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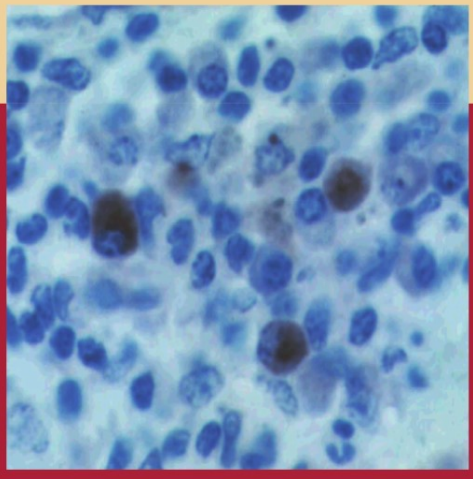

Th 17 Cells: Role in Inflammation and Autoimmune Disease

Valerie Quesniaux
Bernhard Ryffel
Franco Di Padova

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Preface

The IL-17 cytokines represent a novel family of cytokines, which defines a new effector T cell, the Th17 cell, and extend the Th1-Th2 paradigm. Th17 cells in part co-express at least IL-17A and IL-17F, IL-21 and IL-22. IL-17 A/F are produced by T cells ($\alpha\beta$ and $\gamma\delta$), iNKT cells, and possibly neutrophils, dendritic cells and Paneth cells. The regulation of IL-17 family member's expression, and the identification of effector mechanisms are an area of intense current research. Recognized regulators of IL-17A expression include the nuclear receptor RORγt, proinflammatory cytokines such as IL-1, IL-6 with TGF-β, IL-21, IL-23 IL-25 in the absence of IFN-γ and IL-4, which are discussed. Recent data suggest that IL-17A may have a dual function – pro-inflammatory and anti-inflammatory- suggesting that IL-17A may also contribute to terminate inflammation. Further, a reciprocal regulation of Th17 and regulatory T cells including the role of retinoic acid and TGF-β is discussed.

The discovery that patients with rheumatoid arthritis, allergic disorders, psoriasis and inflammatory bowel disease express IL-17A generated interest in the medical community and instigated a flurry of experimental research on the potential role of Th17 in inflammatory diseases.

Experimental studies confirmed that IL-17A is induced and is critical for the development of allergic lung inflammation, arthritis, bacterial sepsis, experimental allergic encephalomyelitis and myocarditis, as well as other inflammatory conditions including organ transplantation. The role of IL-17F and IL-22 is still poorly defined and is only slowly emerging. Importantly, IL-17A neutralization inhibited experimental arthritis opening a new therapeutic possibility to treat rheumatoid arthritis.

Blocking Th17 cell functions such as neutralization of IL-17A, however, might alter host defense to microbial pathogens, such as mycobacteria and opportunistic extracellular pathogens including sepsis, and tumor host responses. Therefore the introduction of neutralizing therapies directed to impair Th17 functions asks for special cautions.

Tools for experimental investigations such as recombinant proteins, antibodies, including neutralizing antibodies and gene deficient mice have been developed.

Neutralizing antibodies may represent novel therapeutics for autoimmune diseases. Clinical studies in rheumatoid arthritis, multiple sclerosis and IBD suggest that IL-17 antibody neutralization may be an interesting addition for severe rheumatoid arthritis and potentially for other autoimmune disorders such as multiple sclerosis.

We believe that this multi-author volume will be very useful for scientists and medical doctors exploring novel mechanisms of inflammation and therapy. Since the first cloning of IL-17 in 1993, the scientific literature on IL-17 expanded exponentially, and more than 1200 scientific publications have been published. This comprehensive review on IL-17 and Th17 cells written by experts provides a timely state of the art review of the literature, summarizing the paradigm shift of a Th17 response which is distinct from Th1 and Th2 responses.

Finally, we wished to thank the authors dedicating their precious time with expert contributions which make this an outstanding volume in Inflammation Research.

Orleans and Basel, September 2008
V. Quesniaux, B. Ryffel and F. Di Padova

Introduction

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Abstract

The identification of IL-17A and Th17 cells have modified the established Th1, Th2 paradigm, led to the definition of a new $CD3^+CD4^+$ Th17 cell subset and introduced a new paradigm to explain the origin of several autoimmune events. However, this paradigm shift tended also to identify the effects of IL-17A with those of Th17 cells and *vice versa*. This view might be insufficient to explain the role of IL-17A in several infection and autoimmune models. IL-17A is in fact produced by several other cell types involved in host defense, autoimmunity and inflammation. Overall, we favor the hypothesis that in an early phase of the immune response γδ T cells are directly involved in the production of IL-17A. This is followed by the involvement of αβ Th17 cells. We cannot exclude that, in some human chronic diseases, other cell types like macrophages or astrocytes may also acquire the capacity to produce IL-17A and be involved in pathology.

IL-17 and Th17 cells

In 1993, Rouvier et al. [1] described the cloning of a rodent cDNA sequence, termed CTLA8, from an activated T cell hybridoma using a subtractive hybridization approach and reported the sequence of the corresponding putative protein. They also recognized that this sequence had homology to an open reading frame encoded within a T cell-tropic c-herpesvirus, Herpesvirus saimiri and suggested that this molecule might be a novel cytokine [1]. Later on it was found that the protein described was a rat protein [2]. The human homologue was reported in 1995 and it was shown to be mainly produced by activated $CD4^+$ cells, to induce the secretion of IL-6 and IL-8 in human foreskin fibroblasts and to enhance the expression of ICAM-1 [2]. This molecule was named IL-17. The mouse homolog was reported a year later [3, 4] and, in one case, was cloned from mouse NKT cells [3].

Subsequently, the mouse and the human IL-17 receptors (IL-17RA) were cloned. IL-17RA was found to be ubiquitously expressed and unique, compared to other cytokine receptors. It was shown to be a single transmembrane receptor with an unusually long cytoplasmic tail [5, 6]. Subsequently the IL-17 family expanded to

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include seven members (IL-17 or IL-17A IL-17B, IL-17C, IL-17D, IL-17E or IL-25 and IL-17F plus the viral homologue ORF13 or vIL-17), while five different receptors were described [7]. As there are several orphan receptors and orphan ligands, future studies are required to assign the respective ligand receptor pairs [8].

Several reports have suggested that IL-17A has a critical role in the protection of the organism against extracellular bacteria and fungi due to the ability of IL-17A to recruit neutrophils to the infected organs/tissues [9, 10]. In addition, a pathological role in several autoimmune models of disease, such as experimental autoimmune encephalomyelitis (EAE) and arthritis became soon evident [11]. Increased production of IL-17 was also reported in various human autoimmune and allergic diseases, such as rheumatoid arthritis [12], multiple sclerosis [13], psoriasis [14] and asthma [15]. All these findings suggested a potential pathogenic role in several human diseases and identified IL-17A as a suitable therapeutic target.

In 2000, Infante-Duarte et al. [16] recognized that IL-17 cannot be categorized as either a Th1 or a Th2 cytokine, but a major revolution in IL-17 research came with the recognition of a novel T cell lineage producing IL-17A [17, 18]. This finding modified the established Th1-Th2 paradigm, led to the definition of the $CD3^+$ $CD4^+$ Th17 cell subset and introduced a new paradigm to explain the origin of several autoimmune events.

Murine Th17 cells

Similar to the other Th cell lineages, Th17 cell functional differentiation is also regulated by cytokines from the environment and by transcription factors. Th17 cells are positively regulated by a few cytokines and negatively regulated by others. *In vitro*, the initial clonal expansion and functional differentiation of murine Th17 cells require transforming growth factor-β (TGF-β) and IL-6 [19–21]. Later on, full acquisition of pathogenic function by effector Th17 cells appears to be mediated by IL-23 [22]. Interestingly, under conditions that mimic an inflammatory stimulus (i.e., in the presence of IL-6), the generation of Th17 cells is favored [21]. In addition, in the absence of IL-6, Th17 cell differentiation can be promoted by IL-21, a cytokine that can be produced by Th17 cells [23–25]. TGF-β, at low concentrations, synergizes with IL-6 or IL-21 to promote expression of the IL-23 receptor (IL-23R), and to favor acquisition of Th17 cell effector functions. However, at high concentrations, TGF-β blocks the expression of IL-23R on Th17 cells and favors the generation of regulatory T cells (Treg) [26]. A fine regulation of IL-17A production in Th17 cells appears to be supported by the observation that, at the single cell level, half of the IL-17-producing cells, stimulated with TGF-β and IL-6, can also produce IL-10, an anti-inflammatory cytokine [22]. These cells, differentiated in the absence of IL-23, appear to be non-pathogenic and to have suppressive activity in experimental autoimmune encephalomyelitis. Therefore, IL-23 has been

considered essential for the switch of Th17 cells to a pathogenic phenotype [22]. In this study, however, it was not clarified whether pathogenic Th17 cells, in addition to IL-10, express a different cytokine profile in comparison to the nonpathogenic Th17 cells. Th17 cells secreting IL-10 might actually represent an additional stage in the differentiation of this cell lineage or an additional subtype. Additional studies are required to reconcile these findings with the observation by Gu et al. [27], who showed that IL-10 has an important direct inhibitory effect on IL-17 production by both T cells and macrophages. The lack of pathogenic potential of this Th17 cell subset suggests that, in host defense or in autoimmunity, the sum of the effects of agonistic or antagonistic cytokines produced by Th17 cells and their respective proportion might be the critical factor.

Moreover, as shown by this nonpathogenic Th17 cell subset, IL-17 secretion is not always dependent on the presence of IL-23 and the expression of the IL-23R. Again, an absolute link between IL-23R expression, IL-23 stimulation, and IL-17 secretion is challenged by additional observations. Liu et al. [28] showed short-term production of IL-17 induced only by TCR stimulation in the absence of IL-23 and Yoshiga et al. [29] reported that iNKT cells can be induced to secrete IL-17 through two pathways, the IL-23-IL-23R pathway and the glycolipid antigen-TCR pathway. Again, IL-17 production is reduced but not absent in IL-23 KO mice [30].

In addition, it has been shown that, *in vivo*, the differentiation of naive CD4 T cells into effector Th17 cells is affected by the costimulatory molecules CD28 and ICOS [18], and that IL-15 is able to favor IL-17 production [31]. All these data suggest that different stimuli can cause IL-17 production by T cells and that Th17 and other IL-17-producing T cells can be engaged in different ways.

On the other hand, many more cytokines such as IL-4, IFN-γ, IL-2, IL-10, IL-12, IL-25 and IL-27 have been described as negative regulators of the differentiation of Th17 cells, suggesting that the generation of Th17 cells is tightly controlled [17, 18, 32–36]. IL-27 appears to have a relevant role in the control of IL-17 production by Th17 cells and represents an important check point under the control of dendritic cells and innate immunity. In particular, IL-27, not only has direct immunosuppressive effects on various T cell subsets, but is also able to induce the release of IL-10 by $CD4^+$ and $CD8^+$ T cells. However, the extent of the involvement of IL-10 in explaining the effect of IL-27 needs to be further characterized [37]. The relationship between Th17 cells, Treg and IL-2 has also received special attention. Treg are able to scavenge IL-2 from Th1 responder cells and to dampen Th1-mediated pathology [38]. However, this might actually favor the differentiation of naive T cells toward the Th17 program, by removing an inhibitory cytokine. In conclusion, although Treg are known to dampen Th1- or Th2-mediated pathology, they might actually promote Th17 development and effector function by scavenging a negative regulator such as IL-2 [39]. IFN-γ and IL-4, are other important mediators involved in the control of IL-17A production and Th17 cell differentiation [18]. They probably represent important check points under the control of Th1 and Th2 cells.

In addition to IL-17A, murine Th17 cells produce IL-17F, IL-21, IL-22, TNF-α, and under specific circumstances GM-CSF and IL-6 [16 33] plus, as discussed above also IL-10 [22]. Production of IL-17A and IL-17F [32, 40] appears specific for the Th17 cell lineage. However, at the single-cell level, IL-17A and IL-17F are not always coexpressed, and IL-17A and IL-17F single-positive cells are detected at relatively high frequencies [41, 42]. Moreover, in mice, $CD4^+$ IL-17A single-positive T cells seem to be prevalent in the lamina propria and intestinal intraepithelium [42]. It has also been reported that, even if the large majority of IL-22^+ cells express IL-17A, only a minority of IL-$17A^+$ cells express IL-22 [43], again indicating that at the single-cell level many of these cytokines can be differentially expressed. It is not clear whether this differential expression results from the existence of different cell types, of various stages of maturation or of environmental factors. It appears that the combined activity of different cytokines might potentiate the effects of Th17 cells and of IL-17A. In particular, IL-17A and IL-22 act in a synergistic or additive way to increase the expression of antimicrobial peptides [43].

Another interesting aspect is to try to identify specific cellular markers as only the expression of the IL-23R seems to differentiate Th17 cells from the other T cell lineages. Nakae et al. [40] reported on the expression profile of cell surface molecules on IL-23-derived Th17 cells. They observed that these cells have more similarities to Th1 cells than to Th2 cells. The majority of Th17 cells express several Th1-lineage markers [i.e., IL-18 receptor α, and T cell Ig domain, mucin-like domain-3 (TIM-3)], but not Th2-lineage markers (i.e., T1/ST2, TIM-1, and TIM-2). They reported that the expression of CTLA-1, ICOS, programmed death ligand 1, CD153, Fas, and RANKL is greater on Th17 cells than on Th1 cells. In addition, Th17 cells also express chemokine receptors such as CCR6 and CCR7 and B7 family molecules (CD80, CD86, and PD-L1), but they failed to identify a cell marker unique for Th17 cells, again pointing out the potential link with Th1 cells. Particularly interesting is the observation that RANKL-expressing Th17 cells may contribute to osteoclast-mediated bone resorption by participating in direct RANKL-RANK interactions between Th17 cells and osteoclast progenitor cells [40]. $RANKL^+$ Th17 cells have also been shown in the lesions of arthritis patients [44]. It is reasonable to propose that the function of Th17 cells may also be influenced by the levels of surface expression of molecules in the CD28 or B7 families or the TNF superfamily as these molecules are clearly represented on Th17 cells [40].

Beyond the Th17 cell paradigm

The new paradigm shift, linked to the identification of Th17 cells, also tended to identify the effects of IL-17A with those of Th17 cells and *vice versa*. However, this view appears inadequate to explain the role of IL-17 in several infection and autoimmune models. In the mouse, IL-17A is in fact produced by several other cell types

involved in host defense, autoimmunity and inflammation, including γδ T cells [45], iNKT cells [46], Paneth cells [47] and possibly PMN [31]. In particular, Jensen et al. [45] found that γδ T cells are uniquely suited for the initial IL-17A response, which often is elicited without a clear antigen exposure. They also found evidence for two distinct functional subsets of γδ T cells (Tγδ-17s and Tγδ-IFN-γs). They proposed that Tγδ-17s precursors do not undergo a selection in the thymus, acquire the ability to produce pro-inflammatory cytokines (such as IL-17A) and accumulate in lymph nodes. On the other hand, Tγδ-IFN-γs undergo thymic selection and accumulate in the spleen. They in fact observed that the majority of lymph node γδT cells are Tγδ-17s, whereas a large population of splenic γδT cells are Tγδ-IFN-γs. Moreover, regardless of ligand experience, γδT cells were able to make cytokines immediately upon TCR engagement, making these cells ideally suited to provide IL-17 in the early stages of an inflammatory response, i.e., before the involvement of antigen-specific αβ T cells, which require antigen-induced priming. It is also possible that the use of certain adjuvants in animal models of autoimmune disease, such as EAE or collagen-induced arthritis, can influence the type and ratio of different T cell populations involved.

In the same direction, Yoshiga et al. [29] demonstrated that also iNKT cells synthesize and release IL-17A, accelerate IL-17 production by Th17 cells and play a significant role in the development of collagen-induced arthritis. Moreover, they found that iNKT cells can be induced to secrete IL-17A through two pathways, one involving the direct activation of the TCR by glycolipid antigen and the other the IL-23-IL-23R signaling pathway [29]. It appears, therefore, that IL-17A is produced by different subsets of T cells, which are involved in both innate and adaptive immunity, and it is well suited to participate to both early and late phases of the immune response.

In addition, in IL-10- or IL-10R-deficient mice, even macrophages stimulated with lipopolysaccharide appear to produce high levels of IL-17A and IL-22 [27]. Addition of exogenous IL-10 abolishes IL-17 production both in Th17 cells and in macrophages [27], again suggesting that IL-17 production is highly regulated. The observation that IL-10-deficient mice spontaneously develop colitis [48] and that, in patients with Crohn's disease, macrophages are positively stained for IL-17A [49] is particularly challenging. Recently, Paneth cells have also been shown to produce IL-17A, in an IL-23 independent fashion and to be one of the causes of a systemic inflammatory response syndrome [47].

In humans, IL-17 has been detected in several T cell subsets, such as naive, central memory and effector memory $CD4^+$ $IL\text{-}17A^+$ T cells [50], NKT-like cells [51], macrophages [49], astrocytes [13], oligodendroglia [13], Langerhans cells [52] and also in a human myeloma cell line [53]. Conversely, blockade or loss of IL-17A does not equate with a complete loss of Th17 cell function, as Th17 cells produce several other inflammatory cytokines, including IL-17F, IL-21, and IL-22 [54].

In conclusion, we favor the hypothesis that in an early phase of the immune response γδ T cells are directly involved in the production of IL-17A. This is followed by the involvement of αβ Th17 cells. Later on, in some human chronic diseases, other cell types like macrophages or astrocytes may also acquire the capacity to produce IL-17A and be involved in pathology.

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Discovery and IL-17 family

IL-22 and IL-17: Common and different properties

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Abstract

Many studies over the last few years have shown that a subpopulation of T helper cells called Th17 cells play a major role in both the defense against certain microbes and the development and maintenance of chronic inflammatory diseases. IL-22, IL-17A, and IL-17F may be the most important effector mediators of this novel cell population. This chapter illuminates the common and differing properties of IL-22, IL-17A, and IL-17F with respect to their genes, protein structure, cellular sources, receptors, target cells, and biological effects. Surprisingly, with the exception of a few similarities (in part identical producing cell types and responding cells), most basic aspects of IL-22 and IL-17A/IL-17F are different.

Introduction

Cytokines are proteins secreted by numerous cells that play an important role in intercellular communication. Not only do they serve in the reaction of the immune system to pathogens, they also regulate hematopoiesis, wound healing, angiogenesis, and physiological and pathological tissue re-organization. Cytokines elicit biological effects by binding to the extracellular moiety of specific, transmembrane receptor proteins in the outer membrane of cells. Mediated by the intracellular moiety of such receptors, this binding induces a coordinated series of intracellular events leading to functional changes in these cells. Interleukins are cytokines that classically serve to establish communication particularly between immune cells. The repression of the function of interleukins sets the most successful strategy for therapy of autoimmune diseases developed in the last 10 years. Due to their similarities in regards to genome location, gene structure, secreted protein structure, and receptors used, various cytokines have been grouped into families. It is important to note that members of the same family do not always exhibit similar biological effects.

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It is currently generally accepted that the most important effector cytokines of Th17 cells are IL-22, IL-17A, and IL-17F. IL-22 is a member of the IL-10 cytokine family. In contrast, IL-17A and IL-17F belong to the IL-17 cytokine family.

The IL-10 family comprises IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28α, IL-28β, and IL-29. IL-28α, IL-28β, and IL-29 (also designated as interferon-λs) are the youngest members and form a theoretical bridge to the type I interferon cytokine family. Interestingly, the members of the IL-10 family are encoded by genes that have similar exon–intron structures [1]. These genes are located in the human genome in three clusters: the first comprises the genes for IL-10, IL-19, IL-20, and IL-24 on chromosome 1q; the second comprises the IL-26 and IL-22-encoding genes located on chromosome 12q; and the third comprises the genes for IL-28α, IL-28β, and IL-29 on chromosome 19q [2–4]. The amino acid (aa) identity between the members of the IL-10 family is approximately 13–25%, whereby characteristic aa positions are conserved. Despite the relatively low sequence identity, all family members show a strikingly similar secondary structure; their α-helical proteins form an anti-parallel conformation with six to seven helices. All IL-10 family members exert their biological effects *via* heterodimeric receptor complexes composed of a type 1 receptor chain (R1) and a type 2 receptor chain (R2). These receptor chains belong to the cytokine receptor family class 2 (CRF2), which additionally comprises the receptors of the type I and type II interferons and tissue factor, a receptor for the coagulant component VIIa [2–6]. They are related by their extracellular moieties, which include the ligand-binding residues. These 200 aa extracellular domains are composed of two tandem fibronectin type III (FNIII) domains. Each of these domains has a structural framework of seven β-strands connected by loops. Additionally, the position of several conserved cysteines and a completely conserved tryptophan characterize the CRF2. In almost any case, the R1 chains have the longer intracellular moiety able to bind signal transducers and activators of transcription (STAT) molecules. For IL-10, it is believed that ligand binding initially occurs to the R1 chain (IL-10R1). This induces a conformational change in the IL-10 protein that enables it to bind secondarily to the R2 chain (IL-10R2), leading to an aggregation of the two receptor chains and initiation of signal transduction. The interaction of IL-22 with its receptor complex may occur similarly (IL-22R1 is the R1 binding chain, and IL-10R2 is the R2 second chain). However, in the case of IL-19, IL-20, and IL-24, the initial binding occurs to the R2 chain. Importantly, the IL-10 family members share receptor chains (the IL-10 family comprises nine cytokines, there are only four R1 chains and two R2 chains for these cytokines; Tab. 1). For instance, the IL-10R2 chain is also part of the receptor complexes for IL-10, IL-22, IL-26, IL-28α, IL-28β, and IL-29. Moreover, not only single receptor chains are shared among different IL-10 family members but even whole receptor complexes (Tab. 1). For instance, the complex composed of IL-20R1 and IL-20R2 is used by IL-19, IL-20, and IL-24. The situation regarding the assignment of receptors in this family becomes even more complicated considering that some cytokines are actually able to

Table 1. Combination of receptor chains in receptor complexes enabling the effects of the IL-10 family members.

