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# Regulation of innate and adaptive immune responses by the related cytokines IL-12, IL-23, and IL-27

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## Abstract

The functional characterization and subsequent purification of T cell growth factor/interleukin (IL)-2 in the early 1980s established this secreted protein as a key mediator of immune cell activation and provided the prototype that enabled the discovery of numerous cytokines over the ensuing two decades. While soluble immunoregulatory factors were initially identified functionally as biological activities present in the culture supernatants of activated lymphocytes/monocytes, this methodology shifted radically following the completion of the human genome sequence. Computer-generated structural modeling algorithms have replaced functional assays and biochemical purification as the initial means of discovering new cytokines. To date, a total of 31 interleukins, as well as over a dozen other related hematopoietic factors, have been identified. These cytokines and their receptors may be grouped on the basis of structural homologies as well as by shared ligand and receptor subunits. The challenge now at hand is to define the biological functions of the newly identified cytokines and to elucidate the common and divergent roles of related family members. This point is well illustrated by the IL-12/IL-23/IL-27 family, whose members share ligand and receptor subunits and play somewhat overlapping roles in innate and adaptive immune responses. These three cytokines are not entirely redundant, as they may preferentially activate naïve or memory T cells, induce discrete T cell cytokine profiles, contribute to distinct stages of host immune responses to infectious agents, and differentially promote autoimmunity. Further elucidation of the unique functions of the IL-12 family members may lead to improved immunodiagnostics and therapies.

**Key words:** immune regulation, T cell activation, cytokines.

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## CYTOKINE DISCOVERY: FUNCTION-BASED AND COMPUTATIONAL STRATEGIES

The first cytokines were identified based on biological activities present in the supernatant of cultured cells. For example, T cell growth factor (TCGF) was initially described based on mitogenic activity present in the culture supernatants of lectin-stimulated peripheral blood mononuclear cells, and lymphocyte activating factor (LAF) was described as a macrophage product that augments production of TCGF [48]. The term interleukin (IL) was adopted in 1979 to define proteins acting between leukocytes, so that LAF was designated IL-1 and TCGF became IL-2 [1]. The initial characterization and cloning of IL-2 provides a prime example of the early methodology used to identify cytokines based on an observed biological function, and this preents a striking contrast to the identification of the latest interleukin, IL-31, which began by computer mediated homology searches of the human genome.

A quantitative biological assay was essential for the initial purification of the first cytokines. In the case of IL-2, the development of IL-2-responsive T cell clones was critical to enable purification of the factor from activated lymphocyte culture supernatants [23]. These clones proliferated in response to IL-2, so that individual fractions of samples generated during the biochemical purification process could be tested for stimulatory activity and the fractions containing bioactivity could be readily identified. Monoclonal antibodies were raised against purified IL-2, and these antibodies were used to affinity purify the cytokine and also to show that neutralization of this protein blocked IL-2 bioactivity. This biological assay was subsequently used for the expression cloning of IL-2. The characterization and cloning of this cytokine took approximately 5 years and was described in over 20 publications [48].

In contrast to IL-2, the latest cytokine to receive a designated interleukin number is IL-31, a cytokine that was cloned and purified before its biological functions were known [17]. The IL-31 receptor was identified by searching the human genome for sequences with homology to known cytokine receptors. The ligand was then cloned, using cell lines transfected with the IL-31 receptor (IL-31R) in an expression cloning method analogous to that used to clone IL-2. Several experimental approaches were then used to discern the function of IL-31, including real-time PCR assays quantifying IL-31 and IL-31R RNA expression in various tissues and lymphocyte subsets, microarray analysis of IL-31--regulated target genes, generation of IL-31 transgenic mice, implantation of mini-osmotic pumps expressing recombinant IL-31 in mice, and IL-31R gene-deficient mice. Together, these analyses found a function for IL-31 as a protein secreted by activated T cells that induces the expression of chemokines and inflammatory cell recruitment to the skin. Notably, this entire analysis, including the description of the sequences of both the IL-31R and its ligand, was described in a single landmark publication [17].

Thus, early cytokines were initially identified as mediators of a biological activity, and that activity was used as a basis to subsequently purify and clone the factors. In contrast, this process has been reversed in the identification of the most recent cytokines. The availability of the human genome sequence, as well as sophisticated computational modeling of structural homologies, have enabled the identification of new cytokines "*in silico*" in the absence of information on biological function. Experimental technologies including real time PCR and DNA microarray assays for measuring gene expression, as well as transgenic mice and gene-knockout methodologies for *in vivo* analysis, now make it possible to start with putative cytokine or cytokine receptor cDNA sequences and rapidly define their expression patterns and biological functions [19].

