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The IL-12 Family of Heterodimeric Cytokines: New Players in the Regulation of T Cell Responses

Minireview

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Originally the only known heterodimeric cytokine, IL-12 is now part of a family of five cytokines and shares important functions in the regulation of both innate and adaptive immunity with two of them, IL-23 and IL-27. Although initially these three cytokines were considered to have largely overlapping immunological functions, more recent studies, including two articles in this issue of *Immunity* (Hamano et al., 2003; Villarino et al., 2003), indicate that they mediate complex and well-differentiated functions.

When the cytokine Interleukin-12 (IL-12) was first purified in 1989, it was discovered, unlike all other known cytokines, to have the unique molecular structure of a covalently linked heterodimer composed of two chains, p40 and p35 (Trinchieri, 2003). The sequence of p35 is homologous to that of IL-6 and G-CSF and indicates a four-α-helix bundle structure, typical of cytokines. The sequence of the p40 chain is homologous to the extracellular portion of members of the hemopoietin receptor family, particularly IL-6 receptor α chain (IL-6Rα) and cyliary neutrotrophic factor receptor (CNTFR). The extracellular portions of the receptor α chain of the IL-6 family of cytokines (e.g., IL-6, IL-11, CNTF) can be secreted as soluble molecules able to form complexes with the cytokines. These complexes bind to the other transmembrane chains of the receptor complex (usually containing the gp130 β-receptor chain), inducing signal transduction. Thus, it was proposed that IL-12 evolved by a cytokine of the IL-6 family covalently bound to the extracellular portion of its primordial α chain receptor (Trinchieri, 2003). The membrane receptor complex of IL-12 is formed by the two chains IL-12Rβ1 and IL-12Rβ2, which are homologous to gp130. IL-12Rβ1 binds IL-12 p40, and it is associated with Tyk2, whereas IL-12Rβ2 recognizes either the heterodimer or the p35 chain and is associated with Jak2. Signaling through the IL-12 receptor complex induces phosphorylation, dimerization, and nuclear translocation of several STAT transcription factors (1, 3, 4, 5), but the predominant response and most of the biological responses to IL-12 are mediated by STAT4 (Trinchieri, 2003). IL-12 is mostly produced by activated hematopoietic phagocytic cells (monocytes, macrophages, neutrophils) and dendritic cells. IL-12 p40 is often secreted in large excess over the p70 heterodimer; p35 is only secreted as a part of the heterodimer when p40 is also produced by the same cells. IL-12 is a potent inducer of interferon-γ (IFN-γ) production from T, NK, and other cell types, and it has been shown to be a potent inducer of differentiation of T helper 1 (Th1) cells. A very large number of studies in experimental animals, using genetically deficient animals or neutralizing antibodies, have shown that IL-12, due to its Th1-inducing activity, is required for resistance to bacterial and intracellular parasite infections and for the establishment of organ-specific autoimmunity (Trinchieri, 2003). However, patients with complete deficiency of IL-12 p40 chain or the IL-12Rβ1 chain of its receptor complex have a limited susceptibility to mycobacteria or salmonella infections only, not the expected complete lack of resistance to most infections (Fieschi and Casanova, 2003). Also, in the mouse, IL-12 was shown not to be required for Th1 responses, although its expression was essential for optimal responses (Jankovic et al., 2002).

For more than 10 years, IL-12 has remained the only known heterodimeric cytokine. However, recently, it has been joined by four new cytokines with a similar structure to form a family of heterodimeric cytokines (Figure 1). Within the neuropoietic cytokines of the IL-6 family, CNTF was known to associate with the soluble extracellular part of its specific receptor (sCNTFR) to form a biologically active heterodimer signaling through a receptor complex composed of gp130 and leukemia inhibitory factor receptor (LIRF). More recently, it was found that CNTFR induces the secretion and is required for functional responses to the cardiothrophin-like cytokine (CLC) (Lelievre et al., 2001). CLC can be secreted either in association with sCNTFR or with another soluble α receptor-homolog, the cytokine-like factor-1 (CLF-1), thus forming two different heterodimeric cytokines. sCNTFR/CLC is active on cells expressing gp130 and LIFR, whereas CLC/CLF-1 requires also the presence of the transmembrane CNTFR.