	IL-10R1	IL-20R1	IL-22R1	IL-28R1
IL-10R2	IL-10	IL-26	IL-22	IL-28 IL-29
IL-20R2	?	IL-19 IL-20 IL-24	IL-20 IL-24	?

bind to more than one receptor complex. This is the case for IL-20 and IL-24, which signal *via* both the IL-20R1/IL-20R2 complex and the IL-22R1/IL-20R2 complex [2–4]. Despite the structural relation and the use of similar or partly identical receptors, the IL-10 family members exhibit different biological effects [5, 6].

The IL-17 family consists of six members: IL-17, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F [7, 8]. The genes encoding these mediators have an only moderately similar structure that is completely different from that of the IL-10 family's genes. The genes for IL-17A and IL-17F are located on chromosome 6q. The gene encoding IL-17B is located on chromosome 5q, for IL-17C on chromosome 16q, for IL-17D on chromosome 13q, and for IL-17E on chromosome 14q. The IL-17 family members are also structurally related proteins. The characteristic motive is the C-terminal, so-called cysteine knot formation. The greatest sequence similarity exists between IL-17A and IL-17F (47.8% identity between mature proteins). The similarity between other members is about 20% only. The IL-17 family members also act *via* receptor complexes that are composed of two type I transmembrane proteins. In the case of IL-17A and IL-17F, the chains are called IL-17RA and IL-17RC. Interestingly, IL-17A and IL-17F mediate in part similar biological effects.

As far as it is currently known, IL-22, IL-17A, and IL-17F are the most important effector cytokines of Th17 cells. This chapter illuminates the common and differing properties of these mediators with respect to their basic aspects.

The IL-22 and IL-17 encoding genes

The IL-22 and IL-17A/IL-17F encoding genes are located on different chromosomes and have different structures (Fig. 1).

The human *IL22* gene is located on the longer arm of chromosome 12, on 12q15, approximately 52 and 99 kbp upstream from the *IL26* and *IFNG* locus, respectively, and has the same transcriptional orientation as these two adjoining genes (reverse strand) [6]. The human genomic sequence for *IL22* can be found

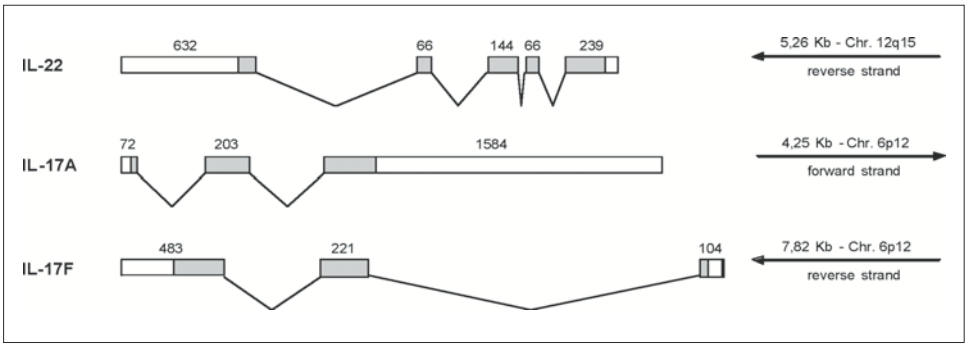

Figure 1
The IL-22 and IL-17A/IL-17F coding genes have different structures. Shown are the exon-intron structures of the genes. Exons (0.68 cm correspond to 250 bp) are presented as boxes comprising non-coding regions (in white) and coding regions (in gray). Introns (0.68 cm correspond to 875 bp) are presented as lines. Genomic localization is also indicated.

in the National Center of Biotechnology Information (NCBI) database under the accession number NT_029419. The *IL22* gene is 5.257 bp long (according to the February 2008 release of the NCBI human genome database) and has the same basic structure as most other members of the IL-10 family [6]. It is comprised of five exons: exon 1, 239 bp; exon 2, 66 bp; exon 3, 144 bp; exon 4, 66 bp; and exon 5, 632 bp. The IL-22 mRNA straddles 1.147 bp (NM_020525, NCBI, April 2008). The first 53 bp of exon 1 encode the 5'-untranslated region. The other portion of exon 1 (186 bp), the exons 2 to 4, and the first portion (75 bp) of exon 5 contain the protein-coding part. The rest of exon 5 (554 bp) encodes the 3'-untranslated region, which includes six single and two overlapping copies of the ATTTA motif known to be involved in the regulation of mRNA degradation. Altogether, the open reading frame is comprised of 537 bp (without the stop codon), predicting a length of 179 aa for the encoded protein.

The human IL-17A- and IL-17F-encoding genes are located on the longer arm of chromosome 6, on 6q12, and have similar structures [7, 8]. The distance between these genes is 46 kbp. *IL17A* is located on the forward strand and has a length of 4.252 bp (NT_007592, NCBI, February 2008) and *IL17F* is located on the reverse strand and has a length of 7.815 bp (NT_007592, NCBI, February 2008). Both genes are comprised of three exons: exon 1, 72 (*IL17A*) / 104 (*IL17F*) bp; exon 2, 203 (*IL17A*) / 221 (*IL17F*) bp; exon 3, 1.584 (*IL17A*) / 483 (*IL17F*) bp. The IL-17A mRNA straddles 1.859 bp (NM_002190, NCBI, May 2008) and IL-17F mRNA 808 bp (NM_052872, NCBI, April 2008). The first 45 and 71 bp of *IL17A* and of *IL17F* exon 1, respectively, encode the 5'-untranslated region. The other portion of exon 1, exon 2, and the first portion of exon 3 contain the protein-coding part.

The rest of exon 3 encodes the 3'-untranslated region. Consequently, the open reading frame is comprised of 565 bp (*IL17A*) and 489 bp (*IL17F*) (without the stop codon), predicting a length of 155 aa (IL-17A) and 163 aa (IL-17F) for the encoded proteins.

The structures of secreted IL-22 , IL-17A, and IL-17F

The secondary structures of IL-22 and IL-17A/IL-17F are very different. As described above, the human *IL22* gene encodes a protein of 179 aa in length, which, after splitting off the signal peptide, is secreted as a polypeptide of 146 aa [9]. Amino acid sequence alignment (mature proteins) revealed that human IL-22 has an 80.8% identity to murine IL-22 [6]. Like all other IL-10 family members, IL-22 has an α-helical structure. In fact, the six helices (referred to as helices A–F) are arranged in an anti-parallel conformation resulting in a monomeric, bundle-like protein. This structure was proved upon its resolution by X-ray diffraction [10, 11]. Monomeric conformation of IL-22, at least at physiologically relevant concentrations, was also confirmed by gel filtration chromatography and dynamic light-scattering studies [10, 12]. In IL-10, there is a 90° angle between the first four helices and the helices E and F that leads to an entwining of two monomers forming a V-shaped dimer in which each domain of the V shape is formed by helices A–D from one partner and helices E and F from the other partner [13, 14]. The IL-22 primary structure contains four Cys, which form two intramolecular disulfide bridge bonds (Cys40-Cys132 linking the N terminus to the DE loop, and Cys89-Cys178 linking helix C to helix F) as deduced from the X-ray structure analysis of IL-22 [10].

The theoretical molecular mass of human IL-22 is 16.7 kDa. However, the recombinant protein expressed in eukaryotic cells migrated as several bands of more than 17 kDa [15, 16]. In fact, glycosylation was found on all three potential N-linked glycosylation sites in the IL-22 sequence: one located in helix A (Asn54-Arg55-Thr56), one in the AB loop (Asn68-Asn69-Thr70), and one in helix C (Asn97-Phe98-Thr99) [16]. Interestingly, the glycosylation is not associated with a noteworthy change of the tertiary structure of IL-22 [10, 11]. However, the glycosylation on Asn54 has been found to be important for IL-22's interaction with IL-10R2, whereas no influence of any glycosylation was observed for IL-22R1 binding [16].

IL17A and *IL17F* encode proteins of 155 and 163 aa, respectively, in length. After splitting off the signal peptides (23 aa for IL-17A and 30 aa for IL-17F) the secreted polypeptides are 132 aa (IL-17A) and 133 aa (IL-17F) long. Amino acid sequence alignment revealed identities (with respect to the mature proteins) between human and murine IL-17A and between human and murine IL-17F of ca. 60% and 55%, respectively.

The crystal structure of IL-17F has been resolved and shows that the protein forms a disulfide-linked dimeric glycoprotein [17]. The core of the IL-17F monomer

is composed of two pairs of anti-parallel strands; one pair includes strands 1 and 2, while the other includes strands 3 and 4. Two disulfide bridges (Cys72-Cys122 and Cys77-Cys124) connect strands 2 and 4. A third disulfide bridge (Cys17-Cys107) connects the loop between strands 3 and 4 of one monomer to the N terminus of the other monomer [17]. The structure of the IL-17F homodimer includes a classical cysteine knot motif, which is also found in the transforming growth factor (TGF)-β and nerve growth factor super families [18]. One difference in the cysteine knot motif of IL-17F compared with the other known cysteine knot protein families is that it only utilizes four Cys instead of the classical six Cys to form the knot. Although the crystal structure of IL-17A is lacking so far, the structure of IL-17A is very probably similar to IL-17F. In addition to IL-17A and IL-17F homodimers, an IL-17A-IL-17F heterodimer also exists. The theoretical molecular mass of secreted human IL-17A and IL-17F is 15.1 and 14.9 kDa, respectively.

The cellular sources of IL-22 and IL-17

Currently, it is generally accepted that IL-22, IL-17A, and IL-17F can be produced by the same cells – a subpopulation of T helper cells called Th17 cells [19]. However, various studies hint that, at least in some acute inflammation models in the murine system, T cells are not cellular sources of IL-22 and that in the human systems both Th1 and Th17 cells are important cellular sources of this cytokine. Furthermore, it seems that the production of IL-22 and IL-17 is differently regulated.

IL-22 mRNA expression was initially described in 2000 in the murine system in IL-9-stimulated T cell lines, to a small extent in an IL-9-stimulated mast cell line, and in concanavalin A (Con-A)-activated spleen cells [20], as well as in the human system in T cells isolated from the peripheral blood and activated with anti-CD3 antibody (Ab) or Con-A [9]. Shortly after these publications, more systematic and quantitative analyses on primary human monocytes, B, T, and NK cells demonstrated that IL-22 expression can only be found in activated (here done by anti-CD3 Ab) T cells and, at lower levels, activated (here done by IL-2/IL-12) NK cells [21]. Among the T cell subsets, IL-22 was preferentially expressed by the $CD4^+$ fraction as opposed to the $CD8^+$ fraction, and within the $CD4^+$ subset by the $CD45RO^+$ memory cells [21]. Additional co-stimulation (*via* CD28) strongly increased IL-22 expression. Moreover, polarization towards the T1 phenotype [IL-12 / anti-IL-4 monoclonal (m)Ab] further increased this expression, whereas polarization to the T2 phenotype (IL-4 / anti-IFN-γ mAb), or regulatory (IL-10 / TGF-β) cells decreased it [21]. The data on IL-22 protein production substantiated that IL-22 is exclusively expressed in T and NK cells [22].

In 2005, two independent groups demonstrated that the activation of murine naive T cells with mature dendritic cells (DCs) in the presence of IL-6 and TGF-β up-regulated the receptor for IL-23 (p19/p40) and, together with this cytokine,

induced development of Th17 cells [23, 24]. Th17 cells primarily produce IL-17A, IL-17F, and IL-6, but not IL-4 or IFN-γ. They express the transcription factor RORγt and IL-23R. In the human system, the combination of IL-23, TGF-β, IL-1β, and IL-6 appears to be necessary for full development of Th17 cells. In fact, the removal of any of these four cytokines decreased IL-17 production as well as RORγt and IL-23R expression by at least 50% [25]. The Th1 cell cytokines IL-12 and IFN-γ as well as Th2 cell cytokine IL-4 actively suppress the development of Th17 cells [23, 24].

After the discovery of the Th17 subset, several groups analyzed IL-22 expression in murine Th17 cells generated *in vitro* [26–28]. The results of these groups clearly demonstrated that, although Th1 cells produce more IL-22 than naive T cells, Th2, or regulatory T cells, the Th17 cells are the major IL-22 cellular sources among T cells. However, various, very recent observations suggest that Th17 cells are not always the main producers of IL-22 *in vivo*. For example, the high IL-22 levels, found in certain organs during acute systemic inflammation after LPS application in mice, are very similar in wild-type (WT) mice and T cell-deficient SCID/beige mice (R. Sabat and K. Wolk, unpublished data). The actual producing cell type in this model is not yet clear. However, no expression of IL-22 was found in monocytes, macrophages, or immature or mature DCs that were generated *in vitro* from human blood monocytes, or in mast cells that were isolated from the skin of healthy donors [21, 29]. In addition, we also screened a variety of primary non-immune human tissue cells and cell lines, but did not find any IL-22 mRNA expression in tissue cells ([30] and R. Sabat and K. Wolk, unpublished data). Furthermore, during intestinal infection with *Citrobacter rodentium*, IL-22 expression levels were similar in the inflamed colon of WT mice and T cell-deficient (Rag2-deficient) mice to those demonstrated by Ouyang's group [31]. Interestingly, in contrast to IL-22, the IL-17A expression was reduced in T cell-deficient animals in both models (systemic inflammation and mucosal infection).

Regarding the human system, a very recent study by Volpe et al. [25] showed convincingly that IL-22 production by Th17 cells was less pronounced than that by Th1 cells. Additionally, Scriba et al. [32] investigated mycobacteria-specific T cells in peripheral blood of healthy participants and found that IL-22- and IL-17-producing T cells were distinct from each other and had phenotypic characteristics of long-lived central memory cells. These data suggest a profound species difference and imply caution in extrapolating mouse data to the human system.

The preferential production of IL-22 by Th1 and Th17 memory cells suggests that elevated levels of this cytokine exist in chronic, T cell-mediated diseases, such as psoriasis, Crohn's disease (CD), and rheumatoid arthritis. Indeed, high IL-22 mRNA levels can be found in lesional skin from patients with psoriasis, whereas IL-22 was completely absent in the healthy skin of control donors [22, 30]. Interestingly, this up-regulation was even more pronounced than that of IL-1β, IL-12, TNF-α, or the prototypical Th1-cytokine IFN-γ ([22, 30] and R. Sabat and K. Wolk,

unpublished data). Moreover, the high cutaneous levels of IL-22 were associated with increased levels of this cytokine in circulation, whereas blood levels of IFN-γ and IL-1β did not appear to be elevated in these patients, and TNF-α levels were only minimally up-regulated [22]. Anti-psoriatic therapy significantly reduced the cutaneous IL-22 mRNA expression and blood IL-22 levels. A correlation analysis of IL-22 plasma levels revealed a clear positive correlation between IL-22 plasma concentrations and the disease severity of psoriasis [22]. In addition to the expression of IL-22 in psoriasis, high cutaneous levels of this cytokine were detected in another T cell-mediated skin disease, atopic dermatitis [30]. The samples used for these analyses were derived from patients in the chronic disease stage that, in contrast to the acute stage, was described as lacking the dominance of Th2 polarization. Furthermore, in contrast to the normal colonic mucosa, $CD4^+$, IL-22-expressing T cells were shown to be present in inflamed regions of the gut in patients with inflammatory bowel disease, particularly in active lesions of patients suffering from CD [33]. Whereas in active ulcerative colitis (UC) these IL-22-expressing cells were localized mainly within the lamina propria, in active CD they were scattered throughout the submucosa [33]. IL-22 is also present in high quantities in the blood of CD patients in comparison to healthy persons, and IL-22 serum levels positively correlated with disease activity [34]. In contrast to IL-22, IL-17, IFN-γ, and IL-1β were only scarcely detectable and showed no differences in blood levels between these patients and control. Interestingly, in a mouse colitis model, IL-22 mRNA expression was elevated predominantly in the inflamed intestine, but also in the mesenteric lymph nodes [34]. It should be mentioned that Fujiyama's group did not find IL-22 expression in the gut mucosa of patients with infectious colitis [33]. IL-22 expression has also been described in rheumatoid arthritis. Nojima's group demonstrated IL-22 expression in such patients both in synovial tissues (both in the lining and the sublining layers) and in mononuclear cells of the synovial fluid [35]. Although no control patients could be included in this study, the authors suggest that the expression was increased compared to the healthy situation since no IL-22 expression was detected in deeper regions of the synovia. Additionally, IL-22 expression was found in lung tissue of normal subjects and patients with interstitial lung disease [36]. A study that performed Western blot analysis using an anti-IL-22 Ab from Santa Cruz biotechnology, which apparently recognizes only one of the glycosylation forms of IL-22, showed decreased IL-22 expression in bronchoalveolar lavage from patients with sarcoidosis compared to that from patients with idiopathic pulmonary fibrosis or normal subjects.

IL-22 is expressed in certain inflammatory murine models. For example, high IL-22 levels were detected in models for: acute systemic inflammation (LPS model mentioned above), colitis, hepatitis, mucosal and systemic infections [31, 34, 37–44].

In contrast to the production of other members of the IL-17 family, the major source of IL-17A and IL-17F appears to be the Th17 cells. Recently, Acosta-Rodri-

guez et al. [45] undertook a characterization of Th17 cells present in the human blood. They found IL-17A-producing cells both in the central memory ($CCR7^+$) and the effector memory ($CCR7^-$) CD4 T cell subsets, but not in naive CD4, naive or memory CD8 subsets, or among γδ T cells. Interestingly, the IL-17A-producing cells represented a subset within CCR6-expressing cells. The majority of *in vivo*-generated IL-17A-producing cells were $CCR6^+/CCR4^+/CXCR3^-$, which produced neither IL-4 nor IFN-γ. In addition, $CCR6^+/CCR4^-/CXCR3^+$ cells produced IL-17A, but they also secreted IFN-γ [45]. It should be mentioned that most Th2 cells are $CCR6^-/CCR4^+/CXCR3^-$ and that most Th1 cells are $CXCR3^+$ (they are either $CCR6^+$ or $CCR6^-$ but always $CCR4^-$). In mice, IL-17 is produced during certain infections (such as with *Klebsiella pneumonia*, *Bacteroides fragilis*, *Borrelia burgdorferi*, *Mycobacterium tuberculosis*, and fungal species) and during chronic tissue inflammation [such as experimental autoimmune encephalomyelitis (EAE) and collagen-induced arthritis] [19, 46]. In line with these observations, in murine models, IL-17 expression has been detected in the target tissue during the progression of various human autoimmune diseases such as multiple sclerosis, rheumatoid arthritis, and inflammatory bowel disease [19, 46]. However, in contrast to IL-22, IL-17 does not appear to be present in blood of patients suffering from such chronic inflammatory disorders ([22, 29] and R. Sabat and K. Wolk, unpublished data).

Interestingly, it seems that the production of IL-22 and IL-17A/IL-17F is regulated differently. During acute inflammation in mice, the production of IL-22 is IL-23 dependent, but independent of IL-6. In contrast to IL-22, IL-17A expression in mice is clearly IL-6 dependent but IL-23 independent ([31] and R. Sabat and K. Wolk, unpublished data). Also, in the human system, the IL-22 and IL-17A production were modulated differently by IL-23, TGF-β, IL-1β, TNF-α, and IL-6 [25].