## CYTOKINE AND CYTOKINE RECEPTOR FAMILIES

A combination of functional and computational approaches has led to the identification of 31 proteins designated as interleukins as well as numerous related cytokines [9]. Linear sequence homologies observed in the respective receptors initially provided the basis for defining distinct cytokine families [5]. However, when more sophisticated structural modeling algorithms were developed, the homologies in these receptors were found to be more accurately reflected in the tertiary structures of the ligands even though linear sequence homologies among the ligands are generally very low [10, 49]. Cytokines, as well as their receptors, may be comprised of more than one subunit, and individual subunits may be shared among distinct ligand/receptor complexes [39]. This redundancy may necessitate the re-evaluation of functions initially ascribed to a particular cytokine or receptor subunit if it is later found that the specific protein under study is also a component of additional cytokines or receptors.

The vast majority of interleukins and related cytokines are categorized within two related groups, the type I and type II hematopoietic cytokine/receptor families [9]. A third group, the IL-1/Toll-like family, contains the microbial product-binding Toll-like receptors (TLR) and only two interleukins, IL-1 and IL-18 [18]. The receptors in this latter group contain a conserved ~200-amino-acid cytoplasmic Toll-IL-1R domain, and the IL-1 and IL-18R fall within a subgroup that additionally contains an extracellular Ig-like domain. The IL-1 and IL-18 ligands are cleaved from precursors to form the active cytokines, comprised of 12 β-strands aligned in a trefoil structure [6].

The type I and type II hematopoietic cytokines/ /receptors are closely related to one another and are very distinct from the IL-1/TLR members. There are 34 human cytokine receptor chains and 27 ligands in the type I hematopoietic group [9] (Table 1). This gene family was initially defined by sequence similarities observed between the IL-2R β chain and the erythropoietin receptor, including an extracellular ~200--amino-acid fibronectin-like domain that contains 4 conserved cysteine residues in the membrane-distal region and a membrane-proximal WSXWS (Trp-Ser-X-Trp--Ser) motif [5, 14]. These motifs are completely conserved in 25 of the 34 receptor chains. Active receptors may be formed as single-chain homodimers or as heterocomplexes comprised of multiple chains. In several cases, an individual receptor subunit may be involved in more than one distinct complex. For example, the gp130 protein may pair with any one of several distinct partners, resulting in the receptor complexes of at least 8 different cytokines, including IL-6, IL-11, leukemia inhibitory factor, oncostatin M, novel neurotrophin-1/B cell-stimulating factor-3/cardiotrophin-like cytokine, cardiotrophin-1, and IL-27 [9, 39].

The majority of the type I hematopoietic ligands function as monomers and contain a conserved 4-fold α--helical core structure [9, 10, 49]. A subgroup of heterodimers has also been described which includes IL-12, IL-23, and IL-27 [20, 51]. These heterodimeric ligands are comprised of an α subunit with helical structure similar to the monomeric ligands and an additional β subunit. Some of the type I receptors contain soluble subunits, and these soluble receptors are structurally related to the β subunits of the dimeric ligands. For example, IL--6Rα is a soluble protein that heterodimerizes with the transmembrane gp130 subunit to form the active IL-6R. The soluble IL-6Rα is closely related to the β subunits of the dimeric cytokines IL-12, IL-23, and IL-27 [9].

The type II hematopoietic cytokine/receptor family includes interferons and the related interleukins IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28, and IL-29 [31, 47] (Table 1). Similar to type I members, type II cytokines exhibit only 15–25% amino-acid homology,

**Table 1.** Characteristics of type I and type II hematopoietic cytokines and cytokine receptors

| Type I | Type II |
|---|---|
| 27 human cytokine genes<br>e.g. IL-2, IL-3, IL-4, IL-5, IL-6, IL-7, IL-9, IL-11, IL-12α, IL-13, IL-15, IL-21, IL-23α, IL-27α, CNTF, CT-1, EPO, G-CSF, GH, GM-CSF, LIF, LPT, NNT-1, OSM, PRL, TPO, TSLP | 27 human cytokine genes<br>e.g. IFN-α (13 genes), IFN-β, IFN-ω, IFN-ε, IFN-κ, IFN-γ, IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, IL-29 |
| Monomers: 4-fold α-helical core | Monomers: α-helical core |
| Dimers: 4-fold α-helical subunit plus soluble receptor-like subunit | Dimers: homodimers of α-helical monomers |
| 34 human receptor chains | 12 human receptor chains |
| Extracellular fibronectin-like domain:<br>4 membrane distal Cys<br>Membrane proximal Trp-Ser-X-Trp-Ser | Extracellular fibronectin-like domain:<br>4 membrane proximal Cys<br>Lack membrane proximal Trp-Ser-X-Trp-Ser |

