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Role of IL-6 in Mycobacterium avium–Associated Immune Reconstitution Inflammatory Syndrome

Daniel L. Barber,* Bruno B. Andrade,† Cortez McBerry,* Irini Sereti,‡ and Alan Sher†

Immune reconstitution inflammatory syndrome (IRIS) is a major adverse event of antiretroviral therapy in HIV infection, and paradoxically occurs as HIV viremia is suppressed and CD4 T cell numbers recover. IRIS reflects pathogenic immune responses against opportunistic infections acquired during the period of immunodeficiency, but little is understood about the mechanisms of inflammatory pathology. In this study, we show that IL-6 and C-reactive protein levels transiently rise at the time of the IRIS event in HIV-infected patients, unmasking *Mycobacterium avium* complex infection after starting antiretroviral therapy. To directly test the role of IL-6 in IRIS pathology, we used a model of experimentally inducible IRIS in which *M. avium*–infected T cell–deficient mice undergo a fatal inflammatory disease after reconstitution with CD4 T cells. We find that IL-6 neutralization reduces C-reactive protein levels, alleviates wasting disease, and extends host survival during experimental IRIS. Moreover, we show that combined blockade of IL-6 and IFN-γ further reduces IRIS pathology, even after the onset of wasting disease. The combination of these clinical and experimental–model data show that the IL-6 pathway is not only a biomarker of mycobacterial IRIS but also a major mediator of pathology distinct from IFN-γ and may be a useful target for therapeutic intervention. The Journal of Immunology, 2014, 192: 676–682.
We have previously described a model of experimentally inducible *M. avium* IRIS in mice (23, 24). In the system used, T cell–deficient mice harboring a disseminated *M. avium* infection are injected with CD4 T cells to mimic the ART-induced recovery of Th cells in a mycobacterial coinfection, T cell–deficient AIDS patient. In this robust model of severe IRIS, adoptive transfer of CD4 T cells into chronically infected TCRαKO mice leads to a rapid, IFN-γ–dependent wasting disease, and most of the mice succumb 2–4 wk following T cell transfer. In this study, we perform both patient and animal model studies to examine the role of IL-6 in *M. avium*–associated IRIS. We show that IL-6 levels rise sharply in HIV/MAC–coinfected patients at the time of an unmasking IRIS event and use the experimental animal model to demonstrate that IL-6 blockade greatly reduces IRIS disease. Moreover, coblockade of IFN-γ and IL-6 in the experimental model further reduced IRIS associated disease and was effective even when therapy was initiated after the onset of wasting. These data strongly support the hypothesis that IL-6 is a major mediator of disease during IRIS in *M. avium*–coinfected HIV+ individuals.

### Materials and Methods

#### Human subjects and sample collection

Plasma samples were obtained from seven HIV+ patients (five male/two female; median age of 45 y; range, 27–50 y) with a median CD4 T cell count of 24 cells/μl (range, 1–86 cells/μl) recruited as part of a cohort study at the Clinical Center of the National Institutes of Health and who developed unmasking MAC IRIS after ART initiation. All participants provided written informed consent at the Clinical Center of the National Institute of Allergy and Infectious Diseases, National Institutes of Health, under an Institutional Review Board–approved protocol (NCT00286767). All patients were ART naive or had interrupted ART for >6 mo after receiving <5 mo of therapy (n = 1) and had less than or equal to 100 CD4+ T cells/μl at baseline. IRIS was defined according to the AIDS Clinical Trials Group criteria. The median time to unmasking MAC IRIS was 19 d (range, 7–99 d).

#### Mice, bone marrow chimeras, and *M. avium* infections

C57BL/6 and athymic nude mice were obtained from Taconic Farms, and IL−/− mice were purchased from The Jackson Laboratory. Ag85b–specific TCR transgenic (Tg) P25 mice (25) were bred in our animal facility. To generate bone marrow (BM) chimeric mice, animals were lethally irradiated with 950 rad and reconstituted with 8–10 × 10^7 BM cells isolated from wild-type (WT) or IL−/− mice the same day after irradiation. Thy1.2-depleting Ab (500 μg) was added to the BM cell inoculum to ensure depletion of contaminating mature CD4 T cells. Mice were allowed to reconstitute for 10–12 wk before they were exposed to *M. avium*. For bacterial infections, the mice were inoculated i.v. in the lateral tail vein with 1 × 10^9 CFU *M. avium* SmT 2151. All Kaplan–Meier graphs of mouse survival represent mice that were found dead or reached moribundity and were euthanized. All animals were bred and maintained in an American Association for the Accreditation of Laboratory Animal Care–approved facility at the National Institute of Allergy and Infectious Diseases, National Institutes of Health, according to the Guide for Care and Use of Laboratory Animals, and all procedures were conducted according to an animal study proposal that was approved by the National Institute of Allergy and Infectious Diseases Animal Care and Use Committee.