Receptors

Both IL-22 and IL-17A/IL-17F conduct their biological effects *via* receptor complexes that are composed of two different transmembrane proteins. However, the chains of the IL-22 receptor complex are completely different from the chains of the IL-17 receptor complexes.

The IL-22 receptor complex is composed of IL-22R1 and IL-10R2 (Tab. 1 and [9, 15, 47]). In accordance with the CRF2 characteristics, both transmembrane chains have an extracellular moiety containing two tandem FNIII domains with several aa positions conserved within this receptor family [3, 4]. The human IL-22R1-encoding gene (*IL22RA1*; sequence under NT_004610) is located on chromosome 1p36.11 (reversed strand), near the IL28RA locus, whereas the IL-10R2-encoding gene (*IL10RB*; sequence under NT_011512) is located on 21q22.11 (forward strand), near the *IFNAR1*, *IFNAR2*, and *IFNGR2* loci. *IL22RA1* and *IL10RB* have similar structures, and are comprised of seven exons, with exons 2–7 containing

the sequences for the 574-aa (sequence under NP_067081) and 325-aa (sequence under NP_000619) mature proteins. The transmembrane moieties are predicted to be encoded by sequences derived from exon 6 of the corresponding genes. The longer intracellular moiety of IL-22R1 (predicted 325 aa *versus* 79 aa in the IL-10R2) contains four Tyr-X-X-Gln motives indicating putative STAT recruitment sites [48]. The extracellular domains of IL-22R1 and IL-10R2 contain three and four putative N-linked glycosylation sites (Asn-X-Thr/Ser), respectively.

With the identification of the components of IL-22 receptor complex, the question arose as to which receptor chain is the primary IL-22-binding chain. The initial studies published by Xie et al. [9] and Kotenko et al. [48] described direct IL-22 binding to IL-10R2 and to both IL-22R1 and IL-10R2, respectively. In contrast, Logsdon et al. [12, 16] determined the kinetic binding data by surface plasmon resonance techniques showing that IL-22 has a high affinity towards IL-22R1 ($K_D < 20$ nM) but no actual affinity for IL-10R2. However, IL-10R2 showed a measurable affinity for the IL-22/IL-22R1 complex. Moreover, Fouser's group reported that biotinylated IL-22 binds soluble IL-22R1-Fc but not IL-10R2-Fc in an ELISA-based format, although IL-10R2-Fc stabilized an established association of IL-22 with IL-22R1-Fc [49]. This work additionally provided the initial evidence for a conformational change of the IL-22 molecule during the interaction with its receptor chains. Using scans of overlapping peptides derived from the protein sequence, our own experiments aimed at identifying the possible binding sites for the interaction between IL-22 and IL-10R2. We showed a missing interaction between native IL-22 and the peptide scan derived from the IL-10R2 aa sequence, although native IL-10R2 was able to bind defined peptides of the peptide scan derived from the IL-22 aa sequence (see below) [43]. This underpins the notion that the IL-22 binding site for IL-10R2 may include sequence residues of this cytokine that are normally not accessible on the surface, but become accessible after a conformational change induced by initial interaction with IL-22R1 (Fig. 2).

So far, no crystal structure of a complex composed of IL-22 and components of its receptor complex has been described. The current knowledge on residues in these molecules that are involved in their binding is based for IL-22/IL-22R1 on homology models and for IL-22/IL-10R2 on experimental data. However, there are no published data clarifying whether there is any interaction between the receptor chains in the ternary IL-22/IL-22R1/IL-10R2 complex. Based on the data on the IL-10/IL-10R1 complex, Walter's group generated a homology model for the IL-22/IL-22R1 complex, and predicted that the IL-22R1 loops L2–L6 contact IL-22 residues located on helix pre-A (residues 47–48), helix A and the AB loop (residues 53, 57, 61–73), and helix F (residues 162–175) [11, 12]. In IL-22, a very similar binding site was also postulated for IL-22R1 by Nagem et al. [10]. To map the IL-10R2 binding site of human IL-22, we recently analyzed the binding of soluble IL-10R2 to a scan of immobilized overlapping peptides derived from the aa sequence of IL-22 [43]. Our data revealed distinct binding of IL-10R2 to the N-terminal end of helix

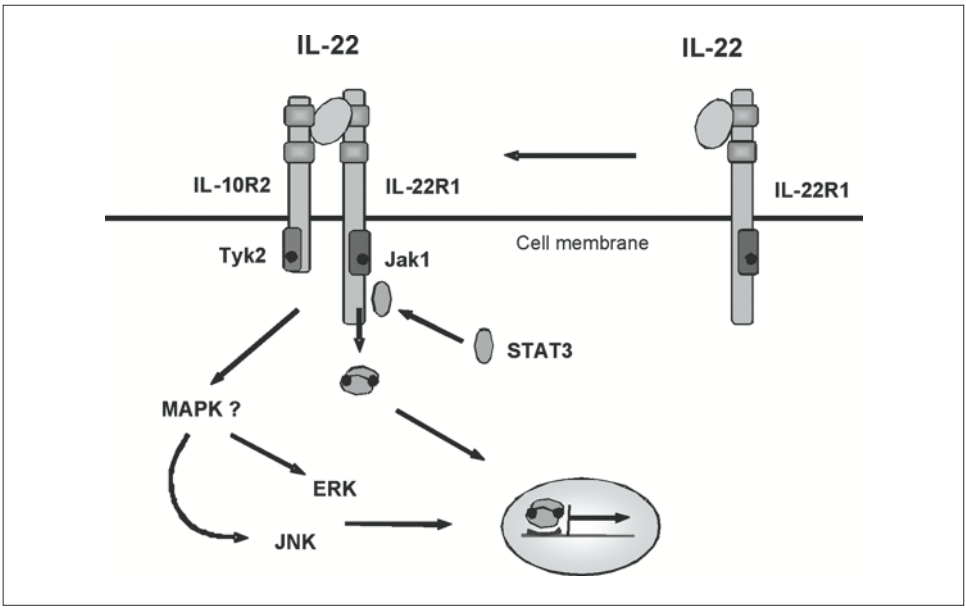

Figure 2
The interaction between IL-22 and the IL-22 receptor complex. IL-22 affects their target cells via *the IL-22 receptor complex comprising IL-22R1 and IL-10R2. There is evidence that IL-22 binds first to the high-affinity chain IL-22R1. This induces a conformational change in the cytokine allowing its interaction with IL-10R2. These interactions lead to signal transduction* via *JAK–STAT pathways. In some cell types, activation of MAP kinase pathways has also been described.*

A, and a region comprising the helix D and the DE loop of IL-22. As deduced from the IL-22 X-ray structural data [10], these two separate segments in the primary sequence form a discontinuous epitope in the IL-22 molecule, which is located next to a region that has been proposed as a binding site for IL-22R1. Our data are basically in good accordance with the data from Walter's group that showed that IL-22 mutations at Tyr51, Asn54, or Arg55 (N-terminal end of helix A), to a greater extent, and at Tyr114 and Glu117 (helix D), to a lesser extent, impaired its affinity to soluble IL-10R2 in the presence of soluble IL-22R1 [16]. As noted above, Asn54 represents the residue whose glycosylation is important for the interaction between IL-22 and IL-10R2 [16]. It is currently not clear whether this carbohydrate interacts with IL-10R2 or whether it functions as a lever with which IL-22R1 creates the binding site for IL-10R2 in IL-22.

The receptor complex for IL-17A and IL-17F is composed of IL-17RA and IL-17RC [50–52]. IL-17RA and IL-17RC, together with IL-17RB, IL-17RD,

and IL-17RE, are five transmembrane proteins that do not fall into any known receptor families. The human IL-17RA-encoding gene (*IL17RA*; sequence under NT_011519) is located on chromosome 22q11.1 (forward strand), whereas the IL-17RC-encoding gene (*IL17RC*; sequence under NT_022517) is located on 3p25.3-p24.1 (forward strand), near the *IL17RE* locus. Multiple, alternatively spliced transcript variants of *IL17RC* encoding isoforms have been detected [53]. *IL17RA* and *IL17RC* have limited similarity and contain the sequences for the 866-aa (sequence under NP_055154) and 791-aa (sequence under NP_703191) premature proteins, respectively. IL-17RA is composed of a 288-aa extracellular domain, 21-aa transmembrane domain, and 525-aa intracellular moiety. IL-17RC shares 23% aa identity with IL-17A. IL-17RC is the binding chain in the IL-17 receptor complex. In fact, both IL-17A and IL-17F interacted with IL-17RC with comparable, high affinity (K_D ca. 0.5 nM). In contrast, IL-17RA binds IL-17A effectively (K_D ca. 2 nM), but binds IL-17F with an approximately 1000-fold lower affinity [50]. Currently, IL-17RA and IL-17RC are assigned to the so-called 'SEFIR' ('similar expression to fibroblast growth factor genes, IL-17 receptors and Toll–IL-1R') protein family, defined by a conserved cytoplasmic SEFIR domain similar to the conserved motif found in Toll–IL-1R. Using computational modeling, Kramer et al. [54] recently identified two FNIII domains in the extracellular part of IL-17RA connected by a non-structured linker. The membrane-distal domain seems to be required for interactions with IL-17.

Target cells

The targets cells of IL-22 and IL-17A/IL-17F are only partially identical. The big difference is that immune cells do not bear the IL-22 receptor complex but are sensitive towards IL-17A/IL-17F. In addition, the number of different tissue cells that IL-22 acts on is smaller than the number of tissue cells whose function is regulated by IL-17A/IL-17F.

Several research groups, including our own, have extensively studied the expression of the IL-22 receptor complex components. IL-10R2 has been shown to be ubiquitously expressed, which can be explained by its function as part of several cytokine receptors [30]. Therefore, the expression of the IL-22R1 chain should determine whether a cell is an IL-22 target or not. The close relationship of IL-22 to IL-10 initially suggested extensive effects of IL-22 on various populations of immune cells. However, our studies using real-time RT-PCR did not detect any expression of IL-22R1 in bone marrow, blood mononuclear cells, thymus, or spleen [30]. Moreover, we did not detect any IL-22R1 expression in a variety of resting or activated primary immune cells including monocytes, B cells, T cells, NK cells, macrophages, immature and mature DCs [21, 22, 30]. In accordance with the results obtained with primary blood immune cell subpopulations, corresponding cell lines

(Jurkat T-lymphoblast cells, THP-1 monocytic leukemia cells, Raji B cell lymphoma cells, and K562 chronic myelogeneous leukemia cells) expressed IL-10R2 but not IL-22R1 [21, 43]. Lecart et al. [55] and Boniface et al. [56] later confirmed the missing IL-22R1 expression in B cells and PBMCs, respectively.

In contrast to immune cells, our data showed that a few organs expressed the IL-22R1 chain and, therefore, contain putative target cells of IL-22. These organs include the skin and kidney, and those from the digestive (pancreas, small intestine, liver, colon) and the respiratory (lung, trachea) systems with the highest expression found in the skin and pancreas [30]. Interestingly, most IL-22R1-expressing tissues form outer body barriers and contain epithelial cells. These quantitative results are in line with the semi-quantitative data published earlier by Aggarwal et al. [57] and Parrish-Novak et al. [58]. A pattern similar to that observed in tissues was found in corresponding cell lines (e.g. HaCaT keratinocytes cell line, BxPC-3 pancreatic adenocarcinoma cells, C170 colon tumor cells, HepG2 hepatocyte carcinoma cells, and A549 lung carcinoma cells) [30, 43]. Interestingly, the keratinocyte expression of IL-22R1 and IL-10R2 strongly increased in a dose- and time-dependent manner in the presence of IFN-γ, suggesting an elevated sensitivity of keratinocytes to IL-22 under T1 conditions [22].

Contrary to our assumption that epithelial cells themselves constitute the major cell population within the IL-22R1-expressing tissues, Fujyama's group demonstrated IL-22R1 expression in normal colonic mucosa exclusively in subepithelial myofibroblasts [33]. Moreover, Nojima's group described IL-22R1 expression in synovial fibroblasts *in situ* and in synovial fibroblast lines derived from patients with rheumatoid arthritis [35]. However, no data are available regarding the IL-22R1 expression of such fibroblasts from healthy donors.

Upon discovery of IL-17RA, Yao et al. [52] found them in the spleen, kidney, lung, liver, and the brain. IL-17RC is also known to be expressed in a variety of tissues [53]. Unfortunately, systematical studies on the specific IL-17 receptor expressing cells are lacking so far. However, it appears that basically any cell type is sensitive towards IL-17 action.

Signal transduction

IL-22 and IL-17A/IL-17F use different signal transduction pathways to activate target cells. Like other members of the IL-10 family, after interaction with its receptor complex, IL-22 activates the JAK/STAT pathway. Most studies investigating IL-22-induced signal transduction in cells with endogenous receptor expression refer to tumor cell lines and show activation of STAT3, and depending on the system used, often STAT1 and/or STAT5 as well (see below). Using the H4IIE rat hepatoma cell line, Lejeune et al. [59] investigated the JAKs that are responsible for the IL-22-induced STAT activation. These authors used Western blot analysis in cell lysates

and immunoprecipitations using specific anti-JAK Abs to show that IL-22 led to tyrosine phosphorylation of Jak1 and Tyk2, but not Jak2. The essential role of Jak1 for IL-22-induced signaling was confirmed by the absence of IL-22-induced STAT-responsive reporter gene activity in Jak1-deficient cells [59]. Since Tyk2 has been known to be associated with IL-10R2, these studies suggest Jak1 as the kinase associated with the IL-22R1. In addition to tyrosine phosphorylation of STAT molecules, activation of the three major MAP kinase pathways (p38 kinase, JNK, and ERK1/2) and serine phosphorylation of STAT3 was suggested to be required for maximum IL-22-induced transactivation of the STAT-responsive promoter in the H4IIE cell line [59]. The IL-22-induced signal transduction was also investigated in detail in primary cells such as in human keratinocytes, human colonic subepithelial myofibroblasts, and human synovial fibroblasts from patients with rheumatoid arthritis. Using Western blot analysis, our group found IL-22-induced tyrosine phosphorylation of STAT3 in human keratinocytes, but only minor and non-distinctive activation of STAT1 and STAT5, respectively [22, 30]. In cells from one of four donors, IL-22 additionally induced tyrosine and threonine phosphorylation of ERK1/2 and JNK. No phosphorylation of p38 kinase or IκB-a was observed for any donor [22]. Andoh et al. [33] demonstrated that IL-22 in human colonic subepithelial myofibroblasts induced activation not only of STAT3 (STAT1 and STAT5 were not accessed in this study) but also of ERK1/2, JNK, p38 kinase, and NF-κB. A third group addressing this issue, Ikeuchi et al. [35] described activation of ERK1/2 and p38 (STAT molecules, JNK and NF-κB pathways were not included in this study) upon IL-22 stimulation of human synovial fibroblasts from patients with rheumatoid arthritis. The differences between these three studies are most likely due to cell specificity, but may also result from the different IL-22 concentrations used (10 ng/mL in the keratinocyte study, 200 ng/mL in the myofibroblast study, and 100 ng/mL in the synovial fibroblast study).

Following interaction with its receptor complex, IL-17A/IL-17F preferentially activated the NF-κB and MAP kinase (p38 kinase, JNK, and ERK1/2) pathways [60]. The binding of IL-17 to its receptor complex leads to recruitment of the adaptor protein Act1 to IL-17RA. For this recruitment the conserved cytoplasmic 'SEFIR' domain of IL-17RA is required. Interestingly, in its coiled-coil region at the C terminus, Act1 also contains a SEFIR domain. Afterwards, the recruitment of the TAK1 kinase (TGF-beta-activated kinase 1) and the E3 ubiquitin ligase TRAF6 (TNFR-associated factor 6) to the IL-17 receptor complex follow [61]. In 2002, it was demonstrated that TRAF6 is necessary for IL-17 activation of NF-κB and JNK [62]. Interestingly, IL-17 treatment of cells also increased the phosphorylation of JAK1/2-activated PI3K signaling. The inhibition of either JAK or PI3K had no effect on the DNA-binding activities of p65 and p50 to NF-κB consensus sequences, but decreased IL-17A-induced gene expression [63]. This result suggests that the JAK-associated PI3K signaling axis acted independently of NF-κB and MAP kinase pathways.

Biological effects of IL-22 and IL-17A/IL-17F

The biological effects of IL-22 and IL-17A/IL-17F differ in many aspects. IL-22 seems to be a novel type of immune mediator that increases the innate immunity of tissue cells, protects tissues from damage and enhances their regeneration. IL-17A/IL-17F are typical pro-inflammatory mediators (Fig. 3).

As described above, immune cells are not the target cells of IL-22. This conclusion is based on a range of different observations from our laboratory [21, 29, 30, 43]. As mentioned, neither resting nor stimulated immune cells express IL-22R1 [21, 29, 30]. Additionally, no STAT tyrosine phosphorylation was induced by IL-22 in any of these cells [30]. Finally, no influence of IL-22 was found *in vitro* or *in vivo* upon the screening of a large range of immunological parameters in the presence or absence of cell-specific stimuli [30, 43]. In addition to these studies, other groups were subsequently unable to show that IL-22 regulated the function of immune cells [55–57].

The IL-22R1 and IL-10R2 expression patterns suggest that the most important target cells of IL-22 are cells of the digestive tract, skin, lungs, and kidney [30]. In

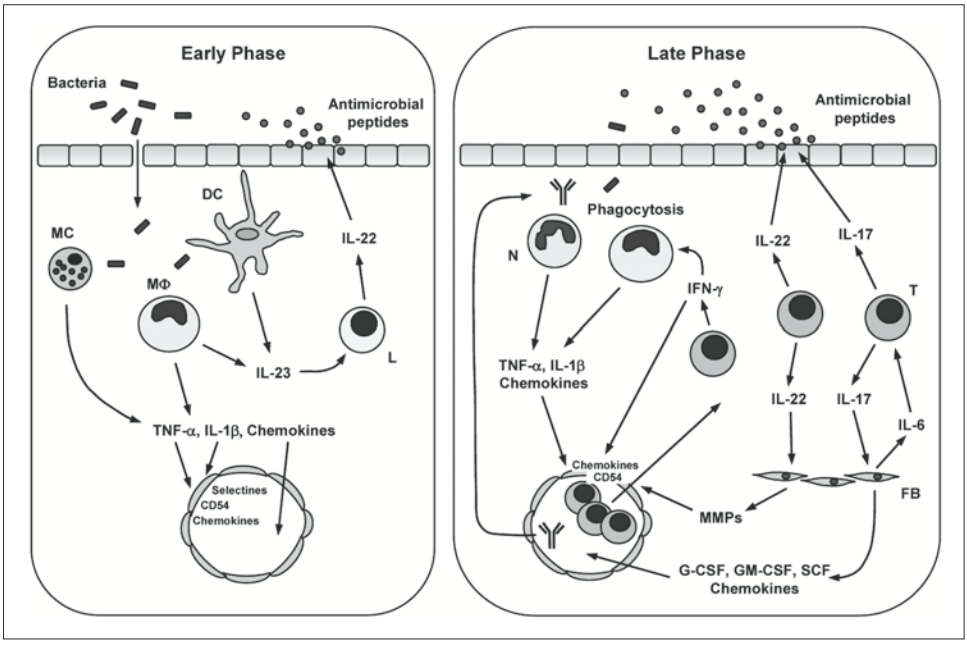

Figure 3
The role of IL-22 and IL-17A/IL-17F during mucosal infections (see text). MC, mast cell; MΦ, macrophage; DC, dendritic cell; L, lymphocyte; N; neutrophil granulocyte; T, T cell; FB, fibroblast.

the year that IL-22 was discovered, Renauld's group demonstrated that IL-22 was able to induce mRNA expression of acute phase proteins such as serum amyloid A (SAA), α1 anti-chymotrypsin, and haptoglobin in the HepG2 human hepatoma cell line [47]. Moreover, IL-22 injection in mice increased hepatic SAA mRNA expression in these animals [47]. Later, we confirmed this SAA induction at the protein level *in vitro* and *in vivo* and found that IL-22 also induced LPS-binding protein (LBP) [30, 34, 43]. Additionally, IL-22 possesses a protective role in liver injury. Radaeva et al. [40] used the model of Con-A-induced hepatitis, which is associated with massive infiltration of activated T cells and IL-22 production. The authors demonstrated that pretreatment with IL-22 alleviated the Con-A effect, whereas the injection of Con-A together with IL-22 neutralizing Ab worsened it [40]. Zenewicz at al. [44] confirmed the observation that IL-22 provides protection to hepatocytes during acute liver inflammation by means of IL-22-deficient mice. These authors also demonstrated that IL-17 had no such role in liver inflammation. The molecular mechanisms of the protective IL-22 action during liver inflammation remain unclear.

