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Interleukin-12: an update on its immunological activities, signaling and regulation of gene expression

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Abstract

Interleukin-12 (IL-12) is a heterodimeric cytokine composed of the p35 and p40 subunits. It is produced by antigen-presenting cells and plays a critical role in host defense against intracellular microbial infection and control of malignancy via its ability to stimulate both innate and adaptive immune effector cells. The potency of IL-12 renders itself to stringent regulation of the timing, locality and magnitude of its production during an immune response. Subversion of the delicate control and balance frequently leads to immunologic disorders. In this article, we provide an update, since our last review of the subject four years ago, on recent advances in: (1) uncovering of novel activities of IL-12 and related molecules in various immunological settings and models; and (2) dissection of the physiological pathways involved in the modulation of IL-12 production by pathogens and immune regulators. The increased understanding of IL-12 immunobiology and expression will likely benefit the development of therapeutic modalities to correct immune dysfunctions.

Keywords

Interleukin-12; antigen-presenting cell; macrophage; dendritic cell; T helper cell; natural killer cell; cytotoxic T lymphocyte

I. Interleukin-12, the Molecule

Interleukin-12 (IL-12) was identified in the late 1980s from the culture media of Epstein-Barr virus (EBV)-transformed lymphoblastoid B cell lines, which was able to stimulate natural killer (NK) cell activity, to generate lymphokine-activated killer (LAK) cells, and to induce the production of Interferon-γ (IFN-γ) from NK and T cells [1]. It was found to be a heterodimeric molecule, and the genes encoding the two-subunit chains of IL-12, p35 and p40, were cloned [2,3]. The heterodimeric IL-12 is often referred to as IL-12 p70. The two genes encoding IL-12 p40 and p35 are located on separate chromosomes (chromosomes 11 and 6, respectively, in humans and mice, respectively) [4–7] and are not evolutionary duplicates. The p35 cDNA encodes a 209 amino acid polypeptide corresponding to a mature protein of 27.5 kDa. It contains seven cysteine residues and 3 potential N-glycosylation sites. The p40 cDNA sequence encodes a 328 amino acid polypeptide with a 22 amino acid signal peptide which generates a mature protein of 34.7 kDa. It contains ten cysteine residues and four potential N-glycosylation sites, and one consensus heparin-binding site [2,8]. The p35 gene has some homology to IL-6 and granulocyte colony-stimulating factor (G-CSF) [9] whereas the p40

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chain is homologous to the extracellular portion of the α chain of ciliary neurotropic factor (CNTF) receptor as well as to those of the IL-6 and G-CSF receptors [10,11]. The p40 chain carries the hallmarks of the hematopoietin receptor family: one tryptophan and four cysteine residues in conserved positions and a WSEWAS sequence, similar to the consensus motif WSXWS in the hematopoietin receptor family [12]. Most of the members of this transmembrane receptor family can be released from the producer cells in soluble forms containing the extracellular portion truncated after the WSXWS motif by either alternative splicing of the mRNA transcripts or by proteolytic digestion of the receptor [12].

Heterodimers of p40 and p35 are formed via disulfide bonds and secreted, usually upon stimulation of producer cells. However, IL-12 may also exist as a preformed membrane associated molecule that can be quickly released (within minutes) from phagocytic cells upon contact with intracellular microbes such as *Leishmania* in the absence of *de novo* transcription [13]. This is in contrast to the production of IL-12 induced by bacterial products such as lipopolysaccharide (LPS), which takes place with much slower kinetics (several hours) and which depends on *de novo* transcription. Another study demonstrated that IL-12 production by neutrophils, which mobilize rapidly to the site of infection by the protozoan pathogen *Toxoplamsa gondii*, appear to be derived from prestored pools [14].

In addition to forming heterodimers with p35, both mouse and human p40 are secreted in large excess as free p40 monomers and can also form homodimers ($p40_2$), which exhibit biological activities antagonistic to heterodimeric IL-12 p70 [15,16]. The production of immunosuppressive IL-12p40 homodimers was also induced in DCs and macrophages exposed to ultraviolet radiation [17]. Surprisingly, Jana et al. found that IL-12 p70, $p40_2$ (the p40 homodimer) and p40 (the p40 monomer) all induced the production of TNF-α in BV-2 microglial cells and in mouse primary microglia and peritoneal macrophages [18].

In 2000, Oppmann et al. reported a novel gene, p19, discovered in a computational screen of genomic databases, as a p35 homologue and dimerization partner with p40. The resulting cytokine, named IL-23, has biological activities both similar to and distinct from those of IL-12. In particular, IL-23 can induce strong proliferation of mouse memory ($CD4^+CD45Rb^{low}$) T cells [19], resulting in elevated IL-17 secretion [20], while IL-12 does not manifest such activities. IL-23 also proved to be the critical cytokine for autoimmune inflammation in the brain, rather than IL-12, which had long been suspected to be the main culprit [21]. Production of natural IL-23 heterodimers has been shown both in mice and in humans. Although the full spectrum of cell types producing IL-23 is not known yet, dendritic cells (DCs) that are potent producers of IL-12 are also able to produce IL-23.

Homodimers of p35 have not been reported to date. However, p35, which is not secreted in the absence of a second chain, may heterodimerize and be secreted together with a second cellular protein, EBV-induced gene 3 (EBI-3) with limited homology to IL-12 p40, although no biological function of this novel heterodimer has yet been demonstrated [22]. Searching sequence databases with a computationally derived profile of members of the IL-6 helical cytokine family led to the identification of yet another novel hematopoietic cytokine, p28, which is distantly related to IL-12 p35 [23]. IL-27 is an early product of activated antigen-presenting cells (APCs). It drives rapid clonal expansion of naïve but not memory $CD4^+$ T cells [23], in contrast to IL-23.

II. Cell Types That Produce IL-12

II.1. B lymphocytes

Although IL-12 was originally identified and purified from EBV-transformed B cell lines, normal B lymphocytes are poor producers of IL-12 even in the activated state. Schultze et al.

demonstrated that a subset of human tonsillar B cells can be induced to secrete bioactive IL-12 mainly via CD40 ligation facilitated by activated Th1 cells [24]. Expression after CD40 activation is restricted to $CD38^{-}IgD^{\pm}$, non-GC B cells. IL-12 produced from these cells is postulated to provide a positive feedback during T-B interactions, thereby maintaining the differentiation pattern of the T cells during amplification of the immune response [24]. Using CpG oligodeoxynucleotides (ODN) conjugated with an Ag (ovalbumin), Shirota et al. showed that murine B cells could serve as efficient APCs independently of surface Igs [25]. The B cells cultured with CpG-conjugated Ag not only enhanced IFN-γ formation by Th1 cells, but also induced Th1 differentiation from unprimed T cells. These effects were associated with an increase in the expression of CD40, CD86, and class II molecules on B cells and the coordinated production of IL-12 [25].

II.2. Macrophages

It has been now firmly established that the major physiological cell types that produce IL-12p70 are APCs such as monocytes/macrophages [26] and DCs [27]. Within macrophages, the so-called classically activated and alternatively activated macrophage populations differ in their ability to produce IL-12. For instance, chronic helminth infection induces alternatively activated macrophages to express high levels of CCR5 and low levels of IL-12, associated with a poor ability to induce antigen-specific proliferation of $CD4^{+}$ T cells [28].

II.3. Dendritic cells

Humans have two distinct types of DC precursors. Peripheral blood monocytes give rise to immature myeloid DCs after culturing with granulocyte-macrophage colony-stimulating factor (GM-CSF) and IL-4 [29,30] or after transmigration through endothelial cells and phagocytosis [31]. These immature cells become mature myeloid DCs (designated DC1s) after stimulation with CD40 ligand (CD40L) or endotoxin [32,33]. The $CD4^{+}CD3^{-}CD11c^{-}$ plasmacytoid cells from blood or tonsils give rise to a distinct type of immature DC with features of the lymphoid lineage after culture with IL-3 [34–36]. These cells differentiate into mature DCs (designated DC2s) after CD40L stimulation [36]. Rissoan et al. demonstrated that DC2s produce low levels of IL-12 and direct Th2 differentiation, whereas DC1s produce high levels of IL-12 and skew T cell differentiation toward Th1 [37].

Chang et al. described a phenotypically and functionally novel monocyte-derived DC1 (mDC1) subset that lacks IL-12 synthesis, produces high levels of IL-10, and directs differentiation of Th0/Th2 cells. Like conventional mDC1s, this novel subset expressed high levels of CD11c, CD40, CD80, CD86, and MHC class II molecules, but lacked expression of CD1a [38]. This population could be matured into $CD83^{+}$DCs in the presence of anti-CD40 mAbs and LPS plus IFN-γ, but it remained $CD1a^{-}$ and lacked IL-12 production even upon maturation. The lack of IL-12 and CD1a expression by these cells did not affect their APC capacity. However, while the conventional mDC1s strongly favored Th1 differentiation, the novel subset directed differentiation of Th0/Th2 cells when cocultured with purified human peripheral blood T cells, further indicating functional differences between the two subsets of mDC1 [38].

Martin et al. reported a new $B220^{+}$ subpopulation of immature-like human DCs ($B220^{+}$DCs) with low levels of expression of major histocompatibility complex (MHC) and costimulatory molecules and markedly reduced T-cell stimulatory potential, located in the thymus, bone marrow, spleen, and lymph nodes. $B220^{+}$ DCs display ultrastructural characteristics resembling those of human plasmacytoid cells and accordingly produce IFN-α after virus stimulation. $B220^{+}$DCs acquired a strong APC capacity upon incubation with CpG ODN, concomitant with a strong upregulation of MHC and costimulatory molecules and the production of IL-12 and IL-10. The unstimulated $B220^{+}$DCs may represent a subset of physiological tolerogenic DCs endowed with the capacity to induce a nonanergic state of T-cell unresponsiveness, involving the differentiation of T regulatory cells capable of suppressing antigen-specific T-cell proliferation [39].