#### CD4 T cell adoptive transfer and in vivo Ab treatments

Spleen and lymph node CD4 T cells were isolated from naive WT, IL−/−, or P25 TCR Tg mice as indicated using MACS magnetic beads, according to the manufacturer’s instructions (Miltenyi Biotec). Cells were routinely >90% pure CD4 cells after column separation. For adoptive transfer, cells were suspended in saline, and 2 × 10^7 T cells were injected i.v. in a volume of 300 μl. Control Ab (clone HRPN), anti–IL-6 (clone MP5-20F3), anti–IL-6R (clone 15A7), anti–IFN-γ (clone XMG1.2), and anti-Thy1.2 (clone 30H12) mAbs were purchased from BioXcell. For in vivo blockade experiments, 200–500 μg Ab was administered every third day for the indicated amount of time. No difference was observed between mice given PBS or control Ab.

#### Flow cytometry

CD4 T cell frequencies in blood were determined by flow cytometry. Blood was collected in EDTA tubes, and RBCs were lysed with ACK lysing buffer. In different experiments, cells were stained with various combinations of CD4, TCRβ, CD3e, Vβ11, and fixable viability dye eFlour780. All Abs and live/dead dyes were purchased from eBioscience and BioLegend. All samples were acquired on a BD LSR Fortessa and analyzed with FlowJo version 9.5.

### Human and mouse cytokine measurements

Cytokine measurements were performed on cryopreserved human plasma samples or mouse serum samples stored at −80°C. Mouse CRP, mouse IL-6, mouse TNF-α, and human IL-6 was measured in with ELISA kits purchased from R&D Systems. Human CRP and mouse IFN-γ was measured with a high-sensitivity ELISA purchased from eBioscience.

#### Weight loss and core body temperature measurements

Weight loss was represented as a percentage of initial weight before T cell transfer as follows: percentage of weight change = ([current weight/preweight] × 100) − 100. Core body temperature was measured by rectal thermometry on nonanesthetized mice with a murine probe thinly coated with a petroleum-based lubricant.

#### Statistical analysis

The statistical differences between groups were analyzed by Student t test, and the difference between survival curves was calculated with a log-rank Mantel–Cox test in GraphPad Prism software version 6. Groups were considered significantly different when p ≤ 0.05.

### Results

#### IL-6 and CRP increase during unmasking IRIS in HIV/MAC–coinfected individuals

Increases in the plasma concentrations of IL-6 and CRP after ART are frequently found to distinguish HIV patients who develop IRIS from those who do not. To characterize the dynamics of IL-6 and CRP expression during IRIS, we performed a detailed kinetic analysis of these proteins in a cohort of seven HIV-infected individuals who developed unmasking MAC/IRIS. The patients developed IRIS at time points ranging from 2 to 10 wk following ART. In patients 1, 2, 3, 5, and 6, CRP was at or close to normal concentrations (<3 mg/l) before ART and spiked at the time of the IRIS event (Fig. 1A). This peak in CRP was then followed by a rapid decline afterward. In patient 4, the plasma CRP concentration was high even before ART but increased further at the time of the IRIS episode.

We next measured IL-6 in these patient samples. In patient 1, the concentration of plasma IL-6 was within the range found in healthy donors but then rapidly increased at the time of the IRIS event at week 2 and then dropped back to normal by week 4 (Fig. 1B). Moreover, in patients 3, 4, 5, 6, and 7, the IL-6 concentrations increased between the time point immediately preceding and the IRIS event. Interestingly, in patients 3, 4, 5, and 7, the concentration of IL-6 initially dropped in the first weeks following ART but then rose sharply during IRIS. In patient 2, the concentration of IL-6 was high before ART and remained elevated until after the patient developed IRIS at week 2. These data indicate that CRP and IL-6 increase in HIV-infected individuals at the time of unmasking MAC/IRIS.