Regarding the exocrine pancreas, Gurney's group showed IL-22-induced up-regulation of pancreatitis-associated protein 1 (PAP1) and osteopontin mRNA expression in mouse primary acinar cells *in vitro* [57]. In the last few years, an increasing amount of evidence has been provided that PAP1 plays an important role in the protection against tissue injury.

The effects of IL-22 that have been most precisely characterized to date concern those on the biology of keratinocytes and other epithelial cells. Following our initial study demonstrating that human keratinocytes are important target cells and the preliminary effects of IL-22 on these cells [30], two independent studies were conducted in parallel that identified a broad range of IL-22 effects on these cells using gene expression arrays specifically designed for keratinocytes and inflamed skin [22, 55]. These studies from Morel's and our group found that IL-22 influenced only a very limited number of the analyzed mRNA species. The gene products can be divided into three functional categories: (i) anti-microbial proteins, (ii) differentiation-associated proteins, and (iii) mobility-regulating proteins. In fact, IL-22 enhanced the expression of the anti-microbial molecules, reduced the expression of the differentiation-associated molecules, and regulated the expression of molecules assumed to lead to increased cellular mobility. Interestingly, IL-1β enhanced the IL-22 effects [22]. The first group of IL-22-regulated gene expressions in keratinocytes includes those of the β-defensins (hBDs) 2 and 3, as well as S100A7, S100A8, and S100A9. The second group of IL-22-sensitive genes concerns profillagrin, keratins 1 and 10, calmodulin-like 5, keratinocyte differentiation-associated protein, and kallikrein 7. These proteins are involved in the terminal differentiation of keratinocytes, which is a particular apoptotic process that results in the formation of the stratum corneum. The last group of IL-22-sensitive gene expressions concerns the expression of the matrix metalloproteinases (MMPs) 1 and 3, which are increased by IL-22,

and annexin A9, which is decreased by IL-22. Interestingly, these three functions are also altered in keratinocytes from psoriasis patients. These data, together with the high IL-22 expression levels in psoriatic plaques and in the blood of psoriasis patients, suggest that IL-22 plays a major role in the dramatic keratinocyte functional alterations in psoriasis [6, 30, 64]. This postulated major role of IL-22 in the pathogenesis of psoriasis was further supported by two recently published studies in mice: Zheng et al. [28] found that repeated cutaneous application of IL-23 induced acantosis in mice in an IL-22-dependent way; and recently, Fouser's group published a psoriasis-like disease model in mice, which is induced by the transfer of memory T cells to pathogen-free scid/scid mice [39]. In this model, neutralization of IL-22 reduced acanthosis, inflammatory infiltrates, and expression of some cytokines. Furthermore, the authors demonstrated that IL-22 application into the skin of normal mice induced anti-microbial peptide gene expression [39]. Two further studies in mice confirmed the significance of the IL-22-induced anti-microbial proteins in epithelial cells. Zheng et al. [31] demonstrated that IL-22 induced RegIII proteins in intestinal epithelial cells and IL-22-deficient mice died as a result of intestinal infection with *C. rodentium*. Furthermore, the Kolls group found that IL-22 up-regulated expression of lipocain 2 in lung tissue, and neutralization of IL-22 during pulmonal *K. pneumoniae* infection led to animal death [37].

Similar to the studies with keratinocytes, Fujiama's group used a cDNA microarray to detect all possible effects of IL-22 on human colonic myofibroblasts [33]. Colonic subepithelial myofibroblasts are known to play important roles in the organogenesis of the intestine. Fujiama's group found that stimulating human colonic subepithelial myofibroblasts with a high dose (200 ng/mL) of IL-22 altered the expression of 28 genes (out of the ca. 16 000 sequences present on the used array), which could also be pooled into functional groups. The first group identified in the study includes mediators that regulate inflammation. IL-22 induced the expression of two anti-inflammatory or protective acting proteins: follistatin and IL-11. However, IL-22 also induced the expression of many pro-inflammatory mediators, i.e., chemokines (CCL7, CXCL1, CXCL2, CXCL3, CXCL6, and CXCL8) and IL-6. At this point, it could be said that no systemic level of IL-6 could be detected after the application of IL-22 in mice. It is possible that (i) the IL-22 anti-inflammatory effects dominate locally (i.e., in the gut), or that (ii) the IL-22 induction of IL-6 in colonic subepithelial myofibroblasts is an effect specific for humans, and/or (iii) the induction of IL-6 required a high concentration of IL-22. In fact, the IL-6 induction discovered *in vitro* by Andoh et al. became significant only with 100 ng/mL IL-22 [33]. The second group of modified expressions that was identified in this study as being influenced by IL-22 includes some MMPs (MMP1, MMP3, and MMP9). The third group composes mediators that regulate the proliferation of epithelial cells. In fact, IL-22 induced the expression of amphiregulin and leukemia inhibitory factor (LIF). In addition to these effects, Fujiama's group demonstrated that IL-22 promotes the expression of regulators

of signal transduction (SOCS2) and metabolism (stanniocalcin 1), and reduced the expression of elements from signaling pathways (PPAR-γ, IRAF-1). The Fujiama's study suggests that IL-22 can play a role in the pathogenesis of inflammatory bowel disease [33]. Two other studies attempted to illuminate this role [34, 41]. Since we found a decrease of the constitutive IL-22BP expression in the inflamed intestine simultaneously to a strong increase of IL-22 in the inflamed intestine and the mesenteric lymph nodes in the colitis mice model, we assumed a potential systemic role of IL-22 in CD patients. This idea was supported by the fact that, in line with the mouse results, IL-22 blood levels of CD patients are strongly elevated in comparison to healthy persons. In our study, we demonstrated that IL-22 induces LBP blood levels reaching concentrations known to neutralize LPS [34]. These data suggest that systemic IL-22 may contribute to the prevention of systemic inflammation provoked by LPS present in the blood of CD patients through its induction of hepatic LBP. Mizoguchi's group recently demonstrated, by means of a novel microinjection-based local gene-delivery system, that IL-22 is capable of targeting the inflamed intestine in a mouse model of Th2-mediated colitis [41]. This mouse model is similar to UC. IL-22 gene delivery induced both STAT3-dependent expression of mucus-associated molecules and restitution of mucus-producing goblet cells. Importantly, IL-22 gene delivery led to rapid amelioration of local intestinal inflammation [41]. Interestingly, both studies independently postulated a protective role of IL-22 in inflammatory bowel disease. Finally, the Becher group investigated the role of IL-22 in experimental autoimmune encephalomyelitis (EAE). IL-22-deficient mice were fully susceptible to EAE [38].

In contrast to IL-22, which acts on only certain tissue cells and functions by promoting the anti-microbial defense and by protecting against damage of non-immune tissues, both IL-17A and IL-17F have clear pro-inflammatory properties and act on a broad range of cell types.

IL-17A and IL-17F induced the production of many neutrophil-attracting chemokines, such as CXCL1, CXCL2, CXCL5, and CXCL8 in fibroblasts, epithelial cells, and endothelial cells [65, 66]. In DCs, IL-17A reduced the production CCL17, a chemokine that is responsible for recruitment of Th2 cells to the site of inflammation [67]. Furthermore, IL-17A and IL-17F enhanced the expression of G-CSF, GM-CSF, and stem cell factor in tissue cells and thereby led to strengthened granulopoiesis [68, 69]. Interestingly, adenovirus-mediated gene transfer of the murine IL-17A cDNA resulted in a transiently transgenic phenotype, with dramatic effects on *in vivo* granulopoiesis. There was a significant increase (tenfold) in the absolute neutrophil count in the peripheral blood. This was associated with a doubling in the spleen size [70]. IL-17, through enhancement of chemokine production and granulopoiesis, caused a significant increase in the immigration of neutrophilic granulocytes to the site of inflammation, and is thereby a key cytokine for the recruitment, activation, and migration of neutrophils. Additionally, IL-17A time- and dose-dependently induced the expression of hBD2 in primary human airway

epithelial cells [71]. The IL-17A-initiated enhancement of hBD2 expression was not attenuated by the IL-1R antagonist or by neutralizing anti-IL-6 Abs. In primary human tracheobronchial epithelia, IL-17A also enhanced the expression of the mucin genes MUC5B and MUC5AC [72]. The treatment of tissue cells with IL-17A and IL-17F also induced the expression of IL-6 and metalloproteinases [65]. Further investigation of MUC5B expression demonstrated that the IL-17 effect is, at least partly, mediated by the autocrine/paracrine loop through IL-6. IL-17A up-regulated IL-1β and TNF-α expression and synthesis in human macrophages in a dose- and time-dependent fashion [73]. Interestingly, IL-17 also influences $CD4^+$ T cell and B cell interactions required for the formation of the germinal center. Indeed, mice lacking the IL-17RA have reduced B cell development and humoral responses [74]. All these observations suggest that IL-17 has an important role in host protection against specific pathogens (gram-negative bacteria and fungi). Indeed, as demonstrated using IL-17RA-deficient animals, IL-17 plays an important role in host protection against *K. pneumoniae* [75], *Toxoplasma gondii* [76], and systemic candidiasis [77]. It should be noted that IL-17A/IL-17F does not play an essential role in every infection since IL-17RA-deficient mice do not show an increased susceptibility to infection with intracellular pathogens such as *M. tuberculosis* or *Listeria monocytogenes*.

IL-17A/IL-17F are important mediators of certain autoimmune diseases. In 2002, Nakea et al. [78] generated IL-17A-deficient mice and found that contact, delayed-type, and airway hypersensitivity responses as well as T cell-dependent Ab production were significantly reduced in this mutant. Lubberts et al. [79] showed that blocking of endogenous IL-17A/IL-17F with an IL-17RA-Fc fusion protein in the autoimmune collagen-induced arthritis model resulted in suppression of arthritis, whereas overexpression of IL-17 enhanced collagen-induced arthritis. These clear pro-inflammatory effects of IL-17 seem to be TNF-α- and IL-1β-independent under arthritic conditions [80]. Furthermore, the treatment of rats suffering from adjuvant-induced arthritis with IL-17RA-Fc fusion protein also significantly reduced paw volume and radiographic scores [81]. Komiyama et al. [82] found that the development of EAE was significantly suppressed in IL-17A-deficient mice. These animals exhibited delayed onset, reduced maximum severity scores, ameliorated histological changes, and early recovery. Interestingly, IL-17A, but not IL-17F, seems to be required for the initiation of EAE [83]. The role of IL-17 in experimental colitis in mice is less clear. It has been reported that IL-17F deficiency resulted in reduced dextran sulfate sodium-induced colitis, whereas IL-17A deficiency caused more severe colitis [83, 84]. Zhang et al. [85] found that IL-17RA-deficient mice were significantly protected against TNBS-induced weight loss and colonic inflammation. Moreover, IL-17R-deficient animals showed reduced tissue myeloperoxidase activity. IL-17 may also play a role in myocarditis. Sonderegger at al. [86] demonstrated that the neutralization of IL-17A in experimental autoimmune myocarditis reduced cardiac inflammation and heart autoantibody responses.

Interestingly, some effects of IL-22 and IL-17 are additive. This is the case for the induction of anti-bacterial proteins in epithelial cells [27]. However, there are many other effects where these cytokines do not work additively, such as in the induction of psoriatic-like keratinocyte changes or the induction of acute-phase proteins ([34] and R. Sabat and K. Wolk, unpublished data).

It becomes clear from all these data that we do not yet have a complete understanding of the biological functions of IL-17 and, in particular, of IL-22. Regarding IL-22, it is completely unknown which functions are regulated by this cytokine in the kidney. Our knowledge on how IL-22 affects pancreas biology is also poor. It is reserved for us as researchers to explore this expansive and exciting new field of mediators. What we currently do know, however, is that IL-22 and IL-17A/IL-17F are different in many of their basic aspects and in their biological importance.

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Other sources of IL-17: Invariant natural killer T cells

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Abstract

Interleukin-17 (IL-17) plays a major role in various models of immune-mediated tissue injury, including organ-specific autoimmunity, allergic disorders and microbial infections. Th17 cells are currently the most thoroughly characterized source of IL-17 credited for causing and sustaining the tissue damage mediated by this cytokine. Similarly to their Th1 and Th2 counterpart, Th17 cells depend on specific factors for their differentiation from naïve T cell precursors, before acquiring their typical cytokine profile. However, this is not the case for all IL-17-producing cells, particularly for a subset of invariant natural killer T cells, termed iNKT17, which are ready to produce this cytokine immediately upon stimulation, in keeping with their capacity to intervene during early stages of the inflammatory response.

Introduction

Naïve $CD4^+$ T lymphocytes proliferate and differentiate into two distinct effector subsets, T helper type 1 (Th1) and Th2 cells, characterized by their distinct cytokine profile. Th1 cells produce large amounts of interferon-γ (IFN-γ), whereas Th2 cells are a source of interleukin-4 (IL-4), IL-5 and IL-13 [1]. More than 2 years ago, a new effector population called Th17 was identified by its capacity to produce IL-17A (or IL-17), IL-17F and IL-21 [2]. IL-17 is the founder member of this newly identified cytokine family composed of six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25) and IL-17F [3]. IL-17 is characterized by its potent pro-inflammatory action resulting from several pleiotropic activities, namely the ability to induce pro-inflammatory cytokines (IL-6 and TNF-α) and chemokines (KC, MCP-1 and MCP-2), as well as proliferation, maturation and chemotaxis of neutrophils that ultimately mediate tissue infiltration and destruction. Th17 cells recognize peptide antigens like their Th1 and Th2 counterparts. In contrast, a particular set of T cells can be stimulated specifically by lipids. The understanding of this latter mode of activation has been greatly improved by the discovery of CD1d-dependent

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antigen-presentation pathways that enable T cells to recognize endogenous and foreign lipids, mainly glycolipids, and to participate in the onset and regulation of the immune response. Invariant natural killer T (iNKT) cells are the most widely studied T cell population responding to this type of antigen presentation [4–6], raising the question whether they can produce IL-17 upon exposure to glycolipids. We have recently demonstrated that this is effectively the case for a particular subset of iNKT cells [7], and this subset forms the focus of the present review.

Natural killer T cells: Generalities

The term "natural killer T (NKT) cells" was initially used to designate T lymphocytes expressing NK markers, such as NK1.1 (NKR-P1A or CD161). Subsequently, this population turned out to be quite heterogeneous and comprises several subsets with distinct functional capacities [4–6]. The most abundant and better characterized population expresses a highly restricted T cell receptor (TCR) repertoire composed of a single invariant Vα14Jα18 chain in mice and a Vα24Jα18 chain in humans, preferentially paired with limited TCR Vβ chains. These unconventional T cells have been termed iNKT cells. Their semi-invariant TCR reflects a positive selection by glycolipid antigens presented by the non-polymorphic MHC class I-like molecule, CD1d. Among the CD1 family, composed of five members CD1a–e, CD1d is the only one that is conserved in mice and humans [8]. Compared with classical MHC molecules, CD1d has a narrower and more hydrophobic groove that facilitates presentation of lipid and glycolipid molecules to T cells, mainly iNKT cells. This unconventional T cell population is most frequent in murine liver (10–40% among the lymphocytes), but is also present in thymus, bone marrow, spleen, lymph nodes, lungs, and blood [4–6].

iNKT cells recognize a limited number of synthetic or naturally occurring α- and, to a lesser extent, β-glycosylated sphingolipids bound to CD1d expressed on antigen-presenting cells (APCs), such as dendritic cells (DCs). These CD1d-restricted antigens are believed to be generated during steady-state conditions (for instance in the thymus) but also in peripheral sites during injury, infection and/or inflammation. The first glycolipid identified as a specific stimulant of iNKT cells was α-galactosylceramide (α-GalCer), originally isolated from a marine sponge. Until now, this molecule remains the most common tool for evaluating the functional capacities of iNKT cells. It has also been useful for the construction of the first CD1d tetramers, which provide the most sensitive and specific means to identify and purify iNKT cells [9].

The autoreactivity ascribed to iNKT cells prompted several investigators to search for self-glycolipids. Isoglobotrihexosylceramide, a lysosomal glycosphingolipid, has been claimed to be one of these natural endogenous ligands of CD1d-restricted T cells [5, 6] followed by other endogenous glycolipids such as charged

β-linked glucosylceramide(s) [10]. Exogenous lipids like galacturonic acid-containing glycosphingolipid from non-pathogenic *Sphingomonas* bacteria, but also from *Borrelia burgdorferi* spirochetes, a Gram-negative bacterium responsible for Lyme disease, are also potent iNKT cell activators. This study established that iNKT cells not only respond to antigens derived from pathogenic microbes but can recognize other classes of glycolipids, such as the galactosyl diacylglycerols from *B. burgdorferi* [11]. Moreover, there is growing evidence that other microorganisms, including mycobacteria, *Schistosoma* sp., *Leishmania* sp., and *Plasmodium* sp. may also produce CD1d-restricted ligands capable of activating iNKT cells.

The interest in iNKT cells arose first from their unique capacity to produce simultaneously large amounts of both Th1 (IFN-γ) and Th2 (IL-4) cytokines conferring the ability to influence the outcome and/or the development of several inflammatory diseases. iNKT cells are effectively implicated in tumor immunity, infections, autoimmune diseases and allergic asthma. In most of these pathologies, iNKT cells play a protective role, even though in some cases, they can become deleterious. It is likely that this contrasting effect results from the cytokine profile generated by iNKT cells in each situation since IFN-γ production is required for their protective role in anti-tumor responses, while IL-4 is necessary for their detrimental effect in experimental allergic asthma [12–14]. These observations raise two fundamental questions: (1) which factors induce the particular cytokine profile of iNKT cells that determine their influence on diverse pathologies, and (2) does the iNKT population inherently comprise distinct subsets with distinct cytokine profiles? There are some data in favor of either assumption since cytokines present in the microenvironment as well as specific ligands can shift the cytokine profile towards predominant IL-4 or IFN-γ production [15–17]. Moreover, it has been demonstrated in humans that $CD4^+$ and $CD4^-$ iNKT cells preferentially produce IL-4 and IFN-γ, respectively, supporting the existence of intrinsically distinct iNKT cell populations [5, 6].

We have recently identified a particular subset of iNKT cells that generated high amounts of IL-17 and showed low IL-4 and IFN-γ levels in response to both synthetic and physiological iNKT cell ligands [7]. Because of these functional characteristics, which differ from those shared by the majority of iNKT cells, we called this subset iNKT17 cells.

$NK1.1^{neg}$iNKT IL-17-producing cells

The majority of iNKT cells expresses the NK1.1 marker and promptly produces high levels of both IL-4 and IFN-γ [7]. By contrast, we found that the capacity to produce high levels of IL-17 but only small amounts of IL-4 and IFN-γ, in response to both synthetic (α-GalCer or PBS-57) and natural ligands (lipopolysaccharides (LPS) or glycolipids derived from *Sphingomonas* sp. and *B. burgdorferi*) was associated exclusively with the iNKT cells that lack NK1.1 [7].

iNKT activation downregulates NK1.1 expression, raising the question whether iNKT NK1.1^{pos} and iNKT NK1.1^{neg} actually represent two functional subsets or two stages of activation. To address this issue, we tried to modify the cytokine profile of the NK1.1^{pos} and NK1.1^{neg} iNKT cells in culture conditions known for driving Th17 differentiation of naïve T cells. We could not transform NK1.1^{pos} iNKT cells into IL-17-producing cell by culturing them in presence of both TGF-β and IL-6, which are required for driving the differentiation of naïve mouse CD4 T cells into Th17 cells. Furthermore, addition of IL-23, which enhances IL-17 production by conventional T cells, also did not increase their IL-17 production, suggesting that NK1.1^{neg} and NK1.1^{pos} cells are indeed functionally distinct (Fig. 1). In further support of this assumption, NK1.1^{neg} iNKT cells respond immediately to glycolipid stimulation by producing IL-17 without prior polarization, in contrast to conventional naïve T cells.