In mice, DCs express CD11c molecules on the cell surface. DCs had been previously subdivided on the basis of CD4 and CD8α expression [40]. Several studies suggested that CD8α$^+$ DCs preferentially induce Th1 responses, whereas CD8$^-$ DCs induce Th2 development. Using a prototypic Th1-inducing adjuvant, heat-killed *Brucella abortus* (HKBA), to assess stimulation of murine DC subsets and the relationship between Ag burden and IL-12 production, Huang et al. showed that DCs were the sole producers of IL-12, although most HKBA uptake was by splenic macrophages and granulocytes. However, more CD8α$^-$ than CD8α$^+$ DCs produced IL-12 after HKBA challenge, whereas only CD8α$^+$ DCs produced IL-12 in response to challenge with another Th1-promoting antigen, soluble *Toxoplasma gondii* Ags [41]. These findings challenge the notion that CD8α$^+$ and CD8α$^-$ DCs are destined to selectively induce Th1 or Th2 responses, respectively. They suggest that the nature of the stimulating substance is important in determining which DC subsets are activated to produce IL-12. Recently, murine DCs were re-categorized with respect to function, especially in terms of cytokine production and *in vitro* T cell allo-proliferation activity. Similar to the functional classification for human DCs, murine DCs are now classified as either DCs that possess high T cell allostimulatory activity and produce high levels of IL-12 (similar to human DC1), or as plasmacytoid DCs (pDCs) that have low T cell allostimulatory activity and produce high levels of IFN-α or IL-12 following viral or CpG challenge [42,43].

Henri et al. reported a hierarchy of susceptibility of murine splenic DC subsets to infection by *Leishmania major* and an inverse relationship to IL-12 production [44]. CD4$^+$ CD8α$^-$ DCs are the most permissive host cells for *L. major* amastigotes, followed by CD4$^-$ CD8α$^-$ DCs; CD4$^-$CD8α$^+$ DCs are the least permissive. However, the least susceptible CD4$^-$ CD8α$^+$ DC subset was the best IL-12 producer in response to *L. major* infection.

Three populations of DCs have been identified in the murine Peyer's patch (PP). CD11b$^+$ CD8α$^-$ (myeloid) DCs are localized in the subepithelial dome, CD11b$^-$ CD8α$^+$ (lymphoid) DCs in the interfollicular regions, and CD11b$^-$ CD8α$^-$ (double-negative; DN) DCs at both sites [45]. Furthermore, Iwasaki and Kelsall described the presence of a novel population of intraepithelial DN DCs within the follicle-associated epithelium and demonstrated a predominance of DN DCs only in mucosal lymphoid tissues [46]. All DC subpopulations maintain their surface phenotype upon maturation in vitro, and secrete a distinct pattern of cytokines upon exposure to T cell and microbial stimuli. In an effort to understand the functional relevance of the three DC subsets, these researchers purified DC from spleen and PP, and stimulated them in vitro either through CD40 cross-linking (to mimic a mature T cell stimulus) or with *Staphylococcus aureus* (a microbial stimulus). Only myeloid DCs from the PP produced high levels of IL-10 upon stimulation with soluble CD40L trimer, or *Staphylococcus aureus*. In contrast, lymphoid and DN, but not myeloid DCs, produce IL-12 p70 following microbial stimulation, whereas no DC subset produces IL-12 p70 in response to CD40 ligand trimer. These findings thus suggest that DC subsets within mucosal tissues have unique immune inductive capacities.

Edwards et al demonstrated that microbial and T cell-derived stimuli can synergize to induce production of high levels of IL-12 p70 or IL-10 by individual murine DC subsets, but that the choice of cytokine is dictated by the microbial pattern recognition receptor engaged [47]. Bacterial components such as CpG-containing DNA or extracts from *Mycobacterium tuberculosis* predispose CD8α$^+$ and CD8α$^-$ CD4$^-$ DCs to make IL-12 p70. In contrast, exposing CD8α$^+$, CD4$^+$ and CD8α$^-$CD4$^-$ DCs to heat-killed yeasts leads to production of IL-10. In both cases, secretion of high levels of cytokine requires a second signal from T cells, which can be replaced by CD40L. Moreover, *M. tuberculosis* extracts promote IL-12 production primarily via the Toll-like receptor 2 (TLR2)- and MyD88-dependent pathway, whereas heat-killed yeasts activate DCs via a TLR2-, MyD88-, and Toll/IL-1R domain-containing protein-independent pathway. This study suggests the notion that T cell feedback amplifies innate signals for cytokine production by DCs and that pattern recognition rather than ontogeny determines the production of cytokines by individual DC subsets.

Murine thymic DC subsets are comprised of two subpopulations based on CD8α chain expression (CD8α^+ and CD8α^- DCs). These thymic DCs synthesize IL-12 p70 when stimulated with a combination of lipopolysaccharide (LPS), anti-CD40 monoclonal antibody (mAb), GM-CSF, and IFN-γ [48]. Okada et al. further reported the identification of a heterogeneous murine thymic cell subset expressing CD11c and B220 (CD45R), following the previous identification of the population of CD11c$^+$ B220$^-$ DCs [49]. The CD11c$^+$ B220$^+$ subset expresses Ly6C^{high} and MHC class II^{low} in contrast with the CD11c$^+$ B220$^-$ subset. Freshly isolated thymic CD11c$^+$ B220$^+$ cells show typical plasmacytoid morphology and differentiate to mature DCs in vitro upon stimulation with CpG ODN 2216 (TLR9 ligands); they are thus termed thymic plasmacytoid DCs (pDCs) [49]. These thymic pDCs are highly sensitive to spontaneous apoptosis in vitro and induce low T cell allo-proliferation activity. Thymic pDCs express low TLR2, TLR3 and TLR4 mRNA, and high TLR7 and TLR9 mRNA. Thymic pDCs also produce high amounts of IFN-α following culture with CpG ODN 2216 as compared with the CD11c$^+$ B220$^-$ thymic DC lineage, which expresses low TLR9 mRNA and produces high IL-12p40 with CpG ODN 2216.

When monocytes differentiate into DCs, their ability to respond to different commensal bacteria dramatically changes, and they become unresponsive to probiotic gram-positive bacteria. Karlsson et al. demonstrated this by stimulating purified human monocytes and monocyte-derived DCs with UV-inactivated Gram-positive (*Lactobacillus plantarum* and *Bifidobacterium adolescentis*) and Gram-negative (*Escherichia coli* and *Veillonella parvula*) bacterial strains that are normal gastrointestinal bacterial flora. Monocytes produced higher levels of IL-12 p70 in response to *L. plantarum* than in response to *E. coli* and *V. parvula*. In contrast, DCs secreted large amounts of IL-12 p70 in response to *E. coli* and *V. parvula* but were practically unresponsive to *L. plantarum* and *B. adolescentis*. The lack of a response to the Gram-positive strains correlated with lower surface expression of TLR2 on DCs than on monocytes [50].

The immunobiology of IL-12 is briefly summarized in Fig 1.

III. IL-12 Receptors and Signaling

IL-12 receptors, IL-12Rβ1 (low affinity, Kd = 2–6 nM, 1000–5000 sites per cell) and β2 (high affinity, Kd = 5–20 pM, 100–1000 sites per cell) chains, are primarily expressed on activated T and NK cells [51]. Both of these subunits have extensive homology with gp130, the common receptor β chain of the IL-6-like cytokine superfamily. Using flow cytometry, Vogel et al. showed that freshly isolated murine peritoneal B-1 and conventional B lymphocytes bound IL-12, but splenic B cells failed to react unless first stimulated with LPS. All murine B cell sources were found to express the IL-12Rβ1 subunit transcripts. IL-12 binding was also detected on *S. aureus*/IL-2-stimulated B cell blasts but not on freshly isolated peripheral blood lymphocytes [52]. In human primary B cells, Durali et al. found a functional IL-12 receptor (IL-12R) that internalizes following IL-12 binding. IFN-γ and, to a lesser extent, IL-12 positively regulated the IL-12Rβ2 subunit but had no effect on IL-12Rβ1. IL-12 induced the phosphorylation and nuclear translocation of STAT4 while having no direct effect on STAT1 activation or T-bet (T-box expressed in T cells) expression in primary B cells [53]. These findings indicate that B cells represent another major target for IL-12 in addition to T and NK cells, and that IL-12 can directly affect humoral immunity.