IL-12 p40 was shown to associate not only with the IL-12 p35 chain, but also with a p19 chain to form a novel covalently linked heterodimeric cytokine, IL-23. p19, like p35, has homology with IL-6 and G-CSF, and is secreted only when associated with IL-12 p40 (Oppmann et al., 2000). The IL-23 receptor complex is composed of IL-Rβ1 and of a novel gp130-like chain, the IL-23R (Parham et al., 2002). Similarly to IL-12, IL-23 signaling activated Tyk2, Jak2, and STAT 1, 3, 4. However, the activation of STAT4 is not as predominant as for IL-12, and STAT3/4 heterodimers, rather than STAT4 homodimers, are induced by IL-23. IL-23 is produced by similar cell types as IL-12, and the receptor complex is expressed or upregulated on T and NK cells, as well as phagocytic and dendritic hematopoietic cells. IL-23 has been proposed to have similar but not overlapping functions with IL-12 in inducing IFN-γ production, T helper 1 (Th1) cell differentiation, and activation of the antigen-presenting functions of dendritic cells (Parham et al., 2002). Unlike IL-12, which is a relatively poor T cell mitogen preferentially affecting naive T cells, IL-23 selectively induces proliferation of memory T cells.

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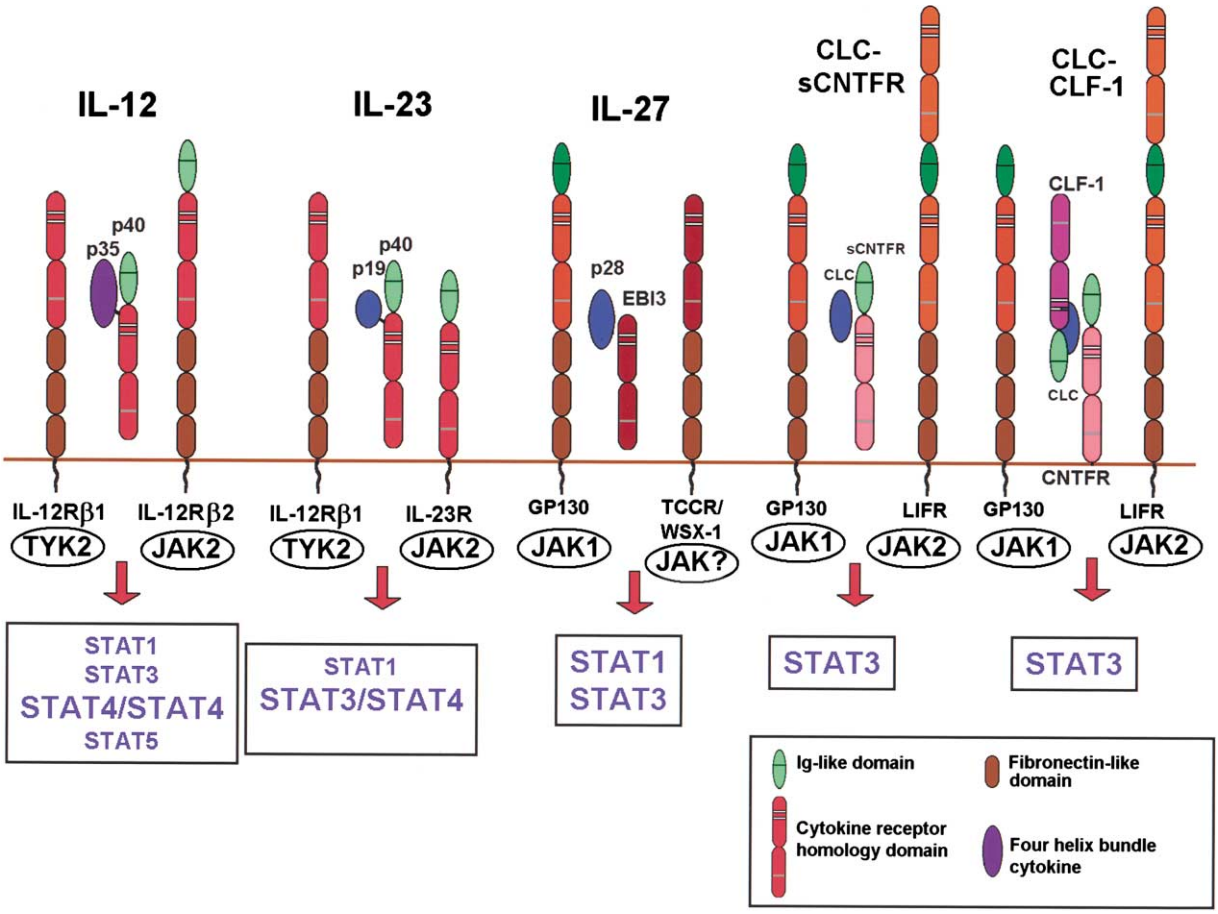

Figure 1. Schematic Depiction of the Family of Heterodimeric Cytokines, Their Receptors, and Main Signaling Pathways