It is noteworthy that the lack of NK1.1 expression is also a distinctive feature of peripheral IL-17-producing iNKT cells, and can be used for their analysis and purification. The NK cell receptor protein 1 (NKR-P1) (NK1.1 or CD161) belongs to a family of type II transmembrane C-type lectin-like receptors. It is not clear how

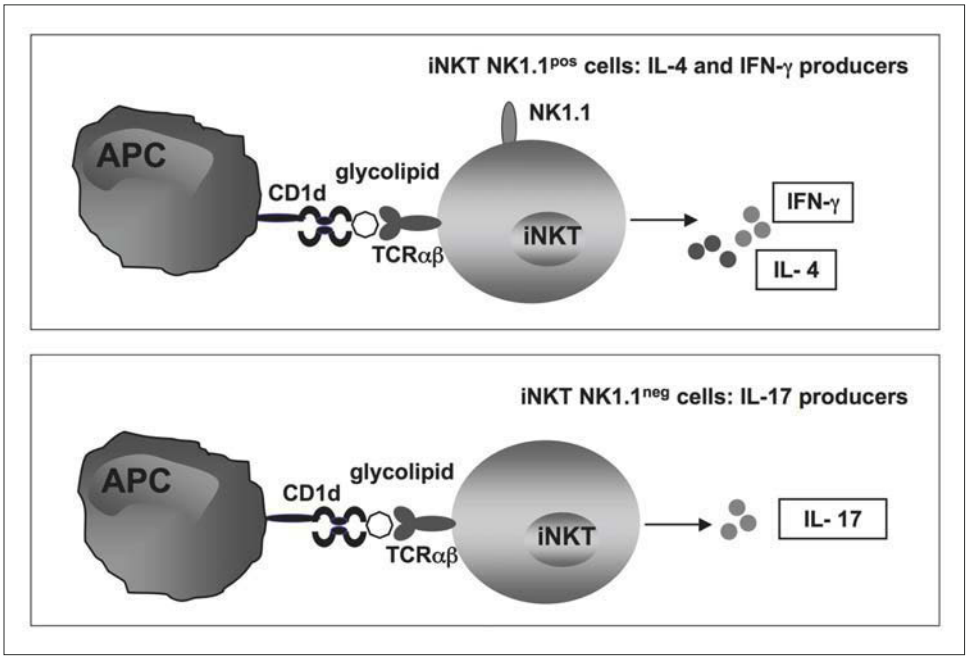

Figure 1
Two subsets of invariant natural killer T (iNKT) cells. (Upper panel) iNKT NK1.1^{pos} cells: IL-4 and IFN-γ producers; (lower panel) iNKT NK1.1^{neg} cells: IL-17 producers.

it affects the functional properties of iNKT cells, but according to recent reports this marker is acquired during iNKT cell differentiation in the thymus and expressed by mature iNKT cells that produce IFN-γ [18]. Indeed, unlike conventional autoreactive T cells, autoreactive iNKT cells are not deleted in the thymus but are positively selected by CD1d molecules expressed by $CD4^+CD8^+$ cortical thymocytes. Moreover, cytokine analysis in NKT cell populations at intermediate differentiation stages between the $NK1.1^{neg}$ and $NK1.1^{pos}$ phenotype are in favor of Th2 to Th1 conversion since as they progress through the $CD44^{low}NK1.1^-$, $CD44^{high}NK1.1^-$, and $CD44^{high}NK1.1^+$ stages, their IL-4:IFN-γ production ratio decreases [18]. In the thymus, $NK1.1^-$ iNKT cell precursors produce high levels of IL-4 but no IFN-γ. The expression of NK1.1 receptor and the capacity to produce IFN-γ are late events observed either in the thymus or after emigration into peripheral tissues [18]. Thus, it will be important to determine whether iNKT17 cells exist in the thymus, whether the iNKT $NK1.1^-$ precursors, which produce IL-4, can also produce IL-17, or whether distinct cells produce these cytokines and, in this case, perform the phenotypic identification and functional characterization of developmental iNKT17 intermediates. Our unpublished data shows that a particular thymic iNKT cell subset can produce IL-17 (M.L. Michel and M.C. Leite-de-Moraes).

IL-17, iNKT cells and infections

$NK1.1^{neg}$iNKT IL-17-producing cells represents up to 10% of iNKT cells in spleen and liver while they account for at least 30% of iNKT cells in lungs [7]. Their high frequency in the lung is consistent with a role in the natural immunity to inhaled antigens. Indeed, airway neutrophilia induced by α-GalCer or LPS instillation is significantly reduced in iNKT-cell deficient $J\alpha18^{-/-}$ mice, which produce significantly less IL-17 in their bronchoalveolar lavage fluid than wild-type controls [7]. Furthermore, airway neutrophilia is abolished by a single treatment with neutralizing mAb against IL-17 before α-GalCer administration, suggesting that this new population of IL-17-producing iNKT cells can contribute to neutrophil recruitment through preferential IL-17 secretion.

Both iNKT cells and IL-17 have been implicated in several infection disorders, suggesting that iNKT cells can represent at least one of the sources of this cytokine. For instance, it was demonstrated that IL-17 was critical for host defense against *Klebsiella pneumoniae* and that protective immunity against this pathogen obtained by CpG ODN administration resulted in enhanced bacterial clearance in lung and blood [19, 20]. In this model, a correlation exists between the higher numbers of pulmonary neutrophils, γδ-T cells and NKT cells and the protection observed. However, it remains to be determined whether iNKT17 cells are be implicated in the protection afforded by CpG treatment. Knowing that iNKT cells can be indirectly stimulated by CpG to produce IFN-γ [10], it will be important to determine

whether this or others TLR ligands can induce IL-17 production by iNKT cells. This is expected because we have already demonstrated that LPS, a TLR 4 ligand, can induce IL-17 production by iNKT cells [7].

IL-17, iNKT cells and autoimmune diseases

For several years the Th1/Th2 paradigm has been used to explain the mechanisms implicated in T cell-mediated tissue damage. In fact, there is evidence supporting the involvement of Th1 cells in the induction of autoimmune inflammation and tissue injury. However, some predictions of the Th1/Th2 hypothesis were completely wrong and the results were just the opposite [21], e.g., the supposed harmful effect of IFN-γ on experimental allergic encephalomyelitis (EAE). In fact, IFN-γ administration protected mice from this disorder, while EAE was worse following anti-IFN-γ treatment or in IFN-γ-deficient mice. Some erroneous conclusions have been published probably because IL-23 was only identified in 2000 [22]. IL-23, which comprises the p40 subunit of the IL-12 but a different p19 subunit, does not induce Th1 but favors Th17 differentiation. Several recent reports demonstrated that Th17 cells are implicated in the severity of both EAE and collagen-induced arthritis (CIA). Since IFN-γ impairs Th17 differentiation, it is now accepted that the protective effect of IFN-γ treatment in EAE depends on its action on Th17 cells.

iNKT cells have also been implicatied in these autoimmune pathologies. In fact, α-GalCer treatment ameliorates EAE induced by myelin basic protein in C57BL/6 mice, CIA and also spontaneous autoimmune diabetes in non-obese diabetic (NOD) mice probably by shifting the immune response toward an anti-inflammatory Th2 type [4–6, 23]. Furthermore, it has been demonstrated that NOD mice present a numerical and functional deficit in IL-4-producing iNKT cells and enhanced amounts of IL-17 [24]. Thus, it is possible that IL-4-producing iNKT cells are reduced while IL-17-producing iNKT cells, which favor inflammation, are enhanced in these autoimmune diseases. This proposition merits further examination.

IL-17, iNKT cells and allergy

It was recently reported that IL-17 contributes to experimental allergic asthma [25] as assessed by the reduction of the major disease symptoms in IL-17R-deficient mice. Paradoxically, neutralization of IL-17 in wild-type mice augmented established allergic asthma, while it attenuated the allergic response when administered exogenously, revealing its previously unknown regulatory functions in this model. These data provide evidence that IL-17 can exert a dual effect in experimental allergic asthma. It is noteworthy that iNKT cells can also exert opposing functions during the pathogenesis of this pathology. In fact, iNKT cells enhance asthma severity by

its capacity to produce IL-4 and IL-13 [13, 14]. However, a single α-GalCer treatment to sensitized mice can abrogate asthma hallmarks through an IFN-γ-dependent pathway. In this pathology, the role of both IL-17 and iNKT cells is intriguing probably because allergic asthma is a Th2 inflammatory disease that also requires Th1-like inflammation in its onset to obtain the major asthma symptoms. Thus, a better understanding of the role of IL-17-producing iNKT cells, and the mechanisms by which these cells can influence the characteristic asthma symptoms is required to potentially lead to new approaches to inhibit asthma progression.

IL-17 production by γδ T cells

In addition to Th17 and iNKT17 cells, it has been reported that γδ T cells are IL-17 producers. Like iNKT cells, these are innate cells, which predominantly express recurrent families of TCR with reactivity to a small set of conserved self-structures. The specificity of most innate γδ T cell subsets remains unclear, although they have a tendency to recognize stressed, activated or transformed cells and are implicated in various infectious conditions.

Recent studies demonstrated that TCR $\gamma\delta^+$ T cells were an important source of IL-17 in the lung of both *Mycobacterium tuberculosis* and *M. bovis* bacillus Calmette-Guérin-infected mice [26, 27], thus participating in the host defense against this mycobacterial infection. Moreover, in another model of host inflammation, after intraperitoneal infection with *Escherichia coli*, which also induces neutrophil influx, IL-17 was rapidly generated by γδ T cells, especially those bearing the Vδ1 chain [28]. IL-17 production by resident peritoneal γδ T cells occurred in response to IL-23 alone, suggesting that TCR-mediated recognition of bacterial antigens followed by expansion are not required for this activity. This is an important point because it suggests that IL-17 production by γδ T cells can be triggered by TLR-mediated pathogen recognition that will induce IL-23 production by tissue-resident APCs such as peritoneal macrophages. In this respect, γδ T cells and iNKT17 cells are very similar because they both produce IL-17 after stimulation with LPS, a TLR-4 ligand, in the presence of APCs [7].

In addition to their involvement in inflammatory responses in several models of infection, γδ T cells can also contribute to the development of some autoimmune diseases such as CIA [29]. These T cells reside in the synovium of mice and their proportion in the joints rises dramatically when mice develop CIA. However, the role of γδ T cells in this pathology is not clear since even though their depletion before immunization significantly delayed the onset of arthritis and diminished its severity, no major differences were observed in γδ T cell-deficient mice. In contrast, depletion of γδ T cells after immunization resulted in rapid and severe exacerbation of CIA. This differential effect on disease development could be explained by the intervention of two distinct γδ T cell subsets at different time points. In support of

this conclusion, it has been reported that most of $V\gamma4^+V\delta4^+$ but not $V\gamma1^+$ T cells from draining lymph nodes produce IL-17, thereby increasing the severity of the disease [30].

Concluding remarks

Recent studies have highlighted the phenotypic and functional diversity of IL-17-producing cells. Even though most of these investigations dealt with Th17 cells, new sources of IL-17 such as iNKT and γδ T cells should no longer be ignored. These innate cells can take part in the first line of host defense against pathogens but in some cases they may facilitate the development of autoimmune diseases since they can also be stimulated by endogenous, still unidentified, antigens. Thus, the identification of these antigens that could be associated with tissue damage, cell stress, injury and death will provide essential clues to the understanding of the immunoregulatory functions of these cells. It is now well established that iNKT cells respond to glycolipids rather than peptides, a mode of recognition that provides new insights into the mechanisms through which the innate immune system controls certain pathologies. Another important point is that neither iNKT nor γδ T cells depend on prior polarization to become IL-17 producers and that in some cases they can even be directly stimulated by cytokines like IL-23 to secrete IL-17.

The complex regulatory mechanisms involved in the control of infections, allergies and autoimmunity are the object of an ever-increasing number of investigations. The way in which IL-17 and its producer cells, namely iNKT and γδ T cells, are involved in these pathologies is the object of intensive research that will certainly provide new approaches to manipulate the immune system to prevent the development of these diseases or to attenuate their severity.

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Contributions of IL-22 to Th17 responses: Repairing and protecting peripheral tissues

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Abstract

IL-22 is a cytokine mainly produced by Th17 cells under the control of IL-23. Although this cytokine is structurally related to IL-10, it does not share any activity with IL-10 and is, so far, completely devoid of activity on immune and hematopoietic cells. IL-22 responsive cells are mainly found in peripheral tissues and include keratinocytes, lung and intestinal epithelial cells as well as hepatocytes. *In vivo*, IL-22 expression fits with the spectrum of inflammatory processes related to Th17 activation, including multiple sclerosis, inflammatory bowel disease and psoriasis in human. However, its pathophysiological significance varies in each of these diseases. IL-22 does not seem to play any major role in multiple sclerosis, at least based on the classical mouse model for this disease. By contrast, this cytokine appears to play a protective role in mucosal inflammation both in lungs and colon. Finally, IL-22 turns out to be one of the main proinflammatory mediators responsible for inappropriate activation of keratinocytes in psoriasis lesions, raising some promising perspectives for future clinical applications.

Introduction

Interleukin-22 (IL-22) was originally identified as a gene up-regulated by IL-9 in murine T cell lymphomas and mast cell lines [1]. It was first named IL-TIF for IL-10-related T cell-derived inducible factor, because the amino acid sequence of the protein encoded by this gene showed 22% identity with IL-10. In addition, IL-10 and IL-22 share a common cytokine receptor chain, as the IL-10Rβ chain is part of both receptor complexes when it associates with the IL-10Rα or the IL-22R chain [2, 3]. Because of these similarities, it was tempting to speculate that IL-22 might recapitulate some of the anti-inflammatory activities of IL-10 or, at least, play some role in the fine-tuning of inflammatory processes. However, it rapidly turned out that IL-22 does not have any common biological activity with IL-10, mainly because the second chain of their respective receptor complexes have completely distinct patterns of expression with very little or no overlap [4, 5]. Whereas the IL-10Rα chain

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is mainly expressed by cells from the hematopoietic and immune systems, such as monocytes, macrophages, mast cells, and B and T lymphocytes, the IL-22R chain is mainly expressed by non-hematopoietic cells such as hepatocytes, keratinocytes, and lung and intestinal epithelial cells [4, 6, 7].

Over the last few years, the pathophysiological significance of IL-22 has been highlighted by the finding that this cytokine is produced by Th17 lymphocytes, which are considered to be responsible for major autoimmune diseases. In this chapter, we review the data that support the idea that IL-22 could represent a valuable target for this type of pathological processes, starting from the regulation of IL-22 expression and its association with disease, and including *in vivo* observations in preclinical models of autoimmune diseases.

IL-22 production

T helper cells represent a major source of IL-22, although other cell types can also produce this cytokine. Originally, murine IL-22 was cloned as a gene induced by IL-9 in T lymphocytes and mast cells, but IL-22 was also shown to be expressed in freshly isolated T lymphocytes activated by polyclonal stimuli such as anti-CD3 antibodies or ConA [1–3]. Although IL-9 is associated with Th2-type immune responses, Th2 lymphocytes produce little IL-22 and this cytokine was rather considered as a Th1 cytokine [4, 8], until it became apparent that IL-23 is a potent regulator of IL-22 and that Th17 cells differentiated in the presence of IL-23 were even better at producing IL-22 *in vitro* [9]. In the mouse system, TGF and IL-6 are the main factors promoting Th17 cell differentiation and the exact function of IL-23 in Th17 responses remains a subject of debate. Although IL-23-deficient mice were reported to lack Th17 cells and to be protected against autoimmune diseases such as experimental autoimmune encephalitis (EAE) and collagen-induced arthritis, this cytokine is neither sufficient nor required to induce the differentiation of Th17 cells from naïve T cell precursors. The current dogma is that TGF-β and IL-6 are the key factors that promote the polarization of murine Th17 cells, whereas IL-23 may provide survival signals for differentiated Th17 cells [10].

When mouse Th17 cells were differentiated in the presence of a classical Th17-promoting cocktail including TGF-β, IL-6, IL-1β, TNF-α and IL-23, they were shown to express 100 times more IL-22 than Th1 and 1000 times more than Th2 cells [9]. Under these conditions, 80–90% of IL-22$^+$ cells also expressed IL-17A [9, 11, 12]. However, there are some significant differences in the optimal conditions for production of IL-22 or IL-17A. In one report where naïve CD4$^+$ cells were activated by anti-CD3 and anti-CD28 antibodies in the absence of antigen-presenting cells (APC), IL-6 alone was able to promote IL-22 production, whereas

addition of TGF-β actually inhibited this effect [11]. By contrast, IL-17A production required both IL-6 and TGF-β. However, in another experimental setting, TGF-β was found to be important for optimal expression of IL-22 induced by IL-6, IL-1β and TNF-α [9], and the reasons for this apparent discrepancy remains unclear.

Another unique feature characterizing IL-22 production by Th17 cells is its high dependence on IL-23. IL-23 on its own induces IL-22 production even under APC-free conditions *in vitro*, or *in vivo* after injection into the ear [11, 12]. In the human, the combination of TGF-β and IL-6 do not seem to induce Th17 differentiation of naïve $CD4^+$ cells, but IL-23 is a major inducer of IL-17A and IL-22 [13, 14]. Alternatively, IL-1 and IL-6 can also promote the differentiation of human T cells producing both IL-17A and IL-22 [15]. By contrast, TGF-β rather inhibits the production of these cytokines by human Th17 cells [13]. As opposed to these reports that focused on Th17 differentiation *in vitro* from naïve precursors, another experimental setting focusing on IL-17A and IL-22 production by BCG-specific $CD4^+$ T cells led to the conclusion that these cytokines are mostly produced by distinct lymphocyte subsets [16].

Altogether, these data indicate that, although IL-22 and IL-17 can be produced by the same Th17 cells, regulation of their production at the molecular level may be distinct. In this respect, STAT3 was shown to be an important regulator of IL-22 production in response to IL-9 in initial studies [1], and STAT3 is required both for IL-22 production and for Th17 differentiation [17]. More recently, another transcription factor, the aryl hydrocarbon receptor (AhR) was found to play a critical role in IL-22 production by Th17 cells [18]. AhR mediates a wide range of cellular events in response to aromatic hydrocarbons such as tetrachlorodibenzo-p-dioxin (TCDD). When the expression of this ligand-dependent transcription factor was analyzed in different T helper cell subsets, it turned out to be specifically expressed in Th17 cells. Moreover, AhR-deficient T lymphocytes could still differentiate into Th17 cells, but completely lacked IL-22 production. Finally, molecules that activate this receptor promote differentiation of T lymphocytes towards the Th17 lineage in general, and IL-22 production in particular. These observations that link a transcription factor responsive to environmental pollutants to the Th17 differentiation program opens interesting perspectives regarding the influence of environmental factors on autoimmune processes [18].

It must also be stressed that Th17 T lymphocytes are not the only source of IL-22. Beside mast cells that produce IL-22 in response to IL-9 [1], myeloid cells such as monocytes, macrophages and dendritic cells can also produce this cytokine either *in vitro* in response to IL-23 [11], or *in vivo* in response to inflammatory stimuli such as LPS [2]. As underscored in the next section of this chapter, the main source of IL-22 differs from one pathological situation to another, in contrast to IL-17A, which appears to be more strictly a Th17 product.

Association between IL-22 and diseases

Role of IL-22 in psoriasis

Psoriasis is a chronic inflammatory skin disease that affects approximately 2% of the general population and which is characterized by hyperplasia of epidermis, infiltration of leukocytes and dilatation of blood vessels. Psoriasis was previously considered as an autoimmune disease mediated by Th1 lymphocytes, but accumulating data point to IL-23 and Th17 cells as important mediators of this disease [19]. Several reports have shown that IL-22 production is also associated with this disease. Using quantitative RT-PCR, Wolk and colleagues [20] have shown that high IL-22 levels were present in skin from patients suffering from psoriasis and correlated with the expression of β-defensins, which are well described disease markers, whereas IL-22 is not detectable in healthy skin. High IL-22 levels in psoriatic skin were also associated with strongly up-regulated cutaneous S100A7, S100A8, S100A9 and MMP1 expression. Moreover, psoriatic patients showed elevated IL-22 plasma levels, which correlated with disease severity [21, 22]. T lymphocytes infiltrating the skin were the main source of IL-22, in line with the production of this cytokine by Th17 cells *in vitro* [22]. Treatment with cyclosporine A down-regulated both IL-17 and IL-22 gene expression in the skin of psoriasis patients [23].

One of the main features of psoriasis lesions is an inappropriate activation of keratinocytes leading to increased proliferation of these cells, alteration of their maturation and production of various mediators that cause further inflammation and promote angiogenesis [19]. Interestingly, keratinocytes constitutively express the IL-22 receptor and IL-22 activates STAT3 in these cells [20, 22, 24]. The importance of STAT3 activation in this disease is highlighted by the fact that transgenic mice with keratinocytes expressing a constitutively active STAT3 develop a skin phenotype that closely resembles psoriasis [25]. *In vitro*, IL-22 up-regulates the expression of a series of proinflammatory molecules including S100A7, S100A8, S100A9 and β-defensins by keratinocytes [20, 24], alone or in cooperation with IL-17 [9]. In addition, IL-22 promoted keratinocyte migration in an *in vitro* injury model, down-regulated the expression of genes associated with keratinocyte differentiation, and induced hyperplasia of reconstituted human epidermis [24].