Signal transduction through IL-12R induces tyrosine phosphorylation of primarily the Janus family kinases JAK2 and TYK2 [54], which in turn phosphorylate and activate STAT4 [55]. In addition to tyrosine phosphorylation, it has been demonstrated that STAT4 is phosphorylated on serine residue in response to IL-12 [56]. The IL-12-dependent STAT4 serine phosphorylation is mediated by stimulation of p38 mitogen-activated protein kinase (MAPK) through its upstream activators, MAPK kinase (MKK) 3/6, growth arrest and DNA damage inducible (GADD)45-β and -γ [56,57]. It is not mediated by stimulation of extracellular signal-regulated kinases (ERK) 1/2 or c-Jun N-terminal kinase (JNK). Serine phosphorylation is required for full transcriptional activity of STAT4 and IFN-γ production, but not for proliferation. Moreover, the phosphatidylinositol 3-kinase (PI3K)/Akt pathway has been demonstrated to be activated by IL-12, and plays an important role in proliferation, but not in IFN-γ production [58]. Using a yeast two-hybrid screening, Yoshimoto et al. identified mouse sphingosine kinase 2 (SPHK2) as a molecule associated with the mouse IL-12Rβ1 cytoplasmic region [59]. SPHK is a key enzyme catalyzing phosphorylation of sphingosine to form sphingosine 1-phosphate (S1P), an important lipid messenger that is implicated in the regulation of a wide variety of important cellular events, including cell growth, survival, motility, cytoskeletal changes, and the release of calcium from intracellular stores [60]. Reciprocal mutagenesis analyses of the two interacting molecules revealed that the region including the proline-rich domain in SPHK2 is probably responsible for the binding to IL-12Rβ1, while the regions including the carboxyl terminus and Box II in the IL-12Rβ1 cytoplasmic region appear to be involved in the binding to SPHK2. Transient expression of wild-type SPHK2 in T cell hybridoma augmented IL-12-induced STAT4-mediated transcriptional activation. Ectopic expression of dominant-negative SPHK2 in Th1 cell clone significantly reduced IL-12-induced IFN-γ production, while that of wild-type SPHK2 enhanced it. In contrast, the expression of dominant-negative SPHK2 minimally affected IL-12-induced proliferation. A similar decrease in IL-12-induced IFN-γ production was observed when dominant-negative SPHK2 was expressed in activated primary T cells using a retroviral expression system [59].

During IL-12-induced signaling, activated STAT4 is recruited to phosphorylated Tyr-800 in the human IL-12Rβ2. Yamamoto et al. demonstrated that suppressor of cytokine signaling-3 (SOCS-3) is also recruited to IL-12Rβ2 by the interaction involving the SOCS-3 SH2 domain and phosphorylated Tyr-800 in IL-12Rβ2 [61]. Furthermore, SOCS-3, but not its SH2 domain-defective mutant, inhibited the IL-12-induced activation of DNA-binding and transcriptional activities of STAT4 [61]. These results suggest that SOCS-3, expressed at high levels in Th2 cells, plays an inhibitory role in STAT4-mediated IL-12 signaling by binding to the STAT4 docking site in IL-12Rβ2.

To note, the newly discovered IL-12-related cytokine IL-27 is reportedly able to set the early stage of Th1 differentiation by potently inducing the expression of the major Th1-specific transcription factor T-bet and its downstream target IL-12Rβ2 independently of IFN-γ, thus conferring upon the naïve T cells responsiveness to IL-12 [62].

IV. Immunological Activities of IL-12

IV.1. IL-12 in infectious diseases

IL-12 is produced by phagocytic APCs in response to intracellular bacterial and parasitic infections. The importance of IL-12 in host resistance to infectious agents is underscored by IL-12Rβ1 expression deficiency found in otherwise healthy individuals highly susceptible to mycobacterial [63] and *Salmonella* [64] infections. IL-12 p40 expression deficiency in a young female patient was associated with recurrent episodes of *pneumococcal pneumonia* with sepsis and other infections in the absence of fevers [65]. Because of the shared cytokine chain of IL-12 p40 and the β1 chain of the receptor between IL-12 and IL-23, it is difficult to discern the relative importance of IL-12 and IL-23 in host defense against intracellular microbes. Although the relative roles of IL-12 and IFN-γ in Th1-cell priming may be to a significant extent pathogen-dependent, in most infections IL-12 regulates the magnitude of the IFN-γ response at the initiation of infection, thus potentiating natural resistance, favoring Th1-cell development, and inhibiting Th2 responses. Treatment of animals with IL-12, either alone or as a vaccine adjuvant, has been shown to prevent diseases caused by many of the same infectious agents, by stimulating innate resistance or promoting specific reactivity [66].

IV.2. IL-12 in malignant diseases

Recombinant human IL-12 has been studied as a single agent for systemic treatment of various types of cancer in patients. Following the first phase II clinical trial that unexpected resulted in severe toxicity and deaths [67], improvements have been made with respect to the insertion of a treatment-free period of one week after the first administration of IL-12, conforming with most phase I studies, which reduced toxicity of subsequent injections [68,69]. However, two recent phase II studies performed in patients with advanced renal cell and ovarian cancer yielded disappointing results, with overall response rates of only 7% and 4%, respectively [70,71]. The lack of efficacy in these studies is postulated to be due to endogenous IL-10 production which occurs at relatively high dose levels of IL-12, countering the biological effects of IL-12. However, a strong CTL response was observed in patients with advanced melanoma after IL-12 administration. The number of tumor-specific CTL increased in the circulation, and influx of specific memory $CD8^+$ T cells into metastasized lesions was demonstrated [72].

Alternatively, greater efficacies have been achieved using IL-12 in different types of cancer vaccines as an adjuvant. Many animal studies have shown that IL-12 plus tumor vaccine was more effective and less toxic than either component alone [73–79]. The effects of IL-12 as a vaccine adjuvant are believed to be related to its ability to induce multiple inflammatory cytokines such as GM-CSF, TNF-α, IL-8, IL-6, IL-15 and IL-18 [80,81], and to activate NK cells [82], to enhance the function of DC such as their maturation and antigen presentation [83], and to prime naïve T cells. The use of IL-12 can also induce tumor-specific humoral immunity. In evaluating the efficacy of DC-based and/or IL-12 gene-based therapy in the treatment of 38C13 B cell lymphoma using a hydrodynamic transfection-based technique to deliver a high and persistent level of IL-12 from a plasmid encoding IL-12 (pIL-12), it was found that either treatment alone was ineffective. However, a combined treatment induced 100% long-term survival [84]. Furthermore, a long-lasting anti-tumor immunity was induced in the mice which resisted further tumor challenge 58 days after initial inoculation. The surviving mice showed a strong IFN-γ-producing Th cell response and humoral antibody response, without detectable CTLs. The antibody from the immune sera mediated a complement-dependent lysis of tumor cells that was tumor specific. Furthermore, immunization of mice with DC-based vaccine and pIL-12 treatment elicited higher levels of anti-idiotype IgG titer and an enhanced IgG2a response which increased the efficacy in mediating 38C13 tumor lysis [84].

A phase II human study of immunization with Melan-A peptide-pulsed PBMC plus recombinant human IL-12 was conducted in 20 patients with advanced melanoma (who had received prior therapy and had visceral metastases). Two patients achieved a complete response, five patients achieved a minor or mixed response, and four patients had stable disease. There were no grade 3 or 4 toxicities. There was a significant increase after vaccination in Melan-A-specific $CD8^+$ T-cell responses by IFN-γ production. There was a correlation between the magnitude of the increase in Melan-A-specific T cells and clinical response [85]. Evidence that IL-12 may increase the immune response was also obtained in a phase I study involving 48 patients with high-risk resected stage III or IV melanoma, who were immunized with melanoma-specific peptides with or without a low dose of IL-12 [86]. These data suggest that even in advanced cancer patients, IL-12 can stimulate antigen-specific immune responses and supporting further development of IL-12 as a vaccine adjuvant.

IFN-γ mediates most of the well-known immunological activities of IL-12. Shi et al. recently reported IFN-γ-independent activities induced by therapeutic application of recombinant IL-12 in restricting tumor growth and metastasis in the 4T1 murine mammary carcinoma model [87]. IFN-γ-deficient mice carrying 4T1 tumor exhibited no gross defect in the number of tumor-infiltrating lymphocytes but have exaggerated angiogenesis in the tumor. Administration of IL-12 significantly restricted neoangiogenesis in the tumor in the absence of IFN-γ, and retained certain therapeutic efficacy even when applied late during tumor progression. IL-12 exposure in vivo did not irreversibly modulate the immunogenicity of the tumor. A global gene expression analysis of primary tumors revealed interesting IL-12-induced molecular patterns and changes, implicating a number of novel genes potentially important for IFN-γ-independent immune responses against the tumor, as IL-12-mediated anti-proliferation, anti-metastasis, and anti-angiogenesis activities [87].

IV.3. IL-12 in autoimmune diseases

The property of IL-12 to strongly promote the development of Th1 cells is a double-edged sword. It endows IL-12 with the ability to orchestrate host defense against intracellular infectious agents and malignant growth on the one hand, and the possibility to cause or exacerbate inflammatory cell-mediated diseases such as autoimmune disorders on the other. IL-12-mediated inflammatory pathogenesis has been reported in such autoimmune diseases as inflammatory bowel disease (IBD), collagen-induced arthritis (CIA), and insulin-dependent diabetes mellitus (IDDM). IL-12 was for many years thought to be responsible for the T cell-mediated pathogenesis in experimental autoimmune encephalomyelitis (EAE) until recently when IL-23 was shown to be the “culprit” [21]. IBD, including Crohn’s disease (CD) and ulcerative colitis (UC), is a chronic inflammatory disease that causes destruction of gastrointestinal tissue. It is characterized by an exaggerated immune response. In CD, IL-12 plays a pivotal role in driving increased expression of Th1 cytokines and the associated pathology [88].

Mutations in *CARD15*, which encodes nucleotide-binding oligomerization domain 2 (NOD2), underlie the occurrence of intestinal inflammatory disease in a substantial subgroup of patients with Crohn’s disease [89]. NOD2 is a member of the NOD-leucine-rich repeat (LRR) protein family, whose members share a tripartite domain structure consisting of a C-terminal peptide recognition (LRR) domain, a central NOD domain, and an N terminus made up of protein-protein interaction domains, such as caspase recruitment domains (CARDs) or pyrin domains [90]. NOD2 is expressed intracellularly in APCs [91] and through its C-terminal LRR it allows these cells to recognize and react to a component of bacterial peptidoglycan (PGN), muramyl dipeptide (MDP) [92,93]. Watanabe et al. used *Card15*$^{-/-}$ mice to show that intact NOD2 signaling inhibited TLR2-driven activation of NFκB, particularly of the NFκB subunit c-Rel. Moreover, NOD2 deficiency or the presence of a CD-like Card15 mutation increased Toll-like receptor TLR2-mediated activation of NFκB-c-Rel, IL-12 production, and enhanced Th1 responses [94].

