Discovery and Biology of IL-23 and IL-27: Related but Functionally Distinct Regulators of Inflammation

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Abstract

Long-term resistance to many infections depends on the innate ability of the immune system to coordinate the development of antigen-specific adaptive responses. Deficiencies in these events can result in increased susceptibility to pathogens, whereas an inability to regulate an appropriate response can lead to devastating pathological conditions. For over a decade, interleukin (IL)-12 has been recognized as the canonical cytokine that links innate and adaptive immunity, and with the discovery of IL-23 and IL-27 as cytokines related to IL-12, there has been a concerted effort to understand the relationship between these factors. The results emerging from these studies have provided fundamental new insights into the developmental pathways that promote the differentiation and function of $CD4^+$ T helper cells and offer a dramatically altered perspective on the cause and prevention of autoimmune disease. In this review, we aim to highlight the discoveries that have led to our current understanding of the biology of IL-23 and IL-27 in the context of their role in resistance to infection, immune-mediated inflammation, and cancer.

INTRODUCTION

In higher eukaryotes, exposure to pathogens induces a series of events that engages the innate immune system and subsequena adaptive responses. These mechanisms of resistance operate together to efficiently eradicate or control pathogens and to establish long-lived immunological memory. There is growing recognition that the ability of innate cells to recognize conserved molecular patterns shared among large classes of pathogens provides specific information required to tailor the development of appropriate adaptive responses. Thus, the inflammatory environment established by the innate response influences the activation, expansion, and selection of pathogen- (antigen-) specific T and B cells. Although these latter events can be initiated rapidly, an effective adaptive response is usually not established for days to weeks after primary challenge. Many of these same events are involved in the development of chronic inflammatory conditions, whether resulting from a failure to mount a protective response or an inability to regulate these activities appropriately. An understanding of the factors that coordinate these events offers the opportunity to design therapies for infectious or atopic diseases. As a consequence, this topic remains among the most compelling issues in immunology today.

In the past 15 years, researchers have learned much about the innate signals that coordinate subsequent adaptive responses. Besides cell-cell contacts that provide activation signals via peptide-MHC/TCR and classical costimulatory interactions (B7/CD28), antigen-presenting cells communicate with T cells via cytokine production. As a consequence of interacting with various microbial products, antigen-presenting dendritic cells (DCs) and macrophages, as well as other cell types, produce a variety of these soluble factors that are responsible for the expansion and differentiation of naive T cells to generate mature phenotypes such as Th1 and Th2 cells, effective against intracellular and extracellular pathogens, respectively. IL-12, discovered in 1989, is recognized as the signature cytokine produced by cells of the innate system that influences adaptive cell-mediated immunity. IL-12 has a central role in promoting the differentiation of naive $CD4^+$ T cells into mature Th1 effector cells and is a potent stimulus for natural killer (NK) cells and $CD8^+$ T cells to produce interferon (IFN)-γ. Numerous murine model studies illustrate the importance of this pathway by establishing that IL-12 is required for the development of protective innate and adaptive responses to many intracellular pathogens.

Given the central role of this factor in the development of cell-mediated immunity, it is not surprising that IL-12 has also been implicated in the development of various autoimmune inflammatory conditions. The discovery of two IL-12-related cytokines in our laboratory, which we have named IL-23 and IL-27, has dramatically altered our perspectives on the cause and prevention of autoimmune disease. Although these discoveries initially set us on a path to reexamine the immune regulatory role of IL-12 and in particular its role in autoimmunity, the identification of these molecules has now clearly opened the door to a much deeper understanding of how our immune system responds to pathogenic challenges.

IL-12: THE PROTOTYPE OF A SMALL FAMILY OF HETERODIMERIC CYTOKINES

The type I cytokines are a superfamily of immune modulators defined by the structural motifs common to these ligands and their receptors, such as the common four-helix bundle and the hematopoietin receptor domain, respectively. Many of these factors are involved in the development and regulation of immune responses. For instance, IL-6 is closely associated with the regulation of innate and adaptive immunity. The receptor for IL-6 contains a unique IL-6Rα chain as well as gp130, a shared component of the

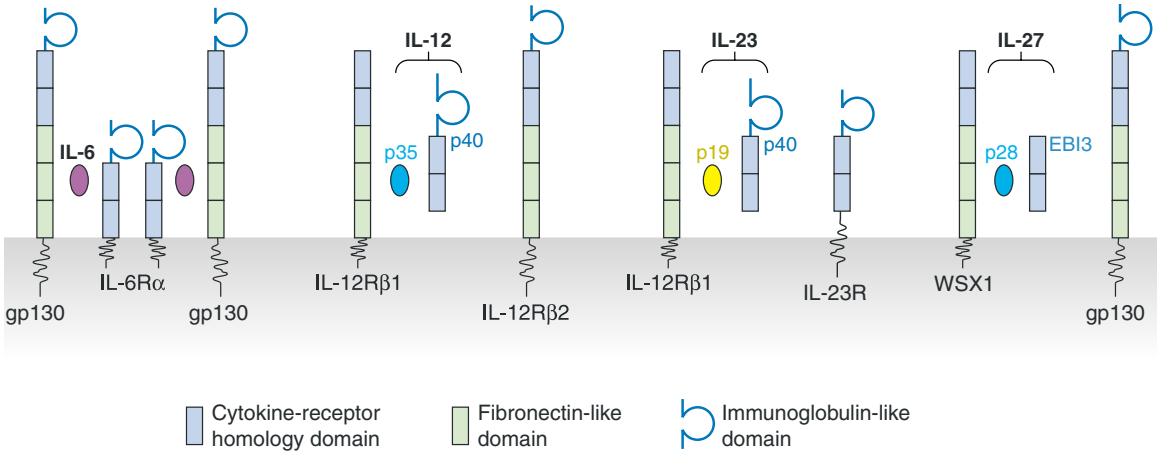

Figure 1

The IL-6/IL-12 family of cytokines.

receptors for several other family members, including IL-11, LIF (leukemia inhibitory factor), G-CSF (granulocyte colony-stimulating factor), and oncostatin M. IL-12, a product of phagocytic cells and DCs in response to microbial stimulation, is a member of this family (see **Figure 1**). It differs from cytokines such as IL-6 and G-CSF in that it is a heterodimeric complex, comprising two disulfide-linked proteins. The IL-12p35 component is homologous to type I cytokines, such as IL-6, and the IL-12p40 component is related to the soluble IL-6R and the extracellular domains of other hematopoietic cytokine receptors. This structural relationship implies that IL-12 evolved from a soluble cytokine/cytokine receptor complex.

