Regulation of IL-10 and IL-12 production and function in macrophages and dendritic cells [version 1; referees: 3 approved]

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Abstract
Interleukin-10 and Interleukin-12 are produced primarily by pathogen-activated antigen-presenting cells, particularly macrophages and dendritic cells. IL-10 and IL-12 play very important immunoregulatory roles in host defense and immune homeostasis. Being anti- and pro-inflammatory in nature, respectively, their functions are antagonistically opposing. A comprehensive and in-depth understanding of their immunological properties and signaling mechanisms will help develop better clinical intervention strategies in therapy for a wide range of human disorders. Here, we provide an update on some emerging concepts, controversies, unanswered questions, and opinions regarding the immune signaling of IL-10 and IL-12.

Keywords
IL-12, IL-10, immune signaling
**Interleukin-12 signaling**

Interleukin-12 (IL-12) is the first member of a family of heterodimeric cytokines identified\(^1\). It is a pro-inflammatory molecule produced primarily by professional antigen-presenting cells (APCs), including monocytes/macrophages and dendritic cells (DCs)\(^2\). IL-12 is composed of p35 (encoded by \(\text{Il12a}\)) and p40 (encoded by \(\text{Il12b}\)) chains, and it principally activates natural killer (NK) cells and induces the differentiation of naive CD4\(^+\) T lymphocytes to become interferon-gamma (IFN-\(\gamma\))-producing T helper 1 (Th1) effectors in cell-mediated immune responses to intracellular pathogens\(^3\). IFN-\(\gamma\), in turn, acts on APCs to augment IL-12 secretion in a positive feedback loop\(^4,\(^5\). The p40 chain can also form a dimer with p19 to give rise to IL-23\(^6\), which is required for Th17 differentiation, function, and maintenance\(^6\). Similarly, the p35 chain can combine with Epstein-Barr-induced 3 (EBI3) to form IL-35\(^7\), the latest addition to the IL-12 family, in induced regulatory T-cell population (referred to as iTreg)\(^8\) and in tolerogenic human DCs\(^9\). IL-12 and IL-23 have overlapping as well as distinct immunostimulatory activities\(^10\). IL-12 signals through the IL-12 receptor (IL-12R) comprised of the IL-12\(\beta_1\) and IL-12\(\beta_2\) subunits that are expressed on T cells, NK cells, and DCs\(^11,\(^12\). IL-12 stimulates non-receptor Janus kinase 2 (JAK2) and tyrosine kinase 2 (TYK2) activities, leading to the phosphorylation of signal transducers and activators of transcription (STATs) (in particular, STAT4 homodimers)\(^13,\(^14\). IL-35 is an immunosuppressive cytokine that signals through IL-12\(\beta_2\) and gp130, resulting in the heterodimeric formation and activation of STAT1 and STAT4, which turn in bind to the unique promoter regions of Ebi3 and \(\text{Il12a}\)\(^15\).

**Regulation of interleukin-12 production**

Both \(\text{Il12a}\) and \(\text{Il12b}\) genes need to be expressed coordinately in the same cells to produce biologically active IL-12\(^16\). Paradoxically, the mRNA of \(\text{Il12a}\) is widely expressed in many cell types, albeit at low levels in some cells, most of which do not even produce IL-12. The \(\text{Il12b}\) mRNA is restricted to cells that can produce biologically active heterodimer\(^16\). Synthesis of the p35 chain was proposed to be a rate-limiting step for IL-12 production for its low abundance of transcripts in cells under steady-state conditions\(^17\). Over the past 20 years, a large number of molecular analyses have identified numerous transcription factors that bind to the promoter regions of \(\text{Il12a}\) and \(\text{Il12b}\). The promoters of \(\text{Il12a}\) have been shown to bind transcription factors such as nuclear factor kappa B (NFkB) c-Rel (in DCs)\(^18\), c-Maf (as an inhibitor)\(^19\), and IFN regulatory factor 1 (IRF-1)\(^20\) in activated macrophages. Goriely et al. showed that lipopolysaccharide (LPS)- and IFN-\(\gamma\)-induced human \(\text{Il12a}\) gene activation was immediately preceded by a selective and rapid remodeling of a single positioned nucleosome within the -396/-241 region of the promoter containing critical Sp1-binding sites\(^21\). The same group also reported that, in human DCs activated through Toll-like receptor 3 (TLR3) and TLR4 but not TLR2, IRF-3 was recruited to an IFN-stimulated response element (ISRE) between -251 and -242 in the \(\text{Il12a}\) gene promoter. Accordingly, DCs from IRF-3-deficient mice were impaired in TLR4-induced \(\text{Il12a}\) mRNA expression and IL-12p70 synthesis\(^22\).

