

Updates on cells and cytokines

(Supported by a grant from Hoechst Marion Roussel, Inc., Kansas City, Mo.)

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The regulation of IL-12: Its role in infectious, autoimmune, and allergic diseases

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IL-12 is a heterodimeric molecule (IL-12 p70) composed of the p35 and p40 subunits. Its physiologic source in vivo has primarily been from antigen-presenting cells (APCs), including monocytes/macrophages and dendritic cells; however, neutrophils, keratinocytes, and B cells have also been shown to be involved in its production. Functionally, IL-12 is important in influencing the differentiation of naive CD4⁺ T cells toward an interferon (IFN)- γ -producing T_{H1}-type cell, as well as in enhancing cytotoxic T cell-mediated lysis and natural killer (NK) cell activity.¹ In recent years, IL-12 has been shown to play a critical role in the regulation of immune responses in various infectious and autoimmune disease models,² as well as in the allergic asthma model.³ Thus because of its potent effects on cellular immunity and its role in such a wide spectrum of clinical disease, it is of considerable interest to understand the mechanism or mechanisms by which IL-12 is regulated. In this review, we outline three important factors that regulate IL-12 production.

ROLE OF IFN- γ IN THE PRODUCTION OF IL-12

Although IL-12 can have multiple effects on the immune response, its major role is to augment IFN- γ production.⁴ In addition, it is clear from several studies that IFN- γ itself has an important role in IL-12 regulation. Thus a major challenge addressed by multiple investigators was to define the role that IFN- γ had on IL-12 induction and to determine whether IFN- γ precedes the production of IL-12 or vice versa. It was initially reported that human monocytes stimulated with IFN- γ alone did not induce IL-12 p40 mRNA or protein production, although IFN- γ was able to prime cells for lipopolysaccharide

Abbreviations used

APC:	Antigen-presenting cell
IFN:	Interferon
LPS:	Lipopolysaccharide
NK:	Natural killer
PBMC:	Peripheral blood mononuclear cell
PHA:	Phytohemagglutinin
SAC:	<i>Staphylococcus aureus</i> Cowan strain

(LPS)-induced transcription of the IL-12 p40 gene.⁵ In similar studies,⁶ it was shown that priming of monocytes with IFN- γ enhanced expression of both p35 and p40 mRNA in response to LPS. Finally, it was reported that induction of IL-12 p40 from macrophages in response to mycobacterial infection was dependent on the presence of both IFN- γ and tumor necrosis factor- α .⁷ Taken together, these data suggested a requisite role for IFN- γ in IL-12 production from monocytes^{5,6} or from bone marrow-derived macrophages.⁷

Additional evidence, however, suggested that IL-12 could be induced in an IFN- γ -independent manner. First, it was shown that IFN- γ receptor-deficient mice infected with *Leishmania monocytogenes* produced significant amounts of both IL-12 p40 and IFN- γ after restimulation of spleen cells in vitro.⁸ This study suggested that production of these T_{H1} cytokines could occur in the absence of IFN- γ responsiveness. Moreover, IFN- γ -deficient mice infected with *Toxoplasma gondii* were also shown to produce IL-12 p40 and p70 after in vitro stimulation.⁹ It thus became clear that IFN- γ may not be essential for induction of IL-12 in mice infected with *L. monocytogenes* or *T. gondii*.

To conclude, the ability of IFN- γ to enhance IL-12 production occurs by the following mechanisms: (1) IFN- γ directly induces IL-12 p35 transcription from peripheral blood mononuclear cells (PBMCs) and primes monocytes for LPS-induced transcription of the IL-12 p35 and p40 genes. (2) IFN- γ increases expression of CD40 on human monocytes,¹⁰ which likely enhances monocyte responsiveness to CD40 ligand (CD40L) stimulation. (3) IFN- γ down-regulates IL-10 (a known negative regulator of IL-12) from monocytes (see below).

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J Allergy Clin Immunol 1998;102:11-5.

