HOT TOPICS

Protective and detrimental roles of IL-10 in HIV pathogenesis

Douglas S. Kwon[1,2,3], Daniel E. Kaufmann[1,3]

[1] Ragon Institute of MGH, MIT and Harvard, Massachusetts General Hospital, Charlestown, Massachusetts, USA
[2] Division of Infectious Diseases, Brigham and Women's Hospital, Boston, Massachusetts, USA
[3] Division of Infectious Diseases, Massachusetts General Hospital, Boston, Massachusetts, USA

Correspondence: D.E. Kaufmann, MD, Massachusetts General Hospital East, Ragon Institute of MGH, MIT and Harvard, Room 5239, 149 13th Street, Charlestown, MA 02129, USA
<dkaufmann@partners.org>

Accepted for publication June 24, 2010

ABSTRACT. Successful pathogen clearance depends on a finely orchestrated equilibrium between inflammatory immune responses and immunoregulatory mechanisms that limit collateral tissue damage. The cytokine interleukin 10 (IL-10) has been shown to play a critical role in this balance in numerous infectious diseases. Studies in animal models have revealed that IL-10 gene-knockout or signaling blockade can enhance resistance to pathogens, and substantially facilitate viral clearance. These same interventions in other infections however, result in more severe disease due to the inability of the immune system to adequately contain the pathogen load, and to control immune-mediate damage. This IL-10-regulated balance is also apparent in human infectious diseases. This review summarizes evidence that IL-10 impacts many aspects of HIV pathogenesis, including the regulation of HIV-specific CD4 and CD8 T cell functions, as well as modulation of HIV-replication in PBMC subsets. Genetic polymorphisms in the IL-10 gene promoter that lead to decreased IL-10 expression have been associated with more rapid disease progression in late stages of HIV infection, suggesting that the anti-inflammatory effects of IL-10 may be protective in the setting of chronic immune activation. We conclude with a discussion of important questions remaining, and the potential for therapeutic intervention based on manipulation of the IL-10 pathway.

Keywords: interleukin-10 (IL-10), antigen-presenting cell, CD4 T cell, CD8 T cell, regulatory T cell, HIV, T cell exhaustion, immune activation

In the setting of chronic infections, the immune system must adapt to the ongoing presence of inflammatory responses driven by the persisting pathogen in order to control its replication at an acceptable level while limiting immune-mediated damage (immunopathology) (reviewed in 1). The majority of humans are chronically infected by several viruses, *e.g*. from the *Herpesviridae* group, with no apparent clinical manifestations [1]. In contrast, in HIV infection, the lack of pathogen clearance leads to continuous viral replication and disease progression in the large majority of infected individuals, with the rare exception of subjects who control the virus in the absence of therapy (HIV controllers or long-term non-progressors [2]). Numerous studies have shown that pathogens apply diverse strategies to avoid immune control, although the critical mechanisms determining pathogen persistence versus clearance, after the acute phase of infection, remain largely unknown (reviewed in [1, 3]). One important factor leading to impaired viral control in the setting of chronic viral infections is T cell exhaustion, defined as the progressive functional impairment of pathogen-specific, T cell responses upon persistent exposure to antigen [4]. Studies of gene-expression profiles of exhausted cytotoxic T cells (CTL) in murine models [5] and humans [6], suggest that T cell exhaustion is due to both active suppression and passive defects in metabolism and cell signaling. Consistent with this model, recent studies have highlighted that HIV successfully evades immunity by exploiting inhibitory regulatory networks that play an important role in maintaining peripheral tolerance to self-antigen and avoiding excessive immune activation under physiological conditions (reviewed in [7]).

In this article, we review studies that examine the involvement of the major immunoregulatory cytokine interleukin-10 (IL-10) in HIV-specific T cell dysfunction and disease progression. We also discuss important, unanswered questions, and the potential for therapeutic intervention based on manipulation of this inhibitory pathway, including the specific therapeutic challenges relating to maintaining the balance between successful antiviral immunity and collateral immunopathology during HIV infection.

THE IL-10 PATHWAY AND ITS ROLE IN INFECTIOUS DISEASES

IL-10 is a type II cytokine and the prototypic member of a family of cytokines that includes IL-19, IL-20, IL-22, IL-24, IL-26, IL-28 and IL-29 (reviewed in [8]). IL-10 is

doi: 10.1684/ecn.2010.0201

primarily an anti-inflammatory cytokine, with critical functions in preventing inflammatory and autoimmune diseases; it has emerged as a key immunoregulator during infection with viruses, bacteria, fungi, protozoa, and helminthes (reviewed in [9-11]). Inflammatory bowel disease and other excessive inflammatory responses occurring in IL-$10^{-/-}$ mice indicate that IL-10 is critically involved in limiting deleterious inflammatory responses *in vivo* [12, 13].