Interestingly, a novel nuclear protein called GC-binding protein (GC-BP) was found in macrophages that engulf apoptotic cells via phagocytosis. GC-BP is activated via tyrosine phosphorylation induced by interactions between the phagocyte and the apoptotic cell expressing externalized phosphatidylserine. GC-BP has a direct and selective inhibitory activity on the transcription of the \(\text{Il12a}\) gene and IL-12 production\(^23\). It is speculated that this is part of the mechanisms that help suppress autoimmune responses to self-antigens during the clearance of apoptotic cells. This notion is consistent with the converse observation of the induction of IL-10 production during phagocytosis of apoptotic cells\(^24\).

Compared with \(\text{Il12a}\), the \(\text{Il12b}\) promoter has been more extensively studied, and numerous transcriptional factors have been identified as regulators for \(\text{Il12b}\) transcription. When murine macrophages are stimulated with LPS, nucleosome 1 is selectively remodeled so that the transcription factor CCAAT enhancer-binding protein \(\beta\) (C/EBP\(\beta\))/LAP could gain access to this region\(^25\). However, remodeling of nucleosome 1 alone is not sufficient for \(\text{Il12b}\) transcription and more factors are required for its induced expression. These factors include NFkB\(^26,\(^27\), PU.1\(^28\), IRF-1\(^29\), nuclear factor in activated T cells (NFAT)\(^30\), and IFN consensus sequence-binding protein (ICSBP, also called IRF-8)\(^31\) in human or murine macrophages or both. Activation protein 1 (AP-1) has been reported to be an activator of \(\text{Il12b}\) transcription in LPS-stimulated macrophages\(^32\), whereas in tumor-derived prostaglandin \(E_2\) (\(PGE_2\))-treated macrophages, it appears to play the opposite role: inhibiting \(\text{Il12b}\) transcription and promoting tumor progression in vivo\(^33\). The controversy has not been resolved to date.

Goodridge et al. observed that whilst LPS-induced p38 mitogen-activated protein kinase (MAPK) activation is required for the induction of both p40 and p35 subunits, extracellular signal-regulated kinase (ERK) signaling mediates negative feedback regulation of p40, but not p35, production\(^34\). Such ERK activation is downstream of calcium influx and targets LPS-induced \(\text{Il12b}\) transcription by suppressing the synthesis of the transcription factor IRF-1. In contrast, the negative regulation of the p35 subunit of IL-12 occurs via a calcium-dependent, but ERK-independent, mechanism, which was thought to involve NFkB signaling.

CpG oligodeoxynucleotides (ODN) activates the TLR9/MyD88/ TRAF6 (TNF receptor-associated factor 6) cascade leading to the activation of I kappa B kinase (IKK) -NFkB and JNK, which are critical for the production of pro-inflammatory cytokines. Ma et al. reported that the catalytic subunit of DNA-dependent protein kinase (DNA-PKcs) is involved in this activation process\(^35\). DNA-PKcs-deficient DCs exhibited a defect in the IL-6 and IL-12p40 expression in response to CpG-ODN in a dose- and time-dependent manner. Loss of DNA-PKcs impaired phosphorylation of IKK, IkB\(\alpha\), NFkB, and JNK in response to CpG-ODN\(^35\). TLR2-mediated production of IL-12p40 in monocytes and macrophages triggered by the synthetic ligand Pam3csk4 has been shown to activate the phosphorylation of JNK-1/2. Blocking JNK with a chemical inhibitor resulted in inhibition of Pam3csk4-induced p40 production\(^36\). However, the further downstream signaling is not clear.

At the transcriptional level, the differential regulation of \(\text{Il12a}\) and \(\text{Il12b}\) genes is well illustrated in macrophages derived from C/EBP\(\beta\)-deficient mice. In sharp contrast to the enhanced induction of \(\text{Il12b}\) mRNA, C/EBP\(\beta\)\(^37\) primary macrophages derived from
both the bone marrow and the peritoneal cavity displayed a totally defective production of II12a mRNA. This may explain the defective production of bioactive IL-12 and the impaired Th1 responses of C/EBPβ-deficient mice to Candida albicans, a pathogen that requires Th1-mediated control. The enhanced p40 production in C/EBPβ-deficient macrophages is in direct contradiction to an earlier molecular study. It cautions against directly extrapolating in vitro data for its in vivo relevance.