CIA is an experimental autoimmune model for human rheumatoid arthritis. Administration of an anti-IL-12 antibody significantly reduces the symptoms of arthritis and abrogates the humoral and Th1 cytokine response to the autoantigen collagen type II [95]. Paradoxically, these effects are observed in IFN-γ receptor-null mice [95], suggesting that endogenous IL-12 can promote Th1 and disease pathology through a pathway independent of IFN-γ production. In the insulin-dependent diabetes mellitus (IDDM) model, endogenous IL-12 appears to be inessential for spontaneous development of the disease, since IL-12 p40-null non-obese diabetic (NOD) mice developed IDDM and insulitis as well as wild type mice [96]. However, Th1 development in peripheral tissues, but not in the inflamed pancreas, was impaired in IL-12 p40-knockout NOD mice. Moreover, the pancreas-infiltrating T cells in NOD mice treated with IL-12 antagonists were skewed to a Th2 phenotype and the treated mice were protected from IDDM [96]. Interestingly, a similar pattern of pathogenic and protective effects of IFN-γ was seen in this model [97].

V. Receptor-mediated Regulation of IL-12 Gene Expression

V1. Induction of IL-12 production via Toll-like receptors (TLRs)

Microbial recognition and differentiation are mediated in part by pattern recognition receptors (PRRs). The Toll-like receptor (TLR) family is the best characterized class of PRRs in mammals. There are an estimated 10–15 TLRs in different mammalian species [98]. TLRs detect multiple pathogen-associated molecular patterns (PAMPs), including LPS (by TLR4), bacterial lipoproteins and lipoteichoic acids (LTA) (by TLR2), flagellin (by TLR5), the unmethylated CpG DNA of bacteria and viruses (by TLR9), double-stranded RNA (by TLR3), and single-stranded viral RNA (by TLR7) [99–101]. Qi et al. evaluated the role of TLR4 and TLR2 in the induction of IL-12 and IL-10 by their respective ligands, LPS, PGN and yeast zymosan, respectively, in bone marrow-derived mouse DCs. LPS induced low-levels of IL-10 but high-levels IL-12 p70 production. In contrast, DCs exposed to PGN produced low levels of IL-12 but high levels of IL-10. Zymosan-exposed DCs produced high levels of both IL-10 and IL-12 [102]. This observation suggests that LPS, PGN, and zymosan have inherently distinct abilities to induce DC IL-10 and IL-12 production. Alternatively, this phenomenon may reflect different sensitivities of DCs to these microbial stimuli. In addition, TLR2 functions by heterodimerizing with TLR1 and TLR6 in order to recognize ligands [103]. Lore et al. analyzed the effects of different TLR ligands to enhance immune responses induced by human APCs, including $CD123^+$ plasmacytoid DCs (PDCs), $CD11c^+$ myeloid DCs (MDCs), monocytes, and B cells. PDCs, which express TLR7 and TLR9, responded to imidazoquinolines (imiquimod and R-848, synthetic ligands for TLR7) and to CpG-ODN (for TLR9) stimulation, resulting in enhancement in expression of costimulatory molecules and induction of IFN-α and IL-12 p70. In contrast, MDCs, which express TLR3, TLR4, and TLR7, responded to poly(I:C), LPS, and imidazoquinolines with phenotypic maturation and high production of IL-12 p70 without producing detectable IFN-α [104]. Agrawal and coworkers demonstrated that *E. coli* flagellin, which engages TLR5, triggers immature human monocyte-derived DCs to stimulate Th1 responses via IL-12 p70 production in a manner that depends on the phosphorylation of p38 and JNK 1/2 [105].

V2. Induction of IL-12 production viaCD40

In addition to the innate pathway of induction of its synthesis through TLRs, IL-12 production by macrophages or DCs can also be induced during a memory immune response via contact with activated T cells by the CD40/CD40L interaction. Kennedy et al. first showed that the expression of CD40L by activated T cells is critical for T cell-dependent IL-12 production by mouse macrophages [106]. However, Padigel et al. recently reported that $CD40L^{-/-}$ mice could control *Leishmania major* infection when inoculated with low numbers of parasites and that cells from these mice produce IL-12 [107,108]. Moreover, in vivo treatment with a TNF-related activation-induced cytokine (TRANCE, also known as RANK-L)-receptor fusion protein in $CD40L^{-/-}$ mice results in a decrease in the number of IL-12-producing cells as well as a shift from a dominant Th1 to Th2 type response in infected mice [109]. The interaction of TRANCE and its receptor RANK (TRANCE-R) is important for bone remodeling and essential in the development and activation of osteoclasts [110]. TRANCE, expressed on activated T cells, can induce IL-12 production via its interaction on Dcs with RANK, and also enhance DC survival [111]. These results demonstrate that in $CD40L^{-/-}$ mice the TRANCE-RANK costimulatory pathway is alternatively used to promote IL-12 production and the activation of a protective Th1 type response. Yu et al. studied the role of three signaling pathways, p38MAPK, ERK, and PI3K, in CD40L-induced monocyte-derived DC activation, survival, and expansion of virus-specific $CD8^+$ T cell responses. The study showed that the p38MAPK pathway was critical for CD40L-mediated up-regulation of CD83, a marker of DC maturation, and that CD40L-induced monocyte-derived DC IL-12 production is mediated by both the p38MAPK and PI3K pathways [112]. Paradoxically, IL-10 is also induced by the CD40/CD40L interaction in macrophages. How CD40 signaling regulates the secretion of these counteractive cytokines is the subject of another study, which showed that weak CD40 signals induce ERK-1/2-dependent IL-10 expression, whereas stronger signals induce p38MAPK-dependent IL-12 production [113].

V3. CCR5-mediated induction of IL-12 production in dendritic cells

The activation of DCs to produce IL-12 is thought to be a key step in the initiation of cell-mediated immunity to intracellular pathogens. Aliberti et al. first showed that chemokines were rapidly induced in the spleen of mice injected with soluble toxoplasma antigen (STAg) of tachyzoites of *Toxoplasma gondii* [114]. Ligation of the C-C chemokine receptor (CCR) 5 can provide a major signal for the induction of IL-12 synthesis by the $CD8^+$ subset of DC by *T. gondii* and this pathway appears to be important in establishing interferon-dependent resistance to the parasite. Purification of the STAg extract showed that cyclophilin-18 (C-18) was its principal component [115]. Antibodies generated against recombinant C-18 inhibited STAg-induced synthesis of IL-12. Recombinant C-18 showed high affinity for and triggered cell signaling through CCR5. These findings suggest that the unusual potency of *T. gondii* in inducing IL-12 from DCs results from its synthesis of a unique chemokine mimic that signals through CCR5. The ability to generate this strong protective response may benefit parasite transmission by preventing the protozoan from overwhelming its intermediate hosts.

However, a seemingly opposite role of CCR5 in the regulation of IL-12 was observed in an oral tolerance experimental autoimmune encephalomyelitis (EAE) mouse model [116]. DePaolo et al. showed that two CCR5 ligands, CCL4 and CCL5, are expressed in gut tissues after feeding of the antigen myelin oligodendrocyte glycoprotein (MOG). $CCR5^{-/-}$ mice were unable to be tolerized by feeding a high dose of MOG and were not protected from developing EAE. Moreover, $CCR5^{-/-}$ mice fed with MOG displayed higher IL-12 production in the intestinal mucosa compared to the wild type mice. A selective CCR5 antagonist, methionine (Met)-RANTES inhibited CCL2 expression, resulted in enhanced IL-12 production and the inability for mice treated with Met-RANTES to become orally tolerized [116]. This study suggests that CCR5 ligands may function inhibit IL-12 levels in the gut after Ag feeding, promoting a cellular environment that favors tolerance rather than immunity.

V4. IFN-γ priming for IL-12 production

IL-12 production in primary monocytes is strongly dependent on the activation state of the cells. IFN-γ provides a powerful stimulation signal for monocytes to become activated macrophages and bactericidal with a much enhanced potential to produce IL-12, although it alone does not induce IL-12 p40 gene expression. This is referred to as the “priming” effect of IFN-γ on monocytes or monocytic cell lines [117]. IFN-γ’s enhancing effect is likely mediated through activation of some members of the Interferon Regulatory Factor (IRF) family induced by IFN-γ, particularly IRF-1 and Interferon Consensus Sequence Binding Protein (ICSBP or IRF-8) that interact directly with the IL-12 p40 and p35 promoters in a synergistic manner [118,119]. The priming effect of IFN-γ for augmented IL-12 production may represent a mechanism by which a robust IL-12-induced Th1 response is invoked and sustained in vivo.

V5. Complement receptor-mediated inhibition of IL-12 production

Marth and Kelsall reported that cross-linking of complement receptor (CR) 3 (also known as CD11b or Mac-1) with antibody or certain particulate ligands (including particles coated with the complement C3 activation product iC3b) inhibits IL-12 production by both murine and human monocytes/macrophages with little if any down regulation of the production of other proinflammatory cytokines or chemokines [120]. On the other hand, ligation of iC3b to CR3 on antigen-presenting cells leads to the sequential production of TGF-β2 and IL-10, which is essential for the induction of tolerance in an immune-privileged site after intraocular antigen injection [121]. IL-10, and possibly TGF-β, can inhibit IL-12 production in an autocrine manner. The ability of CR ligation to specifically inhibit IL-12 production suggests that complement activation products can directly regulate the type of immune response through interaction with APCs.