Both type I and ype II hematopoietic cytokines are comprised of an α-helical core structure. The respective receptors are similar in containing an extracellular fibronectin-like domain, but these may be distinguished by the different spacing of 4 conserved Cys residues and the presence (type I) or absence (type II) of a Trp-Ser-X-Trp-Ser motif. Abbreviations: CNTF – ciliary neurotrophic factor, CT-1 – cardiotrophin-1, EPO – erythropoietin, G-CSF – granulocyte colony-stimulating factor, GH – growth hormone, GM-CSF – granulocyte-macrophage colony-stimulating factor, IFN – interferon, LIF – leukemia inhibitory factor, LPT – leptin, NNT-1 – novel neurotrophin-1, OSM – oncostatin M, PRL – prolactin, TPO – thrombopoietin, TSLP – thymic stromal lymphopoietin.

but contain a common 4-fold α-helical structure. The type II receptors also possess fibronectin-like extracellular domains. However, they may be distinguished from the type I receptors by the location of the 4 conserved Cys residues in the membrane-proximal region and a lack of the WSXWS motif [5, 47]. Type II ligands may function as monomers or homodimers, and receptor complexes may also be formed by heterodimerization of distinct subunits. In some cases, a single ligand may bind more than one receptor complex and, conversely, one receptor complex may bind more than one ligand. For instance, human interferon (IFN)-α, IFN-β, and IFN-ω all bind to a receptor complex consisting of proteins designated IFNAR1 and IFNAR2. In addition, IL-20 can bind to two distinct receptors comprised of either IL-22R1 and IL-20R2, or IL-20R1 and IL-20R2 [31, 47].

Overall, the initial classification of the hematopoietic cytokines and receptors into related families laid the foundation for subsequent cytokine characterization. Once sequence and structural homologies were observed among founding family members, it became possible to use conserved motifs to mine DNA sequence databases to identify novel members. This facilitated the transition from function-based to sequence-based cytokine and cytokine receptor identification. However, as more cytokines and receptors were identified, it became apparent that many cytokine and receptor subunits were shared in distinct complexes. This redundancy adds a substantial level of complexity to the analyses of cytokine function and underscores the need to evaluate the function of a given subunit in the context of all possible complexes.

One subgroup within the type I hematopoietic family illustrates these points well. The founding member, IL-12, was initially identified and cloned by virtue of its biological functions as a natural killer (NK) cell stimulatory factor [30, 58] and cytotoxic lymphocyte maturation factor [28, 50]. The two additional family members, IL-23 [38] and IL-27 [44], were later cloned by homology searches of DNA sequence databases. It was subsequently discovered that the IL-12 and IL-23 heterodimeric ligands and their respective receptors share common subunits, and that these are closely related to IL-27 and its receptor [20, 51, 56]. This structural redundancy has been reflected to some degree in overlapping functions among the three cytokines in regulating cellular innate and adaptive immune responses, although studies of microbial infection and autoimmunity indicate that the three cytokines are not entirely redundant. Notably, the discovery that IL-12 and IL-23 share common ligand and receptor subunits has brought into question early studies involving targeted disruption of the IL-12 gene and its receptor, because some of the functions first ascribed to IL-12 may have unknowingly been mediated by IL-23.

## THE IL-12 FAMILY: IL-12, IL-23, AND IL-27

IL-12 was initially characterized as a factor secreted by phorbol ester/calcium ionophore-stimulated B lymphoblastoid cell lines [30, 50]. This factor could stimulate cells of both the innate and adaptive immune system by inducing NK cell cytotoxicity and IFN-γ production as well as enhancing T cell proliferation induced by mitogens [21, 30, 51]. In a manner analogous to the purification of IL-2, biological assays were used to biochemically purify this activity to homogeneity. IL-12 was

thereby defined as a heterodimer consisting of 40-kD (p40) and 35-kD (p35) subunits [58]. By comparison, novel subunits comprising IL-23 (p19) and IL-27 (p28) were identified in a homology search of the human DNA sequence database, using the highly conserved "D" helical structure of IL-6-related cytokines as a probe [38, 44]. Like IL-12, both IL-23 and IL-27 are heterodimers (Fig. 1). Active IL-23 is comprised of the IL-23p19 and IL-12p40 subunits [38], while IL-27 is made up of IL-27 p28 and Epstein Barr virus-induced gene 3 (EBI3), an IL-12p40-related protein [15, 16, 44]. In each case, the cytokine dimer is composed of an α subunit that resembles the 4-fold helical structure of monomeric cytokines (IL-12p35, IL-23p19, and IL--27p28) and a β subunit that more closely resembles soluble cytokine receptors (p40 and EBI3) [9, 38, 44].