An important pathway in robust IL-12 induction is the requirement for “priming” of LPS-activated macrophages and DCs by IFN-γ for the expression of maximal amounts of II12a and II12b mRNAs and for IL-12 production. The IFN-γ priming is a positive feedback mechanism for more robust IL-12 production in certain immune responses, as the primer IFN-γ is derived principally from NK cells and activated Th1 lymphocytes, cells that are initially activated by APC-derived IL-12 upon pathogen infection. Overall, inadequate investigations have been performed to elucidate this important feedback amplification mechanism in a comprehensive manner.

Negishi et al. reported that MyD88-associated IRF-1 migrates into the nucleus more efficiently than non-MyD88-associated IRF-1. The critical role of MyD88-dependent “IRF-1 licensing” is underscored by the observation that the induction of a specific gene subset downstream of the TLR-MyD88 pathway, such as IFN-β, inducible nitric oxide (NO) synthase, and IL-12p35, is impaired in Ifr1-deficient cells. The study places IRF-1 as an additional member participating in MyD88 signaling and provides a mechanistic explanation for the enhancement of the TLR-dependent IL-12p35 induction program by IFN-γ.

The TLR-NFkB-dependent pathway inducing IL-12 and the IFN-dependent pathway inducing type I IFN (α and β) and IFN-regulated genes have also been shown to cooperate for the robust production of IL-12 in DCs. Gautier et al. reported that R-848/Resiquimod (TLR7 ligand and the TLR7/8 ligand in human) synergized with poly (I:C) (TLR3 ligand) or LPS (TLR4 ligand) in inducing high levels of bioactive IL-12p70 secretion and IFN-β mRNA accumulation by mouse bone marrow-derived DCs (BMDCs). Strikingly, IL-12p70, but not IL-12p40, secretion was strongly reduced in BMDCs from STAT1−/− and IFNAR−/− mice. STAT1 tyrosine phosphorylation, IL-12p35, and IFN-γ mRNA accumulations were strongly inhibited in IFNAR−/− BMDCs activated with the TLR ligand combinations. Similar observations were made by using neutralizing anti-IFNAR2 antibodies in human TLR8-expressing peripheral blood monocyte-derived DCs. This study suggests that TLR engagement on DC induces endogenous IFNs that cooperate with the NFkB-inducing machinery for optimal IL-12p70 secretion.

Signaling events from distinct classes of pathogen recognition receptors (PRRs) affect each other in modulating innate and adaptive immunity through modulating IL-12 production. Activation of cytosolic RIG-I-like receptors (RLRs) results in the selective suppression of TLR-induced transcription of the II12b gene through the binding of RLR-activated transcription factor IRF-3 to the II12b promoter, while it competitively edges out IRF-5, a transcriptional activator of II12b that binds to the same sequence motif, the ISRE.

IFR-5 binding in this region is usually accompanied with chromatin remodeling of both regulatory regions and the formation of a productive transcriptional complex containing other transcription factors. Consequently, the activation of RLRs in mice attenuated TLR-induced Th1 and Th17 responses against viral infection of mice. Similarly, Kim et al. identified a crosstalk between TLR4- and nucleotide-binding oligomerization domain 2 (NOD2)-mediated activities in the regulation of intestinal mucosal defense and tissue homeostasis via NOD2 signaling selectively interfering with TLR-induced II12a gene expression and IL-12 production via the transcriptional regulator C/EBPβ.