#### IL-6 increases and drives CRP expression during experimentally induced *M. avium* IRIS

To model IRIS in mice, we developed a system that recapitulates the immunological scenario underlying IRIS in HIV-infected individuals: T cell reconstitution of a lymphopenic host harboring a microbial infection. TCRαKO mice are infected with *M. avium* and, after at least 60 d of infection, the mice are injected with purified CD4 T cells (Fig. 2A). Although CD4 T cells are normally critical for control of mycobacterial infections, in this setting of immune reconstitution, CD4 T cell transfer triggers a rapid wasting disease that results in the death of most of the animals (Fig. 3) (24). We found that CD4 T cell transfer into *M. avium*–infected TCRαKO mice induces increases in the serum concentration of IL-6 (Fig. 2B). IFN-γ production by the CD4 T cells was not required for IL-6 increase.

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independent experiments from day 11 to 12 post-CD4 T cell transfer. CRP and IFN-γ concentrations were measured before and at several time points following ART. Arrowheads represent the time points when the IRIS event occurred. The dotted line in (A) represents a normal CRP value for reference. The horizontal light gray shaded region and dotted line in (B) represents the range and the geometric mean of IL-6 values in healthy donors. The dark gray bars in patient 6 and 7 represents the time points were prednisone was administered to control inflammatory disease.

induction because transfer of IFN-γ knockout (KO) CD4 T cells induced similar amounts of IL-6 compared with WT T cells (Fig. 2B). Serum CRP was elevated in *M. avium*–infected TCRαKO mice compared with naïve animals, and during IRIS following adoptive transfer of CD4 T cells, CRP increased further (Fig. 2C). CRP expression can be induced by IL-6, and CRP and IL-6 concentrations positively correlated in the serum (Fig. 2D). Therefore, we next examined the role of IL-6 in inducing CRP expression during IRIS. IL-6 neutralization with mAbs decreased serum IL-6 concentrations back down to the concentrations seen in uninfected mice (Fig. 2C). IFN-γ-deficient CD4 T cells also failed to drive increases in CRP above what is found in TCRαKO mice that did not receive T cells (Fig. 2C). Collectively, these data indicate that IL-6 and CRP increase during experimentally induced *M. avium*–IRIS. Furthermore, these commonly observed biomarkers of IRIS are directly linked as elevated CRP expression requires IL-6 and IFN-γ.

**IL-6 blockade prevents wasting disease and prolongs survival of experimentally induced *M. avium*–IRIS**

Given the association of IL-6 with disease during *M. avium*–IRIS in both humans and mice, we sought to directly evaluate the role of IL-6 in the pathogenesis of IRIS. *M. avium*–infected TCRαKO mice were reconstituted with purified CD4 T cells and treated with anti–IL-6-neutralizing mAb starting on the day of T cell transfer. Mice that received CD4 T cells alone developed a severe wasting disease starting around day 7 posttransfer and lost >30% of their body weight by day 12 (Fig. 3A). In contrast, mice that received CD4 T cells and anti–IL-6 first began to show signs of weight loss around day 10 posttransfer, and by day 20 when blockade was stopped, the mice had lost ∼20% of their body weight (Fig. 3A). CD4 T cell transfer also induced a dramatic drop in core body temperature. TCRαKO mice harboring a chronic *M. avium* infection were ∼35°C, and on day 12 posttransfer of CD4 T cells, untreated control mice had dropped to ∼32°C, whereas mice receiving CD4 T cells and anti–IL-6 had dropped to ∼34°C (Fig. 3B). Moreover, IL-6 blockade significantly extended the survival of mice following CD4 T cell transfer (Fig. 3C). The protective effect of IL-6 blockade was not due to differences in the degree of the expansion of the donor CD4 T cells as untreated, and anti–IL-6–treated mice had similar percentages of differences in the degree of the expansion of the donor CD4 T cells (Fig. 3C). TNF blockade also had no effect on IL-6 induction (data not shown). Taken together, these data indicate that IL-6 production not only correlates with IRIS in humans and mice but also directly contributes to the pathology seen in *M. avium*–infected T cell–deficient mice following CD4 T cell reconstitution. Interestingly, the protective effect of IL-6 blockade in experimentally induced IRIS is even greater than what we previously reported with TNF neutralization.

**Radio-resistant cells are the major source of pathogenic IL-6**

Cells of both hematopoietic and non-hematopoietic origin can produce IL-6, so we next examined the cell types involved in...
mediating IL-6–dependent disease in experimentally induced IRIS. To test the role of CD4 T cell–derived IL-6 in IRIS pathogenesis, we transferred either WT or IL-6 KO CD4 T cells into \textit{M. avium}–infected TCR\textsuperscript{a} KO mice. WT and IL-6 KO CD4 T cells induced identical courses of wasting diseases, indicating that CD4 T cells are not a significant source of IL-6 in murine IRIS (Fig. 4A).