The IL-12R is comprised of two subunits, IL-12Rβ1 and IL-12Rβ2, which were first isolated by screening an expression library with radiolabeled ligand [45]. Since IL-23 and IL-27 are structurally similar to IL-12, and the dimeric structure of the IL-12R had been previously described, the IL-23 and IL-27 receptors were identified by testing the IL-12R and related receptor subunits for the ability to bind the more recently discovered ligands. IL-23 was found to interact with IL-12Rβ1, but in conjunction with a distinct second receptor subunit, IL-23R [41]. IL-27 binds a heterodimeric receptor comprised of the "orphan" cytokine receptor WSX-1/TCCR and gp130 [43] (Fig. 1).

Patterns of ligand and receptor expression are similar among the three cytokines [32, 51, 56]. All three cytokines are predominantly produced by activated monocytes, macrophages, and dendritic cells. Monomeric ligands are not functionally active, and heterodimers must be produced in the same cell to generate active cytokines [28, 38, 44, 58]. The respective receptors are broadly expressed in many lymphocyte subsets and show some variation in expression levels on naïve- versus memory-phenotype $CD4^+$ T cells. All three receptors are expressed on macrophages, dendritic cells, NK cells, and activated T cells [20, 51, 56]. The IL-27R WSX-1/TCCR and gp130 mRNAs are also readily detected in B cells, mast cells, and endothelial cells [43]. Naïve murine $CD4^+$ T cells, defined as $CD45RB^{hi}$, express relatively high levels of IL-12Rβ2 and low levels of IL-23R. The converse is true in T cells with a memory phenotype ($CD4^+CD45RB^{lo}$), which express relatively high levels of IL-23R and lower levels of IL-12Rβ2 [41]. Thus there is considerable overlap in the cell subsets that produce IL-12, IL-23, and IL-27, as well as in the distribution of the respective receptors. However, differences in receptor expression patterns have been observed among different cell types, as well as upon differentiation of $CD4^+$ T cells, indicating that responsiveness to these cytokines is not static but instead may be modified in the course of an active immune response.

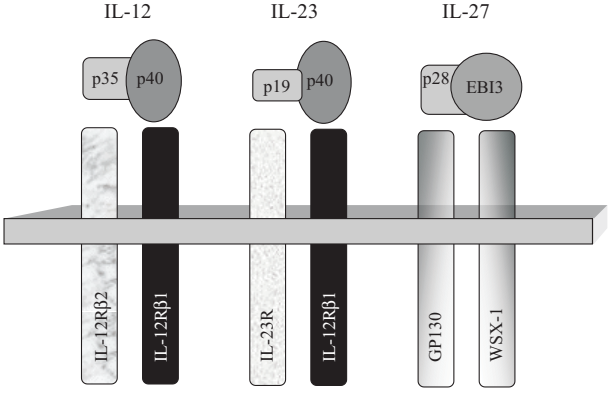


**Fig. 1.** Relationship between IL-12 family members and their shared or unique cytokine/cytokine receptor subunits. Biologically active IL-12 is comprised of p35 and p40 subunits that together form the IL-12p70 heterodimer, which binds specifically to the IL-12Rβ1/IL-12Rβ2 receptor. IL-23 is comprised of the IL-12 p40 subunit paired with a p19 subunit protein. The IL-23 heterodimer binds to IL-12Rβ1 paired not with the IL-12Rβ2 subunit, but with the unique IL-23R. IL-27 is a heterodimeric cytokine containing the Epstein-Barr virus-induced gene 3 (EBI3) subunit (related to the IL-12 p40 subunit) paired with a novel p28 subunit with homology to the IL-12 p35 subunit [15, 16, 44].

Consistent with the expression of receptors for IL-12 family members on cells of the innate and adaptive immune system, these cytokines have been found to regulate diverse functions of several lymphocyte subsets. They play a role in NK cell activation, as co-factors for T cell receptor (TCR)-induced T cell proliferation, as promoters of T cell cytokine production, and as regulators of B cell antibody production.