Emerging evidence has demonstrated that mammalian target of rapamycin (mTOR) is an important regulator of immunity by modulating the differentiation, activation, and function of lymphocytes and APCs. In exploring the long-held “puzzle” of low levels of IL-12 induced through TLR4 signaling in macrophages and DCs, which implied the existence of stringent regulatory mechanisms, He et al. identified the critical regulatory roles of three protein kinases, mTOR, phosphoinositide-3 kinase (PI3K), and ERK, in TLR-induced Th1 responses by reciprocally controlling IL-12 and IL-10 production in innate immune cells of murine origin. Moreover, it was revealed that c-fos was a key molecule that mediated the kinase-regulated IL-12 and IL-10 expression in TLR4 signaling by regulating c-fos expression and NFκB binding to the promoters of IL-12 and IL-10 in a differential manner. These findings confirmed the role of c-fos in this capacity reported in an earlier study by Mitsuhashi et al. and were corroborated by a similar study in human DCs with an additional delineation of the opposing activities of the two components of the mTOR complex, mTORC1 and mTORC2, in this signaling pathway. Thus, by controlling the balance between IL-12 and IL-10, mTOR can specifically regulate the TLR-induced T-cell response in vivo. Indeed, blockade of mTOR by rapamycin efficiently boosted TLR-induced antigen-specific T- and B-cell responses to hepatitis B virus and hepatitis C virus vaccines. This study links a ubiquitously present and fundamentally important pathway of cellular survival, proliferation, and function to the production of a highly restricted specialist molecule in the immune system. Notably absent from the study is the answer to an obvious question: is the induction of IL-10 via mTOR signaling responsible for the inhibition of IL-12 production? Figure 1 summarizes our current understanding of the transcriptional mechanisms regulating the IL-12p40 promoter.

Interleukin-10 signaling

IL-10 was first discovered by complementary DNA clone-based screening for secreted factors by established Th2 cells that regulate cytokine production by activated Th1 cells. IL-10 is a major immunosuppressive cytokine. It is a critical component in the maintenance of the fine balance between swift and potent immune responses against invading pathogens and the control of detrimental pathological injury. Almost all cells of the innate and adaptive arms of the immune system can produce IL-10, including DCs, macrophages, mast cells, NK cells, eosinophils, neutrophils, B cells, CD8+ T cells, CD4+ Th1, Th2, and Th17 cells, and regulatory T (Treg) cells. The major role of IL-10 is to limit the extent of the activation of both the innate and the adaptive immune cells to maintain a homeostatic state. This role of IL-10 is vitally important.
in protecting the host from infection-associated immunopathology, autoimmunity, and allergy, such as sepsis, arthritis, insulitis, inflammatory bowel disease (IBD), and so on. In addition to these activities, IL-10 regulates growth or differentiation (or both) of B cells, NK cells, cytotoxic and helper T cells, mast cells, granulocytes, dendritic cells, keratinocytes, and endothelial cells. The IL-10 receptor is composed of at least two subunits that are members of the IFN receptor (IFNR) family, the ligand-binding subunit (IL-10Rα and IL-10R1) and the accessory subunit for signaling, IL-10R2 (IL-10Rβ). IL-10, produced from various cellular sources upon exposure to pathogens and inflammatory insults, binds to its receptor on target cells. Activation of the IL-10 receptor complex induces a tetramer consisting of two IL-10R1 and two IL-10R2 chains, which bind homodimeric IL-10 to the extracellular domains of IL-10R1. Upon the receptor-ligand engagement, phosphorylation of the receptor-associated protein tyrosine kinase JAK1 is recruited to the intracellular domain by the IL-10R1 chain, while non-receptor TYK2 is recruited to the receptor complex by IL-10R2. These kinases serve as a temporary docking site for inactive cytosolic STAT1 or STAT3 or both, which are recruited by JAK1 and TYK2 to the site upon phosphorylation of the IL-10R1 chain at two tyrosine residues. The STATs bind to the IL-10R1 chain via the Src homology 2 (SH2) domain and are tyrosine-phosphorylated by the receptor-associated JAKs. Activation of STAT3 leads to its homodimerization, similarly to STAT1. Translocation of activated STATs to the nucleus renders high-affinity binding to the promoter regions of IL-10-responsive genes. Successful engagement of the IL-10 receptor complex subsequently activates distinct JAK-STAT pathways and downstream signaling events that converge through various mechanisms to influence nuclear transcriptional events such as those mediated by NFκB, resulting in the initiation of extensive anti-inflammatory and homeostatic programs.