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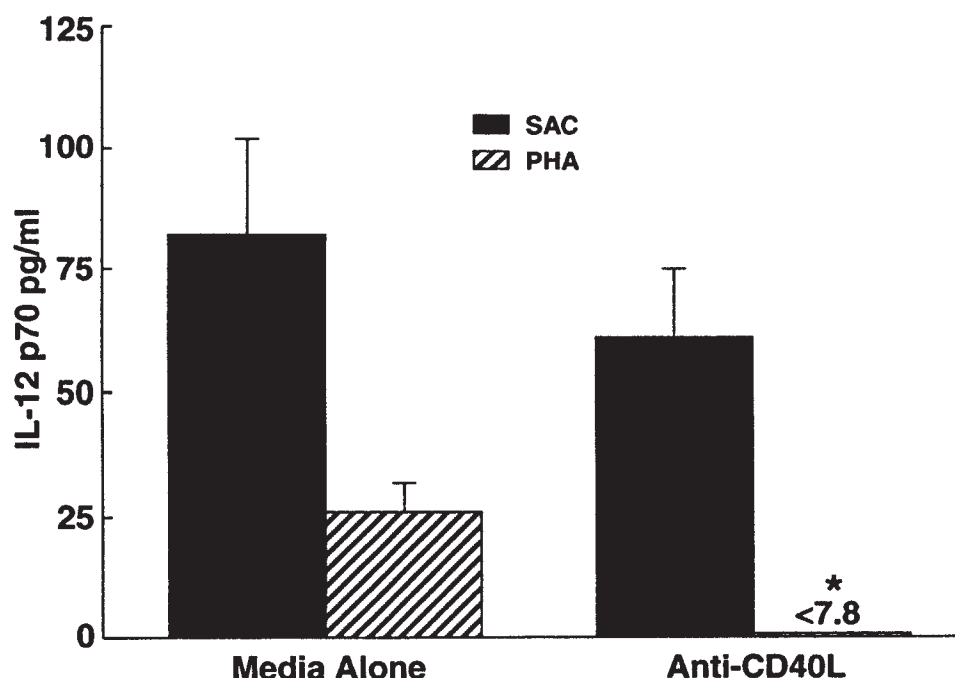


FIG. 1. Fresh PBMCs ($2.5 \times 10^5/250 \mu\text{l}$) obtained from normal donors were added in triplicate to 96-well plates and stimulated with SAC (0.01% wt/vol) or PHA (3 $\mu\text{g/ml}$) in presence or absence of anti-CD40L antibody (10 $\mu\text{g/ml}$). In all experiments, cell culture supernatants were harvested 40 hours after stimulation, and IL-12 p70 protein was measured by specific ELISA (limit of detection, 7.8 pg/ml). Results are represented as mean $\pm$ SEM of six separate experiments. *Statistical significance compared with stimulated cells in media alone.

ROLE OF CD40L/CD40 COSTIMULATION IN THE PRODUCTION OF IL-12

Recent studies have elucidated two distinct pathways for the IL-12 production in vitro.^{11,12} The first pathway is direct induction of IL-12 in response to infectious pathogens (see below). The second is a T cell-dependent mechanism in which CD40L expressed on T cells after activation interacts with its counterreceptor, CD40, on APCs, leading to production of IL-12.^{13,14} These distinct pathways are illustrated in Fig. 1. In this experiment, IL-12 was detected in supernatants of human PBMCs stimulated in culture with heat-killed *Staphylococcus aureus* Cowan strain (SAC) or the T cell mitogen phytohemagglutinin (PHA). The addition of anti-CD40L antibody completely abrogated IL-12 protein production by PBMCs stimulated with PHA but not with SAC, providing evidence that CD40L is required for T cell-dependent induction of IL-12 in human beings.