## NK CELL ACTIVATION

All three IL-12 family cytokines induce IFN-γ production by NK cells, and synergy has been observed in combination with other cytokines. IL-12 induction of NK cell IFN-γ production is augmented by costimulation with IL-18 or IL-2 [55, 58]. Similarly, IL-23 synergizes with IL-18 and IL-2, and the combination of all three cytokines is significantly more potent than either cytokine alone or paired with IL-2 [41]. IL-27 also synergizes with IL-2 and IL-12 [44]. Therefore, all three cytokines have the capacity to stimulate NK cells to produce IFN-γ. This effect may be augmented in the additional presence of IL-2 and/or IL-18. It is not surprising that IL-12 acts synergistically with IL-27, since these cytokines have similar, yet distinctly specific receptors.

## T CELL PROLIFERATION AND CYTOKINE PRODUCTION

T cell proliferation is initiated by antigen stimulation of the TCR and is subsequently driven by cytokines. IL-12, IL-23, and IL-27 all serve as co-factors to augment T cell proliferation, although they may act differentially on naive versus memory T cells. Naive $CD4^+$

and CD8$^+$ T cells activated by initial TCR stimulation undergo augmented proliferation in the added presence of IL-12 [13, 21]. IL-27 is also a potent costimulator of anti-CD3 plus anti-CD28-induced proliferation of naïve CD4$^+$ T cells, defined as CD4$^+$CD45RA$^+$ in the human and CD4$^+$CD45RB$^{high}$ in the mouse [44]. In contrast, IL-23 is a poor cofactor for naïve CD4$^+$ T cell activation [38]. The roles of IL-23 and IL-27 are reversed on memory CD4$^+$ T cells. IL-23 enhances anti-CD3 plus anti-CD28-induced proliferation of memory-phenotype CD4$^+$ T cells, defined as CD4$^+$CD45RO$^+$ in the human and CD4$^+$CD45RB$^{low}$ in the mouse, while IL-27 does not costimulate T cells under these conditions [38].

In addition to effects on T cell proliferation, IL-12 family members also promote T cell cytokine production. IL-12 has been well characterized as an inducer of Th1 cytokines, including IFN-γ, and as a suppressor of Th2 cytokines, such as IL-4 and IL-13 [51]. IL-23 and IL-27 also costimulate CD4$^+$ T cell IFN-γ production, and their preference for regulating cytokine production by naïve- versus memory-phenotype T cells is similar to that observed for stimulating proliferation. Thus, IL-27 synergizes with IL-12 and anti-CD3 to induce IFN-γ production by naïve-phenotype CD4$^+$ T cells [44]. In contrast, IL-23 augments IFN-γ production by PHA blasts as wells as by anti-CD3/CD28-stimulated activated/memory-phenotype T cells [38]. Notably, IL-12 (alone or in combination with IL-18) can induce bona-fide virus-specific memory CD8$^+$ T cells to produce IFN-γ in the absence of TCR engagement [7, 46]. It is not known whether virus-induced memory CD8$^+$ T cells are similarly responsive to IL-23 and/or IL-27.

Despite the observations that IL-12, IL-23, and IL--27 all stimulate IFN-γ production, studies of gene-deficient mice indicate that IL-12 is uniquely required for Th1 differentiation. T cells from IL-12p35-deficient mice immunized with antigenic peptide are less capable of producing IFN-γ after subsequent *in vitro* antigen challenge and instead produce high levels of IL-4 [12]. This indicates a failure to generate Th1 cells in the absence of IL-12, although both IL-23 and IL-27 are still present. By comparison, peptide-immunized IL-23p19--deficient mice are still capable of producing IFN-γ and not IL-4 upon *ex vivo* antigen stimulation [12]. Thus while IL-23 and IL-27 may contribute to inducing a Th1 cytokine phenotype, they are not necessarily required. The absence of IL-12 cannot be compensated by IL-23 or IL-27, again demonstrating that although these cytokines are very similar, their functions are not completely redundant.