V6. Fcγ receptor-mediated inhibition of IL-12 production

Ligating Fcγ receptors (which bind to the Fc portion of IgG often complexed with an immunogen) on macrophages by immune complexes (IC) results in profound and selective suppression of pathogen-activated IL-12 production [122–124]. Cross-linking FcγRI, -II, and -III inhibited IL-12 p70 production in monocytes, whether stimulated by *Staphylococcal* enterotoxin B or LPS. Inhibition of IL-12 by FcγR cross-linking was not mediated by TNF-α, as the presence of an anti-TNF-α Ab could not restore the reduced IL-12 production [125]. However, the inhibitory effect of ICs on IL-12 p40 production can be converted into a stimulatory one when heat-inactivated normal human serum (NHS) devoid of a functionally intact complement system was used [126]. The effect was seen only for IL-12 p40, as production of IL-6 and IL-10 is stimulated by immune aggregates (IA), consisting of heat-aggregated gamma globulin (HAGG) as model IC, in the context of native NHS, whereas the effect was abolished in heat-inactivated NHS [126]. IA-induced IL-12p40 production in a C4 deficient serum was lowered by addition of C4, and addition of the peptide compstatin, which blocks C3 activation and mimicked the effects of heat inactivation on IL-12p40 levels. IA-induced production of IL-10 was partially blocked by anti-Fcγ RII antibodies, whereas Fcγ R or CR blockade had no effect on IL-12p40 production [126]. Since IC and local or systemic complement activation characterize rheumatoid arthritis, systemic lupus erythematosus and many malignancies, different and complement-dependent effects on the production of IL-10 and IL-12 could be of importance in these diseases, where control of the complement system might be a way to direct IC-induced cytokine production in either a type 1 or type 2 direction.

V7. CD47-mediated inhibition of IL-12 production

Thrombospondin 1 (TSP) elicits potent anti-inflammatory activities in vivo, as evidenced by persistent, multi-organ inflammation in TSP-null mice. TSP is believed to be the natural ligand of CD47 antigen, also named integrin-associated protein (IAP) [127], which transiently accumulates at the inflammatory site. Engagement of CD47 by anti-CD47 monoclonal antibodies, by TSP, or by 4N1K, a peptide of the COOH-terminal domain of TSP selectively binding CD47, inhibits IL-12 release by monocytes and DCs [128,129]. Furthermore, CD47 ligation selectively inhibits the development of human naive T cells into Th1 effectors in the presence of exogenous IL-12, suggesting that it also interferes with IL-12 downstream signaling [130]. Human monocyte-derived immature DCs spontaneously produce TSP, which is strongly enhanced by prostaglandin (PG)E2 and to a lesser extent by TGF-β, two soluble mediators secreted by macrophages after engulfment of damaged tissues [131]. Activation of DCs by microbial stimuli increases TSP production. The endogenous TSP produced during early DC activation negatively regulates IL-12, TNF-α, and IL-10 release through its interactions with CD47 and CD36 [131]. DC-derived TSP thus may serve as a negative regulator that contributes to arrest of cytokine production, active resolution of inflammation, and maintenance of homeostasis.

V8. IL-10-mediated inhibition of IL-12 production

IL-10 is a major macrophage-deactivating and immunosuppressive cytokine. It is a critical component in the maintenance of the fine balance between swift and potent immune responses against invading pathogens on the one hand, and the control of detrimental systemic inflammation on the other. IL-10 is a potent inhibitor of IL-12 production in accessory cells, which occurs primarily at the level of transcription of the IL-12 p40 gene [132]. However, the transcriptional mechanism whereby IL-10 inhibits IL-12 p40 expression has remained un-established. Recently, Cao et al. reported that IL-10 and c-musculoaponeurotic fibrosarcoma (Maf) induce their mutual expression in inflammatory macrophages [133]. They demonstrated that c-Maf, an essential transcription factor for development [134] and IL-4 gene expression in Th2 differentiation [135], is also a physiological mediator of IL-10's immunosuppressive activities in macrophages. When overexpressed, c-Maf selectively inhibits transcriptional activation of IL-12 p40 and p35 genes while potently activating IL-10 and IL-4 expression, potentially contributing to the development of a state of anti-inflammation and dichotomy of immunologic polarization [133]. c-Maf induces changes in nuclear DNA-binding activities at multiple sites including the ets (E26), GA-12, NF-κB, C/EBP, and AP-1 elements. Nonetheless, the essential c-Maf-responsive element appears to be located elsewhere. Inhibition of IL-12 p40 gene expression by c-Maf requires the N-terminal transactivation domain, suggesting an indirect mechanism of transcriptional inhibition involving the induction of an unidentified repressor. In c-Maf-deficient murine macrophages, IL-10 production is impaired. However, IL-10-mediated inhibition of IL-12 production remains intact, indicating the existence of alternative mediators in the absence of c-Maf [133].

V9. Effects of type I interferons on IL-12 production

Type I interferons (IFN-α/β) are potent antiviral and immunoregulatory cytokines. Although first noted for their ability to inhibit viral replication, type I interferons are also known to exert multiple immunoregulatory effects on NK and T cells [136], with some of the functions overlapping those of IL-12. Certain viral infections induce IL-12 to elicit NK cell IFN-γ production and antiviral mechanisms. However, high levels of IFN-α/β are dominant in the context of viral infections and act to regulate other innate responses, including induction of NK cell proliferation in vivo and overall negative regulation of IL-12 production [137]. Byrnes et al. showed that type I IFNs are potent inhibitors of IL-12 production by pathogen-activated human monocytes/macrophages. The underlying mechanism involves transcriptional inhibition of the IL-12 p40 gene, marked by downregulation of PU.1 binding activity at the upstream Ets site of the IL-12p40 promoter [138]. However, in a separate study, Heystek et al. investigated the direct effect of IFN-α/β on monocyte-derived DCs at different stages of development, and found that IFN-α/β enhanced IL-12 p70 production by immature DCs but inhibited IL-12p70 production by mature DCs [139]. Interestingly, IFN-α/β strongly counteracted the IL-12-enhancing effect of IFN-γ on DCs regardless of their maturation status [139]. The differential modulatory effect of IFN-α/β on the IL-12-producing capacity of DCs and their cross-regulatory effect on IFN-γ may reduce inflammatory processes. The IL-12-enhancing effect of IFN-α on DCs was corroborated in an independent study where double-stranded RNA was used to induce DC maturation and cytokine production. IFN-α enhanced the production of IL-12 and TNF-α induced by double-stranded RNA but had no effect on IL-10 production [140].

V10. Adrenoceptors-mediated regulation of IL-12 production

Catecholamines are a class of endogenous mediators that may potentially direct the responsiveness of macrophages through α- or β-adrenoceptors. Expression of α_2- and β-adrenoceptors on macrophages can be activated by the endogenous ligand norepinephrine and by adrenergic drugs frequently used in clinical practice [141,142]. Stimulation of these receptors affects lymphocyte trafficking [143], migration [144] and proliferation [145]. They also modulate cytokine production and the functional activity of different lymphoid cells [146]. Accumulative evidence indicates that agents such as catecholamines that stimulate the β_2-adrendoreceptor-cAMP-protein A pathway inhibit the production of type 1/proinflammatory cytokines such as IL-12, TNF-α and IFN-γ by APCs or Th1 cells [147–149]. In contrast, the same compounds stimulate the production of type 2/anti-inflammatory cytokines such as IL-10 and TGF-β [150]. Stimulation of cells via the α_2-adrenoceptor by agonists such as clonidine, guanfacine, and oxymetazoline, however, significantly induced IL-12 p40 and p70 production by macrophages in a PKC- and p38MAPK-dependent manner [151].

V11. Apoptotic cell-mediated inhibition of IL-12 production during macrophage phagocytosis

The elimination of apoptotic cells and cell bodies by phagocytes represents an evolutionarily conserved means to prevent exposure of surrounding tissue to potentially cytotoxic, immunogenic, or inflammatory cellular contents [152]. Resolution of inflammation depends not only on the effective removal of apoptotic cells but also on active suppression of inflammatory mediator production. Aberrations in either mechanism are associated with chronic inflammatory conditions and autoimmune disorders [153]. Cytokines play significant roles in the etiology and pathology of many autoimmune diseases. The uptake of apoptotic cells by phagocytes is thought to suppress autoimmune responses in part through the release of IL-10, TGF-β, platelet activating factor (PAF), and PGE2, and inhibition of TNF-α, GM-CSF, IL-12, IL-1β, and IL-18 production [154]. Suppression of the production of inflammatory cytokines such as IL-12 during the clearance of dead cells by professional phagocytes is a critical mechanism to generate a tolerant state in the immune system to autoantigens [155]. Recently, Kim et al. explored how apoptotic cell-derived signals regulate IL-12 gene expression [156]. They demonstrated that cell-cell contact with apoptotic cells is sufficient to induce profound inhibition of IL-12 production by activated macrophages. The cell membrane lipid molecule phosphatidylserine (PS), which becomes externalized during apoptosis and which serves as a critical recognition molecule on apoptotic cells for clearance by phagocytes, could mimic the inhibitory effect. The inhibition does not involve autocrine or paracrine actions of IL-10 and TGF-β. These researchers identified a novel zinc finger nuclear factor, named GC-binding protein (GC-BP), that is induced following phagocytosis of apoptotic cells by macrophages or by treatment with PS. GC-BP selectively inhibits IL-12 p35 gene transcription by binding to its promoter in vitro and in vivo, thus decreasing IL-12 production. GC-BP itself undergoes functionally significant tyrosine dephosphorylation in response to apoptotic cells. These findings significantly enhance the understanding of an essential physiological process in which cytokine responses are tightly regulated, with implications in the development and pathogenesis of inflammatory and autoimmune diseases.