It is important to note that the cellular source of IL-10 production is critical to its immunological activities in a cell-specific manner. Mice with a specific deletion in T cells generated by Cre/loxP-mediated targeting showed heightened contact hypersensitivity reactions and succumbed to severe immunopathology upon infection.
with Toxoplasma gondii. Splenocytes from these mice secreted increased amounts of pro-inflammatory cytokines after activation in vitro compared with wild-type (WT) control splenocytes. However, in contrast to complete IL-10 deficiency, sensitivity to endotoxic shock and skin irritant responses of the skin in the T-specific IL-10-deficient mice were not greater than those of the WT controls. A critical role of B cell-derived IL-10 has been demonstrated in the mouse model of experimental autoimmune encephalomyelitis (EAE). Mice with a disruption in the Ig µ heavy chain (µMT), which results in a lack of B cells, develop a non-self-nature by phagocytes results predominantly in anti-inflammatory reactions characterized by the production of immunoregulatory cytokines IL-10, PGE₂, and transforming growth factor beta (TGFB)β, which are critical to ensuring cellular homeostasis and suppression of autoimmunity as an evolutionarily well-preserved mechanism. Chung et al. reported that the production of IL-10 in response to apoptotic cells is dependent on CD36, p38 MAPK, and the transcription factor TALE homeoprotein Pre-B-cell leukemia homeobox 1 (Pbx1). The study establishes a novel role of a developmentally critical factor in the regulation of homeostasis in the immune system and opens up a new area for future exploration at the intersection between cellular homeostasis and immune responses to exogenous pathogens as well as to endogenous danger signals.

**Regulation of interleukin-10 production**

IL-10 production by macrophages and DCs through pathogen-associated molecular patterns (PAMPs) has been most widely studied. Macrophages produce IL-10 as a consequence of the recognition of PAMPs by its PRRs. Several classes of PRRs are expressed by macrophages, including TLRs, C-type lectin receptors, RIG-1 (retinoic acid-inducible gene 1) receptors, and NOD-like receptors. The PAMPs bind to the TLRs with its TLR-interacting (TIR) domain, initiating signaling into macrophages with the help of intracellular adaptors that lead to the activation of multiple members of the MAPKs and subsequently transcription factors Sp1, C/EBPβ, and SMAD4. In macrophages, including TH17 and TH1, IL-10 production is enhanced by TLR3 or TLR4 activation results in the production of IFNβ, which sets up a feedback loop to sustain IL-10 mRNA induction.

B cells express a number of TLRs. Agonists that act via TLR2, TLR4, or TLR9 have all been shown to promote IL-10 production. TLR9 activation in B cells stimulates activation of Bruton’s tyrosine kinase (Btk), and B cells from Btk knockout mice fail to secrete IL-10 following TLR9 stimulation. However, the molecular mechanism downstream of Btk is not clear. The role of Btk is not restricted to B cells, as Btk-deficient macrophages also secrete less IL-10 than WT cells.

CD4⁺ T cells have been identified as an important source of IL-10 in vivo. Various transcription factors have been reported to induce IL-10 in T cells, including SP1, c-Jun, c-Maf, SMAD4, GATA3, and STAT5. However, the molecular signaling pathways that regulate IL-10 induction have not been fully delineated. The studies in this area have been complicated by the existence of multiple Th cell subsets, many of which can produce IL-10, including Th1, Th2, Th17, and Treg cells, albeit with different capacities. These observations have prompted the hypothesis that the IL-10 locus becomes differentially modified during Th cell polarization, which then invokes subtly different molecular mechanisms that drive IL-10 transcription in a quantitatively variable manner in the various T-cell subtypes.

In contrast to the host response to infectious agents, clearance of apoptotic cells of a self-nature by phagocytes results predominantly in immunoregulatory cytokines IL-10, PGE₂, and transforming growth factor beta (TGFB)β, which are critical to ensuring cellular homeostasis and suppression of autoimmunity as an evolutionarily well-preserved mechanism. Chung et al. reported that the production of IL-10 in response to apoptotic cells is dependent on CD36, p38 MAPK, and the transcription factor TALE homeoprotein Pre-B-cell leukemia homeobox 1 (Pbx1). The study establishes a novel role of a developmentally critical factor in the regulation of homeostasis in the immune system and opens up a new area for future exploration at the intersection between cellular homeostasis and immune responses to exogenous pathogens as well as to endogenous danger signals.

**Regulation of interleukin-12 production by interleukin-10**

The potency of IL-12 in host defense makes it a target for stringent regulation. Indeed, the temporal, spatial, and quantitative expression of IL-12 during an immune response in a microenvironment contributes critically to the determination of the type, extent, and ultimate resolution of the reaction. Breaching of the delicate control and balance frequently leads to immunologic disorders and pathogenesis. One of the most important and well-studied negative regulators of TLR-induced IL-12 production is IL-10. IL-10 suppression of both IL12a and IL12b genes is seen primarily at the transcriptional level, and the inductions of the two genes have different requirements for de novo protein synthesis. How IL-10 suppresses Il12a transcription is unknown at present. IL-10 targets an enhancer 10 kb upstream of the Il12b transcriptional start site that is bound by nuclear factor, interleukin 3-regulated (NFI/L3), a B-ZIP transcription factor. Myeloid cells lacking NFIL3 produce excessive IL-12p40 and increased IL-12p70. Thus, the STAT3-dependent expression of NFIL3 is a key component of a negative feedback pathway in myeloid cells that suppresses pro-inflammatory responses.