Recently, a population of CD4$^+$ T cells has been described that appears distinct from the Th1 or Th2 type. These cells produce high levels of IL-17, and their generation may be selectively driven by IL-23 [8, 32]. *In vitro* culture of murine splenic CD4$^+$ T cells with a combination of anti-CD3, anti-CD28, IL-2, and IL-23 generates cells with the capacity to produce IL-17 upon TCR restimulation. In the absence of IL-23, these IL-17-producing cells are not produced [2]. A selective effect of IL-23, and not IL-12, in driving formation of this IL-17--producing subset has been observed in a mouse model of collagen-induced arthritis (CIA). In this case, stimulation of lymph node cells from collagen-immunized animals with IL-23 triggers predominantly IL-17-producing CD4$^+$ T cells, while IL-12 stimulation drives IFN-γ production [35]. This selectivity is further supported by the finding that IL-17-producing CD4$^+$ T cells can be generated from splenocytes of mice deficient in only IL-12 (IL-12p35-deficient), but not from mice lacking both IL-12 and IL-23 (IL-12p40-deficent) [2]. Thus, IL-12 may be most critical for driving the differentiation of CD4$^+$ T cells to IFN-γ production, while IL-23 stimulates the formation of IL-17-producing CD4$^+$ T cells. Given that IL-23 has been reported to be a poor stimulator of naïve T cells *in vitro*, it is possible that the IL-17--producing CD4$^+$ T cells are not derived directly from naïve precursors, but possibly from a subset of early--activated Th1 or Th2 cells [8].

## REGULATION OF ANTIBODY RESPONSES

The predominant effects of IL-12 or IL-27 on humoral responses include a decrease in IgG1 and IgE production and preferential class switching to form IgG2a. These effects may result from direct actions of these cytokines on B cells or indirectly via regulation of IFN-γ production. By comparison, IL-23 appears to affect antibody responses only indirectly via T cells, because IL-23-deficient mice have defective T cell--dependent antibody responses but normal T cell-independent antibody responses.

Both IL-12 [61] and IL-27 [60] antagonize *in vitro* anti-CD40-, IL-4- or LPS-induced production of IgG1 and IgE by purified B cells and trigger the formation of IgG2a antibodies. Similar promotion of IgG2a production by IL-12 and IL-27 has been observed *in vivo*. IgE production induced by *Nippostrongylus brasiliensis* infection is diminished by additional *in vivo* administration of IL-12, whereas IgG2a production is augmented [61]. Similarly, IL-27R-deficient mice exhibit reduced basal levels of serum IgG2a, and upon immunization with ovalbumin show a specific defect in IgG2a production [11]. It is possible that IL-12 and IL-27 are acting indirectly via IFN-γ, because IFN-γ itself can trigger IgG2a class switching. Indeed, analyses of IFN-γ-deficient mice indicate that IL-12-induced IgG2a production does require IFN-γ, whereas IL-27 acts independently of IFN-γ [60]. Thus, both IL-12 and IL-27 promote IgG2a class switching, and this may occur through IFN-γ--dependent or -independent mechanisms, respectively.

In contrast to the specific induction of IgG2a production by IL-12 and IL-27, IL-23 appears to broadly induce T cell-dependent antibody responses of all isotypes [22]. Basal serum Ig levels in IL-23 p19-deficient mice are normal for all isotypes. However, upon immunization with the T-dependent antigen ovalbumin, reduced amounts of all antibody isotypes are produced,

ranging from only 7–16% of wild-type control levels. Nevertheless, responses to T-independent antigens are normal, indicating that the defect in the humoral response is indirectly due to defects in T cell activation. Consistent with this hypothesis, IL-23 stimulation of purified B cells does not induce proliferation or class switching, and B cells from IL-23p19-deficient mice proliferate normally *in vitro* in response to LPS, anti-IgM, and anti-CD40 [22]. Thus it appears that IL-23 does not act directly on B cells, but rather affects antibody responses indirectly via T cells. Overall, IL-12, IL-23, and IL-27 clearly contribute to the regulation of the humoral immune response. While there is evidence that IL-27 may directly co-stimulate class switching, it appears that the effects of IL-12 and IL-23 may be indirect and more dependent on IFN-γ and/or T cells.

## ROLE OF IL-12 FAMILY MEMBERS DURING INFECTION OR AUTOIMMUNITY

IL-12, IL-23 and IL-27 have all been implicated in the Th1-type host responses to intracellular pathogens, including *Toxoplasma gondii* [33, 53], *Leishmania major* [3, 42, 59], *Trypanosoma cruzi* [29], and *Listeria monocytogenes* [11]. These cytokines have also been reported to down-regulate Th2 responses to pathogens such as the helminth *Trichuris muris* [4]. However, the three cytokines are not entirely redundant in these responses, as a deficient host response caused by the selective lack of one of the cytokines cannot always be compensated by the presence of the other cytokines. Moreover, in some cases one cytokine may be required early in the generation of the antimicrobial response, while another cytokine may be involved later in terminating the response after the microbe has been cleared.