In vivo, the role of IL-22 in psoriasis is supported by recent data from two murine models related to the human disease. In a first model, injection of IL-23 into mouse ear induced the development of epidermal acanthosis with a mixed inflammatory infiltration, as well as STAT3 activation in epidermal keratinocytes. IL-23 induced the production of both IL-22 and IL-17 after injection into the ear, and IL-23-induced ear swelling was significantly decreased in IL-22$^{-/-}$ mice in comparison with control mice [11]. Histologically, both epidermal acanthosis and dermal inflammation were reduced in IL-22$^{-/-}$ mice. In a second model, the transfer to scid mice of CD4^{+}CD45RB^{hi}CD25^{-} cells, a population of T lymphocytes depleted of

Treg, induced psoriasis-like lesions with elevated levels of anti-microbial peptides and inflammatory cytokines including IL-22 and IL-17A. Treatment of these mice either with anti-IL-23p40 or with anti-IL-22 blocking antibodies led to a significant reduction of the lesions and of S100A8, S100A9 and β-defensin gene expression [26]. Although one can still argue that such mouse models do not perfectly recapitulate the pathophysiological processes underlying human psoriasis disease, the data obtained in these two models strongly support the hypothesis that IL-22 indeed plays a significant role in the development of psoriasis lesions.

IL-22 and liver: From the acute-phase response to liver protection

Hepatocytes were the first biological targets identified for IL-22. Activation of STAT3 in these cells is well known to induce an acute-phase response and IL-22 was indeed found to up-regulate the expression of typical acute-phase genes such as serum amyloid A, α1-antichymotrypsin and haptoglobin [2]. The signal transduction pathways activated by IL-22 in hepatocytes are very similar to those activated by IL-6 [27]. It is therefore not surprising that these two cytokines play a similar protective role in mouse models of hepatitis or liver injury. IL-22 promotes liver regeneration *in vitro* [28]. *In vivo*, IL-22 injection or gene delivery significantly protects mice against liver injury, necrosis and apoptosis induced by administration of ConA or carbon tetrachloride (CCl4), by enhancing STAT3 activation and expression of Bcl-xL, Bcl-2 and Mcl-1 anti-apoptotic molecules [29, 30]. Moreover, administration of anti-IL-22 antibodies worsens liver injury in the ConA-induced hepatitis model [30], and similar results have been obtained in IL-22$^{-/-}$ mice [31]. However, the clinical relevance of this particular mouse model of hepatitis for human disease remains elusive at this stage.

IL-22 in the intestinal mucosa: A regulator of innate immune response and inflammation

The acute-phase response is not a unique characteristic of hepatocytes. In response to IL-22, intestinal epithelial cell lines such as Colo205 up-regulate the expression of typical acute-phase genes such as serum amyloid A and α1-antichymotrypsin, suggesting that this cytokine could modulate intestinal inflammation [32]. The biological significance of the acute-phase response for the regulation of inflammatory processes remains elusive as the various acute-phase response proteins can have either pro- or anti-inflammatory activities. Among those genes, LPS-binding protein (LBP) is definitely an anti-inflammatory mediator, which is both up-regulated in the serum of patients with Crohn's disease and inducible by IL-22, raising the hypothesis that this cytokine might thereby protect patients with Crohn's disease against a massive

inflammatory reaction to LPS present in their serum or by promoting phagocytosis of bacteria opsonized by LBP [33].

Interestingly, IL-22 gene expression is increased in inflamed colonic lesions of patients with Crohn's disease and, to a lesser extend, in patients with ulcerative colitis [34, 35], whereas high IL-22 serum concentrations have also been described in Crohn's disease patients [33]. In the mouse, experimental colitis can be induced by adding dextran sodium salts (DSS) to the drinking water, and development of colitis also correlates with increased IL-22 expression [33, 34]. IL-22 expression has been reported in other mouse colitis models including spontaneous colitis in TCRα KO mice, where IL-22 expression remained modest, and in the $CD45RB^{hi}$ transfer model characterized by higher levels of this cytokine [36]. In the TCRα KO model, further IL-22 gene delivery significantly reverted goblet cell depletion typically associated with colitis and stimulated the expression of mucin genes MUC1,-3, -10 and -13 by colon epithelial cells in a STAT3-dependent manner [36]. The fact that coadministration of the mucolytic agent *N*-acetyl-cysteine abolished the protective effect of IL-22 in this model supports the hypothesis that restitution of mucus-producing goblet cells was a critical mechanism underlying this activity. In the DSS mouse colitis model, anti-IL-22 antibodies significantly delayed the recovery from acute colitis as judged by body weight loss. Disease scores were exacerbated in the anti-IL-22-treated mice, indicating that endogenous IL-22 plays an anti-inflammatory role in this model [36].

A similar anti-inflammatory and protective effect of IL-22 has been recently reported during intestinal infection by *Citrobacter rodentium* [37]. A few days after infection, IL-22 is induced in the colon of wild-type mice, together with IL-17A and IL-23. IL-23-deficient mice were known to show a high susceptibility to this bacterial infection, and expressed decreased levels of IL-22, while IL-17 expression was not affected. When $IL\text{-}22^{-/-}$ mice were infected, they showed an increased submucosal and transmural inflammation, with large numbers of bacteria penetrating deeply in the colonic crypts. By contrast both wild-types and mice deficient for the IL-17 receptor IL-17RC survived infection without any significant histological difference. Wild-type mice receiving anti-IL-22 antibodies at the same time as bacterial inoculation continued to lose weight and all became moribund 12 days after inoculation, whereas all control mice survived. Interestingly, IL-22 production induced by *C. rodentium* and the effect of anti-IL-22 treatment were not affected in Rag2 knockout mice, indicating that most of IL-22 produced in this model was not the result of the specific immune system, in contrast to IL-17A production, which was abolished in these mice [37].

During *C. rodentium* infection, the expression of a few genes was found to be strictly dependent on IL-22, including RegIIIβ and RegIIIγ, which belong to a family of secreted C-type lectin proteins. Most importantly, administration of exogenous RegIIIγ protected $IL\text{-}22^{-/-}$ mice from weight loss upon *C. rodentium* infection and approximately 50% of RegIIIγ-treated $IL\text{-}22^{-/-}$ mice survived infection,

whereas all control-treated IL-22$^{-/-}$ mice became moribund or died [37]. RegIII proteins have been reported to kill some Gram-positive bacteria by interacting with peptidoglycan carbohydrate, but there is no evidence that these proteins could have any direct killing activity against a Gram-negative bacteria such as *C. rodentium*. Alternatively, RegIII proteins might act as autocrine growth factors involved in epithelial repair processes, thereby preventing the extension of the inflammatory reaction.

IL-22 and rheumatoid arthritis

Rheumatoid arthritis (RA) is another autoimmune disease in which Th17 lymphocytes are supposed to play a significant role. However, very little information is available at this stage to evaluate the putative activity of IL-22 in this disease. IL-22 mRNA has been detected by RT-PCR in RA synovial tissues and mononuclear cells isolated from RA synovial fluid samples. Expression of the IL-22R was also detected in synovial tissues and IL-22 was shown to up-regulate the expression of the MCP-1 chemokine by synovial fibroblasts from RA patients, suggesting that IL-22 plays a pro-inflammatory role in this disease [38]. Preliminary results obtained with IL-22$^{-/-}$ mice in a collagen-induced arthritis model seem to support this hypothesis, but the underlying mechanisms remain unclear (Geboes et al., manuscript in preparation).

IL-22 and experimental autoimmune encephalomyelitis

Multiple sclerosis (MS) is the most common inflammatory disease of the central nervous system and autoreactive T cells of the Th17 subset are responsible for most of the pathological features in its animal model, EAE. In human MS patients, numerous CD45RO+ T cells immunopositive for IL-17 or IL-22 were detected in highly infiltrated multiple sclerosis lesions, but not in normal-appearing white matter or non-inflamed brain specimens [39]. Moreover, both IL-17R and IL-22R receptors were highly expressed on brain vessels within heavily infiltrated multiple sclerosis lesions, but not in subject without MS. *In vitro* primary cultures of brain-blood-barrier (BBB) endothelial cells also expressed these two receptors, and both IL-17 and IL-22 induced an increase in the BBB permeability *in vitro*. In addition, IL-22 promoted transmigration of CD4^{+} lymphocytes, possibly through enhanced secretion of MCP-1 by endothelial cells [39]. Based on these observations, it was tempting to speculate that IL-22 plays a significant role in the development of the disease. However, although high IL-22 expression correlate well with the disease, IL-22$^{-/-}$ mice develop EAE exactly as control mice, suggesting that this cytokine does not exert any non-redundant activity in this mouse autoimmune model [12].

IL-22 in lung inflammatory processes

Bronchial epithelial cells express significant levels of IL-22R and respond to IL-22 with STAT3 activation [7], suggesting that IL-22 might play a regulatory role for pulmonary inflammation, reminiscent of its activity in the digestive tract. IL-22 and IL-17 cooperatively induce the expression of host defense genes in primary human bronchial epithelial cell cultures, including those encoding β-defensin2, S100A7 and inflammatory cytokines [40]. In line with previous observations with hepatocytes or intestinal epithelial cells, IL-22 promotes the recovery of transepithelial resistance in injured lung epithelium *in vitro*. A model of pulmonary infection by *Klebsiella pneumoniae* was used by Aujla and colleagues [40] to assess the involvement of IL-22 in mucosal host defense *in vivo*. In this model, the IL-22 mRNA was detectable as early as 6 h after infection of wild-type but not of $Rag2^{-/-}Il2rg^{-/-}$ mice (deficient in natural killer, T and B cells) nor in IL-23-deficient mice, suggesting that IL-22 was produced mainly by Th17 lymphocytes. Whereas $IL\text{-}17^{-/-}$ mice show a moderately increased susceptibility to the infection, injection of anti-IL-22 antibodies dramatically affected the survival of *K. pneumoniae*-infected mice, and favored the dissemination of the bacteria [40]. However, inhibition of IL-22 did not affect IL-17 production in this model, indicating that IL-22 does not control the development of the Th17 response. It is likely that IL-22 exerts its protective activity in this experimental model by up-regulating the expression of anti-microbial genes such as lipocalin-2 by epithelial cells [40]. Taken together, these observations suggest that IL-22 plays a more crucial role than IL-17A in experimental bacterial pneumonia.

Lung inflammation frequently results in fibrosis, as highlighted by silica- and bleomycin-induced lung fibrosis experimental models. In these models, expression of both IL-17A and IL-22 expressions are up-regulated, but IL-17 seems to play the major role in the development of the inflammatory stage as lung neutrophil infiltration was reduced in IL-17R-deficient mice but not in $IL\text{-}22^{-/-}$ mice. As far as fibrosis is concerned, neither IL-17 nor IL-22 seem to play any significant role (Huaux et al., manuscript in preparation).

Concluding remarks

Our understanding of the biology of IL-22 has been boosted during the last 2 years by the demonstration that this cytokine is mainly produced by Th17 cells and is strongly associated with IL-23-mediated inflammatory processes. Interestingly, it is not yet easy to answer the apparently simple question whether IL-22 is a pro- or anti-inflammatory cytokine. In psoriasis disease, experimental evidence point to a proinflammatory role, whereas this cytokine has rather an anti-inflammatory effect in hepatitis and inflammatory bowel disease models. In fact, it looks like the common theme in all these different inflammatory processes where IL-22 is involved is

that this cytokine aims at promoting the integrity of biological barriers and favoring wound healing either in liver, in lung or intestinal mucosae or in skin. This wound healing activity is not surprisingly coupled with an anti-microbial response, which either limits or promotes the cascade of inflammatory reactions depending on the balance with the intensity of the aggression. The ongoing challenge consists in developing new tools and learning more about how to finely tune this subtle balance to help IL-22 to keep focusing on its beneficial wound healing and protective activities.

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Retinoic acid in mucosal immune-regulation

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Abstract

The vitamin A metabolite, retinoic acid (RA), and transforming growth factor-β (TGF-β) are both abundantly produced in the gut and are known to play significant roles in a variety of developmental processes, including the differentiation of lymphocyte lineages. TGF-β mediates the direct inhibition of Th1 and Th2 cytokine polarization concomitant with the generation of regulatory T cells (Tregs). Paradoxically, along with inflammatory cytokines such as IL-6, it also induces the differentiation of pro-inflammatory IL-17-producing CD4 helper T cells (Th17). RA, in contrast, is able under certain conditions to stimulate Th2 differentiation and it is a profound inhibitor of IFN-γ synthesis. Additionally, RA has been shown to efficiently promote gut tropism. We recently described RA as a key modulator of TGF-β-driven immune deviation capable of suppressing Th17 differentiation while promoting Foxp3$^+$Treg generation. Here we discuss how RA can affect mucosal immune regulation.

An effective immune regulation is a condition *sine qua non* for the gut physiology. The constantly stimulated gut mucosa generates distinct regulatory mechanisms that control the activation and migration of lymphocytes and also induce different types of regulatory DCs and T cells. In the last 25 years, it has become clear that antigen-presenting cells (APCs) perform crucial functions in the generation of both optimal immune responses and immune regulation. Through the expression of tight junctions, epithelial-associated DCs are able to establish rigid contacts with the neighboring epithelial cells, while extending their dendrites to sample luminal antigens, including whole bacteria [1]. Intestinal lymphocytes, DCs and epithelial cells constantly interact and their cross talk is reinforced by cell surface receptor-ligand interactions, including expression of integrin $\alpha_E\beta7$/E-cadherin and CD8αα/TL contact [2–4]. Both DCs and epithelial cells express Toll-like receptors (TLRs) that induce cellular activation and lead to the migration of DCs to regional lymph nodes, where they can present processed antigens to the naïve T cells.

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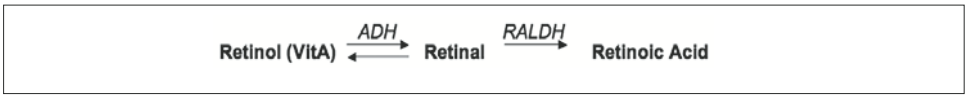
Regarding their regulatory capacity, it was shown that antigen presentation by mucosal DCs in the mesenteric lymph nodes (MLNs) plays crucial roles in the development of oral tolerance, which can be defined as diminished systemic immune responses for an antigen previously contacted *via* the oral route [5]. Also, mucosal plasmacytoid CD8αα$^+$ DCs are inefficient at inducing CD4 T helper proliferation but instead they can promote the differentiation of IL-10-producing CD4 cells [6]. In addition to their regulatory role, it has also been demonstrated that mucosal DCs from MLNs and Peyer's Patches (PPs) are unique in their capacity of degrading vitamin A to generate retinoic acid (RA) [6] (Scheme 1). Vitamin A (or provitamin-A carotenoids, such as beta-carotene) is absorbed from the diet in the form of retinol, which can be converted to retinal (RAL) through a reversible catalytic process mediated by alcohol dehydrogenases (ADHs). The subfamily of ADH enzymes is ubiquitously expressed in the body, but different classes of ADH present particular distribution. For instance, in the liver, the major storage site for vitamin A, both basic (class I) and acidic (class III) ADH are expressed, while DCs from intestinal tissues express preferentially class II ADH [7]. The irreversible conversion of retinal to RA is catalyzed by retinal dehydrogenases (RALDH) [8].



Scheme 1
Production of retinoic acid (RA). Vitamin A is absorbed from the diet in the form of retinol, which is converted to retinal (RAL) through reversible catalytic process mediated by alcohol dehydrogenases (ADH). The irreversible conversion of retinal to RA is catalyzed by retinal dehydrogenases (RALDH).

RA and TGF-β are both abundantly produced in the gut and are known to play significant roles in a variety of developmental processes, including the differentiation of lymphocyte lineages. TGF-β mediates the direct inhibition of Th1 and Th2 cytokine polarization concomitant with the generation of Tregs [9]. Although the so-called thymus-derived naturally occurring Treg cells (nTregs) are important for the control of a variety of autoimmune processes, it has been shown using monoclonal non-self-antigen-specific TCR transgenic mice (such as OVA-specific TCR transgenic mice) that lack nTregs that peripheral neoconverted Foxp3$^+$ Treg cells are efficient and sufficient for oral tolerance induction [10]. Additionally, blocking of TGF-β during feeding of the antigen (OVA) inhibited both the establishment of oral tolerance and the peripheral conversion of OVA-induced Tregs [10]. RA, in contrast, is able under certain conditions to stimulate Th2 differentiation, whereas it strongly

inhibits IFN-γ synthesis [11]. More recent reports, however, have shown that RA can efficiently suppress both Th1 and Th2 differentiation *in vitro* [12, 13].

The RA produced by MLN, lamina propria (LP) and PP DCs mediates up-regulation of gut specific molecules on the T cells that they prime, such as integrin α4β7 and chemokine receptor CCR9, which interacts with MAdCAM-1 and CCL25, respectively, expressed on intestinal epithelium [7, 14–16]. Consistently, mice reared on vitamin A-deficient diet (VAD) have a severe decrease in the number of LP immune cells in the intestine [7]. RA is involved in both B cell IgA-class switching and migration to the LP of the intestine [17]. Interestingly, it was recently reported that HIV-1 envelope protein GP120 can bind to α4β7 expressed by mucosal T cells or T cells previously exposed to RA [18]. It is not known, however, whether targeting α4β7 expression affects HIV-1 replication *in vivo*.

The functions of TGF-β and RA are also known to merge in a variety of biological processes, including embryogenesis, organ development and carcinogenesis [19]. For example, TG-interacting factor (TGIF) is a transcriptional repressor common to the TGF-β and RA signaling pathways [20]. Moreover, mice with a deficiency in RALDH-2 die before birth with several developmental defects and reduced TGF-β1 production [21–23]. On the other hand, RA can also inhibit TGF-β-mediated effects, such as lung fibrosis [24].

Our recent finding and those of others, showing that RA also plays a central role in directing the immunological functions of TGF-β, expands the consequences of their interrelationship to the adaptive immune system as well [12, 25–30]. The abundant production of TGF-β and RA in the mucosa and the ability of RA to promote TGF-β-dependent Treg differentiation may thus be directly related to the increased frequency of Foxp3-expressing Tregs in the LP in normal mice, to oral tolerance induction and, therefore, to mucosal immune regulation [25–27]. We have presented evidence that the signaling through RA receptors may play an important role in the control of inflammation in the gut [25]. Using an infectious mouse model, we showed that exogenous RA inhibits the induction of Th17 cells *in vivo*, whereas injection of RA receptor (RAR) antagonists resulted in a decrease of Foxp3$^+$Treg cells in the LP [25]. Whether the production of RA by mucosal DCs is crucial for the development of oral tolerance and for the conversion of naïve T cells into Foxp3$^+$Tregs in the gut, as well as for the mucosal *in situ* control of Th17 cells, is not yet known.

Two groups have shown that CD103$^+$ DCs are the main population involved in the RA production. Coombes et al. [28] showed that RA production by CD103$^+$, but not CD103$^-$ MLN DCs, efficiently converted naïve CD4$^+$ T cells into FoxP3$^+$ T cells in a TGF-β-dependent fashion. Sun et al. [27] demonstrated that, while LP CD103$^+$ DCs could induce Foxp3$^+$ T cells in the absence of exogenous TGF-β, both CD103$^+$ and CD103$^-$ LP DC-populations were highly efficient at inducing Tregs in the presence of exogenous TGF-β, when compared to spleen DCs. More recently, Denning et al. [31] extended and added complexity to these findings. They reported

that LP macrophages are potent regulatory APCs able to convert naïve T cells into Foxp3$^+$Treg cells in an IL-10-, RA- and TGF-β-dependent manner. On the other hand, LP CD11b$^+$CD11c$^+$ cells are highly pro-inflammatory, inducing high levels of IL-17-producing T cells but few Tregs. It was also reported that CD11b$^-$CD11c$^+$ cells express high levels of CD103. Although this population induces much lower levels of Th17 cells as compared to the CD11b$^+$ DCs, these authors could not find any increased ability to induce Tregs. Therefore, the diversity of mucosal APCs is more extended and more complex than previously appreciated.

Our study shows that RA signaling through RARs in the T cell blocks the inhibitory effects of inflammatory cytokines, such as IL-6, on the TGF-β-mediated Foxp3 induction. Similarly to the RA and TGF-β interactions, several studies have shown that RA may synergize or antagonize with IL-6 signaling or production [17–24]. Moreover, RA has been shown to improve clinical symptoms and reduce the levels of inflammatory cytokines, including IL-6, TNF-α and IFN-γ in a model of arthritis [32], an autoimmune disease shown to correlate with increased production of IL-17 [33]. Finally, we have showed that RA directly inhibits RA orphan receptor gamma T (RORγt) that is involved in Th17 differentiation [34]. It is not know, however, whether RA antagonistic effects on IL-6 signaling extend to the recently described IL-21 pathway of Th17 differentiation [35–37].