Coexpression of both subunits of IL-12 in one cell is required to generate biologically active IL-12 (1). When p35 is expressed without p40, it is not secreted. In contrast, in the absence of p35, p40 can be secreted as a disulfide-linked homodimer or as a monomer. Because p35 is present in many cell types at low levels and p40 expression is usually much higher and restricted to cells that produce the biologically active dimer, p35 expression likely determines the amount of heterodimer produced. Because p40 is expressed in 10- to 1000-fold excess over p35, secretion of IL-12 by activated macrophages and DCs is always accompanied by the secretion of free p40. The biological effects of IL-12 are mediated by a high-affinity IL-12 receptor comprising two subunits, IL-12Rβ1 and IL-12Rβ2, that activate the Jak/Stat pathway of signal transduction, a common feature of this class of receptors. Structurally, both receptors resemble members of the class I cytokine receptor family and within this family are most closely related to the signal-transducing receptors gp130 and the receptor for G-CSF. The IL-12 receptor chains are coexpressed primarily on activated T cells and NK cells, but they can also be found on DCs. Consistent with the biology of this family of cytokines, IL-12 prominently activates the Jak/Stat pathway, and the effects of this cytokine have been largely attributed to Stat4 (signal transducer and activator of transcription 4).

DISCOVERY OF IL-23: A HETERODIMERIC CYTOKINE THAT SHARES ITS p40 SUBUNIT WITH IL-12

In the late 1990s, advances in the ability to search sequence databases with structure-based alignment tools led to the identification of a number of novel sequences that were

related to the IL-6 family of cytokines, in the laboratory of Fernando Bazan and Robert Kastelein at DNAX Research Institute. One of these sequences was a novel four-helix bundle cytokine, which we named p19, with an overall sequence identity of approximately 40% to the p35 subunit of IL-12. Initial attempts to purify p19 from the supernatant of transiently transfected cells were unsuccessful. This observation, combined with the knowledge that IL-12p35 requires IL-12p40 for secretion, led to studies that revealed that secretion of p19 depends on its ability to partner with IL-12p40 (2). The resulting heterodimeric cytokine was named IL-23. Similar to IL-12, IL-23 is expressed predominantly by activated DCs and phagocytic cells, and natural IL-23 was purified as a disulfide-linked heterodimer from activated mouse and human peripheral blood–derived DCs.

Given the structural similarities between IL-12 and IL-23, it was not surprising that this close relationship was also apparent in the composition of their receptors. The p40 subunit of IL-12 binds to IL-12Rβ1, and we demonstrated that this receptor is also a component of the receptor for IL-23. Identification of a second, unique IL-23 receptor subunit, which we named IL-23R, followed when we used a functional cloning strategy to isolate a cDNA that encoded a novel member of the IL-6/IL-12 cytokine receptor family (3). Although structurally this transmembrane protein belongs to the class I cytokine receptor family, it lacks the three membrane-proximal fibronectin type III–like domains characteristic of the tall subclass that includes gp130, as well as both IL-12 receptor subunits. The human IL-23 receptor chains are predominantly coexpressed on activated/memory T cells, T cell clones, and NK cell lines, but also at low levels on monocytes, macrophages, and DC populations. Mouse IL-23 receptor subunits are coexpressed on activated T cells, bone marrow–derived DCs, and activated and inflammatory macrophages. IL-23R signal-transduction studies indicate a constitutive association with Jak2 (Janus kinase 2) and the activation predominantly of Stat3 with limited Stat4 phosphorylation.

DISCOVERY OF IL-27: A THIRD IL-12-RELATED HETERODIMERIC CYTOKINE

At the time of p19's discovery, we identified an additional member of the long-chain four-helix bundle cytokines, which we named p28, according to its apparent molar mass as determined by SDS-PAGE. p28 aligns well with the IL-6/IL-12 family, with the exception of a unique stretch of 13 glutamic-acid residues present in human and mouse p28, which in mice is interrupted by a DK dipeptide. Because it is predicted to be part of the loop region between helix C and D, this highly charged stretch of amino acids is not likely to interfere with the overall helical fold of the protein. Similar to our experience with IL-23p19, we were unsuccessful with our initial attempts to purify p28 from the supernatant of transiently transfected cells. This experience led us to search for potential partners among the small family of secreted type I cytokine receptors, which include p40, CLF-1, and Epstein-Barr virus–induced molecule 3 (EBI3). Of these candidates, only coexpression of EBI3 and p28 led to secretion of a stable heterodimeric protein complex that we designated IL-27 (4). Subsequent work revealed that WSX-1, an orphan type I cytokine receptor expressed by lymphocytes, partnered with gp130 to form a heterodimer required for IL-27 signaling (5).

IL-12, IL-23, AND IL-27: ARE THERE ADDITIONAL FAMILY MEMBERS?

Why did it take more than 10 years after the initial description of IL-12 before IL-23 and IL-27 were identified? This is a particularly intriguing question for IL-23 because this cytokine shares p40 and one of its receptor components with IL-12. Because all three cytokines are heterodimeric complexes,

the traditional route of functional cloning was not practical. In the case of IL-12, biochemistry led to its identification, whereas for IL-23 and IL-27, the advent of genomics and the development of bioinformatics tools allowed our description of IL-23p19 and IL-27p28. With these sequences in hand and an appreciation of the relationships and structural make-up of this cytokine family, we identified these new family members through the mixing and matching of ligands with receptors. It is possible that additional heterodimeric complexes exist. For example, combinations of EBI3 and IL-12p35, as well as EBI3 and IL-23p19, have been described (6; S. Pflanz & R.A. Kastelein, unpublished observation). However, it remains unclear if these complexes are formed in vivo.

TRIGGERS FOR EXPRESSION OF IL-12, IL-23, AND IL-27

As highlighted above, the innate ability to distinguish different classes of pathogens is critical to establishing inflammatory conditions that influence the adaptive response. We now recognize that IL-12, IL-23, and IL-27 sit at the apex of the regulatory mechanisms that shape these responses. They are produced by skin and mucosal DCs, as well as resident macrophages that act as sentinels of the immune system. Indeed, many pathogens and Toll-like receptor agonists (including LPS, CpG, and PolyI:C) enhance expression of the p40, p35, and p19 subunits, resulting in the release of bioactive IL-12, IL-23, or IL-27. The production of these cytokines can be further augmented by T cell CD40L/CD40 interactions that drive potent positive feedback responses for DC activation.

However, although it was unclear initially whether there was specificity to these activities, more recent studies have begun to uncover differential regulators of IL-12 versus IL-23 production. For example, Smits et al. (7) found that intact gram-positive bacteria preferentially stimulated IL-12 over IL-23. Investigators have also noted differences in IL-12/IL-23 responses when using purified bacterial products, along with whole bacteria. An important unanswered question is whether p35 and p19 can be coexpressed by the same activated DC. Expression of these two genes possibly is reciprocally regulated, just as IFN-γ and IL-4 are in CD4 T cells. The implication is that there may be distinct populations of DCs expressing either IL-12 or IL-23.

