequently positive.

Table 2

Frequent Clinical and Laboratory Features of IRIS by Pathogen

Condition	Clinical Features	Laboratory Features
CMV	blurred vision	Vitritis, uveitis
Cryptococcus	Aseptic meningitis, lymphadenitis, pneumonitis	Sterile cultures, decreased CRAG, elevated CSF pressure in ~75%.
Herpes viruses	exaggerated dermatologic lesions of HSV or VZV of various intensity	PCR positive
Hepatitis B	acute hepatitis	Increased LFTs.
Hepatitis C	rapid development of cirrhosis difficult to distinguish from hepatotoxicity	HBV/HCV viral loads typically decreased.
M. avium complex	lymphadenitis, draining sinuses Pulmonary-thoracic disease Abdominal lymphadenopathy	Granulomatous reaction, paucity of organisms, rare hypercalcemia Fever 66%, Prior diagnosis 25%
M. tuberculosis	lymphadenitis, pneumonitis	Paucity of AFB, PCR positive