Comparative analyses of *T. gondii* infection of mice deficient in either IL-12 or IL-23 show that IL-12 is required to confer protection, and the loss of IL-12 can only be partially compensated by IL-23 [33]. IL-12p40--deficient mice (lacking both IL-12 and IL-23) cannot control *T. gondii* infection and succumb within 18 days. By comparison, IL-12p35-deficient mice (lacking only IL-12) show enhanced resistance to infection compared with IL-12p40-deficient mice, but are unable to clear the infection and succumb within about one month post-infection. The more severe phenotype of mice lacking both IL-12 and IL-23 (compared with those lacking only IL-12) indicates that IL-23 is capable of partially restoring the antimicrobial response of IL-12-deficient mice. This role of IL-23 is further supported by the observation that administration of IL-23 to IL-12-deficient mice reduces parasite burden and prolongs survival, although the mice still eventually succumb to the infection and do not exhibit the survival of wild-type mice. Notably, IL-23p19-deficient mice show the same resistance to *T. gondii* infection as wild-type animals. Therefore, IL-12 appears to be required for an effective host response to *T. gondii* and is able to confer host protection in the absence of IL-23. Although IL-23 may contribute to the host immune response, it can only partially compensate for the absence of IL-12 [33].

IL-27 also contributes to the host response to *T. gondii*, although the role of IL-27 is thought to be in down-modulating the T cell response after the microbe has been cleared [53, 54]. Comparison of IL-12p40-deficient and IL-27R-deficient mice shows that both will succumb to a *T. gondii* infection that is normally resolved by a wild-type host. However, IL-12p40 deficiency results in an inability to reduce parasite loads, leading to an uncontrolled infection, whereas IL-27R deficiency does not result in heightened parasitemia, but in substantial lymphocytic infiltration and local inflammation in the lungs and liver. Because parasites were not readily observed in these organs at 12 days post-infection, the defect in the IL-27R-deficient mice is believed to be due to an inability to attenuate the host response after the parasite burden has been reduced. $CD4^+$ T cells in IL-27R-deficient mice continued to proliferate *in vivo* at a much higher level than wild-type counterparts, and it is suggested that this inability to attenuate the host response may lead to immunopathology manifested by significant immune-cell infiltration and necrosis in the liver and lungs [53]. In contrast to the *T. gondii* model, *Leishmania* infection of IL-27R-deficient mice results in higher parasitemia due to a lower, delayed Th1 T cell response, and uncontrolled T cell expansion/immunopathology is not observed [3, 59]. Increased T cell proliferation is observed in IL-27R-deficient T cells from infected mice, but this may likely be due to an increased state of activation secondary to the increased antigenic load *in vivo* at the time the T cells are examined *ex vivo*. Although IL-27 can inhibit Th2 responses *in vitro* [4], further studies are necessary to determine if IL-27 is required for the down-regulation of T cell responses to other infectious agents *in vivo* or if this is a phenomenon unique to the *T. gondii* model.

These examples demonstrate that while immunomodulatory cytokines are beneficial in protective host responses, they also have the potential to contribute to immunopathology. Indeed, transgenic expression of IL-23 in mice causes systemic inflammation, with lymphocyte infiltration throughout the body and death by 3 months of age [57]. In specific models of inflammatory disease, including CIA and experimental autoimmune encephalitis (EAE), IL-12, IL-23, and IL-27 have all been implicated as contributors to host pathology [24, 25, 27, 32, 35, 40]. For instance, immunization of mice with type II collagen induces symptoms similar to rheumatoid arthritis, with joint inflammation, recruitment of lymphocytes into affected joints, and inflammatory cytokine production. Comparison of IL-12 and/or IL-23 gene-deficient mice demonstrates that IL-23 is required for disease progression, while IL-12 may actually play a protective role [32, 35]. IL-12p40-deficient mice (lacking both IL-12 and IL-23) and IL-23p19-deficient mice (lacking only IL-23) are resistant to CIA. In

contrast, IL-12p35-deficient mice (lacking only IL-12) show more severe disease symptoms than wild-type mice, indicating that IL-23 is required for disease onset in the absence of IL-12. The effects of IL-23 correlate with the generation of a $CD4^+$ T cell population that secretes high levels of IL-17 [35]. IL-17 induces a cascade of inflammatory cytokines and chemokines, including tumor necrosis factor α, IL-6, and monocyte chemoattractant protein, and may contribute to IL-23--driven pathology [8, 32, 35]. This is consistent with the observation that IL-17 gene-deficient mice show significantly reduced susceptibility to CIA [36]. IL-27 may also contribute to arthritis. In a rat model of adjuvant-induced arthritis, administration of neutralizing IL-27p28 antibodies reduced disease severity in terms of diminished joint inflammation and inflammatory cytokine production [24]. Thus, in these models of arthritis, IL-23-deficient mice are resistant to disease, whereas IL-12-deficient mice are more susceptible. IL-27 may also contribute to disease, since neutralizing IL-27 antibodies diminish disease severity.