Additional insights into the differential regulation of IL-12 versus IL-23 production have come from the study of $G_{\alpha i}$-linked G protein–coupled receptors that bind prostaglandin E2 (PGE_2) and ATP. PGE_2 appears to be a strong differential regulator of IL-23 production in mouse DCs. This finding has recently been confirmed and extended using human monocyte-derived DCs. Schnurr and coworkers (8) found that, similar to PGE_2, other cyclic adenosine monophosphate (cAMP)-elevating pathways, including the P2 receptors for ATP, also preferentially enhance IL-23 production. With this finding in mind, it is interesting that pertussis toxin (a virulence factor of *Bordetella pertussis*) blocks $G_{\alpha i}$-linked G protein–coupled receptors, leading to an increase in cAMP. This may account for the ability of *B. pertussis* to preferentially enhance IL-23 expression. These types of studies provide a more comprehensive picture of the factors whereby nonself- (microbial products) and self-signals (CD40, PGE_2, ATP) for danger or injury can regulate the balance between IL-12 and IL-23 production. Understanding these early regulatory mechanisms is of paramount importance to delineate the downstream effects of the immune pathway initiated by these key regulatory cytokines.

IL-23 PROMOTES THE DEVELOPMENT OF A NOVEL T HELPER SUBSET DISTINCT FROM THE CLASSICAL Th1 AND Th2 LINEAGES

In 1986, Mosmann and colleagues (9) described the presence of two types of $CD4^+$

T helper (Th) cell clones that had distinct profiles of cytokine production. The signature cytokine of the Th1 subset was IFN-γ, whereas Th2 cells secreted a variety of soluble factors now recognized as IL-4, IL-5, and IL-13. This seminal observation provided an explanation for the distinct immune responses broadly associated with cell-mediated (Th1) or humoral (Th2) immunity during infection or vaccination. At that time, the authors predicted there would likely be additional Th cell subsets important for driving immune effector functions.

Since then, researchers have identified regulatory populations of T cells (10–12) that suppress the function of Th1 and Th2 cells. However, no additional effector T cell subset was discovered until the recent finding that IL-23 rather than IL-12 is essential for the pathogenesis of autoimmune diseases, including experimental autoimmune encephalomyelitis (EAE), collagen-induced arthritis (CIA), inflammatory colitis, and autoimmune uveitis. For many years, the IL-12-dependent Th1 cells were thought to be essential for the induction of autoimmunity, based on the use of neutralizing p40 antibodies or p40-deficient mice. However, although the inflammatory responses associated with these autoimmune diseases were characterized by IFN-γ production, its contribution to inflammation was less certain. This is illustrated by the importance of p40 for the development of central nervous system (CNS) inflammation during EAE, but animals that lack IFN-γ-mediated signaling (*ifn-*, *ifnr-*, and *stat1*-deficient mice) remain susceptible to this condition and actually develop more severe pathology (13). Similarly, during CIA, treatment with p40-specific antibodies prevented disease, but the absence of the IFN-γ signaling pathway resulted in increased arthritic disease.

Furthermore, the use of p40- or IFN-γ-specific antibodies could antagonize the development of spontaneous inflammatory bowel disease (IBD) in IL-10-deficient mice, but only neutralization of p40, not IFN-γ, ameliorated established colitis. The realization that p40 is shared between IL-12 and IL-23 suggested that the latter cytokine might account for this disparity. Moreover, the finding that stimulation of activated and/or memory T cells in the presence of IL-23 (but not IL-12) led to the production of IL-17, but not IFN-γ or IL-4, provided the first evidence of a unique role for IL-23 in the regulation of a T cell effector function (14, 15).

This discovery suggested that there is indeed an additional T cell subset that has unique functions, and it led to gene-expression studies that revealed that the IL-23-dependent $CD4^+$ T cell population displayed a profile distinct from Th1 cells. For example, IL-12 activated cells expressed increased levels of transcripts for many genes involved in host defense, including IFN-γ, granzyme, TRAIL, FasL, and CCL5, whereas those stimulated with IL-23 expressed IL-17A, IL-17F, IL-6, IL-22, TNF, CXCL1, and α3 integrin. Thus, the IL-12/IFN-γ pathway predominantly induces cytotoxic factors important for the direct killing of microbes or infected cells. Conversely, the IL-23/IL-17 pathway is associated with local tissue inflammation that produces swelling, heat, and pain, and sets up an environment with heightened immune responses (see Evolutionary Significance of IL-23/IL-17 Immune Axis, below). In this sense, the IL-12/IFN-γ and IL-23/IL-17 immune responses may work together to control microbial infections.

After the initial recognition that IL-23 promoted T cell production of IL-17, a series of in vivo studies established their contribution to the development of autoimmune inflammation. Langrish and colleagues (15) demonstrated that the IL-23-driven IL-17-producing cells are highly potent at inducing CNS immune pathology. They induced EAE with as few as 1×10^5 CNS antigen-specific IL-17-producing $CD4^+$ T cells adoptively transferred to naive SJL mice (15). Similarly, CIA studies revealed that the absence of IL-12p35 leads to exacerbated arthritis, and the IL-23-deficient mice are resistant to the

development of bone and joint pathology (16). These latter findings correlated with an absence of $CD4^+$ T cells that make IL-17, a cytokine with a major role in the development of arthritic disease. Finally, the development of spontaneous IBD in IL-10-deficient mice was completely prevented by crossing these mice to IL-23p19-deficient mice, demonstrating that IL-23 also plays an obligatory role in the induction of colitis (17). Although these results clarify the role of the IL-23/IL-17 pathway in autoimmune inflammation, they do not directly explain the enhanced disease associated with the absence of IFN-γ or IFN-γ-mediated signaling in EAE and CIA. These observations led to the idea that IFN-γ is part of a regulatory system that counterbalances the effects of IL-23, consistent with the idea that Th cells can crossregulate each other.

As IL-17 is the signature Th17 effector cytokine, it is important to consider its biological activities. It was initially discovered as a potent proinflammatory cytokine produced by activated $CD4^+$ memory T cells. It is a locally produced cytokine that acts on stromal, epithelial, and endothelial cells and a subset of monocytes to induce secretion of proinflammatory mediators such as IL-8, CXCL1, TNF, and G-CSF that promote rapid neutrophil recruitment, which is important for the control of acute infection (see Evolutionary Significance of IL-23/IL-17 Immune Axis, below). In support of this idea, recent data have also identified a role for IL-17 in the induction of a number of small antimicrobial peptides from keratinocytes (E. Bowman, personal communication). In addition, IL-17 protein has been associated with the regulation of tight junction formation of the intestinal barrier (18), and under normal homeostatic conditions, IL-17 mRNA is constitutively expressed at low levels in the gut (19). During homeostasis, when expressed at low levels, IL-17 likely promotes health of the host and resistance to damage via the mechanisms described above. However, dysregulated production of IL-17 in local organ tissues is pathological and can result in chronic immune-mediated tissue destruction. Indeed, elevated levels of IL-17 are present in the target organ of several human autoimmune diseases, including multiple sclerosis, rheumatoid arthritis, and psoriasis.