IL-10 has a broad range of cellular sources and targets (reviewed in [9, 14]). Multiple cell types of the adaptive immune system can produce IL-10: different CD4 T cell subsets including TH1, TH2 and TH17; both traditional, "natural" CD25+FoxP3+CD4+ Tregs cells and "inducible" Fox-P3-Tr1 Tregs; CD8 T cells and B cells. Additionally, IL-10 is expressed by various cell populations of the innate immune system, including dendritic cells (DCs), monocytes/macrophages, natural killer cells (NKs), mast cells, and polymorphonuclear leukocytes. The IL-10 receptor is widely expressed on most hematopoietic cells and is composed of an α (or R1) subunit specific to IL-10, and a β (or R2) subunit in common with several other cytokine receptors including receptors for IL-22, IL-26, IL-28 and IL-29. Thus, it is likely that different cellular subsets are involved in the production and activity of IL-10, and the role of each may vary significantly, at different stages of an immune response, between tissues compartments, and amongst different pathological conditions. The ability of IL-10 to inhibit cytokine production by T cells and NK cells is thought to be largely indirect, by alteration of monocyte/macrophage functions [15]. IL-10 decreases MHC Class II and CD80/CD86 expression on monocytes and macrophages, and limits production of proinflammatory cytokines, including TNF-α and INF-γ. However, IL-10 can also stimulate the proliferation of B cells and enhance their maturation into plasma cells [16], and may enhance the cytotoxicity of CD8 T cells. Thus, IL-10 can have complex, pleiotropic effects on the immune system, involving multiple cell types and immune compartments (reviewed in [14]).

The impact of IL-10 on disease outcome in the setting of infection is complex and depends on the pathogen and model studied. IL-10 gene-knockout or signaling blockade can enhance resistance to infection by *Listeria monocytogenes* [17], *Mycobacterium avium* [18] and *Trypanozoma cruzi* [19], and IL-10 plays a key role in facilitating the establishment of chronic *Leishmania* major infection [20]. However, the absence of IL-10 signaling results in severe immunopathology in *Toxoplasma gondii* [21] and malaria models [22]. Recent studies in murine LCMV infection [23, 24] have yielded important results on the role of IL-10 in the establishment of chronic viral infection. Treating mice with an IL-10Rα-blocking antibody shortly after acute infection, or infecting IL-$10^{-/-}$ mice, results in enhancement of T cell responses and rapid viral clearance of an LCMV strain that is usually associated with viral persistence [23, 24]. Therefore, IL-10 appears to be a critical modulator of early antiviral responses and, at least in some settings, is a critical determinant for resolving *versus* persisting infection. These findings in murine models suggest that manipulation of the IL-10 pathway might be clinically useful in the treatment of chronic infections in humans. Elevated IL-10 levels have been observed for a number of chronic infectious diseases in humans, including visceral leishmaniasis [25], leprosy [26] and tuberculosis [27]. The observation that the Herpes viruses EBV [28] and CMV [29] encode for IL-10 analogues, suggest that IL-10 can facilitate viral persistence in humans. IL-10 gene promoter polymorphisms, associated with increased IL-10 production, are associated with increased susceptibility to chronic HCV infection [30, 31]. Based upon these data, in particular the results in the LCMV model [23, 24] and studies of antitumoral immunity, interventional studies to block IL-10/IL-10Rα have been proposed as a strategy to enhance underlying immunity in chronic viral infections and cancer, as well as a potential adjuvant in vaccination regimens [32] (reviewed in [11]).

UPREGULATION OF THE IL-10 PATHWAY IN HIV INFECTION

The early description of defective proliferative T cell responses to recall antigens such as tetanus toxoid in HIV-infected individuals [33], led investigators to hypothesize that factors inhibiting immune responses may be expressed at higher levels in HIV infection and contribute to immune deficiency. Gene Shearer's group tested the hypothesis that this cytokine plays a role in HIV-associated immune dysfunction [34], and showed that expression of IL-10 was increased in PHA-stimulated PBMC from HIV-infected persons as compared to HIV-negative controls, and that these elevated IL-10 levels correlated with a more advanced disease stage [35], a lower capacity to produce IL-2, and the frequent presence of syncytium-inducing virus [35, 36]. Other studies have shown that plasma IL-10 levels increased with disease progression and decreased following antiviral therapy [37]. Our recent study [38] further clarified the categories of subjects in whom the IL-10 pathway is upregulated, and also determined the cellular sources of IL-10 in peripheral blood. Spontaneous IL-10 expression, measured as either plasma IL-10 protein or IL-10 mRNA in PBMC, correlated positively with viral load, and diminished following successful antiretroviral therapy. IL-10 levels in "elite controllers" were similar to those in HIV-negative controls, and were significantly lower than in viremic individuals.