The role of CD40L/CD40 interactions regulating the production of IL-12 has also been studied in murine models. Mice deficient in CD40L/CD40 had strikingly diminished proliferative and cytokine responses after in vivo stimulation with a T cell-specific antigen.^{15,16} Furthermore, when CD40- or CD40L-deficient mice with a normally resistant genetic background were infected with *Leishmania* species, they had progressive disease. This was due to the failure of these mice to produce IL-12 and IFN- γ .¹⁷ Moreover, in one of these studies, treatment of CD40L-deficient mice with IL-12 conferred protection, providing evidence for a correlation between CD40L-induced IL-12

and disease susceptibility. In contrast, more recent studies have shown that CD40L-deficient mice infected in vivo with *Histoplasma capsulatum*¹⁸ or *Mycobacteria tuberculosis*¹⁹ are not more susceptible compared with wild-type animals. These data are consistent with the in vitro studies alluded to above that show that microbial stimuli¹¹ readily induce IL-12 in the absence of CD40L/CD40 stimulation. Overall, it appears that, with the exception of *Leishmania* species, most intracellular pathogens can directly induce IL-12 in a CD40L/CD40-independent manner. This paradigm is consistent with the clinical observation that patients with X-linked hyperimmunoglobulinemia M (who have a mutation in CD40L) are not more vulnerable to *H. capsulatum*, *M. tuberculosis*, or *T. gondii*.

MICROBIAL STIMULI AFFECTING IL-12 INDUCTION

As noted above, certain microorganisms or bacterial products can directly induce IL-12. These microbial stimuli induce IL-12 through direct activation of APCs in contrast to discrete protein antigens that require T cell activation and the CD40L/CD40 pathway. An example of this is shown in Fig. 2. In this experiment highly purified human monocytes produced IL-12 in response to SAC stimulation. Furthermore, IL-12 was not diminished by addition of a neutralizing antibody against IFN- γ (from any possible contaminating T cell or NK-cell source). Taken together with the data in Fig. 1, it is clear that SAC induces IL-12 independent of IFN- γ or CD40L. Furthermore, recent in