LINEAGE ORIGIN OF Th1 AND Th17 CELLS

Over the past 15 years, the developmental pathways that promote the differentiation and function of $CD4^+$ Th cells have been a topic of intense investigation. It is well established that IL-12 activation of Stat4 is necessary for optimal differentiation of naive T cells into IFN-γ-producing Th1 cells. IFN-γ then activates the Stat1 transcription factor and subsequent T-bet expression, which are essential for Th1 development (20, 21). Similarly, IL-4 activation of Stat6 is required for naive T cell differentiation into Th2 cells. Stat6 activates GATA-3, which stabilizes and reinforces the Th2 developmental program (22, 23). Given the unique cytokine profile found in the Th17 cells, it is not surprising that these cells do not utilize the classical Th1- or Th2-specific Stat1, Stat4, Stat6, T-bet, and GATA-3 signaling elements. In fact, activation of these transcription factors strongly suppressed Th17 development and function (24, 25).

Since IL-23's initial discovery, Oppmann et al. (2) recognized that this cytokine acts on memory-effector but not naive T cells. This contrasts with IL-12, which can stimulate both naive and activated T lymphocytes. It is well recognized that TCR stimulation of naive T cells is sufficient to induce IL-12Rβ2 but not IL-23R expression; consequently, naive T cells are unresponsive to IL-23 stimulation. This observation suggests that additional cytokines, likely produced by DCs in response to Toll-like receptor signaling, are required to induce the naive T cell expression of IL-23R and subsequent Th17 development. Indeed, three recent reports showed that TGF-β and IL-6 are required for the differentiation of naive T cells into IL-23R-positive IL-17-producing T cells (19, 26, 27).

Although the Th17 cells develop in the absence of IL-23, these cells are not fully committed to the IL-17 production phenotype. When naive T cells are cultured with TGF-β plus IL-6 and the IL-17-producing cells are then restimulated for 3 days with either IL-2 or IL-23, IL-2 converts the TGF-β/IL-6-driven IL-17-producing cells to become IFN-γ-producing Th1 cells. Restimulation with IL-23 and IL-1 is required for further differentiation and maintenance of the IL-17 production phenotype (26, 28). Therefore, although TGF-β and IL-6 are required for lineage commitment of Th17 cells, subsequent exposure to IL-23 and IL-1 is necessary for full effector differentiation and function of Th17 cells.

To search for the immune regulatory factors that control Th17 responses, we performed Affymetrix gene-array analysis of Th17 versus Th1 cells. These studies revealed that *Ctla8* (IL-17A) was the highest elevated gene (>34-fold increase) in cells cultured under Th17 compared with Th1 conditions. Cells grown in Th1 conditions had higher levels of IFN-γ and T-bet. When we queried the Affymetrix data set (containing 60,000 genes and expressed sequence tags) for putative DNA binding proteins, we found a number of potential Th17 cell–specific transcription factors. Of these candidate DNA sequences, the most abundantly expressed Th17-specific gene (>30-fold increase) encoded the orphan nuclear receptor *rorγ*. Using quantitative mRNA expression analysis, we confirmed that the T cell–specific isoform of *rorγ*, RORγt, is highly expressed in Th17 but not Th1 cells (29; B.S. McKenzie, M. McGeachy, N.J. Wison, K.S. Bak-Jensen, C.L. Langrish, J.D. Mattson, B. Basham, R. de Waal Malefyt, T. McClanaha, and D.J. Cua, manuscript submitted). In a collaboration with Littman and colleagues (29), we demonstrated that TCR, TGF-β, and IL-6 stimulation of naive T cells induced RORγt while repressing T-bet expression. Remarkably, RORγt-deficient mice are unable to produce IL-17A and IL-17F in response to TGF-β, IL-6, and IL-23 stimulation.

RORγ is a member of the retinoic acid–related orphan nuclear hormone receptor family that includes RORα and RORβ. RORγt was initially identified in the thymus and is required for the expression of the anti-apoptotic protein Bcl-xL, which promotes the survival of CD4/CD8 double-positive thymocytes (30). As RORγt expression has not been reported in mature peripheral T cells, little is known about the role of this isoform in T cell activation and effector function. To explore the function of RORγt in T lymphocytes, we induced ectopic expression of RORγt using retroviral vectors. We found that enforced RORγt expression induced a proinflammatory Th17 signature that included IL-17A, IL-17F, IL-22, IL-1R1, IL-23R, CCL1 (TCA-3), CXCL1 (GRO-α, KC), XCL1 (lymphotactin), CCR6, and CCL20 (MIP3-α) (B.S. McKenzie, M. McGeachy, N.J. Wison, K.S. Bak-Jensen, C.L. Langrish, J.D. Mattson, B. Basham, R. de Waal Malefyt, T. McClanaha, and D.J. Cua, manuscript submitted). RORγt also induced several family members of TGF-related genes (TGFβ2, TGFβ1i4, TGFα), further implicating the importance of TGF-β signaling in Th17 cell development.

RORγt has important in vivo functions, as illustrated by our EAE experiments in *rorγ*-deficient mice (29) and human-gene-expression studies (B.S. McKenzie, M. McGeachy, N.J. Wison, K.S. Bak-Jensen, C.L. Langrish, J.D. Mattson, B. Basham, R. de Waal Malefyt, T. McClanaha, and D.J. Cua, manuscript submitted). Similar to IL-17-deficient and antibody neutralization studies (15, 31), RORγt-deficient animals displayed only minor clinical signs of EAE. TGF-β and IL-6 were unable to induce IL-17 responses in $rorγ^{-/-}$ mice; consequently, there are no Th17 cells in the CNS, despite a normal induction of Th1 effector cells.

In the human system, we also found that the levels of RORγt mRNA are higher in

peripheral blood mononuclear cell–derived Th17 cells compared with Th1 cells. In addition, analysis of human psoriatic skin samples showed that both IL-17 and RORγt are significantly elevated in lesional skin tissues, further implicating the Th17 immune pathway in human diseases (B.S. McKenzie, M. McGeachy, N.J. Wison, K.S. Bak-Jensen, C.L. Langrish, J.D. Mattson, B. Basham, R. de Waal Malefyt, T. McClanaha, and D.J. Cua, manuscript submitted). On the basis of these findings, we propose a model for Th17 development in which IL-6 and TGF-β induce RORγt expression, which is essential for Th17 differentiation and lineage commitment. RORγt is necessary for the induction of IL-1R1 and for IL-23R expression. IL-1- and IL-23-mediated Stat and NF-κB signaling promote further Th17 development and functional maturation. This model ranks RORγt with other master regulators of $CD4^+$ T cell responses, including T-bet, GATA-3, and Foxp3 (forkhead box P3).