These data raise the question of the source of IL-10 in viremic subjects, given the wide range of cell populations capable of producing this cytokine. In the murine LCMV model, studies by Brooks *et al.* [23] and Erjnaes *et al.* [24] reached different conclusions, either favoring the hypothesis that DC from chronically infected mice induce IL-10 upregulation by CD4 T cells, or that regulatory T cells are the major source of IL-10 during chronic LCMV infection, respectively. Trabattoni *et al.* [39] showed that monocytes are a major source of IL-10 in HIV-infected persons and that they produce this cytokine in significantly higher amounts than in HIV-negative controls. This is consistent with our own assessment of IL-10 production by intracellular cytokine-staining of cells obtained directly *ex vivo* from HIV infected

subjects, which show that CD14+ monocytes appear to be the primary source of IL-10 in untreated infection (unpublished data). However, IL-10 production by other cell subsets has been poorly characterized. Our published results provided a detailed assessment of IL-10 mRNA expression in several, highly purified cellular subsets obtained by cell sorting [38]. Consistent with previous studies [39], our assays suggested that CD14+ monocytes are a major source of IL-10 in both HIV-infected and HIV-uninfected individuals. However, analysis also indicated upregulation of IL-10 mRNA in multiple cell populations, and that CD14+ monocytes showed a less dramatic upregulation of IL-10 mRNA in HIV-infected subjects compared to other cellular subsets, including CD19+ B cells, T cells and NK cells. These data indicate that multiple cell types potentially contribute to IL-10-mediated immune suppression in the presence of uncontrolled HIV viremia.

IL-10 AND CD4 T CELL RESPONSES IN HIV INFECTION

In order to investigate whether the observed increase in IL-10 expression in HIV infection had a functional impact on T helper cell dysfunction, a hallmark of HIV infection, Clerici *et al.* [34] measured T cell proliferation in response to "flu" antigens and HIV Env peptides in the presence or absence of IL-10-blocking antibody. These experiments demonstrated an increase in the proliferative responses in HIV-infected individuals upon blockade of the IL-10 pathway. Further studies showed that IL-10 blockade could restore Env-specific T cell proliferative responses in subjects with relatively preserved CD4 T cell counts, but was lost in individuals with advanced HIV infection [36]. These interesting functional results led to few follow-up studies until recent papers involving the LCMV model, showing the importance of the IL-10 pathway in controlling viral persistence, regenerated interest in the role of IL-10 in viral infections [23, 24]. Our data [38] demonstrated that blockade of the IL-10 pathway with an anti-IL-10Rα antibody significantly increased, not only HIV-specific CD4 T cell proliferation, but also effector functions, as shown by increased secretion of the cytokines IFN-γ and IL-2 by HIV-specific CD4 T cells. It is notable that enhancement of HIV-specific T cell proliferative responses upon IL-10Rα blockade occurred only in individuals with uncontrolled viral replication, and did not occur in 'elite controllers' or individuals with suppressed viral replication with successful antiretroviral therapy (ART). Significant positive correlations were observed between the fold-increase in HIV p24-specific proliferation following IL-10Rα blockade and plasma viral load or IL-10 mRNA. The variable enhancement of T cell responses following IL-10Rα blockade observed in individuals with similar HIV viremia is consistent with a significant heterogeneity in the degree of T cell exhaustion and activity of different inhibitory pathways among HIV-infected subjects, as previously observed for other inhibitory networks acting on HIV-specific CD4 T cells [40, 41]. Additionally, some data suggest that IL-10 differentially modulates apoptosis of the CD4 and CD8 T cell subsets. Studies by Clerici *et al.* [42] showed that IL-10 enhanced programmed cell death of CD4 T cells in HIV-infected subjects. Results from Estaquier *et al.* confirmed these data by demonstrating that IL-10 blockade decreased apoptosis of the CD4 T cell subset [43], whereas CD8 T cell death was not reduced. Whether the impact of IL-10 on HIV-specific T cells follows the same patterns remains to be determined.

IL-10 AND HIV-SPECIFIC CD8 T CELL DYSFUNCTION

Data obtained in the mouse LCMV model showed that virus-specific CTL responses were improved, both quantitatively and qualitatively, in animals treated with anti-IL-10Rα antibody as compared to mice treated with isotype control antibody [23, 24]. Experiments performed with PBMC from HIV-infected individuals likewise showed that HIV epitope-specific CD8 T cell (CTL) proliferative responses could be improved by IL-10Rα blockade *in vitro* [38]. Whether this quantitative increase is also associated with an improvement in effector CTL functions and/or a qualitative change in the proliferating HIV-specific CTL subset remains to be seen.