The IL-12 family cytokines have also been implicated in models of EAE. Similar to the case of CIA, IL-23 appears to be required to induce disease, while IL-12 and IL-27 may play lesser roles. In one murine model of EAE, disease is induced by injection of myelin oligodendrocyte glycoprotein (MOG). This results in lymphocyte infiltration into the central nervous system, inflammation, and demyelination. Transgenic expression of IL-12 in the brain augments antigen-induced EAE [40]. However, IL-12p35-deficient mice (lacking only IL-12) are as susceptible to EAE as wild-type mice, indicating that although IL-12 may contribute to disease, it is not required [27]. In comparison, IL-12p40-deficent mice (lacking both IL-12 and IL-23) are resistant to MOG--induced EAE, suggesting that IL-23 plays the predominant role. This observation has been confirmed and extended by comparison of mice that are deficient in IL-12 and/or IL-23 [12]. IL-23-deficient mice are indeed resistant to MOG-induced EAE. Moreover, administration of IL-23 to disease-resistant IL-12p40-deficient mice (lacking both IL-12 and IL-23) reconstitutes EAE symptoms, confirming that IL-23, not IL-12, is required for disease. An IL-23-induced IL-17-producing $CD4^+$ T cell population appears to contribute to EAE pathology [32]. Adoptive transfer of IL-17-producing T cells confers disease, while comparable transfer of IL-12-induced IFN-γ--producing $CD4^+$ T cells does not. Cytokines other than IL-17 also appear to play a role, since neutralizing IL-17 antibodies only partially block disease progression [32]. Of note, IL-27 may also contribute to EAE pathology; in a rat model of MOG-induced disease, symptom severity is reduced by administration of neutralizing anti-IL--27p28 antibodies [25].

In CIA and EAE models of inflammation and autoimmunity, IL-23 appears to be the dominant cytokine in driving disease. IL-12 and IL-27 likely provide lesser contributions, and IL-12 may even play a protective role in CIA. It is noteworthy that studies involving IL-12p40-deficient mice (now known to also lack IL-23) may have initially indicated that IL-12 played a much more significant role in driving disease. Although IL-12p40-deficient animals do display resistance to these inflammatory conditions, further studies with IL-12- or IL-23-specific deletions clearly show that IL-23 is the required cytokine for promoting disease pathogenesis.

## CONCLUSIONS

Recent advances in computational modeling, coupled with the completion of the human genome sequence, have led to the identification of a total of 31 human proteins designated as interleukins and over a dozen related hematopoietic cytokines. These have been grouped into families based on sequence and structural homologies observed among the ligands and their respective receptors and, in some cases, subgroups have been defined by the sharing of ligand and/or receptor subunits. This is exemplified by IL-12, which shares a ligand subunit and a receptor subunit with IL-23. Both IL-12 and IL-23, and their respective receptors, are also closely related to IL-27 and its receptor. The task now at hand is to fully delineate the roles of these related cytokines in regulating innate and adaptive immune responses.

The structural similarity among IL-12, IL-23, and IL-27 is reflected in overlapping, though not entirely equivalent roles in regulating NK cell activation, T cell proliferation/cytokine production, and antibody class switching. Studies of microbial infections and models of autoimmune disease clearly indicate that these cytokines are not functionally redundant. One area for further investigation will be the systematic evaluation of the roles of these cytokines in defined models of microbial infection. Some of the early studies have indicated that these cytokines may differentially affect naive versus memory T cell responses, but these results relied mainly on cell surface markers to define these distinct T cell populations, and some memory markers, such as CD44, may be expressed by a subset of truly naïve T cells during homeostatic proliferation [26, 34]. It will therefore be important to confirm and extend these studies in models of defined infections, where it is possible to track bona fide antigen-specific T cell and B cell responses. This will enable determination of the roles of IL-12, IL-23, and IL-27, alone and also in combination with other cytokines, in coordinating different stages of host antimicrobial immune responses. Further understanding of these processes will provide new insight into human disorders involving these cytokines [37, 52] and may in turn lead to improved therapeutic treatments to either augment or attenuate immune responses depending on the requirements necessary for prevention or alleviation of disease symptoms.

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