EVOLUTIONARY SIGNIFICANCE OF THE IL-23/IL-17 IMMUNE AXIS

If the price of a dysregulated IL-23/IL-17 immune axis is immunopathology and autoimmune inflammation, there must be strong evolutionary pressures to conserve this immune pathway. This raises several questions about the benefit of the IL-23/IL-17 pathway to the host. Have Th17 cells evolved to provide adaptive immunity tailored to specific classes of pathogens, functions ascribed to the Th1 and Th2 lineages? How important are the innate activities of IL-23 compared with its role in shaping adaptive Th17 responses? IL-23 is produced by sentinel DCs and macrophages within a few hours after exposure to microbial products or endogenous danger signals. This in turn triggers rapid IL-17 responses from tissue-resident T cells—including αβ, γδ, and natural killer T (NKT) lymphocytes—as well as the release of TNF-α and IL-1β from myeloid cells. As highlighted above, IL-17 promotes stromal, epithelial, and endothelial cells and a subset of monocytes to produce a combination of cytokines and chemokines that leads to the rapid recruitment of neutrophils to the site of infection and injury. Thus, IL-23 may play a critical role in driving an early inflammatory immune response to pathogens or injury by directly inducing IL-17 production and early neutrophil recruitment.

Recent work in our laboratory has extended this early work and shown that when delivered intradermally in mice, IL-23 essentially triggers what amounts to a wound-healing response (32). Daily injection of IL-23 in wild-type mice leads to a psoriasis-like phenotype with visually apparent erythema and induration and is associated with prominent dermal papillary blood vessel formation and possibly vasodilation as soon as 2 days after starting treatment. Histological and immunohistochemical examination of IL-23-treated skin show epidermal hyperplasia and a mixed dermal infiltrate consisting of neutrophils, $F4/80^+$ macrophages, $CD11c^+$ DCs, and $CD4^+$ T cells as early as 1 day after IL-23 treatment. Importantly, these activities are unique to IL-23, as neither IL-12 nor TNF-α induces changes in epidermal thickness when injected into skin. The molecular mechanism by which IL-23 induces these responses appears to involve a number of independent innate pathways.

The immediate production of IL-17 from resident $CD4^+$ T cells, as well as other cells, supports the recruitment of neutrophils and may contribute to the antimicrobial response seen in psoriatic lesions. IL-23 also induces the immediate expression of IL-19 and IL-24, which can play a role in the epidermal hyperplasia. This work suggests that IL-23 may have evolved as an innate response to danger—a unique function that has important evolutionary significance. The innate pathways activated by tissue-restricted expression of IL-23 seem aimed at creating an inflammatory environment that provides acute protection from the environment via the

mobilization of innate antimicrobial components and the proliferation of the epidermis, but that also prepares the injured site for heightened immune surveillance.

IL-12 AND IL-23 AS EFFECTOR CYTOKINES WITHIN THE INNATE IMMUNE SYSTEM

Receptors for IL-12 and IL-23 can be found on non-T cells. In a recent collaboration with Powrie and colleagues, we have studied the relative contributions of IL-12 and IL-23 to innate immune responses in a model of IBD (33). In this IBD model, an agonistic anti-CD40 antibody replaces the need for CD40L-expressing activated T cells. Activated CD40 functions on myeloid cells are, without further T cell help, sufficient to induce a pathogenic systemic and intestinal inflammatory response. To our surprise, the CD40-induced colitis was completely dependent on the presence of IL-23, but not IL-12. Treatment of mice with either an IL-23p19-specific antibody or crossing RAG (recombination activating gene)-deficient mice with p19-deficient mice inhibited the mucosal immunopathology but not the systemic immune pathology. Despite the absence of T cells, the IL-23-dependent intestinal inflammation was associated with IL-23-producing intestinal DCs and IL-17 expression within the intestine. Likely local producers of IL-17 in the intestine include neutrophils, macrophages, and lymphocytes. In striking contrast, the systemic inflammatory response as measured by weight loss and by serum proinflammatory cytokine production, but not the mucosal immunopathology, was entirely dependent on the presence of IL-12.

These results highlight several remarkable aspects of the role of IL-12 and IL-23 in the innate immune system. First, the innate immune system represents a potent immune effector arm that can drive pathology independent of the adaptive immune system, and IL-12 and IL-23 are key regulators of this innate pathway. It is likely, however, that inflammation in immune-privileged organs such as the brain and eye still requires infiltration of IL-23-dependent Th17 cells. Second, this work also revealed a striking dichotomy in the regulation of innate systemic and local inflammation, in which IL-23 directs local inflammation and IL-12 promotes systemic responses. These findings have profound therapeutic implications.

THE ROLE OF THE IL-23/Th17 IMMUNE AXIS IN ADAPTIVE IMMUNITY

Have Th17 cells evolved to provide adaptive immunity tailored to specific classes of pathogens? To answer this question, we point out that human clinical data are available on patients with IL-12/IL-23 pathway defects. Humans who either do not make or do not respond to IL-12 and IL-23 owing to mutations in p40 or IL-12Rβ1 have no increased infection risk to gram-positive and gram-negative bacteria, fungi, protozoa, or viruses (34, 35). Originally, these patients were identified as a result of their susceptibility to only two classes of pathogens, nonvirulent mycobacteria and salmonella infections. Because patients with IFN-γ-pathway mutations have mostly overlapping susceptibilities to these pathogens, the IL-12/IFN-γ axis likely is responsible for mediating protection to these pathogens.

In contrast to IL-12/IL-23-deficient humans, p40-deficient mice are highly susceptible to numerous pathogens, including *Listeria*, *Mycobacterium*, *Salmonella*, *Toxoplasma*, *Citrobacter*, *Klebsiella*, *Cryptococcus*, *Leishmania*, and *Francisella*. For most of these pathogens, the IL-12/IFN-γ axis is the dominant pathway in providing protection. In several models, IL-23 provided a limited mechanism of resistance, but its contribution was only detected in the absence of IL-12 (36, 37, 38). A few infection models have shown a significant role for the IL-23/IL-17 axis, including *Klebsiella* infection in the lung (39), intravenous *Candida albicans* infection (40), and infection of the natural rodent pathogen *Citrobacter*

rodentium in the gut (19). The *Klebsiella* study directly compared the infection risk in p40-deficient, IL-12p35-deficient, and IL-23p19-deficient mice, as well as IL-17R-deficient mice, following a 50% lethal dose intranasal infection (39). The results demonstrated independent requirements for IL-12 and IL-23, as well as IL-23-dependent IL-17 in pulmonary host defense against *Klebsiella pneumoniae*. The p19-deficient strain showed substantial mortality from a sublethal dose of bacteria, despite normal IFN-γ induction, and bacterial control was restored in these mice by the administration of IL-17. However, *Klebsiella* infections are not prevalent in patients with IL-12/IL-23 deficiencies.

Similar results were obtained using the natural rodent pathogen *C. rodentium* (19). Whereas wild-type mice were able to clear the bacteria, IL-23p19-deficient mice failed to do so and died within 12 days postinfection. These authors conclude that IL-23 is indispensable for a protective Th17 response, although there is a protective contribution from the IL-12/IFN-γ axis in this model (41).