Kobayashi et al. observed that acetylated histone H4 transiently associated with the Il12b promoter in WT bone marrow-derived macrophages (BMDMs), whereas association of these factors was prolonged in Il10⁻/⁻ BMDMs. Experiments using histone deacetylase (HDAC) inhibitors and HDAC3 short hairpin RNA indicate that HDAC3 is involved in histone deacetylation of the Il12b promoter by IL-10. These results suggest that histone deacetylation on the Il12b promoter by HDAC3 mediates the homeostatic effect of IL-10 in macrophages. More details clearly need to be worked out to understand the important homeostatic regulation of IL-12 production by IL-10. In this context, the IL-4-inducing transcription factor c-Maf is an interesting molecule that can directly and conversely regulate IL-12 and IL-10 gene expression in activated macrophages.

**Interleukin-12 in adoptive cell therapy for cancer**

IL-12 is able to activate all major cytotoxic killer and helper cell types of the immune apparatus (NK, NKT, CD4⁺, and CD8⁺ T cells) that are crucially important for immunosurveillance of and resistance...
to cancer development and progression. The extraordinary anti-
tumor efficacy of IL-12 has been demonstrated in animal mod-
els of cancer of diverse types, and its use in various forms is
now involved in a large number of human cancer clinical trials. Adoptive cell therapy of malignant diseases takes advantage of the
cellular immune system to recognize specific tumor-associated anti-
gens and destroy cancer cells. This is remarkably demonstrated by
redirecting T cells with a chimeric antigen receptor (CAR) toward
CD19, inducing complete remission of leukemia in more than two
thirds of patients in early-phase trials. After initial tumor reduc-
tion by CAR T cells, antigen-negative cancer cells not recognized by
CAR may give rise to tumor relapse. Fortunately, the “quag-
mire” may be overcome by CAR-mediated activation of T cells in
the tumor, releasing inducible IL-12, which augments T-cell acti-
vation and attracts and activates innate immune cells to eliminate
antigen-negative cancer cells in the targeted lesion. Chmielewski
et al. demonstrated the feasibility of this strategy by redirecting
T cells with a carcinoembryonic antigen (CEA)-targeting CAR and
engineering with the inducible recombinant IL-12 expression cas-
tette under the control of the NFAT/IL-2 minimal promoter. In this
context, IL-12 release was triggered by CAR signaling upon tumor
antigen recognition and no IL-12 was detected in vitro without
CAR signaling. The production capacity of such modified CAR
T cells was sufficient to reach therapeutic levels without the need
of repetitive drug application. The therapeutic advantage is indi-
cated by the fact that a dose of 10^5 IL-12 modified tumor-specific
CAR T cells was more effective against established tumors than
10^5 T cells without IL-12 in a pre-clinical model.

To date, despite the enhanced anti-tumor efficacy of IL-12-secreting
CAR T cells in this model, the mechanisms associated with this
enhanced tumor eradication remain unclear. Previous work showed
that IL-12 reversed Treg cell-mediated suppression of CD4^+ Foxp3^−
T-cell proliferation. IL-12 was shown to induce IFN-γ production by
Treg cells in vitro and in vivo. However, IFN-γ expression did not decrease the ability of Treg cells to suppress T-cell prolifera-
tion. Rather, IL-12 treatment decreased Treg cell frequency and
Foxp3^+ levels in Treg cells. Furthermore, IL-12 increased IL-2R expression on effector CD4^+ and CD8^+ T cells, diminished its expression on Treg cells, and decreased IL-2 production by CD4^+
and CD8^+ T effectors. Together, these IL-12-mediated changes
favored the outgrowth of non-Treg cells. Kerkar et al. demon-
strated that engineering tumor-specific CD8^+ T cells to secrete
IL-12 improved their therapeutic efficacy in the B16 mouse model
of established melanoma. Surprisingly, direct binding of IL-12
to receptors on lymphocytes or NK cells was not required. Instead,
IL-12 sensitized bone marrow-derived tumorstromal cells, includ-
ing CD11b^+F4/80^+ macrophages, CD11b^+MHCIIC^CD11c^+ DCs,
and CD11b^+Gr-1^+ MDSCs, causing them to enhance the effects of
adoptively transferred CD8^+ T cells. This reprogramming of mye-
loid-derived cells occurred partly through IFN-γ. MHC I expression
on host cells was essential for IL-12-mediated anti-tumor enhance-
ments. These studies point to the potential immunological
mechanisms of the T cell-secreted IL-12 in tumor models.

