HOT TOPICS

Role of IL-12 in HIV infection and vaccine

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ABSTRACT. Among cytokines that dictate the fate of developing immune responses, IL-12 represents an important nexus for the development of type I cell-mediated immune responses (CMI). This factor is primarily produced by monocytic cell lineages in response to stimuli such as pathogen-associated molecular patterns, dictating the development of naive T cells as they differentiate into antigen-specific T cells. HIV infection results in an early loss of effective TH1 prototype CMI when such responses appear to be precisely the type of CMI needed to control the virus and a host of opportunistic pathogens. Besides CD4 T cell loss, much of the muted IL-12 response has been attributed to direct effects of HIV or its proteins on antigen-presenting cells, while T and NK cell responses to IL-12 appear maintained during chronic HIV infection. However, while IL-12 therapy is unlikely to provide major benefits in the context of an established HIV infection, IL-12 preconditioning of monkeys during acute SIV infection markedly delayed disease progression. These findings suggest that IL-12 may serve as a critical vaccine adjuvant, and as treatment for particular opportunistic agents or neoplasm such as Kaposi's sarcoma; it has already shown promising results in the context of HIV infection.

Keywords: IL-12, immunotherapy, HIV, SIV, vaccine

IL-12, A POTENT IMMUNOMODULATOR

Interleukin 12, originally termed the natural killer cell stimulating factor was identified in 1990 by G. Trinchieri *et al.* [1], as a heterodimer comprised of a p40 and a p35 subunit, covalently-linked, for the biologically active p70 cytokine. Besides activating NK cells, IL-12 was rapidly identified as a master switch for differentiating naïve CD4+ T cells towards the Th1 pathway, a pathway that is antagonized in the presence of IL-10 [2]. Although IL-12 was initially purified from the supernatant of a lymphoblastoid cell line [1], several studies rapidly highlighted the monocytoid lineage, macrophages and myeloid dendritic cells being the primary, physiological sources of IL-12 in response to a large variety of infectious agents that express pathogen-associated molecular patterns (PAMPs) [3]. The triggering of toll-like receptors (TLRs) by these PAMPs, effectively shapes the developing immune response. The role of IL-12 in promoting the generation of potent effector responses was highlighted by several studies in mice. These demonstrated this cytokine's ability to resolve or markedly limit infections that would otherwise linger or remain chronic, such as leishmania, toxoplasmosis, listeria and murine AIDS [4-7]. Interest in the application of such a potent immune modulator in the treatment of various tumors was also rapidly explored, secondary to its unique, dual biological effect of enhancing both the innate and adaptive cellular effector mechanisms along with its anti-angiogenic properties [8]. However, toxicities and death resulted from an ill-fated, phase II trial for the treatment of advanced renal carcinoma [9], which markedly slowed the clinical applications of this cytokine, even though the mechanisms underlying such toxicities have since been ascribed to an inappropriate dose and administration schedule. Another source of clinical caution stems from the fact that IL-12 shares its p40 and p35 chains with other members of the IL-12 family, IL-23 and IL-35, leading to erroneous conclusions regarding the role of IL-12 in select autoimmune disorders or their models (*e.g.* inflammatory bowel disease, experimental autoimmune encephalitis and others). Studies using IL-23 are of particular interest since this cytokine represents another master switch for a CD4 T cell subset synthesizing IL-17, and is thus termed Th17. Results of studies using IL-12 p40 antagonists in a variety of autoimmune disease models led to the conclusion that the perceived deleterious effects of IL-12 need to be revised since the etiology of the inflammatory response were primarily ascribed to IL-23 [10].

IL-12 AND HIV

One of the hallmarks of HIV infection is an early immune dysfunction characterized by the gradual erosion of