The transcription factors STAT5 and STAT3 have been shown to be important for the transcription of Foxp3 and IL-17, respectively [29, 34, 38]. The enhanced expression of Foxp3 in the presence of RA suggests a potential relationship between STAT5 and RARs in a similar fashion as the cooperation between STAT3 and RORγt. It is therefore perhaps not a coincidence that RORγt displays strong homology with the RARs and also appears to function in the context of transcriptional activators and repressors [39]. STAT5 and RARs have even been shown to physically interact *in vivo* to promote RAR-mediated transcription [40]. In addition, the STAT5 consensus binding site directly overlaps with an RAR-response element, which may lead to coordinated transcription activity rather than competition for the same site [40]. The cooperation between STAT5 and RARs results in STAT5-enhanced responsiveness of the RARs to RA-induced transcription of target genes [40]. It was further demonstrated that RAR and STAT5 can bind the same repressor of transcription, SMRT, which can be released by RA [41]. The RA-mediated effects determined in our study may thus reflect the intense communication between the STAT and RAR families of transcription factors, which has not been explored for the differentiation of T lymphocytes. Using high amounts of blocking anti-IL-2 antibody and IL-2-deficient mice, we found that IL-2 signaling could play an important role in the reciprocal regulation of Th17 and Treg differentiation mediated by RA, although the direct effects of RA and IL-2 appear distinct [25]. However, using similar *in vitro* approaches, another study suggested that both STAT3 and STAT5 are not required for this RA-mediated regulation [12]. Another recent finding reports that, similarly to IL-6, the Th2 cytokine IL-4 can also inhibit TGF-β-mediated Foxp3 induction,

via activation of STAT6, which binds to and represses the Foxp3 promoter. Interestingly, RA could reverse this IL-4-mediated effect on Foxp3 expression by activating RARα/RXRα heterodimers, which bind to the same region of the Foxp3 promoter and induce chromatin remodeling, thus reversing the silencing capacity of STAT6 on Foxp3 induction [13]. It is also possible that RA may synergize with Smads that act downstream of TGF-β receptor signaling, and/or with the transcription factor Runx3, which is involved in the induction of CD103 expression and which physically interacts with Smads to cooperate in TGF-β-mediated signaling [42].

The immune regulatory mechanism that we have delineated has particular relevance for the mucosal immune system. The intestinal mucosa forms the largest surface that is exposed to microbes, innocuous and pathogenic, and diet proteins, and also houses the largest proportion of lymphocytes that, under physiological conditions, have an immune quiescent state [43]. Therefore, an improper balance between inflammatory and suppressive immunity can jeopardize mucosal homeostasis. The abundant production of RA by the intestinal epithelium and DCs, and the dominance of RA over IL-6 in controlling the effects of TGF-β, may account for the predominance of $Foxp3^+$ T cells in the intestine, allowing tolerance to prevail in the face of the extensive microbial load.

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IL-25, another promoter of allergy

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Abstract

Although there have been significant insights into the mechanism underlying the initiation of type 1 immune response, how type 2-mediated pathology especially allergic diseases is developed remains unclear. Cytokine environment is important in shaping and initiating Th2 responses. IL-25, also called IL-17E, belongs to the IL-17 family but, unlike other IL-17 cytokine members, possesses a unique function in regulating type 2 immune responses. Here we summarize recent work demonstrating the role of this cytokine in promoting allergy, and discuss its potential producer and responder cells during allergic inflammation. IL-25 thus serves as a novel target for pharmaceutical intervention of allergic asthma disease.

Introduction

Allergic asthma diseases have the characteristics of type 2 immune responses [1]. These are characterized by several changes in the airways, including the local infiltration of Th2 cells and eosinophils, as well as the lung physiological and structural changes, leading to the hyperproduction of mucus and the increase in the airway hyperresponsiveness (AHR) [2]. The pathogenesis of allergic asthma diseases are though to be mainly regulated by Th2-driven immune response to environmental allergens; however, how that response is initiated remains elusive.

IL-17 cytokine family comprises six members, IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F [3–7]. Among them, IL-25 is unique in its structure and function. IL-17 and other structurally related IL-17 cytokine members are known to be potent pro-inflammatory cytokines, while IL-25 has a distinct function in promoting type 2 immune responses. Several studies have linked IL-25 function to the pathogenesis of allergic diseases, especially asthma [8–14]. Multiple cellular source and target cells for this cytokine during allergic diseases have been identified. Its innate function on epithelial cells and fibroblasts in facilitating tissue recruitment of eosinophils and Th2 cells has also been demonstrated. More impor-

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tantly, recent findings indicate that IL-25 also regulates adaptive immune responses by promoting Th2 polarization and amplifying the Th2 cytokine expression profile. Therefore, IL-25 is crucial for the innate and adaptive immune regulation during allergic disease development.

IL-25 and its receptor

Following the cloning of IL-17, five other cytokines, including IL-25, were discovered to constitute a new cytokine family. IL-25 was first identified by Fort et al. [7] and Lee et al. [15] by sequence homology search in genomic DNA using IL-17 cytokine. Among the IL-17 family members, IL-25 is the most divergent with only 16–20% sequence identity to IL-17, suggesting its unique function. Unlike IL-17, which is secreted mainly by T cells, IL-25 was found to be produced by several cell sources, including the first identified cells, Th2 cells [7], as well as innate immune cell types such as lung epithelial cells [12], alveolar macrophages [9], mast cells [16], eosinophils and basophils [13].

The receptor for IL-25, IL-17BR also called EVI27 or IL-17 receptor homolog 1 (IL-17Rh1), is a 56-kDa single transmembrane protein with homology to IL-17 receptor. It shares the same receptor as IL-17B but with a higher affinity of binding [15, 17, 18]. Cross-linking of IL-17BR induced NF-κB activation through TNFR-associated factor (TRAF)6 as well as ERK, JNK, and p38 activation [19]. Moreover, TRAF6-deficient cells had defects in IL-25 receptor-mediated gene expression of IL-6, TGF-β, G-CSF and 'thymus and activation-regulated chemokine' (TARC), implicating the involvement of TRAF6 in IL-25 function. Unlike the broader expression of IL-25, its receptor has been shown to be more restricted in expression, being highest in kidney, liver and small intestine, suggesting more specificity of IL-25 target cells in these tissue. Functional studies suggested that a novel accessory population of non-T/non-B cells (NBNT), characterized as Lin^{-}, MHC class II^{high} and $CD11c^{dull}$, is an IL-25 responder cell [7]. This population was further described to be important for the initiation of *Nippostrongylus brasiliensis* worm clearance *in vivo* [20]. Recent findings also suggested that helper T cells and memory/effector Th2 cells are the direct IL-25 target in promoting Th2 cytokines production. Functional IL-25 producers and their cellular targets, especially T cells, during allergic disease form the focus of our discussion in this article.

The function of IL-25 in pulmonary allergic disease

The biological function of IL-25 was initially assessed *in vivo* by administration of exogenous IL-25 protein and by a systemic transgenic approach. Intraperitoneal

injection with IL-25 protein resulted in type 2-biased immune reaction *in vivo*, including up-regulation of IL-4, IL-5 and IL-13 expression in multiple tissues, increase in serum IgE and IgG1, eosinophil infiltration into the lung and stomach, hypermucus production, and epithelial cell hyperplasia [7]. Similar phenotypes were found in IL-25 transgenic mice systemically overexpressing either human or mouse IL-25, implicating the important role of IL-25 in type 2 immune response [21, 22].

Pulmonary allergic disease is caused by the dysregulation of type 2 immunity. The association of IL-25 function with the development of allergic disease was further supported by several additional findings. While intranasal administration of IL-17 resulted in bronchoalveolar lavage neutrophilia and proinflammatory gene expression, IL-25 intranasal injection led to the massive airway infiltration of eosinophil and profound induction of Th2 cytokine mRNA expression [8]. More importantly, these mice also developed pathological changes in the lung that resemble asthma, including epithelial hyperplasia and mucus hypersecretion, suggesting an important function of IL-25 in allergic disease. In addition, Sharkhuu et al. [10] further demonstrated that intratracheal instillation of IL-25 potently induced AHR, which was dependent on the production of type 2 cytokines. The IL-25-dependent induction of AHR, eosinophilia and mucus hypersecretion is partly mediated by IL-13, IL-4Ra and signal transducer and activator of transcription (STAT)6.

The function of IL-25 in modulating allergic disease was further assessed using genetic approaches. Angkasekwinai et al. [12] found that, at 5 month of age, lung-specific IL-25 transgenic mice spontaneously displayed allergy-like phenotypes, including eosinophil and $CD4^+$ T cell infiltration, mucus hyperplasia, and epithelial hyperplasia. However, Tamachi et al. [11] showed that enforcing expression of IL-25 alone in the lung did not significantly induce allergic inflammation, probably due to the lower expression of their transgene. With their model of chicken ovalbumin protein (OVA)-induced lung inflammation in IL-25 lung transgenic mice, they detected not only infiltration of eosinophil and T cells, but also an significant increase in the levels of IL-4, IL-5, and IL-13 [11].

Conversely, blockade of endogenous IL-25 using an IL-25-specific antibody was reported to attenuate allergic inflammation and the expression of Th2 cytokines, indicating that endogenous IL-25 expression may contribute to the regulation of allergic inflammation [12]. Ballantyne et al. [14] also demonstrated that neutralization of IL-25 during OVA-induced allergic inflammation markedly abrogated the AHR. Likewise, inhibition of IL-25 binding using a soluble IL-25 receptor showed the similar reduction of lung-infiltrating eosinophil, $CD4^+$ T cells, and the level of Th2 cytokines [11]. Taken together, these data support IL-25 as being an important cytokine that functions in modulating allergic airway diseases.

Source of IL-25 during allergic disease

How allergic asthma disease is initiated remains elusive. Several cell types such as eosinophils, basophils, mast cells, and Th2 cells have been shown to be recruited into the airway and involved in the pathogenesis of allergic airway diseases. Lung epithelial cells, the first encounter with environmental allergens, have been suggested to be important for the initiation, maintenance, and the regulation of innate and adaptive immune response in the airways [23]. An initial study by Hurst et al. [8] giving aerosolized fungus *Aspergillus fumigatus* spores showed that the peak of IL-25 mRNA expression in lung occurred in less than 48 h after administration, suggesting that IL-25 secretion early during allergic responses may play an important role in disease development. Moreover, Tamachi et al. [11] found a similar induction of IL-25 transcripts in lung by 24 h following the OVA inhalation in sensitized mice. Taken together, the up-regulation of IL-25 in the airway *in vivo* implicated the crucial role of IL-25 in the regulation of allergic airway disease.

Both lymphoid cells such as Th2 cells and several non-lymphoid cells such as mast cells, alveolar macrophage, eosinophils, basophils, and lung epithelial cells have been reported to be the potential IL-25 producers. IL-25 was first shown to be a Th2-derived cytokine when its mRNA expression was found in highly polarized Th2 cells [7]; however, Th2-derived IL-25 has not yet been shown during allergic disease *in vivo*. Human mast cells are localized in the airway of asthma patients. In a murine model of asthma, IgE-dependent mast cell activation was shown to be important in potentiating the airway response. IL-25 was found to be produced by bone marrow-derived mast cells upon IgE cross-linking [16]. Therefore, mast cell derived-IL-25 might be involved in the regulation of allergic disease. Moreover, using immunohistochemistry, alveolar macrophages were shown to produce IL-25 during particle-induced lung inflammation [9]. The production of IL-25 peaked at 24 h after the particle treatment, suggesting that IL-25 produced by alveolar macrophages may regulate inflammatory responses in the lung. In human, eosinophils and basophils from normal and allergic patients were found to express substantial levels of IL-25 [13]. The expression of IL-25 by eosinophils was further up-regulated by treatment of IL-5 and GM-CSF, while the expression by basophils was induced upon IgE cross-linking. Moreover, eosinophil-derived IL-25 was demonstrated to regulate Th2 cytokine production by activated memory Th2 cells.

In addition to inflammatory cell types, lung epithelial cells exposed to allergens such as ragweeds and the fungus *Aspergillus* up-regulated the mRNA expression of IL-25 along with several other chemokines [12], suggesting that IL-25 may contribute to the initiation of innate allergic cascade. More importantly, IL-25 treatment of lung epithelial cells induced the expression of several chemokines such as eotaxin, TARC and macrophage-derived chemokine (MDC), which were known to be involved in the recruitment of eosinophils and Th2 cells during allergic diseases. Supporting evidence came from data from IL-25 lung transgenic mice [11, 12].

Enforcing the expression of IL-25 by lung epithelial cells resulted in the airway phenotypes similar to pro-allergic reactions. These mice showed airway infiltration of eosinophils, eosinophilic macrophages, and $CD4^+$ T cells as well as the pathological alteration in the lung, including mucus hyperproduction and epithelial cell hyperplasia. Taken together, IL-25 produced from several innate cell types such as eosinophils, basophils, and lung epithelial cells may contribute to the initiation and further progression of allergic diseases.

Cellular targets of IL-25 during allergic airway responses

Although biological effects of IL-25 in mediating type 2 immune responses have been clearly indicated by the above studies, cellular targets of IL-25 and its precise function during type 2 immune responses remain unclear. The first IL-25 responder was initially identified by its function in induction of Th2 cytokine. Fort et al. [7] demonstrated that NBNT cells are the major IL-25 target cells. Total splenocytes that had been depleted of lymphocytes or $RAG^{-/-}$ splenocytes showed a dramatic increase of IL-5 and IL-13. Further phenotypic characterization indicated that these IL-25 responder cells are accessory cells, which do not express any lineage markers but express MHC class II and low levels of CD11c. Intranasal administration of IL-25 led to a major population of IL-5-positive non-lymphoid cells and a small population of IL-5-positive $CD4^+$ T cell. While the NBNT cell population in responding to IL-25 was shown to be involved in controlling intestinal parasite infection, resistance to which requires type 2 immune response in prevention of parasitic infection; the function of this cell type during allergic disease remain to be analyzed. Th2 cells are the major producer of Th2 cytokines and responsible for orchestrating allergic reaction. Recently, there have been several reports indicating that $CD4^+$ T cells might be a cellular target of IL-25 during allergic airway disease *in vivo* [10, 12, 13]. $CD4^+$ T cell depletion inhibited the IL-25-induced enhancement of allergic airway inflammation [11]. The function of IL-25 on $CD4^+$ T cells is reviewed in the following section.

During the allergic airway response, IL-25 functions in the regulation of not only Th2 cytokine production but also type 2 inflammatory chemokine secretions. The receptor for IL-25 was also shown to be constitutively expressed by lung resident cells such as lung fibroblast cells [24] and lung epithelial cells (data not shown), suggesting a direct function on those cells. The expression of IL-17RB by human lung fibroblast was found to be further up-regulated by TNF-α treatment [24]. IL-25 treatment on lung epithelial and fibroblast cells induced expression of several chemokine such as eotaxin, TARC, and MDC, which are important chemokines for recruiting eosinophils and Th2 cells [12]. These data suggest that IL-25 may be capable of initiating the allergic progression through targeting the innate cell types in their tissue recruitment and adaptive $CD4^+$ T cells in the production of Th2 cytokines.

Th2 cells: A novel IL-25 responder

The development of allergen-specific Th2 lymphocytes represents a key event in the allergic inflammatory reaction. Several functional studies imply that, during allergic diseases, $CD4^+$ T cells might be an IL-25 target cell and responds by induction of Th2 cytokine. Naïve peribronchial lymph node cells activated with anti-CD3 and anti-CD28 in the presence of IL-25 enhanced their production of IL-4, IL-5 and IL-13, while there was no effect in IFN-γ secretion [10]. Furthermore, neutralization of endogenous IL-25 expression during allergen-induced allergic airway inflammation led to the reduction of antigen-specific Th2 response [12], indicating the function of IL-25 in regulating helper T cell priming.

The receptor for IL-25, IL-17BR was shown to be highly expressed by *in vitro* generated Th2 cells [25] and circulating human Th2-biased memory cells *in vivo* [26], suggesting a potential function of IL-25 on T cells. Recent studies clearly demonstrated the important function of IL-25 in regulating Th2 differentiation [12] and Th2 effector/memory cells expansion *in vitro* [13]. By characterizing the expression of IL-25 receptor by different T cell subpopulations, Angkasekwinai et al. [12] found that mouse Th2 cells highly expressed IL-17BR transcript, while no expression was found by Th1 or TH17 cells, suggesting a function in directing Th2 differentiation. Treatment of IL-25 during $CD4^+$ T cell differentiation enhanced Th2 cytokine production, including IL-4, IL-5 and IL-13 and inhibited IFN-γ production. The presence of anti-IFN-γ antibody could potentiate the induction of Th2 cytokines by IL-25. Furthermore, time-course analysis of its transcript during T cell differentiation indicated that IL-17BR expression was sustained during Th2 differentiation, suggesting that not only IL-25 may play a role during Th2 differentiation but may also be involved in functional regulation of effector Th2 cells and memory Th2 cells. Treatment of IL-25 on *in vitro* differentiated effector Th2 cells was found to further enhance Th2 cytokine production. In addition, high expression of IL-17BR transcript was found in human Th2 memory cells and the role of IL-25 on memory Th2 cells was further characterized [13]. Wang et al. [13] demonstrated that IL-25 treatment on human $CRTH2^+$ memory cells activated with thymic stroma lymphopoietin-treated dendritic cells (TSLP-DC) enhanced T cell expansion and Th2 cytokine production, including IL-4, IL-5, and IL-13, suggesting the function of IL-25 in inducing further polarization of memory Th2 cells. All functional studies *in vitro* suggest that $CD4^+$ T cells could be a functional cellular target of IL-25 during allergic disease development either by directly influencing naïve T cell differentiation or enhancing effector Th2 cell expansion, and further polarization.

The molecular mechanism by which IL-25 induces Th2 cytokine production by differentiated mouse Th2 cell or human memory Th2 cells *in vitro* might be different. Th2 differentiation induced by IL-25 is dependent on IL-4 expression [12]. IL-25 was shown to up-regulate the early expression of IL-4 gene transcript during naïve T cell polarization, thereby enhancing Th2 cytokine production by day 3 after

activation, and further promoting Th2 differentiation. Treatment of IL-4-deficient naïve T cells with IL-25 or using antagonistic anti-IL-4 antibody in wildtype naïve T cells treated with IL-25 abolished this effect, indicating that endogenous IL-4 is required for IL-25-mediated enhancement of Th2 differentiation. While IL-25 effect on Th2 polarization is IL-4 dependent, the up-regulation of Th2 cytokine production by TSLP-DC-activated Th2 memory cells was shown to be independent of IL-4 [13].

Transcriptional regulation of Th2 cells mediated by IL-25

Transcription factors are crucial in determining the cell fate of helper $CD4^+$ T cells. Several key transcription factors were known to regulate Th2 lineage commitment. While T-bet (T-box expressed in T cells, Tbx21) is the cell lineage-specific factor for Th1 cells, GATA-3 was identified as a master regulator selectively expressed in the Th2 lineage as a consequence of STAT6 activation [27–30]. Nuclear factor of activated T cells (NFAT)c1 and JUNB are transcription factors involved in the early IL-4 expression [31, 32]. IL-25 treatment was found to potentiate the expression of NFATc1 and JUNB, which may be responsible for the regulation of early IL-4 expression [12]. Furthermore, treatment of IL-25 by activated naïve T cell was shown to induce GATA-3 and phosphorylated STAT6 expression. This induction was mediated through IL-4 signaling since neutralizing IL-4 inhibited that expression. Likewise, IL-25 effect on memory Th2 cells was found to regulate several key Th2 transcription factors. Th2 memory cell constitutively expressed GATA binding protein 3 (GATA3), c-maf, and JUNB [13]. Treatment with IL-25 prevented their down-regulation and further activated those transcription factors independent of IL-4 signaling.

During naïve T cell differentiation, IL-25 may regulate Th2 differentiation by enhancing the early IL-4 expression through the direct activation of NFATc1 and JUNB. IL-4 may further activate STAT-6 to induce the upregulation of GATA-3, leading to the Th2 differentiation. Unlike naïve helper T cells, Th2 memory cells constitutively expressed IL-4 and transcription factor GATA-3, c-maf, and JUNB. The maintenance of their expression by IL-25 may not require IL-4 signaling pathway.