IL-10 AND HIV REPLICATION

Besides its effect on immune responses, IL-10 can directly modulate HIV replication in CD4 T cells, monocytes and dendritic cells in complex ways. One study reported CCR5 upregulation and increased HIV infection by the macrophage/CCR5-tropic BaL strain in IL-10-treated monocytes [44], whereas another study found that IL-10 increased replication of a T-tropic CXCR4 strain in MDDC, but inhibited viral replication in macrophages [45]. Investigation of blood samples from pregnant HIV + women showed that IL-10 blocked HIV replication in polyclonally-activated CD4 T cells [46]. The complexity of the IL-10 effects was also illustrated in another report [47]: anti-interleukin 10 (anti-IL-10) reduced CCR5 expression, without influencing CXCR4. Similarly, rIL-10 induced up-regulation of CCR5, but not of CXCR4. The multiple effects of IL-10 on monocytic and dendritic cell phenotype and functions (reviewed in 9) may contribute to the apparently contradictory findings. It is important to note that molecules modulated by IL-10 include both HIV co-receptors and adhesion/signaling molecules involved in the immunological and virological [48] synapses (including ICAM-1, CD80/CD86) [49]. Thus, IL-10 may directly modulate efficacy of cell-to-cell viral transmission too. Additionally, the HIV proteins Tat [50, 51], and gp120 [52, 53] can directly induce IL-10 expression in APCs. Previous reports have indicated that upregulation of IL-10 by monocytes may be due to a direct effect of HIV Env [52], and that the Env of different HIV strains could induce varying degrees of IL-10 secretion by MDDC [53]. However, we found no significant increase in IL-10 mRNA in pDC and mDC in chronically-infected subjects in our recent study [38].

POLYMORPHISMS OF THE IL-10 GENE AND HIV INFECTION

A number of studies in humans have shown correlations between prevalence or severity of various diseases and polymorphisms in the IL-10 or IL-10R genes. Five single-nucleotide polymorphisms (SNPs) tagging the promoter haplotypes of the IL-10 gene, as well as three bi-allelic polymorphisms located in the proximal promoter region of the IL-10 gene have been widely studied [54, 55]. Relative to the transcription initiation site, the promoter polymorphisms are located at positions -1082 (A to G transition), -819 (C to T transition) and -592 (C to A transversion). The -592C>A variant has been associated with lower IL-10 production and the -1082A>G variant with higher production of IL-10 [54]. The -592C>A variant has been associated with more rapid disease progression in late stages of HIV infection [56], and the -1082A>G polymorphism with slower CD4 decline and longer survival in HIV + individuals [57], suggesting that IL-10 may have a protective effect on HIV disease progression. A recent study extended investigations to a total of 21 SNPs and confirmed by haplotype trend regression that the -1082A/-592A haplotype was associated with faster progression to AIDS in Americans of European descent [58], but not in African Americans. Recently, a study by Naicker *et al.* [59] showed in a South African cohort of high-risk women who were HIV negative at the time of enrollment that individuals with genotypes associated with high IL-10 production at two promoter SNPs (-1082G and -592C) were less likely to become HIV infected, but had significantly higher median plasma viral loads during the acute phase. However, as the infection progressed, the association between genotype and median viral load was reversed. Thus, IL-10 may influence HIV susceptibility and pathogenesis, with the effects following transmission differing according to the infection phase and reflecting the net result of positive (*e.g.* on viral replication and immune activation) and negative effects.

UNRESOLVED ISSUES, PERSPECTIVES AND THERAPEUTIC IMPLICATIONS

There is currently a strong interest in novel therapies targeting immunoregulatory pathways in order to enhance immune responses against persistent viruses and cancer cells, or to boost the efficacy of vaccines. In spite of important advances in our knowledge of the IL-10 network in various physiological and pathological conditions, our understanding of the role of this cytokine in HIV infection is still incomplete and several important issues need to be addressed.

First, promising data obtained in well-established murine models cannot be extrapolated to immunodeficiency virus infections in primates. Significant differences exist between the murine and human immune system (for review, see [60, 61]). Studies in the simian immunodeficiency virus (SIV) model in monkeys are therefore necessary before considering interfering with inhibitory pathways in human HIV infection.

Second, the alterations in the activity of the IL-10 pathway in tissues, in the setting of HIV infection, remain almost entirely to be defined. In SIV infection, one study demonstrated marked upregulation of IL-10 RNA in isolated intraepithelial lymphocytes (IEL) and lamina propria lymphocytes (LPL) isolated from jejunal tissue [62]. IL-10 mRNA expression was also shown to be upregulated in the colonic mucosa of HIV-infected individuals as compared to HIV-negative controls [63], but the cell types responsible for this increase in IL-10 were not identified. Investigation of the gut-associated lymphoid tissue (GALT) is of major interest, as involvement of this compartment plays a critical role in HIV pathogenesis and many studies have demonstrated CD4 T cell-derived IL-10 as a key mediator in intestinal immune homeostasis [64].