Based on prior pre-clinical studies demonstrating that IL-12-secret-
ing CAR T cells are protected from inhibition by endogenous Treg
cells (unpublished results), it is conceivable that IL-12-producing
CAR T cells may be refractory to Treg cell-mediated inhibition and
that previously requisite CAR-mediated T-cell “co-stimulation”
(through CD28 or CD40L) may be overcome by CAR T cell-derived
IL-12 secretion. In other words, CAR T cell-derived IL-12 may
render the effectors independent of the “second signal” requirement
“engraved” in classic T-cell activation paradigms. Furthermore, it
is possible that IL-12 secretion within the tumor microenviron-
ment can reverse the anergic state of endogenous tumor-infiltrating
lymphocytes (TILs) and blunt the immune suppression by mye-
loid-derived suppressor cells (MDSCs) as well as modulation of the
tumor-associated macrophages (TAMs) from a suppressive M2
phenotype to a pro-inflammatory M1 phenotype.

**Future perspectives**

IL-10 is a pleiotropic cytokine with a strong role in limiting the scope
and extent of immune activation. Loss of IL-10 function has deleterious
effects. Therefore, IL-10 could be a potential therapeutic agent for
many inflammatory or autoimmune disorders. However, systemic IL-10 administration has proven to be of limited value and
this indicates that IL-10 production would need to be care-
fully targeted to be efficacious therapeutically. This is evidenced
by adoptive transfers of specific types of IL-10-producing immune
cells in some autoimmune disease models that result in protection
against the development of inflammatory pathologies. Thus,
a far more comprehensive and precise understanding of which
IL-10-producing cells are important in vivo, and what the critical
target cells of this IL-10 are would be instrumental in the future
development of the therapeutic potentials of IL-10. The increased
use of conditional gene targeting in mice will help in these future
studies.

In the intestinal mucosa, IL-10 is a well-established regulator of
tissue inflammation and homeostasis. Mutations in the NOD2
gene are strongly associated with Crohn’s disease, a form of IBD
believed to be driven by uncontrolled Th1 and Th17 responses. There
has been long a debate on the nature of the IBD-associated
NOD2 mutations: “loss of function” or “gain of function”? Noguchi
et al. showed that a common disease-related NOD2 mutation,
3020insC, displayed a “gain of function” property in that it sup-
pressed IL10 transcription by blocking the phosphorylation of the
nuclear ribonucleoprotein hnRNPA1 (heterogeneous nuclear ribo-
nucleoprotein A1) via the p38 MAPK. This effect of 3020insC
appears to be unique on the human IL-10 gene but not on its murine
counterpart. The study challenges the present paradigms about
the influence of the 3020insC mutation on Crohn’s disease, cautioning
against deriving conclusions about the human disease on the basis
data from NOD2 knockout mice. It may provide a novel way of
thinking about efforts to identify therapeutic targets for the treat-
ment of Crohn’s disease and other Th1/Th17-mediated autoimmune
diseases associated with the 3020insC mutation.

Although a tremendous amount of knowledge has been gained about
the signaling and function of IL-12 in immune cells since its
discovery in 1989, many important questions remain. It is widely
believed that the majority of the immunological activities of IL-12
are mediated through IFN-γ produced by activated NK and Th1
cells that have been exposed to APC-derived IL-12. However,
considerable levels of IFN-γ-independent activities of IL-12 have
been reported in many infectious disease and cancer models. The cellular and molecular basis of the non-canonical activities of IL-12 await further elucidation. In immunotherapy of cancers, it has long been noted that the repeated administration of recombinant IL-12 could contribute to increased immunosuppressive properties of the tumor by the induction of IL-10 and NO. Although the underlying molecular mechanism for the negative feedback is lacking, the finding that IL-12 is capable of potently inducing its own inhibitor reiterates the concept that the immune system is inherently equipped with an intrinsic negative feedback device that limits ongoing T-cell activation. This also indicates that the kinetics of T-cell responses may be regulated by the ratio of IL-12 and IL-10 levels, which may gradually decline during the immune response.