Presently, it is not clear whether the combination of IL-23 and Th17 is designed to provide protection against unique classes of pathogens. The molecular signature induced by IL-23-responsive cells suggests that the IL-23/IL-17 axis has a limited role in long-term protection against microbial infection. In contrast, as discussed above, the molecular signature induced by IL-12-responsive cells in peripheral tissues is different. IL-12 induces IFN-γ, granzymes, FasL, and a set of chemokines (e.g., MIG, IP-10) that promote antigen-specific cellular immunity against invading pathogens. These two immune pathways likely have evolved to work in synergy with each other to control microbial infections. The initial inflammatory response in an infected or injured peripheral tissue is likely dominated by IL-23. Only later, once the initial danger signal has been processed, is the initial inflammatory response followed by the appropriate immune effector functions, including an influx of activated $CD4^+$ and $CD8^+$ T cells. Depending on the invading pathogen, the IL-12/IFN-γ axis may become the more prominent pathway at this time.

This scenario is consistent with the observed dominance of Th1 or Th2 responsiveness over the IL-23/IL-17 immune pathway, and this dominance may play a role in ensuring that the inflammatory response does not lead to breakdown of tissue-specific immune tolerance leading to autoimmune pathology. A key remaining question is why these regulatory mechanisms fail to shut down the IL-23/Th17 pathway in autoimmune diseases. Additional mechanisms to downregulate IL-23-induced tissue inflammation likely will be identified, and these may be an important function of IL-27.

ANTAGONISTIC ROLES OF IL-12 AND IL-23 IN CANCER

Both IL-12 and IL-23 have essential roles in the interaction between the innate and adaptive arms of immunity. They both are key regulators of inflammatory responses, innate resistance to infection, and adaptive immunity, yet IL-12 and IL-23 drive divergent immunological pathways. Nowhere is this difference more evident—and the consequences perhaps more significant—than in the role each of these cytokines plays in tumor immunity. In preclinical tumor models, IL-12 treatment has a dramatic effect. It vigorously promotes immune surveillance and antitumor responses by inducing IFN-γ-producing Th1 cells and the proliferation and cytotoxic activity of $CD8^+$ T cells and NK cells. As discussed above, IL-23 induces a pathway that leads to the recruitment of a range of inflammatory cells as well as Th17 cells. Under conditions in which the host needs to protect itself against pathogen infections or other forms of insult, IL-23-induced inflammatory processes such as induction of angiogenesis and neutrophil or macrophage infiltration are critical defense mechanisms. However, these same mechanisms can provide a tumor-promoting environment for nascent malignancies.

Because a causal relationship between chronic inflammation and cancer has long been proposed (42), we have recently initiated studies to investigate the role of IL-23 in cancer. Indeed, we show that IL-23 is significantly upregulated in the overwhelming majority of human carcinoma samples (43). Using tumor models in IL-12p35- and IL-23p19-deficient mice, we demonstrate that expression of IL-23 increases inflammatory infiltration in the tumor environment. We also show that an increase in IL-23-dependent inflammatory processes is coupled to suppression of $CD8^+$ T cell infiltration. Thus, although IL-12 promotes tumor infiltration of cytotoxic T cells, local expression of IL-23 in tumor tissue results in exactly the opposite. Because infiltration of cytotoxic effector cells into the tumor tissue is often the stumbling block in tumor therapy, it will be fascinating to pursue the concept that neutralization of IL-23 will result in suppression of tumor-associated inflammation and improved tumor penetration. Regardless of whether IL-23 neutralization or IL-12 treatment protocols ultimately are of clinical benefit in cancer therapy, it is remarkable how these two immune regulators and the immune processes they control have been used either to promote or inhibit tumor incidence and growth.

IL-27: A ROLE IN PROMOTING T CELL ACTIVITY

Prior to the identification of all the components of the IL-27/IL-27R system, there was evidence that implicated the unique IL-27R (TCCR, WSX-1) and EBI3 in the regulation of immunity. As part of the initial cloning of WSX-1, expression studies revealed that high levels of RNA transcripts for this type I receptor were present in $CD4^+$ and $CD8^+$ T cells (44). Subsequent reports that mice lacking the IL-27R had reduced Th1-type responses in a variety of in vitro and in vivo assays suggested this receptor was directly involved in Th cell differentiation (45, 46). Moreover the deletion of *EBI3* revealed that these mice were more resistant to oxazalone-induced colitis (47). With the recognition that the pairing of EBI3 and p28 signaled through the IL-27R, Pflanz et al. (4) demonstrated that IL-27, when used in various combinations with IL-2 and/or IL-12, enhanced the production of IFN-γ by naive $CD4^+$ T cells and NK cells. A molecular basis for some of these events was provided by studies showing that signaling through the IL-27R activated Stat1 and promoted expression of T-bet, a transcription factor whose target genes include IL-12Rβ2 and IFN-γ (48, 49).

Together with reports that T cell stimulation resulted in the downregulation of the IL-27R (46), these findings suggest a model in which IL-27 sensitizes naive $CD4^+$ T cells to the Th1 polarizing effects of IL-12 and indicate a critical role for this cytokine in the early events that influence T cell activation. Consistent with this notion, there are reports that the severity of adjuvant-induced arthritis in rats and EAE in mice can be ameliorated by antibodies specific for IL-27 (50, 51). In the latter model, treatment of $CD4^+$ T cells specific for the autoantigen myelin oligodendrocyte protein with their cognate ligand plus the IL-27p28 component resulted in marked increases in the production of IFN-γ and TNF-α, and increased proliferative responses. However, as discussed below, other studies with EAE have found that IL-27R knockout (KO) mice are more susceptible to disease (52), and initial reports with p28 note that it did not stimulate significant T cell proliferation or IFN-γ production (4). Furthermore, unpublished reports from other groups indicate that blockade of IL-27 worsens CIA (C. Saris, personal communication), and treatment with IL-27 ameliorates this disease (E. Liew, personal communication).

Whereas the role of endogenous IL-27 in the development of autoimmunity is unclear, transgenic overexpression of a hyperlinked form of EBI3 and p28 during viral hepatitis or by immunogenic murine carcinomas leads to increased $CD8^+$ T cell IFN-γ production, cytotoxicity, and tumor clearance

(53–56). This transgenic overexpression also led to the idea that the anticancer effects of IL-27 resulted from its ability to enhance effector function of the immune cells. More recent work in which poorly immunogenic tumor cells were transduced to express IL-27 revealed that they suppressed tumor-induced neovascularization, and this factor could act directly on endothelial cells to induce the production of the antiangiogenic chemokines IP-10 and MIG (57). These studies prompt a reassessment of the mechanisms whereby IL-27 promotes resistance to cancer but also highlight that no studies have assessed the role of endogenous IL-27 in tumor surveillance and control. Indeed, although mice in which the gp130 component of the IL-27R has been modified to allow sustained Stat signaling develop gastric cancer (58), it remains unclear whether the absence of p28, EBI3, or WSX-1 renders mice more likely to develop spontaneous tumors as they age or following treatment with mutagens.