Epstein Barr virus-induced protein 3 (EBI3), an α-receptor-like soluble chain homologous to IL-12 p40, was known for several years, and it has been suggested that it also induces secretion of the p35 and associates with it to form a noncovalently linked heterodimer (Trinchieri, 2003). However, the physiologic relevance of this observation was unclear, and no biological function has been associated with the EBI3-p35 heterodimer. More recently, it was shown that EBI3 induces the secretion of a novel cytokine-like chain, p28, together with which it forms a noncovalently linked heterodimeric cytokine now termed IL-27 (Pflanz et al., 2002). Within the family of long-chain four-helix bundle cytokines, p28 is evolutionary closer to IL-11, CLC, CNTF, and LIF than to IL-6, G-CSF, p35, and p19. One chain of the IL-27 receptor complex is the orphan receptor WSX-1/TCCR. Studies with WSX-1 genetically deficient mice had associated this receptor with IFN-γ production and Th1 responses (Chen et al., 2000; Yoshida et al., 2001). Recently, the second chain of the IL-27 receptor complex has been identified to be gp130 (S.P. et al., submitted). IL-27 activates Jak1, STAT1, and STAT3. IL-27 is produced by human phagocytic and dendritic cells rapidly after activation, possibly earlier than the IL-12 heterodimer. IL-27 synergizes with IL-12 in inducing IFN-γ production by T and NK cells, and it is a very potent mitogen for anti-CD3 activated naive T cells.

The discovery of IL-23 and IL-27, two cytokines that affect IFN-γ production by T and NK cells and that, in the case of IL-23, share with IL-12 part of the receptor complex and of the signaling pathway, has contributed to challenge an essential role for IL-12 in Th1 differentiation. Even in mice genetically deficient for IL-12 p40, IL-12Rβ1, or STAT4, the Th1 response is not completely absent and can be recovered at levels sufficient for protection to intracellular infections when antiinflammatory inhibitory pathways such as those mediated by IL-10 are prevented (Jankovic et al., 2002). The finding that IL-12 is either unable or poorly efficient in inducing the expression of the transcription factor T-bet, considered to be one of the earliest molecular events leading to commitment of T cells to Th1 differentiation and able to upregulate IL-12Rβ2, has led to the hypothesis that IL-12 is required more for the expansion and optimal activation of Th1 cells rather than for the initiation of the Th1 response (Mullen et al., 2001). T-bet expression is dependent on STAT1, and it is efficiently induced by cytokines that efficiently activate STAT1 such as IFN-γ, type I IFN, and IL-27. Th1 responses are completely abolished and Th2 responses are enhanced only in mice deficient for the adaptor molecule MyD88, which is essential for signal transduction in response to the IL-1 family of cytokines (including the IL-1s and IL-18) and for most of the signal transduction in response to Toll-like receptor

(TLR) ligands (Jankovic et al., 2002). This suggests that in addition to IL-12 (and IL-23), other proinflammatory cytokines or signals are important for Th1 response, possibly including IL-27 and the IL-1 family.

On the basis of the early known biology of IL-12, IL-23, and IL-27, a tentative model has emerged that IL-27, by inducing T-bet expression, IL-12Rβ2 chain expression, and proliferation of naive T cells, would be an early inducer of Th1 commitment and differentiation followed by expansion and stabilization of the Th1 response by IL-12, and then by maintenance of Th1 committed memory cells by IL-23, a cytokine able to sustain proliferation of memory T cells. However, recent findings and ongoing studies are seriously questioning such a simple model.

The discovery of IL-23, which shares the p40 chain and the IL-12Rβ1 chain with IL-12 and its receptor complex, has started to provide an explanation to the numerous observations existing in the literature of the different phenotypes of IL-12 p40- and IL-12Rβ1-deficient mice compared to those of IL-12 p35- or IL-12Rβ2-deficient mice. The former two types of mice lack both IL-12 and IL-23 functions, whereas the latter two lack only IL-12. In general, the mice lacking only functional IL-12 are more resistant to infections than those lacking both IL-12 and IL-23, although they are often less resistant than wild-type mice (Trinchieri, 2003).