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cellular effector responses, even prior to extensive CD4+ T cell loss. Although the mechanisms leading to such immune dysfunction are multi-factorial, many studies focused primarily on the function of CD4+ T cells since the CD4+ T cells are the primary target of virus infection. Results from several studies of antigen-specific immune responses conducted on a variety of patient groups published in the nineties, led to the conclusion that both CD4+ and CD8+ T cell responses were markedly enhanced *ex vivo* by the addition of IL-12 [11-13], but that such a capacity to respond decreased in patients with marked CD4 loss and disease progression [14]. The enhancement of CD4+ T cell antigen-specific responses was boosted further in HIV-infected patients by the demonstration that IL-12 inhibited apoptosis in this cell lineage [15], suggesting a potential, prolonged effector function in addition to enhanced magnitude of response. The benefit of IL-12 therapy was tested and confirmed *in vivo* using the non-human primate model of AIDS using the Indian rhesus macaques infected with simian immunodeficiency virus (SIV) [16, 17]. In both studies, viral loads did not appear affected by the therapy, even though IL-12 was administered in the absence of antiretroviral therapy, and a marked increase in the frequency of circulating NK cells and partial restoration of NK lytic functions were noted. Our study also highlighted partial restoration of SIV-specific cytotoxic responses during the chronic SIV infection period [17]. In contrast, IL-12 therapy administered at late stages of SIV infection characterized by low CD4 T cell levels and opportunistic infections showed that most of the cytokine responses were lost during the late stage of the disease, confirming the data obtained with samples from HIV patients analyzed *ex vivo* [14].

Analysis of the mechanisms regulating the IL-12 response potential in PBMCs from HIV infected patients was also conducted, with several reports highlighting a marked diminution in IL-12 production by monocytes, macrophages and dendritic cells from HIV-infected patients upon stimulation with a variety of agents [18-21]. Such findings account for the early dysfunction and loss of cell-mediated responses to HIV and other pathogens long before CD4 T cells numbers have decreased to levels associated with AIDS. While such a reduction in IL-12 production may be secondary to direct infection of monocytes/macrophages or dendritic cells, the relatively low frequency of such infected cells strongly argues against a direct effect of the virus. However, two independent studies uncovered at least two distinct pathways elicited by HIV proteins to inhibit IL-12 production by the monocytic lineages [22, 23]: activation of monocytes/macrophages with *Staphylococcus aureus* in the presence of HIV gp120 resulted in a skew in the cytokine profile produced: IL-12p40 mRNA was markedly diminished while IL-10 production was upregulated, effectively inhibiting IL-12 protein production. The second mechanism is mediated by extracellular HIV vpr, which acts as a glucocorticoid receptor co-activator and represses the production of IL-12 p35 production and release of the biologically active IL-12 heterodimer following activation of monocytic lineages, in a dose-dependent manner [23]. Both mechanisms markedly diminish IL-12 production in response to infection, and likely limit the generation of new and recall type I immune responses.

This latter finding is critical when considering the need to immunize HIV-infected patients and generate effective immune responses, both against HIV and other pathogens, which may cause opportunistic infections. With regards to HIV infection, data from our laboratory have clearly demonstrated the potential of IL-12 in markedly altering the course of disease. Rhesus macaques preconditioned with IL-12 were inoculated intravenously with the highly pathogenic SIVmac251. While the magnitude of the acute viral loads did not differ markedly between IL-12-treated and untreated control monkeys, IL-12-treated monkeys established potent immune responses during the chronic phase capable of maintaining viral load set points substantially lower than control monkeys [24]. The monkeys given IL-12 at the time of infection also maintained a disease-free status for three to four years, while untreated SIV-infected monkeys developed AIDS and had to be euthanized between six to twelve months post-infection. These findings underscore the potency of IL-12 in profoundly shaping immune responses following the initial antigen encounter, markedly altering the host pathogen interactions *in vivo*, an observation seen in murine AIDS [4], simian AIDS [24] and simian malaria [25].

Thus, the use of IL-12 as an adjuvant to vaccines is amply justified, although its incorporation into larger vaccine trials has lagged, in large part due to the early mishap in the renal carcinoma phase II trial [9]. Several studies have used IL-12 as an adjuvant for a variety of immunizations in mice including HIV, showing excellent efficacy. Studies in non-human primates however, have highlighted the value of IL-12 as an adjuvant to DNA prime/virus-like particle protein boost vaccines, where IL-12 administered in the prime and/or in the boost did not prevent infection, but resulted in statistically significant reductions in acute and chronic SIV viral loads (> 2 log), using a challenge virus recognized as difficult to control [26]. Other studies from the Wyeth Lederle team used plasmid DNA-delivered vaccine alone with plasmid-delivered IL-12 and IL-15 [27]. These studies showed improved cellular and humoral responses in the IL-12-adjuvanted groups, as well as improved control of SHIV89.6p, while monkey groups adjuvanted with IL-15 alone, behaved like the animals administered the non-adjuvanted vaccine. In fact, the need for IL-12 in generating HIV env-specific cytotoxic responses was demonstrated more recently in IL-12KO mice in which supplementation with exogenous IL-12 restored such responses [28].