Third, the role of the IL-10 pathway during acute infection needs to be better delineated, as events during acute HIV infection are thought to be critical for subsequent disease course. A recent study in a plasma donor cohort, in which samples were available before and during the earliest stages of infection, showed that HIV-1 infection is followed by an early cytokine storm, in which IL-10 peaks slightly later than several pro-inflammatory cytokines [65]. Interestingly, a study in primary SIV infection showed that expression of an anti-inflammatory profile, including IL-10 and TGF-β, as well as an increase in Tregs, occurred early after infection in African green monkeys, which do not develop AIDS, as compared to the pathogenic infection of rhesus macaques [62], in which expression of IL-10 and TGF-β was comparatively delayed. A protective role of IL-10 in acute HIV/SIV infection would represent a major difference compared to observations in the LCMV model.

Fourth, what is the role of Tregs in IL-10 activity in HIV infection and how would IL-10 blockade affect Treg differentiation and function? This is an important question given the impact of Tregs in multiple infectious disease models and the incomplete understanding of their role in HIV infection and the IL-10 pathway.

Fifth, would blockade of the IL-10 pathway lead to a qualitative improvement in T cell responses *in vivo*, both in peripheral blood and lymphoid tissues, in spite of the fact that multiple inhibitory molecules and complex defects contribute to T cell exhaustion in chronic infections [5, 66]? What would be the effect on B cell and antibody responses, which are stimulated by IL-10?

Sixth, would the detrimental effect of IL-10 blockade on immune activation offset any potential benefit from the restoration of HIV-specific responses or lead to excessive inflammation and autoimmunity? This is a concern given that many studies suggest that continuous immune activation is a crucial factor in the progressive destruction of the immune system (for review, see [67]) and that studies of IL-10 gene promoter polymorphisms suggest a protective effect of high IL-10 levels, at least in later stages of HIV disease. Of note, a placebo-controlled trial investigating the tolerance and impact of recombinant IL-10 therapy on parameters of disease progression in HIV-infected individuals did not show any significant changes in either viral load or CD4 T-cell counts during four weeks of treatment [68].

Seventh, would immune intervention in a pathway such as IL-10 be beneficial to subjects with optimal viral suppression under antiretroviral therapy, which is the current optimal standard of care that can be achieved in the large majority of individuals with current drug regimens?
Eighth, as multiple inhibitory molecules contribute to T cell impairment in chronic viral infections, including HIV, would simultaneous blockade of IL-10 and another pathway enhance efficacy without excessive toxicity, as suggested by the results obtained with combined PD-L1/IL-10 blockade in the LCMV model?
Ninth, what is the potential use of blockade of the IL-10 pathway as vaccine adjuvant? IL-10 blockade has shown promise when used in combination with therapeutic vaccination in a murine model of chronic infection in a setting where the natural T cell response to the pathogen is exhausted [32]. In this regard, local inhibition of IL-10 by siRNA at the site of vaccine delivery has given promising early results [69].
Finally, it appears that the IL-10 pathway is complex, is differentially regulated in tissue compartments, and has pleiotropic effects on the immune system. A potentially better alternative to a relatively blunt, costly and cumbersome tool such as a blocking antibody would be the identification of intracellular targets for small molecules that would act downstream of the IL-10 receptor, potentially with more selective effects.

CONCLUSION

In spite of major advances in antiretroviral therapy that have had a tremendously positive impact on HIV patient care, ways of generating effective HIV-specific immune responses in HIV-infected or uninfected subjects are essentially lacking. There is a critical need for understanding how effective immune responses function against this virus. Blockade of inhibitory pathways in HIV infection, combined with antiretroviral drugs and/or therapeutic vaccination, might offer new therapeutic approaches in the near future. Data obtained on the IL-10 pathway so far suggest that this cytokine has both beneficial and detrimental effects in HIV disease. Although the potential for targeting this pathway for therapeutic purposes in HIV infection is uncertain, the significance of studies of this major immunoregulatory cytokine in HIV/AIDS are directly relevant to our understanding of the pathogenesis and treatment of other chronic infections and cancers.