V12. Other G protein coupled receptor-mediated inhibition of IL-12 production

PGE2 is one of the major immunosuppressive factors derived from many cell types including macrophages and some tumors. Mitsuhashi et al. reported that murine mammary carcinoma-derived PGE2 potently inhibits the production of endogenous IL-12 at the level of protein secretion, mRNA synthesis, and transcription of the constituent p40 and p35 genes. The inhibition can be reversed by NS-398, an inhibitor of the enzymatic activity of cyclooxygenase

2 in PGE2 synthesis. Moreover, PGE2-mediated inhibition of IL-12 production requires the functional cooperation of AP-1, and AP-1 strongly suppresses IL-12 p40 transcription. This study reveals a molecular mechanism underlying the interaction between a progressive malignancy and the immune defense apparatus. PGE2 or IL-4 treatment of IFN-γ and LPS-activated primary human monocytes has been shown to induce a novel binding activity to a repressor element, a purine-rich sequence at −155 termed GA-12 (GATA sequence in the IL-12 promoter) [157].

Prostaglandin D2 (PGD2) and its metabolites are known to be important mediators during acute and chronic inflammation. Faveeuw et al. showed that PGD2 inhibits the CD40- and LPS-induced secretion of IL-12 by murine splenic DCs [158]. The inhibition of IL-12 production is mediated only partially by the cell surface Gαs protein-coupled D prostanoid receptor (DP1) but not by the Gαi protein-coupled DP receptor, DP2. Recruitment of DP1 in DC results in the activation of a cyclic AMP/protein kinase A pathway which is in part responsible for the inhibition of IL-12 production [158].

VI. Intracellular Signaling in IL-12 Gene Expression

Hacker and coworkers [159] analyzed the MAPK pathways triggered by CpG-DNA and their significance for cytokine production in macrophages and DCs, and found that CpG-DNA induced ERK activity in macrophages in a classic MAPK/ERK kinase (MEK)-dependent way. This pathway upregulated TNF-α production, but downregulated IL-12 production. However, in DCs, CpG-DNA and LPS failed to induce ERK activity. Consistent with a specific negative regulatory role for ERK in macrophages, chemical activation of this pathway in DCs suppressed CpG-DNA-induced IL-12 production. These results suggest that differential activation of MAP kinase pathways may be a basic mechanism by which distinct subsets of innate immune cells regulate their effector functions.

In a study to define distinct signaling mechanisms that regulate LPS-mediated induction of IL-12 p40 and p35 in macrophages, Goodridge et al. reported differential regulation of IL-12 p40 and p35 induction via ERK MAPK-dependent and -independent mechanisms [160]. While LPS-induced p38 MAPK activation is required for the induction of both p40 and p35 subunits, ERK MAPK signaling mediates negative feedback regulation of p40, but not p35, production. Such ERK activation is downstream of calcium influx and targets LPS-induced IL-12 p40 transcription by suppressing the synthesis of IRF-1. In contrast, negative regulation of the p35 subunit of IL-12 occurs via a calcium-dependent, but ERK-independent, mechanism likely to involve NFκB signaling [160].

Sugimoto et al. recently identified a serine/threonine kinase, Cot/Tpl2, as a modulator of bacterial DNA-induced IL-12 production and Th cell differentiation [161]. Cot/Tpl2 is indispensable for ERK activation and production of TNF-α and PGE2 in LPS-stimulated macrophages, but is not essential for bacterial CpG-DNA-mediated ERK activation. Peritoneal macrophages and bone marrow-derived DCs from Cot/Tpl2$^{-/-}$ mice produced significantly more IL-12 in response to CpG-DNA than those from WT mice. Enhanced IL-12 production in Cot/Tpl2$^{-/-}$ macrophages is at least partly regulated at the transcriptional level, and the elevated IL-12 p40 mRNA level in Cot/Tpl2$^{-/-}$ macrophages is accompanied by decreased amounts of IL-12 p40 transcription repressors, such as c-Maf and GATA sequence in the IL-12 promoter-binding protein (GA-12-binding protein; GAP-12). Consistently, Cot/Tpl2$^{-/-}$ mice showed Th1-skewed antigen-specific immune responses upon OVA immunization and *Leishmania major* infection in vivo [161]. This work identifies a new negative regulator of IL-12 gene expression. It is yet another example that supports the notion that many oncogenes do not simply promote cell survival and proliferation but are directly involved in suppression of cell-mediated immunity against malignant growth.

The TLR2/MyD88 pathway is important for the production of IL-12 in response to the parasite *Toxoplasma gondii* in NFκB-dependent and independent manners [162]. The adaptor molecule TRAF6 is involved in TLR signaling pathways and associates with serine/threonine kinases involved in the activation of both NFκB and MAPK. Mason et al. investigated the role of TRAF6 in the intracellular signaling pathways involved in the production of IL-12 in response to soluble *toxoplama* antigens (STAg). TRAF6$^{-/-}$ mice and macrophages failed to produce IL-12 p40 in response to STAg. It was also demonstrated that TRAF6-dependent activation of p38 MAPK is required for the production of IL-12 p40 in macrophages in response to toxoplasma antigen. Furthermore, toxoplasma antigen also activates ERK, which leads to the inhibition of IL-12 p40 production, and this may represent a strategy of the parasite to evade early host immune responses [163]. Nevertheless, *T. gondii* possesses molecules that themselves induce eventual IL-12 synthesis through both MyD88- and CCR5-dependent pathways. The balance between activation and interference with proinflammatory signaling is likely to reflect the need to achieve an appropriate level of immunity that allows the host and parasite to maintain a stable interaction [164].

Utsugi et al. investigated the role of JNK in IL-12 production by glutathione redox, which is the balance between intracellular reduced (GSH) and oxidized glutathione (GSSG) [165]. They found that LPS induced IL-12 p40 protein and mRNA in PMA-treated THP-1 human macrophage cell line, and that it activated JNK and p38MAPK, but not ERK, in PMA-treated THP-1 cells. Inhibition of JNK activation using SP600125 enhanced both LPS-induced IL-12 p40 production from THP-1 cells and p70 production by human monocytes. Antisense JNK oligonucleotide augmented IL-12 p40 protein production and mRNA expression. The increase in the ratio of GSH/GSSG induced by glutathione reduced form ethyl ester (GSH-OEt) dose dependently enhanced LPS-induced IL-12 p40 production in PMA-treated THP-1 cells. GSH-OEt augmented p38MAPK activation, but suppressed the JNK activation induced by LPS. These findings indicate that JNK negatively affects LPS-induced IL-12 production from human macrophages, and that glutathione redox regulates LPS-induced IL-12 production through its differential control of JNK and p38MAPK activation.

However, in a separate study, Ma et al. investigated the role of JNK in IL-12 p40 gene expression in LPS-stimulated promonocytic THP-1 cell line stably transfected with CD14, treated also with dexamethasone (DXM), an anti-inflammatory glucocorticoid. A role for JNK in LPS-induced IL-12p40 regulation was demonstrated by using specific inhibitors of JNK activation: SP600125 and a dominant-negative ERK-1 mutant. The study suggests that DXM may inhibit IL-12p40 production in LPS-stimulated human monocytic cells by downregulating the activation of JNK, AP-1, and NFκB transcription factors [166]. The bases for the differences in the role of JNK activation in IL-12 p40 gene expression demonstrated by the above two studies are not understood. They may reflect differences in how the THP-1 cells were activated in the two studies.

Recent studies have shown that PI3K is an endogenous suppressor of IL-12 production triggered by TLR signaling and limits excessive Th1 polarization [167]. Fukao et al. found that numerous stimuli that induced IL-12 production concomitantly elicited PI3K activation in DCs, but both PI3K$^{-/-}$ and PI3K inhibitor-treated DCs showed increased IL-12 production. Consistent with enhanced IL-12 production, an augmented Th1 response was observed upon *Leishmania major* infection in PI3K$^{-/-}$ mice [168]. These findings indicate that a negative feedback mechanism exists that regulates IL-12 production during DC activation and may help prevent the excessive Th1 polarization that causes undesirable immune responses. This study was also supported by the investigation of Martin et al. into the role of the PI3K-Akt pathway in regulating *Porphyromonas gingivalis* LPS-induced production of IL-10, IL-12 p40, and IL-12 p70 by human monocytes [169]. *P. gingivalis* LPS selectively activates the PI3K-Akt pathway via TLR2, and inhibition of this pathway results in an abrogation of ERK1/2 phosphorylation, whereas the activation of p38 MAPK and JNK 1/2 kinases were unaffected. Inhibition of the PI3K pathway resulted in suppressed IL-10 production and enhanced IL-12 production, respectively, accompanied by a pronounced augmentation of NFκB p65 that was independent of IκB-α degradation. Furthermore, the ability of the PI3K-Akt pathway to modulate IL-10 and IL-12 production appears to be mediated by the selective suppression of ERK1/2 activity, as the MEK1 inhibitor PD98059 closely mimicked the effects of wortmannin and LY294002 to differentially regulate IL-10 and IL-12 production by *P. gingivalis* LPS-stimulated monocytes [169].

VII. Transcription Factors That Directly Regulate IL-12 Gene Expression

The study of the regulation of IL-12 gene expression is complicated by the necessity to analyze the coordination of expression of the p40 and p35 genes, which are encoded on different chromosomes. While expression of the p40 gene is restricted to cells that produce IL-12 p70, the p35 gene is more ubiquitously expressed. Many studies have demonstrated that the primary regulatory step for the expression of both IL-12 p40 and p35 genes is at the level of transcription. Thus, this chapter deals exclusively with transcription factors that directly participate in the regulation of IL-12 p40 and p35 genes in macrophages and DCs.