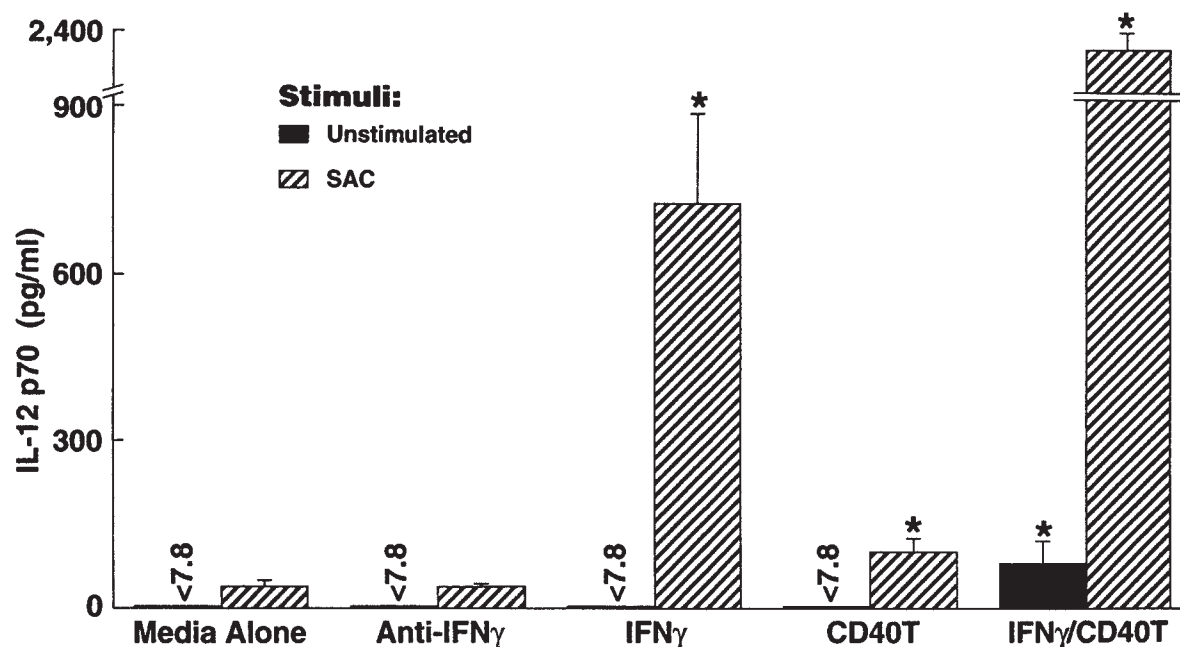


FIG. 2. Fresh elutriated human monocytes ($2.5 \times 10^5/250 \mu\text{l}$) from normal donors were added in triplicate to 96-well plates and stimulated with SAC (0.01% wt/vol) or cultured in media alone in presence of rhIFN- γ (10 ng/ml) and/or CD40T (2 $\mu\text{g/ml}$) or anti-IFN antibody (10 $\mu\text{g/ml}$). Supernatants were assayed at 40 hours by specific ELISA for IL-12 p70 protein. Results are represented as mean $\pm$ SEM of six experiments. *Statistical significance compared with antigen-stimulated or unstimulated cells in media alone.

vivo data have also shown that IL-12 p40 can be induced in an IFN- γ , CD40L-independent manner after injection of mice with a protein derived from *T. gondii*.²⁰

NEGATIVE REGULATORS OF IL-12 PRODUCTION

IL-10 is a potent negative regulatory cytokine capable of inhibiting many proinflammatory cytokines, including IL-12. The cross-regulatory properties of IL-10 are clearly demonstrated in studies showing that IL-10-deficient mice are more vulnerable to LPS-induced endotoxic shock²¹ or succumb to overwhelming lethal inflammatory response after infection with *T. gondii*.²² It should also be noted that IL-12 itself enhances IL-10 production from T cells.^{23,24} This latter finding may provide a mechanism by which T cells autoregulate a proinflammatory response.

In addition to IL-10, there are other protein and infectious agents able to inhibit IL-12 production. In this regard, it was initially reported that IL-12, but not IL-10, was selectively decreased from PBMCs of HIV-infected individuals stimulated with SAC.²⁵ It was concluded from these studies that HIV infection was able to directly inhibit IL-12 production, and that this was independent of IL-10. In subsequent studies, however, although a similar decrease in IL-12 production from cells of HIV-infected individuals was noted, there was also an increase in IL-10 production.²⁶ In both studies, the addition of anti-IL-10 enhanced IL-12 from patients' cells. However, similar increases were also seen in control subjects without HIV infection. Thus HIV infection appears to be a direct negative inhibitor of IL-12. A second viral

agent shown to inhibit IL-12 production was noted after exposure of monocytes to the measles virus.²⁷ In these studies, it was shown that cross-linking of measles receptor CD46 (a complement regulatory protein) or addition of measles virus to monocytes stimulated with SAC and IFN- γ caused significant inhibition of IL-12 production. Furthermore, this effect appeared to be independent of IL-10, suggesting that these factors directly regulate the production of IL-12. In more recent studies, work by Marth and Kelsall²⁸ have shown that CR3 (CD11b/CD18) binding iC3B on human macrophages stimulated with SAC caused a significant decrease in IL-12 production. Additionally, it was shown that *L. major* promastigotes could inhibit IL-12 production in bone marrow-derived macrophages.²⁹ Finally, other factors induced by various stimuli (e.g., PGE2) may also contribute to negative regulation of IL-12 and other proinflammatory cytokines in certain disease states.³⁰