Disclosure*. None of the authors has any conflict of interest to disclose.*

REFERENCES

1. Virgin HW, Wherry EJ, Ahmed R. Redefining chronic viral infection. *Cell* 2009; 138: 30.
2. Deeks SG, Walker BD. Human immunodeficiency virus controllers: mechanisms of durable virus control in the absence of antiretroviral therapy. *Immunity* 2007; 27: 406.
3. Oldstone MB. Anatomy of viral persistence. *PLoS Pathog* 2009; 5: e1000523.
4. Zajac AJ, Blattman JN, Murali-Krishna K, *et al.* Viral immune evasion due to persistence of activated T cells without effector function. *J Exp Med* 1998; 188: 2205 [see comments].
5. Wherry EJ, Ha SJ, Kaech SM, *et al.* Molecular signature of CD8+ T cell exhaustion during chronic viral infection. *Immunity* 2007; 27: 670.
6. Haining WN, Ebert BL, Subrmanian A, *et al.* Identification of an evolutionarily conserved transcriptional signature of CD8 memory differentiation that is shared by T and B cells. *J Immunol* 2008; 181: 1859.
7. Kaufmann DE, Walker BD. PD-1 and CTLA-4 inhibitory cosignaling pathways in HIV infection and the potential for therapeutic intervention. *J Immunol* 2009; 182: 5891.
8. Commins S, Steinke JW, Borish L. The extended IL-10 superfamily: IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29. *J Allergy Clin Immunol* 2008; 121: 1108.
9. Moore KW, de Waal Malefyt R, Coffman RL, O'Garra A. Interleukin-10 and the interleukin-10 receptor. *Annu Rev Immunol* 2001; 19: 683.
10. Couper KN, Blount DG, Riley EM. IL-10: the master regulator of immunity to infection. *J Immunol* 2008; 180: 5771.
11. O'Garra A, Barrat FJ, Castro AG, Vicari A, Hawrylowicz C. Strategies for use of IL-10 or its antagonists in human disease. *Immunol Rev* 2008; 223: 114.
12. Berg DJ, Davidson N, Kuhn R, *et al.* Enterocolitis and colon cancer in interleukin-10-deficient mice are associated with aberrant cytokine production and CD4(+) TH1-like responses. *J Clin Invest* 1996; 98: 1010.
13. Davidson NJ, Leach MW, Fort MM, *et al.* T helper cell 1-type CD4+ T cells, but not B cells, mediate colitis in interleukin 10-deficient mice. *J Exp Med* 1996; 184: 241.
14. Saraiva M, O'Garra A. The regulation of IL-10 production by immune cells. *Nat Rev Immunol* 2010; 10: 170.
15. de Waal Malefyt R, Haanen J, Spits H, *et al.* Interleukin 10 (IL-10) and viral IL-10 strongly reduce antigen-specific human T cell proliferation by diminishing the antigen-presenting capacity of monocytes via downregulation of class II major histocompatibility complex expression. *J Exp Med* 1991; 174: 915.
16. Rousset F, Garcia E, Defrance T, *et al.* Interleukin 10 is a potent growth and differentiation factor for activated human B lymphocytes. *Proc Natl Acad Sci USA* 1992; 89: 1890.
17. Dai WJ, Kohler G, Brombacher F. Both innate and acquired immunity to Listeria monocytogenes infection are increased in IL-10-deficient mice. *J Immunol* 1997; 158: 2259.
18. Denis M, Ghadirian E. IL-10 neutralization augments mouse resistance to systemic Mycobacterium avium infections. *J Immunol* 1993; 151: 5425.
19. Reed SG, Brownell CE, Russo DM, Silva JS, Grabstein KH, Morrissey PJ. IL-10 mediates susceptibility to Trypanosoma cruzi infection. *J Immunol* 1994; 153: 3135.
20. Belkaid Y, Hoffmann KF, Mendez S, *et al.* The role of interleukin (IL)-10 in the persistence of Leishmania major in the skin after healing and the therapeutic potential of anti-IL-10 receptor antibody for sterile cure. *J Exp Med* 2001; 194: 1497.
21. Gazzinelli RT, Wysocka M, Hieny S, *et al.* In the absence of endogenous IL-10, mice acutely infected with Toxoplasma gondii succumb to a lethal immune response dependent on CD4+ T cells and accompanied by overproduction of IL-12, IFN-gamma and TNF-alpha. *J Immunol* 1996; 157: 798.
22. Couper KN, Blount DG, Wilson MS, *et al.* IL-10 from CD4CD25Foxp3CD127 adaptive regulatory T cells modulates parasite clearance and pathology during malaria infection. *PLoS Pathog* 2008; 4: e1000004.

23. Brooks DG, Trifilo MJ, Edelmann KH, Teyton L, McGavern DB, Oldstone MB. Interleukin-10 determines viral clearance or persistence *in vivo*. *Nat Med* 2006; 12: 1301.

24. Ejrnaes M, Filippi CM, Martinic MM, *et al.* Resolution of a chronic viral infection after interleukin-10 receptor blockade. *J Exp Med* 2006; 203: 2461.

25. Ghalib HW, Piuvezam MR, Skeiky YA, *et al.* Interleukin 10 production correlates with pathology in human Leishmania donovani infections. *J Clin Invest* 1993; 92: 324.

26. Yamamura M, Uyemura K, Deans RJ, *et al.* Defining protective responses to pathogens: cytokine profiles in leprosy lesions. *Science* 1991; 254: 277.

27. Zhang M, Gong J, Iyer DV, Jones BE, Modlin RL, Barnes PF. T cell cytokine responses in persons with tuberculosis and human immunodeficiency virus infection. *J Clin Invest* 1994; 94: 2435.

28. Liu Y, de Waal Malefyt R, Briere F, *et al.* The EBV IL-10 homologue is a selective agonist with impaired binding to the IL-10 receptor. *J Immunol* 1997; 158: 604.