FUTURE OF IL-12 USE IN THE CONTEXT OF HIV

As outlined above, IL-12 therapy in the context of HIV infection may need to be revisited. Its most relevant application would be in the context of opportunistic infections that are best met with type I effector immune

responses. In fact, IL-12 therapy is currently administered to patients with Kaposi's sarcoma, for which other therapies have failed [29, 30]. It has to be borne in mind though that most opportunistic infections occur at late stages of HIV infection, following extensive erosion of immune competency of the host, a stage during which primate studies have shown limited clinical benefit of IL-12 therapy alone. It may be possible to explore combination therapies, using additional cytokines such as IL-7, 15 or 21 and or other immune modulatory approaches, to attempt to revive T cell effector responses. The use of ART in conjunction with IL-12 has also not been tested in this model.

Nevertheless, it is our belief that IL-12 may play a critical role as an adjuvant to immunizations delivered to HIV-infected patients. These patients suffer from suboptimal IL-12 production in response to vaccine adjuvants and thus, exogenous, localized delivery of cytokines would palliate the insufficient IL-12 production from dendritic cells and/or macrophages and ensure appropriate T cell differentiation and recruitment. While the investigation of IL-12 in the context of HIV was very active during the nineties, the last decade has seen comparatively less activity. The optimization of cytokine administration for select uses has nevertheless made important progress, and it is likely that targeted and localized administration of cytokines such as IL-12, which have a relatively narrow therapeutic range, remains to be fully explored. Thus, use of encapsulation in micro- or nanoparticles, restricting the delivery to APCs and/or the co-delivery of IL-12 with an antigen delivered *via* transducing vectors, are likely to be more effective than free cytokines and far better tolerated. Thus, we believe that, primarily as an adjuvant to vaccines, IL-12 remains one of the most promising candidates both for HIV-infected patients and developing countries where endemic helminth infections limit the production and release of endogenous IL-12 by resident APCs, leading to suboptimal immune responses to select pathogens.

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REFERENCES

1. Wolf SF, Temple PA, Kobayashi M, *et al.* Cloning of cDNA for natural killer cell stimulatory factor, a heterodimeric cytokine with multiple biologic effects on T and natural killer cells. *J Immunol* 1991; 146: 3074.
2. D'Andrea A, Aste-Amezaga M, Valiante NM, Ma X, Kubin M, Trinchieri G. Interleukin 10 (IL-10) inhibits human lymphocyte interferon gamma-production by suppressing natural killer cell stimulatory factor/IL-12 synthesis in accessory cells. *J Exp Med* 1993; 178: 1041.
3. Macatonia SE, Hsieh CS, Murphy KM, O'Garra A. Dendritic cells and macrophages are required for Th1 development of CD4+ T cells from alpha beta TCR transgenic mice: IL-12 substitution for macrophages to stimulate IFN-gamma production is IFN-gamma-dependent. *Int Immunol* 1993; 5: 1119.
4. Gazzinelli RT, Giese NA, Morse 3rd HC. In vivo treatment with interleukin 12 protects mice from immune abnormalities observed during murine acquired immunodeficiency syndrome (MAIDS). *J Exp Med* 1994; 180: 2199.
5. Khan IA, Matsuura T, Kasper LH. Interleukin-12 enhances murine survival against acute toxoplasmosis. *Infect Immun* 1994; 62: 1639.
6. Sypek JP, Chung CL, Mayor SE, *et al.* Resolution of cutaneous leishmaniasis: interleukin 12 initiates a protective T helper type 1 immune response. *J Exp Med* 1993; 177: 1797.
7. Wagner RD, Steinberg H, Brown JF, Czuprynski CJ. Recombinant interleukin-12 enhances resistance of mice to Listeria monocytogenes infection. *Microb Pathog* 1994; 17: 175.
8. Colombo MP, Trinchieri G. Interleukin-12 in anti-tumor immunity and immunotherapy. *Cytokine Growth Factor Rev* 2002; 13: 155.
9. Leonard JP, Sherman ML, Fisher GL, *et al.* Effects of single-dose interleukin-12 exposure on interleukin-12-associated toxicity and interferon-gamma production. *Blood* 1997; 90: 2541.
10. Damsker JM, Hansen AM, Caspi RR. Th1 and Th17 cells: adversaries and collaborators. *Ann N Y Acad Sci* 2010; 1183: 211.
11. Clerici M, Lucey DR, Berzofsky JA, *et al.* Restoration of HIV-specific cell-mediated immune responses by interleukin-12 in vitro. *Science* 1993; 262: 1721.
12. 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.
13. Dybul M, Mercier G, Belson M, *et al.* CD40 ligand trimer and IL-12 enhance peripheral blood mononuclear cells and CD4+ T cell proliferation and production of IFN-gamma in response to p24 antigen in HIV-infected individuals: potential contribution of anergy to HIV-specific unresponsiveness. *J Immunol* 2000; 165: 1685.
14. Nagy-Agren SE, Cooney EL. Interleukin-12 enhancement of antigen-specific lymphocyte proliferation correlates with stage of human immunodeficiency virus infection. *J Infect Dis* 1999; 179: 493.
15. 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.
16. Watanabe N, Sypek JP, Mittler S, *et al.* Administration of recombinant human interleukin 12 to chronically SIVmac-infected rhesus monkeys. *AIDS Res Hum Retroviruses* 1998; 14: 393.
17. Villinger F, Bucur S, Chikkala NF, *et al.* In vitro and in vivo responses to interleukin 12 are maintained until the late SIV infection stage but lost during AIDS. *AIDS Res Hum Retroviruses* 2000; 16: 751.
18. Chehimi J, Starr SE, Frank I, *et al.* Impaired interleukin 12 production in human immunodeficiency virus-infected patients. *J Exp Med* 1994; 179: 1361.
19. Denis M, Ghadirian E. Dysregulation of interleukin 8, interleukin 10, and interleukin 12 release by alveolar macrophages from HIV type 1-infected subjects. *AIDS Res Hum Retroviruses* 1994; 10: 1619.
20. Chougnet C, Wynn TA, Clerici M, *et al.* Molecular analysis of decreased interleukin-12 production in persons infected with human immunodeficiency virus. *J Infect Dis* 1996; 174: 46.
21. Marshall JD, Chehimi J, Gri G, Kostman JR, Montaner LJ, Trinchieri G. The interleukin-12-mediated pathway of immune events is dysfunctional in human immunodeficiency virus-infected individuals. *Blood* 1999; 94: 1003.
22. Taoufik Y, Lantz O, Wallon C, Charles A, Dussaix E, Delfraissy JF. Human immunodeficiency virus gp120 inhibits interleukin-12 secretion by human monocytes: an indirect interleukin-10-mediated effect. *Blood* 1997; 89: 2842.