VII1. NFκB

An “NFκB half site” at −132/−122 (TAAAATTCCCC) was initially described in the mouse IL-12 p40 promoter [170], which is well conserved in the human counterpart [171]. This site binds p50/p65 and p50/c-Rel heterodimers induced by LPS [170–172]. The two heterodimers bind to this site with comparable affinities and exhibit equivalent transcriptional activities in *in vitro* assays. However, *in vivo*, c-Rel plays a more crucial role than p65 in the regulation of IL-12 p40 gene transcription [173]. Grumont et al. showed that, in contrast to macrophages which require c-Rel for microbe-stimulated p40 transcription, in mouse CD11c$^+$ DCs, the induced expression of p40 by inactivated *S. aureus*, CpG-DNA, or LPS is c-Rel independent [174]. On the other hand, expression of the IL-12 p35 gene is dependent on and regulated directly by c-Rel complexes binding to its promoter.

VII2. C/EBPβ

The transcription factor C/EBPβ is believed to play a fundamental role in regulating activated macrophage functions. Plevy et al. first reported that C/EBPβ plays a crucial and direct role in the transcriptional regulation of mouse IL-12 p40 gene [172]. However, this finding was not corroborated by another study. Gorgoni et al. showed that in immortalized macrophage-like cell lines from C/EBPβ-deficient mice, though IFNγ/LPS-dependent induction of IL-6, IL-1β, TNF-α, inducible NO synthase, and plasminogen activator inhibitor-1 mRNA expression was variably impaired, IL-12 p40, RANTES and macrophage inflammatory protein-1β mRNA expression was upregulated in the absence of C/EBPβ [175]. The differential mRNA expression correlated with differential transcription levels of the corresponding genes, and was in most cases confirmed in primary macrophage populations. Moreover, in sharp contrast to the enhanced induction of IL-12 p40 mRNA, C/EBPβ$^{-/-}$ primary macrophages derived from both the bone marrow and the peritoneal cavity displayed totally defective expression of IL-12 p35 mRNA. Therefore, the IL-12 p35 gene may represent a novel obligatory target for C/EBPβ in macrophages and this may explain the defective production of bioactive IL-12 and the impaired Th1 responses of C/EBPβ-deficient mice to *Candida albicans* infection [175]. Another study also found that IFN-γ, TNF-α, and IL-12 p40 mRNA expression was within the normal range in C/EBPβ$^{-/-}$ mice infected with *Mycobacterium tuberculosis* strains [176].

VII3. PU.1

PU.1 belongs to the *ets* family of DNA binding proteins [177,178]. It is expressed predominantly in macrophages, B-cells, and erythroid cells [179,180]. PU.1 plays important roles in the development of hematopoietic cells. Ma et al. first reported the binding of PU.1 to the human IL-12 p40 promoter constitutively at two sites: immediately upstream of the NFκB half site at −117/−110 [171], and at the *ets* site located at −211/−207[181]. Use of a dominant negative mutant of PU.1 [178] cotransfected with the human IL-12 p40 promoter-luciferase gene into RAW264.7 cells (a murine macrophage cell line) abolished both the reporter activity as well as the endogenous IL-12 p40 protein secretion, suggesting that PU.1 is an obligatory factor for the transcriptional activation of human IL-12 p40 [124]. Type I IFNs are potent inhibitors of IL-12 production by human monocytes/macrophages. The underlying mechanism involves transcriptional inhibition of the IL-12p40 gene, marked by down-regulation of PU.1 binding activity at the upstream Ets site of the IL-12p40 promoter [138]. However, its importance is not confirmed in the mouse IL-12 p40 gene transcription in transient systems [172].

VII4. IRF-1 and ICSBP

Coordinated expression of the two constituent IL-12 genes, p40 and p35, is crucial for appropriate immune responses in timing, location, and magnitude. IFN-γ priming of IL-12 production by macrophages and DCs represents an important physiological process in vivo for escalated cellular response to microbial infections. Liu et al. showed that IRF-1-deficient macrophages have a selective impairment in mRNA synthesis of IL-12 p35 but not the p40 gene, and a strong deficiency in the production of IL-12 p70 but not p40 [182]. They further demonstrated that IRF-1 plays a major role in the transcriptional activation of the IL-12 p35 gene by physically interacting with an inverted IRF element within the IL-12 p35 promoter upon IFN-γ activation. Moreover, IRF-1-mediated transcriptional activation of the p35 promoter requires the cooperation of two adjacent Sp1 elements [182]. Thus, IRF-1 acts as a critical component of IFN-γ signaling in the selective activation of IL-12 p35 transcription in synergy with LPS-mediated events.

The lack of a strong deficiency in IL-12 p40 mRNA expression in IRF-1$^{-/-}$ macrophages shown in this study is in apparent disagreement with the results of two previous studies [183,184]. The possible reasons for this discrepancy were investigated in this study, which indicates that prolonged exposure of macrophages to IFN-γ before LPS stimulation is able to rescue the deficiency in IL-12 p40 production in IRF-1$^{-/-}$ cells via an alternative, uncharacterized mechanism. This unknown alternative pathway is unlikely to play a role in IL-12 p35 and p70 production, because they are not rescued by the IFN-γ pretreatment. The rescue effect of IFN-γ pretreatment on IL-12 p40 expression in IRF-1$^{-/-}$ macrophages was also observed in a previous study by Salkowski et al. [185]. The differential impact of the length of IFN-γ pretreatment on p40 but not on p70 production supports the notion that IRF-1 contributes to the transcriptional regulation of IL-12 p40 and p35 genes through different mechanisms [182].

Recently, Liu et al. reported that ICSBP-deficient macrophages are highly defective in the production of IL-12 [118]. The defect is also observed at the level of IL-12 p40 and p35 mRNA expression. Transcriptional analyses reveal that ICSBP is a potent activator of the IL-12 p35 gene. It acts through a site localized to −226 to −219, named ICSBP-response element (ICSBP-RE), in the human IL-12 p35 promoter through physical association with IRF-1 both in vitro and in vivo. Coexpression of ICSBP and IRF-1 synergistically stimulates the IL-12 p35 promoter activity. Mutations at the ICSBP-RE results in the loss of protein binding as well as transcriptional activation by ICSBP alone, or together with IRF-1 [118]. This study provides novel mechanistic information regarding how signals initiated during innate and adaptive immune responses synergize to yield greater IL-12 production and sustained cellular immunity.

In a study by Zhu et al., ICSBP was found to be associated with NFAT in the absence of DNA, as detected by co-immunoprecipitation of endogenous proteins. A composite NFAT/ICSBP binding site at −68 to −54 was identified which is functionally important for mouse IL-12 p40 promoter activation by LPS and LPS plus IFN-γ. DNA binding of NFAT and ICSBP is demonstrated on the endogenous promoter by chromatin immunoprecipitation. NFAT is required for ICSBP binding to this region [186].

VII5. AP-1

The activation protein-1 (AP-1) transcription factors are immediate early response genes involved in a diverse set of transcriptional regulatory processes [187]. The AP-1 complex consists of a heterodimer of a Fos family member and a Jun family member. This complex binds the consensus DNA sequence (TGAGTCA) sites found in a variety of promoters [188, 189]. The Fos family contains four proteins (c-Fos, Fos B, Fra-1, Fra-2) [190–192], while the Jun family is composed of three (c-Jun, Jun-B, and Jun-D) [193–196]. Fos and Jun are members of the basic leucine-zipper family of sequence-specific dimeric DNA-binding proteins [197]. AP-1 has been shown to be important for the initiation of cell growth [194,197].

The potential link between AP-1 and IL-12 goes back to early observations of a profound effect of trauma and sepsis on IL-12 production. After burn trauma, splenocytes from mice demonstrate aspects of impaired cellular immunity along with diminished production of IL-2, IL-12, and IFN-γ, and increased IL-4 and IL-10 synthesis, which would be consistent with a Th2 phenotype [198]. Importantly, IL-12 treatment after burn injury restores normal resistance to bacterial challenge [198]. Similarly, studies from humans after major injuries demonstrate predominance of the Th2 phenotype and diminished IL-12 and IFN-γ production [199,200]. The murine IL-12 p40 promoter is noted to contain sites for AP-1, GATA, AP3, and PU.1 [6]. The initial *in vitro* studies, however, generated data that did not support an inhibitory role of AP-1 in IL-12 p40 transcription. For example, using a strategy to demonstrate functional activity in a minimal promoter context, Zhu et al. identified a functional AP-1 element in the mouse IL-12 p40 promoter activation at −79 to −74. Mutations at this site significantly reduced LPS-induced promoter activity. Electrophoretic mobility shift assays demonstrate binding of AP-1 family members to this region. Spacing between the previously identified upstream element C/EBP and the AP-1 site is important for promoter activation, suggesting cooperativity between these elements. In this system, overexpressed c-Jun activated the mouse IL-12 p40 promoter and synergistically activated the promoter when co-expressed with C/EBPβ [201].