29. Kotenko SV, Saccani S, Izotova LS, Mirochnitchenko OV, Pestka S. Human cytomegalovirus harbors its own unique IL-10 homolog (cmvIL-10). *Proc Natl Acad Sci USA* 2000; 97: 1695.

30. Persico M, Capasso M, Persico E, *et al.* Interleukin-10 - 1082 GG polymorphism influences the occurrence and the clinical characteristics of hepatitis C virus infection. *J Hepatol* 2006; 45: 779.

31. Paladino N, Fainboim H, Theiler G, *et al.* Gender susceptibility to chronic hepatitis C virus infection associated with interleukin 10 promoter polymorphism. *J Virol* 2006; 80: 9144.

32. Brooks DG, Lee AM, Elsaesser H, McGavern DB, Oldstone MB. IL-10 blockade facilitates DNA vaccine-induced T cell responses and enhances clearance of persistent virus infection. *J Exp Med* 2008; 205: 533.

33. Lane HC, Depper JM, Greene WC, Whalen G, Waldmann TA, Fauci AS. Qualitative analysis of immune function in patients with the acquired immunodeficiency syndrome. Evidence for a selective defect in soluble antigen recognition. *N Engl J Med* 1985; 313: 79.

34. Clerici M, Wynn TA, Berzofsky JA, *et al.* Role of interleukin-10 in T helper cell dysfunction in asymptomatic individuals infected with the human immunodeficiency virus. *J Clin Invest* 1994; 93: 768.

35. Clerici M, Balotta C, Salvaggio A, *et al.* Human immunodeficiency virus (HIV) phenotype and interleukin-2/interleukin-10 ratio are associated markers of protection and progression in HIV infection. *Blood* 1996; 88: 574.

36. Landay AL, Clerici M, Hashemi F, Kessler H, Berzofsky JA, Shearer GM. *In vitro* restoration of T cell immune function in human immunodeficiency virus-positive persons: effects of interleukin (IL)-12 and anti-IL-10. *J Infect Dis* 1996; 173: 1085.

37. Stylianou E, Aukrust P, Kvale D, Muller F, Froland SS. IL-10 in HIV infection: increasing serum IL-10 levels with disease progression--down-regulatory effect of potent anti-retroviral therapy. *Clin Exp Immunol* 1999; 116: 115.

38. Brockman MA, Kwon DS, Tighe DP, *et al.* IL-10 is upregulated in multiple cell types during viremic HIV infection and reversibly inhibits virus-specific T cells. *Blood* 2009; 114: 346-56.

39. Trabattoni D, Saresella M, Biasin M, *et al.* B7-H1 is up-regulated in HIV infection and is a novel surrogate marker of disease progression. *Blood* 2003; 101: 2514.

40. Kaufmann DE, Kavanagh DG, Pereyra F, *et al.* Upregulation of CTLA-4 by HIV-specific CD4(+) T cells correlates with disease progression and defines a reversible immune dysfunction. *Nat Immunol* 2007; 8: 1246.

41. D'Souza M, Fontenot AP, Mack DG, *et al.* Programmed death 1 expression on HIV-specific CD4+ T cells is driven by viral replication and associated with T cell dysfunction. *J Immunol* 2007; 179: 1979.

42. Clerici M, Sarin A, Coffman RL, *et al.* Type 1/type 2 cytokine modulation of T-cell programmed cell death as a model for human immunodeficiency virus pathogenesis. *Proc Natl Acad Sci USA* 1994; 91: 11811.

43. Estaquier J, Idziorek T, Zou W, *et al.* T helper type 1/T helper type 2 cytokines and T cell death: preventive effect of interleukin 12 on activation-induced and CD95 (FAS/APO-1)-mediated apoptosis of CD4+ T cells from human immunodeficiency virus-infected persons. *J Exp Med* 1995; 182: 1759.

44. Sozzani S, Ghezzi S, Iannolo G, *et al.* Interleukin 10 increases CCR5 expression and HIV infection in human monocytes. *J Exp Med* 1998; 187: 439.

45. Ancuta P, Bakri Y, Chomont N, Hocini H, Gabuzda D, Haeffner-Cavaillon N. Opposite effects of IL-10 on the ability of dendritic cells and macrophages to replicate primary CXCR4-dependent HIV-1 strains. *J Immunol* 2001; 166: 4244.

46. Bento CA, Hygino J, Andrade RM, *et al.* IL-10-secreting T cells from HIV-infected pregnant women downregulate HIV-1 replication: effect enhanced by antiretroviral treatment. *AIDS* 2009; 23: 9.

47. Juffermans NP, Paxton WA, Dekkers PE, *et al.* Up-regulation of HIV coreceptors CXCR4 and CCR5 on CD4(+) T cells during human endotoxemia and after stimulation with (myco)bacterial antigens: the role of cytokines. *Blood* 2000; 96: 2649.