23. Mirani M, Elenkov I, Volpi S, Hiroi N, Chrousos GP, Kino T. HIV-1 protein Vpr suppresses IL-12 production from human monocytes by enhancing glucocorticoid action: potential implications of Vpr coactivator activity for the innate and cellular immunity deficits observed in HIV-1 infection. *J Immunol* 2002; 169: 6361.

24. Ansari AA, Mayne AE, Sundstrom JB, *et al.* Administration of recombinant rhesus interleukin-12 during acute simian immunodeficiency virus (SIV) infection leads to decreased viral loads associated with prolonged survival in SIVmac251-infected rhesus macaques. *J Virol* 2002; 76: 1731.

25. Hoffman SL, Crutcher JM, Puri SK, *et al.* Sterile protection of monkeys against malaria after administration of interleukin-12. *Nat Med* 1997; 3: 80.

26. O'Neill E, Martinez I, Villinger F, *et al.* Protection by SIV VLP DNA prime/protein boost following mucosal SIV challenge is markedly enhanced by IL-12/GM-CSF co-administration. *J Med Primatol* 2002; 31: 217.

27. Chong SY, Egan MA, Kutzler MA, *et al.* Comparative ability of plasmid IL-12 and IL-15 to enhance cellular and humoral immune responses elicited by a SIVgag plasmid DNA vaccine and alter disease progression following SHIV(89.6P) challenge in rhesus macaques. *Vaccine* 2007; 25: 4967.

28. Gupta S, Boppana R, Mishra GC, Saha B, Mitra D. Interleukin-12 is necessary for the priming of CD4+ T cells required during the elicitation of HIV-1 gp120-specific cytotoxic T-lymphocyte function. *Immunology* 2008; 124: 553.

29. Yarchoan R, Pluda JM, Wyvill KM, *et al.* Treatment of AIDS-related Kaposi's sarcoma with interleukin-12: rationale and preliminary evidence of clinical activity. *Crit Rev Immunol* 2007; 27: 401.

30. Little RF, Aleman K, Kumar P, *et al.* Phase 2 study of pegylated liposomal doxorubicin in combination with interleukin-12 for AIDS-related Kaposi sarcoma. *Blood* 2007; 110: 4165.