Despite the general properties of AP-1 as a transcriptional activator, overexpression of c-Fos has been shown to have an inhibitory effect on the transcription of several genes [202,203]. Barke et al showed that transcription of the hepatic mitochondrial enzyme carnitine palmitoyltransferase (CPT, the rate-limiting step in long chain fatty acid oxidation) is inhibited after peritoneal sepsis [204], and this inhibition is associated with increased c-Fos mRNA expression and nuclear protein binding to the AP-1 DNA regulatory element in the CPT promoter [202,204]. These observations led to the hypothesis that after LPS stimulation, induction of macrophage c-Fos expression provides inhibitory control of IL-12 p40 and p35 transcription, and removal of c-Fos-mediated transcriptional inhibition will permit increase of macrophage IL-12 p40 and p35 transcription, resulting in elevated IL-12 p70 protein synthesis. This hypothesis was tested in a murine homozygous c-Fos knockout model, which revealed a significant increase in IL-12 p70 protein, p40 mRNA, and transcription rate in peritoneal macrophages stimulated with LPS [205]. Moreover, the priming-induced enhancing effects on IL-12 production by IFN-γ and IL-4 have both been attributed at least in part to the downregulation of c-Fos by these two cytokines during the priming phase [205,206]. Similarly to the mouse gene, the human IL-12 p40 promoter activity stimulated by IFN-γ and LPS is strongly inhibited by overexpression of c-Fos and c-Jun in RAW264.7 cells. Conversely, blocking AP-1 activity using a dominant negative mutant dramatically increases IL-12 p40 transcription and protein synthesis in macrophages [207].

VII6. Erythroid Kruppel-like factor (EKLF)

Recognition and binding to CACCC sequences have been shown to be mediated predominantly by a family of proteins referred to as Kruppel-like factors (KLFs) [208–210]. KLFs have been reported as both activators [211] and repressors [212,213] of gene expression, depending on the type of KLF, cell type, and other transcription factors with which they may interact. For example, erythroid Kruppel-like factors (EKLFs) activate β-globin gene expression when binding to the CACCC element of its promoter [214], but can repress gene expression by recruiting co-repressors such as histone deacetylases [215]. KLFs have a broad biological functions such as cellular proliferation/differentiation, apoptosis, angiogenesis, and tumorigenesis as reviewed recently [216]. Recent evidence suggests that KLFs regulate the promoter activities of complement C4 [217] and iNOS [218], implicating a role of KLFs in immune system due to the gene-regulatory role via the CACCC cis-element. Luo et al. described the expression of EKLF in human primary macrophages and identified a role of EKLF in IL-12 p40 expression [219]. EKLF-specific binding to the CACCC element (−224 to −220) on the human IL-12 p40 promoter was observed in resting human primary macrophages. Functional analysis of the CACCC element revealed a dependent role for EKLF binding in activating IL-12 p40 transcription in resting RAW264.7 cells, whereas EKLF overexpression in the presence or absence of this element repressed IL-12 p40 transcription in IFNγ/LPS-stimulated RAW264.7 cells. Murine endogenous IL-12 p40 mRNA was induced by overexpressed EKLF in resting RAW264.7 cells, whereas EKLF suppressed IL-12 p40 expression in activated RAW264.7 cells. The bi-functional control of IL-12 p40 by EKLF and its modulation of NFκB support a potential function for this factor in regulating homeostatic IL-12 p40 production in macrophages [219].

VIII. Endotoxin Tolerance-Mediated Inhibition of IL-12

Endotoxin tolerance, the deactivation of a subset of endotoxin-driven responses after an initial exposure to endotoxin, may provide protection from uncontrolled immunological activation of acute endotoxic shock. On the other hand, the inhibition of monocyte/macrophage functions associated with endotoxin tolerance can lead to an inability to respond appropriately to secondary infections in survivors of endotoxic shock, a phenomenon known as “immunological paralysis”. IL-12 plays an important role in pathological responses to endotoxin. Karp et al. [220] examined the regulation of IL-12 during endotoxin tolerance and found that pre-exposure of human monocytes to small doses of LPS (priming) ablates IL-12 production induced by secondary challenge with LPS. This suppression of IL-12 production is primarily transcriptional. Decreased IL-12 production in vivo is multifactorial, involving both loss of CD11c(high) DCs as well as alterations in the responsiveness of macrophages and remaining splenic DCs [221]. No demonstrable mechanistic role was found for B or T lymphocytes, the soluble mediators IL-10, TNF-α, IFN-αβ, nitric oxide, or the NFκB family members p50, p52, and RelB [221]. Recently, Kobayashi and colleagues showed that a novel intracellular molecule, IRAK-M, is induced upon TLR stimulation and negatively regulates TLR signaling. IRAK-M prevented dissociation of IRAK and IRAK-4 from MyD88 and formation of IRAK-TRAF6 complexes [222]. IRAK-M$^{-/-}$ cells exhibited increased cytokine production upon TLR/IL-1 stimulation and bacterial challenge, and IRAK-M$^{-/-}$ mice showed increased inflammatory responses to bacterial infection. Endotoxin tolerance was significantly reduced in IRAK-M$^{-/-}$ cells [222]. How this relates to the transcriptional suppression of IL-12 expression induced by endotoxin tolerance remains to be further explored.

IX. Chromatin Remodeling in Induction of IL-12 Gene Expression

Nucleosome positioning, remodeling, and transcription factor binding at inducible mammalian promoters are important for gene regulation. Weinmann et al. first analyzed the chromatin remodeling of the mouse IL-12 p40 promoter induced in macrophages by bacterial products [223]. High-resolution micrococcal nuclease analyses revealed that a positioned nucleosome, nucleosome 1, spans the promoter, with three positioned nucleosomes further upstream. Upon activation, nucleosome 1 was rapidly and selectively remodeled in a protein synthesis-dependent manner. In primary macrophages, IFNγ synergistically enhanced IL-12 p40 expression, but little effect on remodeling or promoter occupancy was observed. These results suggest that remodeling complexes are selectively targeted to a single, promoter-encompassing nucleosome and that IFNγ influences an event that is independent or downstream of remodeling [223].

Albrecht et al. showed that in macrophages and DCs, stimulation by selective TLR ligands CpG-DNA (TLR9), LPS (TLR4) and LTA (TLR2), resulted in striking differences in expression of IL-12, while stimulating similar amounts of TNF-α. Although an IL-12p40 promoter reporter construct was activated equally by CpG-DNA, LPS and LTA, differences in nucleosome remodeling around the endogenous IL-12p40 promoter contributed to the differential IL-12 induction. Upon stimulation, nucleosome architecture was changed to provide increased access to the IL-12p40 promoter [224].

Goriely et al. determined the positioning of nucleosomes within the IL-12(p35) promoter using an indirect end-labeling technique in the THP-1 monocytic cell line [225]. Stimulation with LPS and IFN-γ resulted in hypersensitivity to digestion with DNase I, micrococcal nuclease, and specific restriction enzymes in the region encompassing nucleotides (nt) −310 to −160, indicating selective inducible chromatin remodeling involving disruption of a single nucleosome (named nuc-2). Promoter deletion mutants and reporter gene assays led to the identification of 2 Sp1-binding sites, which acted as key regulatory elements for both basal and LPS/IFN-γ-inducible p35 gene expression: Sp1#1 lies within the remodeled nuc-2 region and Sp1#2 is located in the nucleosome-free region immediately upstream of nuc-2. The same nucleosomal organization and remodeling were observed also in DCs derived from human monocytes. Moreover, in DCs, LPS and IFN-γ synergized in the induction of nucleosomal remodeling and chromatin remodeling at the IL-12 p35 locus immediately preceded its transcription [225].

X. Future Prospects

In the last four years since we reviewed on the subject of IL-12 immunolobiology and gene expression, significant progress has been made with respect to discovery of new IL-12-like cytokines and regulatory mechanisms by which IL-12 gene expression is controlled. Important challenges still lie ahead. First, we need to extensively explore the immunological activities of the newly identified IL-12-related molecules, p19, p23, and EBI3, in their various combinations in the context of their functional relationship to IL-12. Secondly, greater efforts should be taken to explore the adjuvant activities of IL-12 and IL-12-related cytokines in immunotherapy of various infectious and malignant diseases, both in animal models and human clinical applications. This approach looks increasingly promising and necessary given what we already know about IL-12’s “non-specific” effects. Last but not least, we need to intensify investigations into the coordination and disassociation of the expression of the individual constituent genes of IL-12 and IL-12-related cytokines. These will include their cell type distribution, kinetics, and magnitude of expression, and the involvement of the innate TLR pathways that lead to their expression, as well as the combinatorial usage of the limited number of transcription factors that control their expression in a spatial and temporal manner. The potential of IL-12-related cytokines is tremendous, which can be fully and prudently realized only through on an intimate and comprehensive understanding of their immunobiology.

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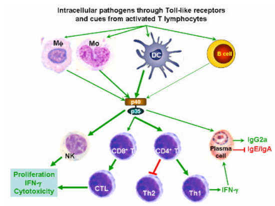

Fig 1. Schematic representation of the immunobiology of IL-12

IL-12 is produced primarily by monocytes/macrophages, DCs, and B cells, typically in response to recognition of intracellular pathogens through by various TLRs. The principal cell types targeted by IL-12 are NK/NKT, T (both $CD4^+$ and $CD8^+$), and B cells. IL-12-stimulated NK cells proliferate, produce IFN-γ and exhibit potent cytotoxicity. CD4+T cells, upon IL-12 stimulation, undergo differentiation to become Th1 effectors at the expense of Th2 differentiation, which is promoted by IL-4/IL-13. IL-12 can also directly activate $CD8^+$ T cells and enhance their cytolytic potential. B cells can respond to IL-12 directly or indirectly through IFN-γ production stimulated by IL-12 to produce cytotoxic immunoglobulins such as IgG2a in mice. Mφ, macrophage; Mo, monocyte; DC, dendritic cell; CTL, cytotoxic T lymphocyte. The color of the arrows indicates stimulation (green) or inhibition (red). The thickness of the arrows is proportional to the potency of the stimulus.

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