48. Piguet V, Sattentau Q. Dangerous liaisons at the virological synapse. *J Clin Invest* 2004; 114: 605.

49. Willems F, Marchant A, Delville JP, *et al.* Interleukin-10 inhibits B7 and intercellular adhesion molecule-1 expression on human monocytes. *Eur J Immunol* 1994; 24: 1007.

50. Badou A, Bennasser Y, Moreau M, Leclerc C, Benkirane M, Bahraoui E. Tat protein of human immunodeficiency virus type 1 induces interleukin-10 in human peripheral blood monocytes: implication of protein kinase C-dependent pathway. *J Virol* 2000; 74: 10551.

51. Huigen MC, Kamp W, Nottet HS. Multiple effects of HIV-1 transactivator protein on the pathogenesis of HIV-1 infection. *Eur J Clin Invest* 2004; 34: 57.

52. Schols D, De Clercq E. Human immunodeficiency virus type 1 gp120 induces anergy in human peripheral blood lymphocytes by inducing interleukin-10 production. *J Virol* 1996; 70: 4953.

53. Shan M, Klasse PJ, Banerjee K, *et al.* HIV-1 gp120 mannoses induce immunosuppressive responses from dendritic cells. *PLoS Pathog* 2007; 3: e169.

54. Edwards-Smith CJ, Jonsson JR, Purdie DM, Bansal A, Shorthouse C, Powell EE. Interleukin-10 promoter polymorphism predicts initial response of chronic hepatitis C to interferon alfa. *Hepatology* 1999; 30: 526.

55. Vidigal PG, Germer JJ, Zein NN. Polymorphisms in the interleukin-10, tumor necrosis factor-alpha, and transforming growth factor-beta1 genes in chronic hepatitis C patients treated with interferon and ribavirin. *J Hepatol* 2002; 36: 271.

56. Shin HD, Winkler C, Stephens JC, *et al.* Genetic restriction of HIV-1 pathogenesis to AIDS by promoter alleles of IL10. *Proc Natl Acad Sci USA* 2000; 97: 14467.

57. Erikstrup C, Kallestrup P, Zinyama-Gutsire RB, *et al.* Reduced mortality and CD4 cell loss among carriers of the interleukin-10 -1082G allele in a Zimbabwean cohort of HIV-1-infected adults. *AIDS* 2007; 21: 2283.

58. Oleksyk TK, Shrestha S, Truelove AL, *et al.* Extended IL10 haplotypes and their association with HIV progression to AIDS. *Genes Immun* 2009; 10: 309.

59. Naicker DD, Werner L, Kormuth E, *et al.* Interleukin-10 Promoter Polymorphisms Influence HIV-1 Susceptibility and Primary HIV-1 Pathogenesis. *J Infect Dis* 2009; 200: 448.

60. Riley JL, June CH. The road to recovery: translating PD-1 biology into clinical benefit. *Trends Immunol* 2007; 28: 48.

61. Mestas J, Hughes CC. Of mice and not men: differences between mouse and human immunology. *J Immunol* 2004; 172: 2731.

62. Smit-McBride Z, Mattapallil JJ, Villinger F, Ansari AA, Dandekar S. Intracellular cytokine expression in the CD4+ and CD8+ T cells from intestinal mucosa of simian immunodeficiency virus infected macaques. *J Med Primatol* 1998; 27: 129.

63. Schulbin H, Bode H, Stocker H, *et al.* Cytokine expression in the colonic mucosa of human immunodeficiency virus-infected individuals before and during 9 months of antiretroviral therapy. *Antimicrob Agents Chemother* 2008; 52: 3377.

64. Groux H, O'Garra A, Bigler M, *et al.* A CD4+ T-cell subset inhibits antigen-specific T-cell responses and prevents colitis. *Nature* 1997; 389: 737.

65. Stacey AR, Norris PJ, Qin L, *et al.* Induction of a striking systemic cytokine cascade prior to peak viremia in acute human immunodeficiency virus type 1 infection, in contrast to more modest and delayed responses in acute hepatitis B and C virus infections. *J Virol* 2009; 83: 3719.

66. Blackburn SD, Shin H, Haining WN, *et al.* Coregulation of CD8+ T cell exhaustion by multiple inhibitory receptors during chronic viral infection. *Nat Immunol* 2009; 10: 29.

67. Silvestri G, Feinberg MB. Turnover of lymphocytes and conceptual paradigms in HIV infection. *J Clin Invest* 2003; 112: 821.

68. Angel JB, Jacobson MA, Skolnik PR, *et al.* A multicenter, randomized, double-blind, placebo-controlled trial of recombinant human interleukin-10 in HIV-infected subjects. *AIDS* 2000; 14: 2503.

69. Singh A, Nie H, Ghosn B, Qin H, Kwak LW, Roy K. Efficient modulation of T-cell response by dual-mode, single-carrier delivery of cytokine-targeted siRNA and DNA vaccine to antigen-presenting cells. *Mol Ther* 2008; 16: 2011.