Endotoxin tolerance, the transient, secondary downregulation of a subset of endotoxin-driven responses after exposure to bacterial products, is thought to be an adaptive response providing protection from pathological hyperactivation of the innate immune system during bacterial infection. IL-12 production is subjected to such a control mechanism. Wysocka et al. examined the development of IL-12 suppression during endotoxin tolerance in mice. The basis for decreased IL-12 production in vivo is clearly multifactorial, involving both loss of CD11c+ DCs as well as alterations in the responsiveness of macrophages and remaining splenic DCs. There is no demonstrable mechanistic role for B or T lymphocytes, the soluble suppressive mechanism for the negative feedback is lacking, the finding that IL-12 is capable of potently inducing its own inhibitor reiterates the concept that the immune system is inherently equipped with an intrinsic negative feedback device that limits ongoing T-cell activation. This also indicates that the kinetics of T-cell responses may be regulated by the ratio of IL-12 and IL-10 levels, which may gradually decline during the immune response.

The acute-phase proteins, C-reactive protein and serum amyloid A (SAA), are biomarkers of infection and inflammation. He et al. reported a novel property of SAA in the differential induction of IL-12 and IL-23 in human peripheral blood monocytes. SAA-induced IL-12p40 production was accompanied by a sustained secretion of IL-23, but not IL-12p35, resulting in preferential secretion of IL-23, but not IL-12. The study identified SAA as a novel endogenous ligand that potentially activates the IL-23/IL-17 pathway, representing a novel mechanism for regulation of inflammation and immunity by an acute-phase protein. The differential production of IL-12 versus IL-23 was also observed in myeloid DCs (mDCs) and plasmacytoid DCs (pDCs) stimulated via TLR ligands. Only mDCs but not pDCs secreted IL-23. Although pDCs produced both mRNA and protein of the p40 subunit, the lack of bioactive heterodimeric IL-23 protein release was due to the absence of translation of the p19 mRNA into protein. These findings support the hypothesis of a coordinated adaptive immune response based on a finely tuned contribution of these cytokines by different DC subsets. How these endogenous and exogenous ligands induce IL-12 and IL-23 differentially at the molecular level bears both great scientific interests and practical implications.

The immunological activities of IL-12 are further complicated by the existence of IL-12p40 homodimer, IL-12p80, which acts as an IL-12 antagonist by binding to the IL-12R but which does not mediate a biological response. Secretion of IL-12 is associated with excess production of IL-12p80. For example, in contrast to the dogma about the restrictive nature of IL-12-producing cell types, meaningful amounts of IL-12p40 monomer and IL-12p80 have been observed in human breast cancer cells, which could potentially thwart the IL-12-induced anti-tumor responses in vivo. Approximately 20% to 40% of the p40 in the serum of normal and endotoxin-treated mice is in the form of IL-12p80. In IL-12-dependent shock models, exogenous IL-12p80 inhibits IL-12-induced cell-mediated immune response and protects mice from sepsis-associated death. However, IL-12p80 has also been reported to stimulate, rather than inhibit, the differentiation of CD8+ Tc1 (type I cytotoxic T) cells in vitro, contrary to its suppressive activity on Th1 function. The divergent functions of the various forms of p40 highlight our lack of full appreciation of its true range of biological activities.

Recent pre-clinical studies demonstrated that treatment with CD19-specific, CAR T cells that secrete IL-12 is able to safely eradicate established disease without the sophisticated and laborious prior conditioning of subjects. Moreover, in severe combined immunodeficient (SCID)-Beige mice with human ovarian xenografts, IL-12-secreting CAR T cells exhibited enhanced anti-tumor efficacy as determined by an increased survival rate, prolonged persistence of T cells, a higher level of systemic IFN-γ, and modulated tumor microenvironment. How the locally released IL-12 contributes to the highly favorable clinical efficacy and immunological modifications to numerous cell types in the tumor environment is an urgent and challenging task for the benefit of further improving this revolutionary therapeutic strategy for cancers of diverse types and progression states.

In summary, the complexity of the heterodimeric nature of both the cytokines and their receptors in the IL-12 family (also including IL-27) associated with the activation of different combinations of tyrosine kinases and STATs underlies the overlapping as well as distinct immunological consequences of the regulation and signaling in this cytokine group. Greater efforts are called for to better decipher the intricacies. In the meantime, more caution is needed in interpreting data derived from studies of individual cytokine or receptor chains.

**Competing interests**

The authors declare that they have no competing interests.

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