We next tested the role of hematopoietic cell–derived IL-6 in IRIS. Athymic nude mice were lethally irradiated and then reconstituted with BM from either WT or IL-6 KO mice (Fig. 4A). Thy1.2-depleting mAb was added to the BM cell inoculum to ensure that any mature CD4 T cells contaminating the BM donor cells were removed. Ten weeks after BM reconstitution, the mice were injected with \textit{M. avium}, and the infection was allowed to progress for an additional 2 mo. Although the injected BM was inherently capable of producing T cells, the use of athymic nude mice recipients prevented the development of T cells allowing us to later study the impact of CD4 T cell reconstitution. This approach yielded T cell–deficient mice that harbor a chronic \textit{M. avium} infection and selectively lack IL-6 in BM-derived cells. Last, TCR Tg CD4 T cells specific for Ag85b (P25 cells) were adoptively transferred to induce IRIS. Interestingly, CD4 T cell reconstitution induced identical wasting disease in both WT and IL-6 KO BM recipient nude mice (Fig. 4C). Although there was a slight trend toward a 2- to 3-d delay in mortality of the IL-6 KO BM recipients, the difference in the survival of WT or IL-6 KO BM recipients during IRIS did not reach statistical significance, and all mice eventually succumbed by day 31 posttransfer (Fig. 4D). Moreover, serum IL-6 concentrations were similar between WT and IL-6 KO BM–recipient mice undergoing IRIS (data not shown). These data indicate that radio-resistant cells are the predominant source of IL-6 that drives wasting disease and death during experimentally induced IRIS in \textit{M. avium}–infected T cell–deficient mice.

\textbf{FIGURE 3.} IL-6 drives experimentally induced \textit{M. avium}–IRIS. TCR\textsuperscript{a} KO mice were intravenously injected with $1 \times 10^6$ CFU \textit{M. avium}. After at least 2 mo postinfection, $2 \times 10^6$ WT CD4 T cells were adoptively transferred i.v., and where indicated, mice were then either untreated or administered IL-6 neutralizing mAb. (A) Weight loss was normalized to the day of T cell transfer. The shaded region indicates the duration of IL-6 blockade. (B) Core body temperature was measured on day 12 after T cell transfer. Uninfected TCR\textsuperscript{a} KO mice displayed a body temperature of 37–37.5°C. (C) Mortality was monitored following CD4 T cell reconstitution. The shaded region indicates the duration of IL-6 blockade. Data are representative of six independent experiments. (D) The frequency of donor CD4 T cells in the blood was measured on day 10 posttransfer. The data are representative of two independent experiments. (E) TNF serum concentrations were measured on day 11–12 posttransfer of CD4 T cells. The data are pooled from four independent experiments.

\textbf{FIGURE 4.} Radio-resistant cells mediate the IL-6 driven pathology of IRIS after reconstitution of \textit{M. avium}–infected T cell–deficient mice. (A) WT or IL-6\textsuperscript{−/−} CD4 T cells were adoptively transferred into TCR\textsuperscript{a} KO mice harboring a chronic \textit{M. avium} infection as described in Fig. 2A, and weight loss was measured. (B) To study IRIS in \textit{M. avium}–infected T cell–deficient mice selectively lacking IL-6 from hematopoietic cells, athymic nude mice were lethally irradiated and constituted with BM from WT or IL-6 KO mice (Fig. 4A). Thy1.2-depleting mAb was added to the BM cell inoculum to ensure that any mature CD4 T cells contaminating the BM donor cells were removed. Ten weeks after BM reconstitution, the mice were injected with \textit{M. avium}, and the infection was allowed to progress for an additional 2 mo. Although the injected BM was inherently capable of producing T cells, the use of athymic nude mice recipients prevented the development of T cells allowing us to later study the impact of CD4 T cell reconstitution. This approach yielded T cell–deficient mice that harbor a chronic \textit{M. avium} infection and selectively lack IL-6 in BM-derived cells. Last, TCR Tg CD4 T cells specific for Ag85b (P25 cells) were adoptively transferred to induce IRIS. Interestingly, CD4 T cell reconstitution induced identical wasting disease in both WT and IL-6 KO BM recipient nude mice (Fig. 4C). Although there was a slight trend toward a 2- to 3-d delay in mortality of the IL-6 KO BM recipients, the difference in the survival of WT or IL-6 KO BM recipients during IRIS did not reach statistical significance, and all mice eventually succumbed by day 31 posttransfer (Fig. 4D). Moreover, serum IL-6 concentrations were similar between WT and IL-6 KO BM–recipient mice undergoing IRIS (data not shown). These data indicate that radio-resistant cells are the predominant source of IL-6 that drives wasting disease and death during experimentally induced IRIS in \textit{M. avium}–infected T cell–deficient mice.
Prophylactic and therapeutic coblockade of IL-6 and IFNγ greatly alleviates IRIS pathology