IL-27 AS AN INHIBITOR OF Th1 RESPONSES ASSOCIATED WITH INTRACELLULAR INFECTIONS

Although the early work described above focused on the proinflammatory activities of IL-27, the general availability of the IL-27R-deficient mice prompted experiments with a range of pathogens that provided unexpected insights into the role of this cytokine in limiting inflammation. For example, resistance to the intracellular parasite *Toxoplasma gondii* depends on IL-12's ability to drive the development of a parasite-specific response dominated by the T cell production of IFN-γ, which is essential for the control of replication of this systemic infection (59). However, rather than having defective Th1 immunity, infected *IL-27R*$^{-/-}$ mice generated normal CD4^{+} and CD8^{+} T cell IFN-γ responses that are sufficient to control parasite replication, but they proceeded to develop a lethal CD4^{+} T cell–dependent inflammatory disease (60). This pathological response was intrinsic to the T cells and was characterized by enhanced T cell proliferation, increased production of IFN-γ and IL-2, and the maintenance of a population of highly activated (CD62L^{low}, CD25^{+}) CD4^{+} and CD8^{+} T cells.

Similarly, investigators have also observed exaggerated T cell responses coupled with the elevated production of inflammatory cytokines that include IL-6, TNF-α, and IFN-γ following challenge with *Trypanosoma cruzi* (61) and *Leishmania donovani* (62), two intracellular pathogens that also cause systemic disease. Similarly, IL-27R-deficient mice infected with *Mycobacterium tuberculosis* have a lower bacterial burden than wild-type counterparts, develop more severe lung pathology, and succumb to this infection, presumably a consequence of immune-mediated pathology (63). For some of the systemic infections discussed above, in the absence of the IL-27R the liver was the site most prominently affected by the development of severe necrosis (60–62). Yamanaka et al. (64) have made a similar observation in a noninfectious model in which *IL-27R*$^{-/-}$ mice display enhanced sensitivity to concanavalin A–induced hepatitis that correlates with the elevated production of IL-4 and IFN-γ by NKT cells.

IL-27 AS AN INHIBITOR OF Th2-TYPE ACTIVITIES

Although the reports discussed above identified a role for IL-27R signaling in limiting infection-induced Th1 effector cells, there is also evidence that IL-27 has a similar effect on Th2 cells. The immune response to *Leishmania major* is characterized by an early burst of IL-4 that allows parasite replication but that in resistant strains of mice is supplanted by a protective IFN-γ response. Initial studies examining how IL-27R KO mice responded to this localized infection revealed that these mice displayed an early susceptibility to *L. major* associated with enhanced Th2 responses and reduced generation of Th1 cells (45). These results were consistent with the

prevailing idea that the IL-27R would promote Th1 activities, but at later time points after infection mice that lack IL-27R or EBI3 develop *Leishmania*-specific Th1 cells and control this infection (45, 65, 66).

One interpretation of these findings is that rather than being required for protective Th1-type immunity to *Leishmania*, IL-27 limits early Th2 activity, and in its absence this primary response is exaggerated. Thus, pretreatment of WSX-1-deficient mice with IL-4-specific antibodies reverses this early susceptibility (66). Further support for this idea came from studies with *Trichuris muris*; resistance to this gut-dwelling nematode depends on $CD4^+$ T cell production of the Th2 cytokines IL-4/IL-13. Following infection with this parasite, *IL-27R*$^{-/-}$ mice develop accelerated Th2 responses and exhibit early expulsion of larval worms (67, 68). Moreover, *IL-27R*$^{-/-}$ $CD4^+$ T cells produce more IL-5 and IL-13 than wild-type counterparts during in vitro Th2 differentiation, and IL-27 can directly inhibit the $CD4^+$ T cell production of IL-4 (66). Similarly, in the absence of the IL-27R, mice develop more severe disease in models of asthma and glomerulonephritis associated with enhanced Th2 responses (69, 70). Therefore, IL-27 appears to have a direct inhibitory effect on Th2 response generation that is independent of its ability to enhance IFN-γ production, and this inhibitory effect may result in part from its ability to suppress GATA-3 expression (71), a transcription factor that promotes Th2 lineage commitment.

Whereas infection with *T. muris* provided insights into the role of IL-27 in mucosal responses, other noninfectious experimental systems have also identified a role for IL-27/IL-27R in the regulation of inflammation in the gut. As mentioned above, the initial studies with mice lacking *EBI3* revealed they are resistant to oxazalone-induced colitis, a form of inflammation associated with type 2 responses (47). That report attributed the lack of disease to an absence of invariant NKT cells, but other groups have found that invariant NKT cell populations are normal in the absence of EBI3 or the IL-27R (H. Yoshida, unpublished observations). In subsequent work, for mice fed a low dose of dextran sulphate sodium (a compound that leads to a loss-of-barrier function and the development of local inflammation), the loss of the IL-27R leads to decreased inflammation (72).

Similarly, with this phenotype IL-10 KO mice develop spontaneous colitis, but in the absence of the IL-27R this pathology is significantly delayed (73). In the former case, the resistance of the IL-27R KO mice has been attributed to the inability of IL-27 to promote a pathological Th1 response, a concept more in line with the original idea that IL-27 was required for Th1 cell activation. However, based on IL-27's ability to inhibit Th2 responses and enhance resistance to *T. muris*, an alternative explanation is that the absence of the IL-27R in the gut allows the establishment of a more Th2-like environment that naturally antagonizes the development of immune pathology. Given some of the paradoxical properties of IL-27, additional experiments are needed to distinguish the types of situations in which the inhibitory versus the stimulatory effects of this cytokine dominate and to determine whether there are tissue-specific effects.

UNDERSTANDING THE INHIBITORY EFFECTS OF IL-27 ON T CELLS

The studies with intracellular pathogens (*Toxoplasma*, *T. cruzi*, *Leishmania donovani*, and *Mycobacteria*) described in the sections above imply that, in the presence of strongly polarizing stimuli, IL-27's ability to promote Th1 responses becomes secondary to its role as a suppressor of effector T cell proliferation and cytokine production. The studies with *Trichuris* and asthma suggest that these effects are not restricted to a particular Th cell subset and that IL-27 could act as a general antagonist of T cell activity. This idea is supported by the finding that IL-27 has a profound suppressive effect on the $CD4^+$ T cell production of IL-2 (74). Although the biology of IL-2 is

complex, this cytokine is a potent growth factor for T cells and promotes the development of Th1 and Th2 cells. Thus, this observation provided the first insight into a property of IL-27 that may explain its broad suppressive effects on T cell responses in multiple models.

Because IL-27 can activate T-bet and because this transcription factor can inhibit IL-2 production (75), one possible mechanism for the inhibitory effects of IL-27 was that it acted through this pathway. However, the ability of T-bet KO T cells to produce IL-2 is still antagonized by IL-27 (74), indicating that other pathways are involved in these events. As a result, the basis for this inhibitory activity is uncertain, and one report suggests that IL-27's ability to activate Stat1 and thereby upregulate SOCS3 (suppressor of cytokine signaling 3) is required for this activity (76). In contrast, we have found that IL-27's ability to suppress IL-2 is Stat1 independent (74), and more recent unpublished observations using SOCS3 KO mice indicate that IL-27 can still antagonize IL-2 production in the absence of this regulatory protein.