SUMMARY OF THE THREE FACTORS REGULATING IL-12

As of this writing, the confluence of the aforementioned factors appears in determining the induction of optimal IL-12 in monocytes/macrophages. Although we have cited examples and shown (Fig. 2) that various pathogens can induce IL-12 in the absence of IFN- γ or CD40L/CD40 stimulation, it is likely that in vivo regulation does involve both of these factors. In this regard, as shown in Fig. 2, stimulation of monocyte cultures with SAC in the presence of IFN- γ strikingly enhanced the production of IL-12, demonstrating the potent priming

effect of IFN- γ . Furthermore, although addition of soluble trimeric CD40L (CD40T) resulted in a comparatively modest twofold increase in IL-12 production compared with SAC alone, the presence of IFN- γ and CD40T caused a demonstrative increase in IL-12 production. These culture conditions may be analogous to an in vivo situation such as the following. After microbial stimulation (e.g., SAC) in the presence of an early source of IFN- γ (either T cell or NK cell derived), there is an influx of activated T cells bearing CD40L. These signals synergize and provide an amplification loop whereby the T cell-dependent pathway augments the microbial pathway for optimal IL-12 induction. Also note that the presence of both IFN- γ and CD40LT in cultures without SAC results in significant amounts of IL-12 production compared with that from unstimulated monocytes or those cultured in either IFN- γ or CD40T alone. In the absence of microbial stimuli, however, substantially less IL-12 is induced, underscoring the importance of this signal to the monocyte/macrophage. Thus the presence of all three cellular signals (i.e., microbial stimuli, IFN- γ , and CD40L/CD40 stimulation) leads to high levels of IL-12 production from human monocytes/macrophages.

IL-12 IN DISEASE STATES

As noted in the introduction, numerous studies have demonstrated that endogenous IL-12 and IFN- γ are required to induce a protective T_{H1} immune response to many intracellular pathogens. Similarly, exogenous IL-12 has been shown to be an effective therapeutic, as well as vaccine, adjuvant in many of these models.³¹ These studies may provide a rationale for using IL-12 alone or combined with chemotherapy in the treatment of various infectious diseases. As a corollary to the protective role of IL-12 in infectious disease, several murine models of autoimmune disease (e.g., experimental autoimmune encephalitis, 2,4,6-trinitrobenzene sulfonic acid-induced colitis, and diabetes) have shown a correlation between a T_{H1} response and the induction of pathology. In each of these autoimmune models, endogenous IL-12 played a crucial role in regulating a pathogenic immune response. These studies may lead to further research for the future treatment of established autoimmune disease with specific IL-12 antagonists (e.g., anti-IL-12 antibody).²

There has also been investigation into the role of IL-12 in allergic disease, in particular allergic asthma, which is characterized by airway hyperresponsiveness and pulmonary eosinophilia and is associated with a T_{H2}-like response. In a murine model of allergic asthma, sensitized mice were shown to have increased levels of IL-4 and IL-5 mRNA and protein after antigen challenge. Administration of IL-12 at the time of antigen challenge abolished airway hyperresponsiveness and pulmonary eosinophilia and generated an increase in IFN- γ and a decrease in expression of T_{H2} cytokines.³ These studies suggest that there may be a future role for IL-12 immunotherapy in the treatment and/or prevention of atopic disorders.

Recent evidence indicates that prokaryotic DNA immunization is a potent inducer of IL-12 and T_{H1}

responses through unique cysteine polyguanine motifs that are not present in mammalian DNA. One promising approach, used in a murine model of *L. major* infection, is to vaccinate animals with antigen (protein or DNA) along with cytokine adjuvant DNA (e.g., IL-12).³² This use of DNA vaccination to induce protective T_{H1} immunity may lead to more durable responses than those obtained with traditional protein plus adjuvant vaccination methods. Strategies using DNA vaccination are not limited to infection. There is evidence that administration of plasmid DNA for a specific antigen could diminish an ongoing T_{H2}-type immune response.³³ Finally, in a model of allergen-gene immunization, DNA vaccination with house dust mite antigen prevented the induction of IgE synthesis, histamine release in bronchoalveolar lavage fluids, and airway hyperresponsiveness in rats challenged with aerosolized allergen.³⁴ This type of study may have future applications for atopic disease as a novel approach toward allergy immunotherapy.

We thank Brenda Rae Marshall for editorial assistance.

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