We previously showed that IFN-γ produced by the reconstituting CD4 T cells is a major mediator of disease in the murine model of M. avium–IRIS (23), so we next asked whether IL-6 blockade affects the production of IFNγ in this system. M. avium–infected TCRαKO mice were injected with CD4 T cells and treated with anti–IL-6 blocking mAb, and IFN-γ was measured in the serum. CD4 T cell reconstitution induced elevations in serum concentrations of IFN-γ equally in mice treated or not with IL-6 blockade (Fig. 5A). Thus, IL-6 and IFN-γ both contribute to IRIS pathology but do not affect the expression of each other in this system.

These observations indicated that IL-6 and IFN-γ are independent mediators of wasting and mortality during M. avium–IRIS, so we next tested the therapeutic efficacy of combined IL-6 and IFN-γ blockade. M. avium–infected TCRαKO mice were injected with CD4 T cells and treated with anti–IL-6/anti–IL-6R, anti–IFN-γ or a mixture of anti–IL-6/anti–IL-6R and anti–IFN-γ starting on the day of T cell reconstitution, and weight loss was measured on day 14 posttransfer. As expected, individual blockade of each pathway with anti–IL-6/anti–IL-6R, or anti–IFN-γ alleviated the extent of wasting (Fig. 5B), but IL-6 and IFN-γ were administered simultaneously at the beginning of T cell transfer, only a slight decrease in weight loss was detected on day 14 post-transfer (Fig. 5B) compared with mice that did not receive T cells, indicating that IL-6 and IFN-γ represent two major mediators of IRIS pathology. Moreover, coblockade of IL-6 and IFN-γ had no effect on serum concentrations of TNF, indicating that the protective effects of this treatment is not likely to be due to suppression of TNF (data not shown).

Finally, we tested the ability of combined IL-6 and IFN-γ blockade to treat IRIS after the onset of wasting disease. To do so, IRIS was induced as before and T cell recipients were treated with a mixture of anti–IL-6/anti–IL-6R and anti–IFN-γ mAbs from day 0 to 16 or from day 10 to 16. Survival after withdrawal of the blocking Abs was monitored. Untreated CD4 T cell recipients showed a median survival time (MST) of 15 d, whereas combined cytokine blockade administered day 0–16 or day 10–16 of T cell transfer extended the MST to 43 d (Fig. 5C). Importantly, mice that began cytokine blockade on day 10 had already undergone ~20% weight loss (data not shown) but still showed an increased MST of 38 d (Fig. 5C). These data indicate that even after the onset of a rapidly progressing wasting disease, blockade of these two effector pathways can dramatically extend survival of this severe M. avium–associated IRIS.

Discussion

Many studies of HIV patients have sought to find correlations between inflammatory mediators and susceptibility to IRIS. Although several pathways have been associated with mycobacterial IRIS, the lack of a preclinical model has made it difficult to ascribe a role of these molecules to the immunopathology of IRIS. This is the first study, to our knowledge to combine human data and a preclinical model to evaluate the potential therapeutic efficacy of targeting an inflammatory mediator for the treatment of IRIS. We show that elevations in serum IL-6 concentrations are associated with disease onset in HIV/MAC-coinfected individuals who develop unmasking IRIS following ART and use the mouse model of experimentally induced M. avium–IRIS to directly test the role of IL-6 in driving IRIS pathology. In fact, the system of experimentally induced IRIS used in this study can even be considered a form of unmasking IRIS similar to the patients described, because the mice are not treated with antimycobacterial drugs and display no overt symptoms resulting from the M. avium infection until after CD4 T cell reconstitution. We find that IL-6 not only correlates with disease but also is a major mediator of IRIS pathology in M. avium–infected T cell–deficient mice. Moreover, we find that IL-6 also likely contributes to the increased levels of the acute-phase reactant CRP, which are so often observed in IRIS patients, indicating that these two biomarkers of IRIS are likely indicative of the same pathway. Although the studies performed here were limited to M. avium–associated IRIS, the observation of increased IL-6 and CRP in IRIS associated with M. tuberculosis and Cryptococcus neoformans infections suggests that the mechanisms delineated here may be of general relevance to multiple manifestations of the syndrome.