IL-27 AND OTHER T CELL AND IMMUNE LINEAGES

Although the initial cloning reports demonstrated the highest level of the IL-27R mRNA in T cells, we are still learning more about the biology of this receptor, knowledge that can provide insights into the cell types affected by IL-27. Early studies led to a model in which resting T cells expressed the highest levels of the IL-27R (46), but detailed analysis revealed that naive T cells have low levels of this subunit and that the highest levels are found on antigen-experienced T cells (77). Indeed, the IL-27R is also expressed on other lymphocytes (including NK and NKT cells, T regulatory cells, and memory populations), and this observation implies that IL-27 will influence the function of these subsets. However, no published studies have identified a biological effect of IL-27 on these last two T cell subsets. Resting NK and NKT cells also express high levels of IL-27R, and $NK1.1^+$ cells from concanavalin A–challenged or *T. cruzi*–infected IL-27R-deficient mice produce elevated levels of IL-4, IFN-γ, and TNF-α (61, 64).

In addition, B cells also express the IL-27R, and IL-27 has diverse effects on these cells, such as promoting proliferation of naive cells (78). Similarly, macrophages and mast cells express the IL-27R. Whereas IL-27 can directly induce mast cells and monocytes to produce IL-1 and TNF-α (5), IL-27 is also a negative regulator of mast cell (66) and macrophage function (63). Together, these latter data suggest that IL-27 can suppress effector functions of a range of immune cell types involved in innate and adaptive immunity, and recent work links IL-27 to the inhibition of macrophage and neutrophil functions during a model of sepsis (79). Even so, the mechanistic basis for these anti-inflammatory activities remains to be explored.

CLOSING THE LOOP: THE RELATIONSHIP BETWEEN IL-23 AND IL-27

Although the initial aim of this review was to highlight the biology of IL-23 and IL-27, recent work has provided new insights into the interactions between them and their family members IL-6 and IL-12. There is already a literature highlighting that many of these members share similar properties. For example, IL-12 is a dominant inducer of IFN-γ, but under certain circumstances IL-23 and IL-27 can also promote IFN-γ (80); IL-6 and IL-27 can both antagonize the production of IFN-γ (60, 81); and IL-12 and IL-27 are both able to downregulate IL-2 production (74, 82). Although these shared properties are likely a consequence of shared signaling pathways, the more unique activities may indicate the major function of these individual members. Thus, the major property of IL-12 appears to be its ability to promote Th1-like activities, whereas IL-23 is involved in the regulation of Th17 cells, and IL-6 can support this latter activity.

The complex biology of these cytokines is further illustrated by recent studies identifying a role for IL-27 in antagonizing the development and/or function of Th17 responses. Two of these reports focus on the development of enhanced CNS inflammation in IL-27R KO mice infected with *T. gondii* or used for EAE (52, 83). In both of these systems, the absence of the IL-27R led to elevated Th17 activity within the brain and more severe clinical disease, and implied that IL-27 was an antagonist of IL-17 production. IL-27 was also able to directly antagonize the development of Th17 cells in vitro, and this inhibitory effect was shown to be Stat1 dependent but independent of T-bet. This work also highlighted the role of SOCS3 in limiting IL-6-induced IL-17, but although some of IL-27's effects have been attributed to the induction of SOCS3 (84), this inhibitory protein was not required for the ability of IL-27 to antagonize this inflammatory pathway. These findings highlight the close relationship of the IL-6/IL-23-driven events and the use of a closely related cytokine (IL-27) to temper this potentially damaging pathway.

CONCLUSIONS

With the initial description of IL-23 and IL-27 as IL-12-like heterodimeric cytokines, there was an expectation that these might have similar effects on the development of Th1-type responses. Indeed, as highlighted above, all three cytokines can enhance IFN-γ production. However, there has been rapid progress in distinguishing the novel or major activities that can be ascribed to these different factors. In particular, the discovery that certain autoimmune disorders are mediated by a novel Th cell subset associated with dysregulated IL-23/IL-17 responses has extended the idea initially proposed by Coffman and Mossman that there were likely to be additional T cell subsets.

Although this finding has opened a new chapter in T cell biology, it has important implications for developing novel therapies to treat organ-specific autoimmune pathologies. Indeed, this is likely a viable target for treatment, given that levels of IL-23p19 and IL-17 are elevated in diseases such as multiple sclerosis, Crohn's disease, psoriasis, ulcerative colitis, cystic fibrosis, asthma, chronic obstructive pulmonary disease, and rheumatoid arthritis. This is illustrated by clinical trials in which administration of a p40-specific human monoclonal antibody resulted in reduced clinical disease, associated with improved mucosal histology and decreased production of cytokines by mononuclear cells isolated from the lamina propria (85, 86). But, as noted by these authors, it is not clear if the therapeutic effects of this treatment result from the neutralization of IL-12 or IL-23.

One general concern of the use of immune modulators is that such approaches may leave patients immunocompromised. For instance, TNF-α blockade as a treatment for rheumatoid arthritis can leave these individuals susceptible to a variety of opportunistic infections. However, although the use of antagonists of IL-12p40 in a clinical situation is in its infancy, early studies have not identified any major side effects. Nevertheless, the loss of the IL-12/IFN-γ pathway in humans is associated with increased susceptibility to viruses, as well as mycobacterial and *Salmonella* species. Therefore, antagonists of IL-23p19 represent good candidates to ameliorate inflammation but would also leave the IL-12/IFN-γ axis intact and thereby would be less likely to compromise immunity to these opportunists. Nevertheless, we are still faced with the question of what is the major function of the IL-23/IL-17 axis in humans. As highlighted above for anti-TNF treatments, efficient immunosuppressive therapies can be associated at some level with adverse events that inform us about how the immune system functions. Perhaps it is only with clinical trials that target IL-23 or the identification of patients with primary genetic defects affecting this pathway that it will become apparent whether IL-23 is required for the control of

particular classes of pathogens and/or as a response to injury.

Studies on IL-27, similar to IL-23, have led to the idea that this cytokine has an unexpected activity in the immune system. There are clearly situations in which its proinflammatory activities are apparent, but there is accumulating evidence from mouse models of a dominant role in the suppression of immune hyperactivity. This knowledge could lead to strategies in which blockade of IL-27 might be useful to augment vaccine-induced immunity, or circumstances in which treatment with IL-27 could suppress inappropriate immune response. Whether this is relevant to humans remains an open question, and presently it is difficult to distinguish whether the increased expression of IL-27 and its receptor associated with chronic inflammatory conditions such as sarcoidosis and Crohn's disease indicates a proinflammatory role for IL-27 or is a sign of an endogenous regulatory mechanism to limit T cell activity. Determining the significance of this pathway may also have to wait for the identification of individuals with relevant primary genetic defects or clinical trials that target this system.

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