Even more striking results were reported in a Th1-mediated autoimmune disease model, the experimental autoimmune encephalomyelitis (EAE). Whereas EAE could not be induced in IL-12 p40-deficient mice, the disease was even more severe in p35- or IL-12Rβ2-deficient mice than in wild-type mice (Zhang et al., 2003). This suggested an essential role for IL-23 in EAE that was fully confirmed in IL-23 p19-deficient mice, which are completely resistant to EAE induction (Cua et al., 2003). However, the generation of a Th1 response to myelin oligodendrocyte glycoprotein in this model required IL-12 and not IL-23. IL-23-deficient mice showed no disease but normal activation of Th1 cells. The expression of IL-23 was required in the CNS apparently in order to induce or maintain macrophage activation. IL-23 in the CNS was produced both by microglia and activated macrophages, but the IL-23 receptor complex was expressed only on activated macrophages. Interestingly, IL-23 p19 has been shown to be highly expressed in sensory ganglia and CNS, either constitutively or upon virus infection (Broberg et al., 2002). The more severe disease in animals lacking IL-12 but not IL-23, in the absence of optimal Th1 response, is probably explained by the dual pathogenic and protective role of IFN-γ in this disease. A very similar role for IL-12 and IL-23 has been observed in the induction of another autoimmune disease, collagen-induced arthritis (C.A. Murphy et al., submitted). In addition to a possible direct proinflammatory role, IL-23 may also promote a distinct T cell activation state characterized by the production of IL-17 (Aggarwal et al., 2003), a cytokine that has a clear role in bone erosion and also may be involved in tissue destruction in other autoimmune pathologies. It is noteworthy that IL-12 antagonizes IL-23-induced IL-17 production by T cells, providing another possible mechanism of the paradoxical protective effect of IL-12 in autoimmunity. The use of p40 transgenic mice has allowed the identification of IL-23 p19 production in the skin, and its ability to induce skin lesions, suggesting a role of IL-23 in cutaneous immunity (Kopp et al., 2003).

WSX-1/TCCR genetically deficient mice show a susceptibility to *Leishmania major* and *Listeria monocytogenes* infection associated with defective or delayed Th1 response, supporting a role for IL-27 in the initiation of the Th1 response (Chen et al., 2000; Yoshida et al., 2001). However, although the early IFN-γ production in response to antigen or even IL-12 was decreased in $CD4^+$ T cells from WSX-$1^{-/-}$ mice, upon secondary restimulation $CD4^+$ T cells from WSX-$1^{-/-}$ mice produced slightly higher levels of IFN-γ than those from wt mice. Similarly, in *L. major*-infected mice, $CD4^+$ T cells from WSX-$1^{-/-}$ mice produced much lower IFN-γ than wt mice at 2 weeks, but at 4 weeks there was no difference between the two groups of mice and, although the footpad lesion did not heal, the progression of the infection was halted. These data are not consistent with a major role of IL-27 in the initiation of the Th1 response but rather with an ability to affect the early inflammatory IFN-γ production without significantly affecting commitment of $CD4^+$ T cells to Th1 differentiation. Interestingly, although IL-27 synergizes with IL-12 in inducing IFN-γ production in human naive $CD4^+$ T cells and in NK cells (Oppmann et al., 2000), IL-27, unlike IL-12 and IL-23, does not induce stable Th1 differentiation and priming for IFN-γ production in human CD4 T cells (R. de Waal Malefyt and R.A.K., unpublished data). The major immunological defect in EBI3$^{-/-}$ mice is a markedly decreased number in invariant natural killer T cells (iNKT) as defined by staining with α-galactosylceramide(αGalCer)-loaded CD1d-tetramers (Nieuwenhuis et al., 2002). In response to αGalCer in vitro or in vivo, the EBI3$^{-/-}$ mice produced much lower levels of IL-4 than wild-type mice, whereas the production of IFN-γ was only moderately and transiently lower than in wt mice. The requirement for IL-27 in the generation of iNKT may perhaps explain the lower level of IFN-γ produced by $CD4^+$ T cells from WSX-$1^{-/-}$ mice in response to IL-12, a potent inducer of IFN-γ production in NKT cells. EBI3$^{-/-}$ mice were resistant to oxazolone-induced colitis, a model mediated prevalently by Th2 cytokine production initiated by iNKT cells, whereas they were at least as susceptible as wild-type mice to trinitrobenzene sulfonic acid-induced colitis, a predominantly Th1-mediated colitis model. Two articles in this issue of *Immunity* (Hamano et al., 2003; Villarino et al., 2003) show that, although WSX-$1^{-/-}$ mice are able to mount a protective Th1 response to infection by *Toxoplasma gondii* and *Trypanosoma cruzi*, the infected WSX-$1^{-/-}$ mice show a much higher lethality compared to wild-type mice or even IL-12p40$^{-/-}$ mice, due to T cell hyperactivity and enhanced production of proinflammatory cytokines, including IFN-γ. These data suggest that IL-27 not only is dispensable for the generation of Th1 responses in strongly polarizing conditions, but, likely due to its ability to activate STAT1 and 3, also exerts a powerful negative feedback mechanism that limits T cell hyperactivity and IFN-γ production.

The present experimental evidence indicates that the simple initial interpretation of IL-23 and IL-27 as copycat cytokines sharing many functions with IL-12 and playing similar roles but active at different times during the Th1 response needs a reevaluation. The biological functions

of IL-23 and IL-27 appear to be much more complex than anticipated and they are not redundant with those of IL-12.

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