Numerous studies have examined many different inflammatory pathways in HIV/tuberculosis-coinfected individuals during ART, and we have experimentally addressed the role of several key cytokines that have been associated with IRIS susceptibility. We showed that IFN-γ and to a lesser extent TNF drive IRIS pathology in M. avium–infected mice, whereas IL-4 and IL-17 have no role (23). Moreover, we have found that IL-7R, IL-1R1, GM-
CSF, M-CSF, and G-CSF blockades have no therapeutic effect in this murine model of IRIS, indicating that these pathways may play little role in the induction of mycobacterial immune reconstitution disease (data not shown). Therefore, although cytokine biomarkers may be found to associate with IRIS, it is critical to functionally examine the role of each molecule in experimental models. Moreover, the accumulating negative data on other inflammatory pathways we are observing emphasizes the major role of IL-6 in IRIS pathology.

Given that IL-6 and IFN-γ represent two major pathways that drive IRIS, we also examined the interplay between these two cytokines. In this study, we found that single neutralization of IL-6 or IFN-γ does not impact the expression of the other, and simultaneous blockade of both cytokines synergistically alleviates wasting disease. Therefore, IL-6 and IFN-γ represent two major and independent pathways of IRIS pathology. Indeed, the pathogenic sources of these two cytokines are different cell types. During experimentally induced IRIS the pathogenic IFN-γ is derived from CD4 T cells. It is possible that many cell types produce IL-6 during IRIS, but our nude mouse BM chimera experiments show that the pathogenic IL-6, which contributes to IRIS, is largely produced by radio-resistant cells. Although we cannot exclude the possibility that IL-6 is derived from a long-lived myeloid cell population, our T cell transfers were performed 18–20 wk following BM reconstitution of the nude mice, so it is more likely that nonhematopoietic cell types are key producers of the pathogenic IL-6 in experimentally induced IRIS. Indeed, multiple nonhematopoietic cell types such as adipocytes, myocytes, fibroblasts, and endothelial cells have all been shown to produce IL-6 in response to a variety of stimuli. Because CD4 T cell transfer induces IL-6 expression, this raises the possibility that the communication between the repopulating CD4 T cells and nonhematopoietic tissues may be a key factor driving the systemic disease during mycobacterial IRIS. Further experiments will be required, however, to determine the relevant nonhematopoietic sources of IL-6 and the mechanisms through which CD4 T cells drive its expression. Likewise, the cell types that are targeted by IL-6 and the downstream activities of IL-6R signaling that lead to pathology will require further investigation.

Although the data indicate that IL-6 is a key determinant of IRIS pathology associated with M. avium infection, the clinical feasibility of blocking IL-6 to treat IRIS HIV patients undergoing ART is not clear. It is possible that IL-6 blockade could exacerbate pathogen replication in HIV patients harboring MAC coinfections, because IL-6 blocking Abs have been shown to impair control of M. avium infections in mice (26, 27). There are, however, indications that IL-6 blockade can be beneficial for the treatment of other inflammatory disorders. For example, the mAb against the IL-6 receptor tocilizumab is prescribed for the treatment of rheumatoid arthritis (28). Our data indicate that IL-6 is a major mediator of the cachexia associated with M. avium–associated IRIS in mice, and IL-6 also has been shown to play a major role in cancer cachexia (29). In a recent case report, a patient suffering from cachexia with an IL-6–overexpressing lung cancer was treated with tocilizumab (30). The elevated CRP in the patient quickly returned to normal concentrations and the patient’s weight loss and other symptoms were greatly alleviated. Cachexia is also frequently observed in HIV patients experiencing mycobacterial IRIS during ART, and it is likely that treatments to prevent wasting would greatly reduce morbidity and mortality in individuals that experience a severe episode of IRIS.

In conclusion, we have shown that CRP/IL-6 elevations occur both in HIV+ persons who develop unmasking MAC-IRIS after ART initiation and in M. avium–infected TCRα KO mice after CD4 T cell transfer. In the mouse model we showed that both CD4 T cell–derived IFN-γ and IL-6 produced by radio-resistant cells have independent pathogenic roles and that blocking both cytokines was synergistically beneficial. These data suggest that the IL-6 pathway may be a reasonable target in therapeutic interventions in HIV+ patients with mycobacterial IRIS.

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**Disclosures**

The authors have no financial conflicts of interest.

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