Summary and future perspectives

IL-25 is a novel IL-17 family cytokine recently shown to be important in the regulation of type 2 immune-mediated diseases, especially allergic asthma disease. Several innate immune cell types related to the initiation of allergic responses such as lung epithelial cells, eosinophils, basophils, mast cells and alveolar macrophages produce IL-25 (Fig. 1). The function of IL-25 in innate immune regulation and down-

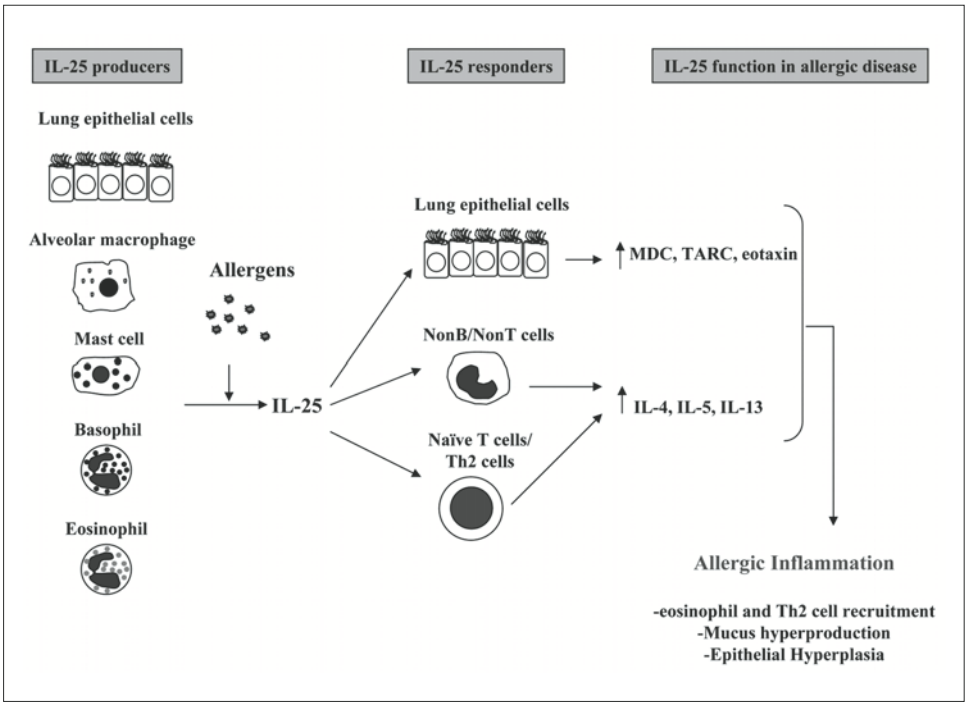

Figure 1.
The involvement of IL-25 during allergic inflammation. IL-25 produced by lung epithelial cells, alveolar macrophages, mast cells, eosinophils and basophils upon allergen sensitization initiates an allergic cascade by regulating both innate and adaptive immune responses, leading to the mucus production, inflammatory cell recruitment, and Th2 cytokine induction.

stream adaptive immune responses may lead to the initiation of allergic diseases. IL-25 may trigger the lung epithelial cells or fibroblasts to produce several type 2 chemokines such as eotaxin, TARC, and MDC, thereby resulting in the recruitment of eosinophils and Th2 cells. More importantly, IL-25 may regulate naïve T cell differentiation toward Th2 lineage and further augment their functions as effector or memory Th2 cells (Fig. 2). The regulation of Th2 differentiation by IL-25 is mediated through the IL-4 signaling pathway, while the effect of IL-25 on effector or Th2 memory cell is independent of IL-4. NFATc1 and JUNB are important in the early IL-4 regulation mediated by IL-25, and the differentiation of Th2 cells is further reinforced *via* the up-regulation of GATA3.

Current studies focusing on the physiological function of IL-25 and its molecular mechanism in regulating Th2 function may benefit the understanding of the complex cellular interactions in immune responses. Since IL-25 has many producer

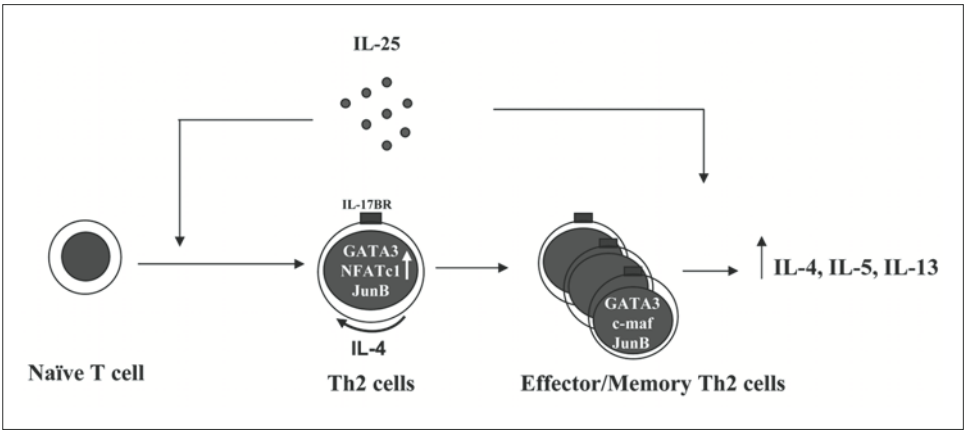

Figure 2.
The function of IL-25 on CD4+ helper T cells. IL-25 promotes Th2 cell differentiation by activating transcription factor JUNB, NFATc1, and GATA3 through the IL-4 signaling pathway, and further amplifies the effector/memory Th2 cell function in augmenting their Th2 cytokine production.

and responder cells, it is crucial to further dissect the contribution of these cells in initiation and propagation of asthma diseases, which may facilitate future development of immunotherapeutic approaches for type 2 immune-related diseases such as allergic asthma.

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Experimental models of disease

Critical role of IL-17 in experimental arthritis

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Abstract

Since the discovery of IL-17 expression in synovial fluid and biopsies in rheumatoid arthritis patients, the role of this pro-inflammatory T cell cytokine in arthritis has been extensively studied using animal models of arthritis. In this chapter, an overview is given of the most important publications elucidating the role of IL-17 in the onset and the progression of experimental arthritis. IL-17 is essential for normal T and B cell development, but is also locally involved in the arthritic process, enhancing the expression of pro-inflammatory cytokines and chemokines. IL-17 also contributes to joint destruction directly by up-regulation of matrix metalloproteinases and stimulating osteoclastogenesis through RANKL induction. The recently discovered Th17 cell is regarded as the main source of IL-17, and the influence of IL-12/IL-23 and other mediators in experimental arthritis is discussed.

Introduction

Experimental models of arthritis and the use of gene-knockout mice, neutralizing antibodies, and overexpression of genes by viral vectors have helped us to position IL-17 in the process of joint inflammation and destruction. The recent discovery of the Th17 cell and new cytokines modulating these IL-17-producing T cells caused quite some turbulence in the scientific world and has resulted in a tremendous comeback of IL-17 in research. This IL-17 hype, more than 10 years after the discovery of the cytokine, has resulted in a wave of new IL-17 papers. To save you from drowning, this chapter provides an overview of the most important publications elucidating the role of IL-17 in the onset and the progression of experimental arthritis. Finally, we summarize some recent findings on factors that drive or inhibit the differentiation and expansion of Th17 cells, like IL-12/IL-23, IL-6, IL-21, and IL-27, and discuss the potential therapeutic value of these mediators.

Th 17 Cells: Role in Inflammation and Autoimmune Disease,
edited by Valérie Quesniaux, Bernhard Ryffel and Franco Di Padova

IL-17 in development of experimental arthritis

IL-17 is a T cell-derived cytokine, a player of the adaptive immunity. Therefore, it was not surprising that a non-immune macrophage-driven arthritis model was not affected in IL-17 receptor-deficient mice. To induce an acute arthritis, IL-17R$^{-/-}$ mice were intra-articularly injected with fragments of streptococcal cell wall (SCW) into the knee. Subsequently the IL-17R-deficient mice developed inflammation and cartilage proteoglycan depletion comparable to wild-type controls [1]. In contrast to acute, non-immune inflammation, IL-17 does play a role in the development of chronic, immune arthritis. IL-17-deficient mice showed a suppressed onset and severity of the collagen-induced arthritis (CIA) model [2]. This suppressed development of CIA was caused by impaired collagen-specific T cell responses and collagen-specific IgG2a production during IL-17-deficiency. An important role for IL-17 was also demonstrated in the spontaneous arthritis development in IL-1Ra$^{-/-}$ mice. IL-17 production was markedly enhanced in T cells from IL-1Ra$^{-/-}$ mice, and development of arthritis in IL-1Ra$^{-/-}$ mice was completely suppressed by a deficiency of IL-17 [3]. Also in this model IL-17 played a crucial role in antigen-specific T cell priming, as shown *ex vivo* using IL-17$^{-/-}$ T cells from DO11.10 mice that carry an ovalbumin (OVA)-specific T cell receptor.

Neutralization of IL-17 prior to the development of experimental arthritis is another approach to determine the role of IL-17 in the onset of disease. Blocking endogenous IL-17 at expected onset of CIA suppressed the arthritis incidence and reduced inflammation scores and bone erosion [4]. Blocking the role of IL-17 at this stage of arthritis development no longer affected T cell immunity, since no differences in T cell responses to murine collagen peptides were found. Also during rat antigen-induced arthritis (AIA), treatment with IL-17 receptor IgG1 Fc fusion protein suppressed arthritis development and severity, while synovial CD3^{+} T cell numbers in these rats were not significantly changed by the anti-IL-17 treatment [5]. These two blocking studies demonstrate that IL-17, as well as being initially important for antigen-specific T and B cell responses, is also directly involved in the process of joint inflammation and destruction. The effect of anti-IL-17 treatment did not seem to affect T cell responses, but the IL-17$^{-/-}$ mice data suggest that prolonged absence of IL-17 signaling might have great effects on (antigen-specific) adaptive immunity.

IL-17: Inducer and accelerator of inflammation and destruction

Overexpression of IL-17 induces joint pathology

IL-17 is a pro-inflammatory cytokine that induces the production of other cytokines like IL-1, TNF, and IL-6 (Fig. 1). Overexpression of IL-17 in a naive knee

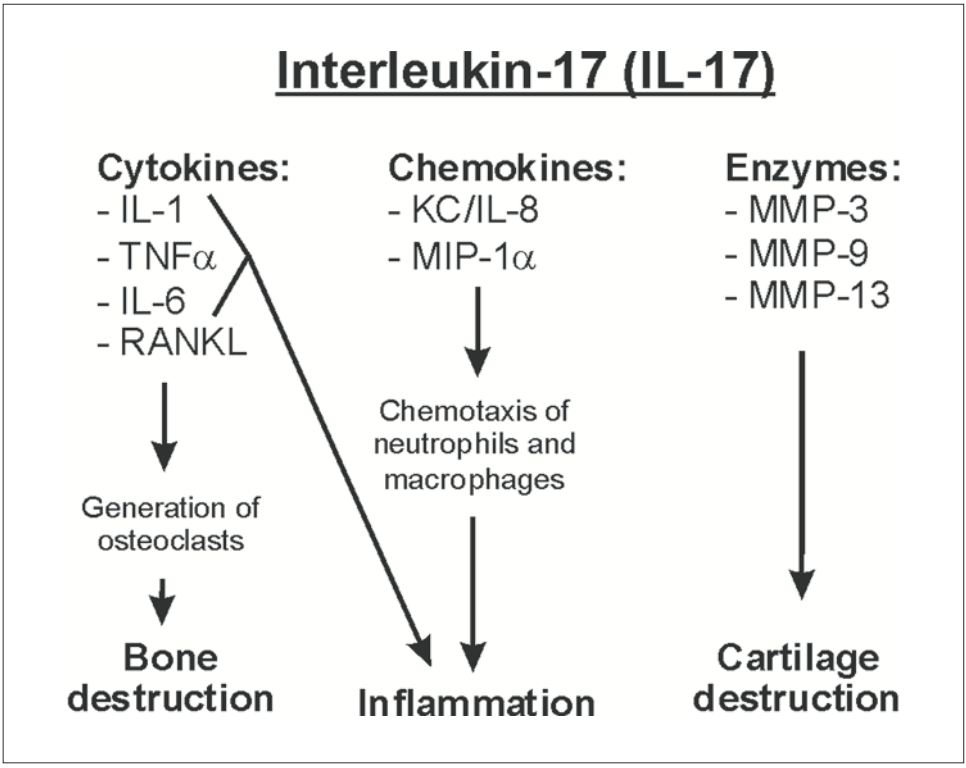

Figure 1
Pathological effects of IL-17 during experimental arthritis.

joint using recombinant protein or adenoviral vectors causes inflammation and cartilage proteoglycan depletion [6, 7]. The IL-17-induced joint inflammation is first characterized by influx of neutrophils, mainly caused by the expression of KC, the murine homologue of the chemokine IL-8. The effect of IL-17 in a naive joint is completely dependent on TNF, since TNF-deficient mice were completely protected against IL-17-induced pathology [7]. In contrast, IL-1$^{-/-}$ mice showed inflammation and proteoglycan (PG) depletion comparable to wild-type controls.

Beside its effect on inflammation *via* the induction of cytokines and chemokines, IL-17 also promotes cartilage destruction (Fig. 1). IL-17 alone is not a very potent inducer of cartilage destruction, but in synergy with TNF IL-17 induces cartilage damage *in vitro* [8]. Because of these strong synergistic effects of IL-17 with TNF, but also with IL-1, it has been suggested that blocking of IL-17 in combination with anti-TNF treatment might be more effective than blocking one of these cytokines alone [9]. When IL-17 is overexpressed during an established arthritis, IL-17 con-

tributes to aggravation of chondrocyte death and cartilage surface erosions [4, 7]. IL-17 enhances the expression of the matrix metalloproteinase (MMP)-3, MMP-9, and MMP-13, and, although IL-1 plays an important catabolic role in experimental arthritis, IL-1$^{-/-}$ studies have shown that IL-17 can mediate cartilage destruction independent of IL-1 [4, 10].

IL-17 also has the potential to cause bone erosion *via* the induction of RANKL (Fig. 1). Local IL-17 gene transfer, especially during CIA, causes marked bone erosion, accompanied by an increase of RANKL and RANK expression in the synovium [11]. The enhanced osteoclast activity caused by IL-17 could be inhibited by osteoprotegerin (OPG) treatment, thereby restoring the RANKL/OPG balance.

Blocking endogenous IL-17 during experimental arthritis

Overexpression studies showed the potential of IL-17 to cause joint inflammation and destruction during arthritis, but only blocking of endogenous IL-17 could elucidate the contribution of this cytokine to the arthritic process. Neutralizing IL-17 after the onset of CIA reduced joint inflammation, cartilage destruction, and bone erosion [12]. This anti-IL-17 treatment resulted in reduced serum IL-6 levels and less synovial IL-1 and RANKL expression. Even late after onset, blocking was still significantly effective. Joint inflammation and cartilage destruction were also markedly suppressed in IL-17R$^{-/-}$ mice during chronic SCW-induced arthritis. In this arthritis model, mice receive repetitive intra-articular injections with fragments of SCW. The arthritis starts as an acute arthritis, and after multiple injections with antigen gradually progresses into a chronic, destructive arthritis with contribution of antigen-specific T and B cells. While the arthritis became more severe after each weekly injection, reduced pathology and suppressed IL-1 and MMP expression were found in IL-17R$^{-/-}$ mice [13]. In comparison to CIA and chronic SCW-induced arthritis, the role of IL-17 in progression of arthritis was even more prominent in the IL-1Ra$^{-/-}$ mice and in mice during AIA flares. IL-1Ra$^{-/-}$ mice, which spontaneously develop arthritis at the age of 6–8 weeks, already have increased numbers of Th17 cells before the onset of arthritis, and systemic IL-17 levels correlate to age and arthritis severity. Blocking of IL-17 in this arthritis model prevented further progression of joint inflammation and destruction (Koenders, *Arthritis Rheum, in press*). During AIA, anti-IL-17 treatment administered shortly before the flare completely prevented cell influx and suppressed the local expression of IL-1, TNF, and RANKL [14]. In this strongly T cell-driven AIA-flare model, the anti-IL-17 treatment seemed far more powerful than during CIA or in arthritic IL-1Ra$^{-/-}$ mice. These findings suggest that, depending on the degree of T cell involvement of the arthritis model, IL-17 plays an important role in the progression of experimental arthritis and contributes to joint inflammation as well as destruction of cartilage and bone.

Manipulating the role of IL-17-producing Th17 cells in experimental arthritis

IL-12 and IL-23: Similar structure, opposite effects

The Th1-driving cytokine IL-12 is a heterodimer that consists of two subunits, the IL-12p35 and the IL-12p40 subunit. IL-23 shares the p40 subunit with IL-12, and in addition has its own unique IL-23p19 subunit. Despite the structural homology between IL-12 and IL-23, these cytokines have clear opposite effect on T cells (Fig. 2). IL-23 was found to stimulate memory T cells to produce IL-17, while IL-12 decreased IL-17 levels [15]. Without the knowledge of Th17 at that time, Murphy et al. [16] studied arthritis in mice lacking only IL-12 ($p35^{-/-}$) or IL-23 ($p19^{-/-}$). In this study it was demonstrated that IL-23 deficiency was protective,

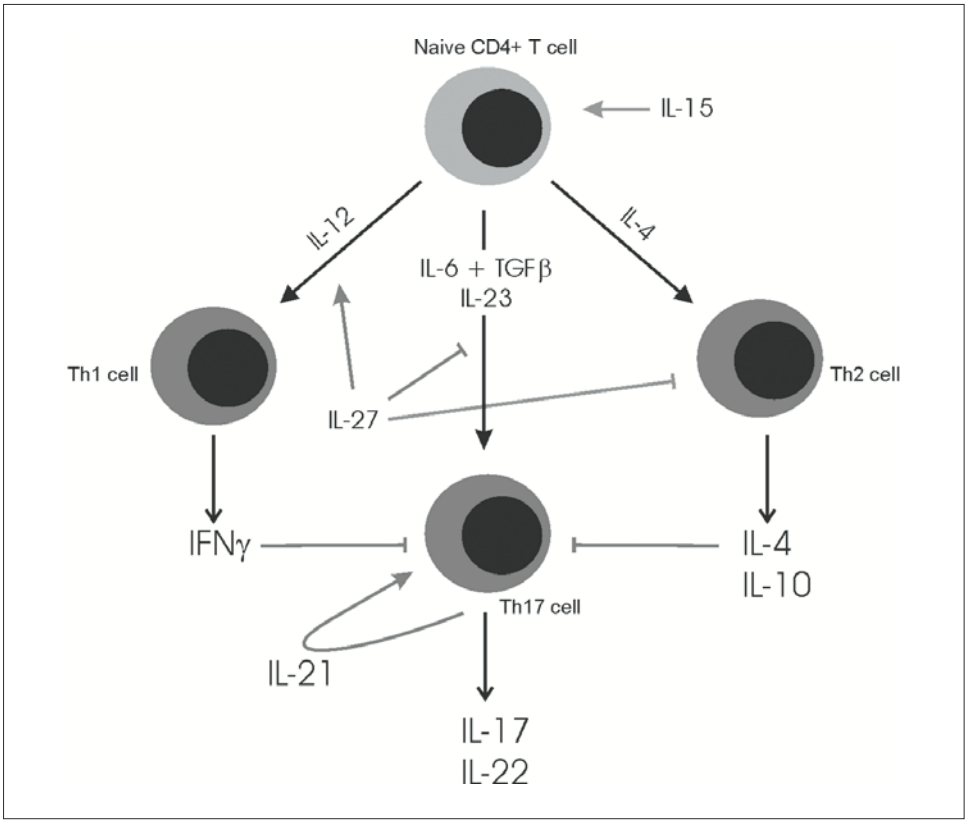

Figure 2
Cytokines influencing T cell differentiation and maturation.

whereas loss of IL-12 exacerbated CIA. While IL-17-producing $CD4^+$ T cells were absent in IL-23p19$^{-/-}$ mice, IL-12p35$^{-/-}$ mice developed more IL-17$^+$ T cells. Also in the spontaneous arthritis in IL-1Ra$^{-/-}$ mice, increased IL-23 expression increases the production of IL-17. IL-23 enhances IL-17 secretion directly, by increasing the IL-17 production *via* STAT3 phosphorylation and activation of the NF-κB pathway in $CD4^+$ T cells, but also indirectly, by inducing the expression of the costimulatory molecule OX40 on $CD4^+$ T cells [17]. The position of IL-23 upstream of IL-17 suggests that targeting IL-23 could be an interesting approach in the treatment of rheumatoid arthritis (RA).

IFN-γ restrains IL-17 production

Not only studies on the Th1-promoting cytokine IL-12, but also on its downstream mediator interferon-γ (IFN-γ) have shown that Th1 cells negatively influence IL-17-producing T cells. In the past, when arthritis was considered a Th1-mediated disease, a lack of IFN-γ paradoxically enhanced CIA [18]. The discovery of Th17 cells led to the rejection of this Th1 concept, and with IFN-γ$^{-/-}$ mice and anti-IFN-γ antibodies it was demonstrated that this Th1 cytokine suppresses IL-17 production during experimental arthritis [19], but also inhibits the expansion of Th cells [20]. Interestingly, DBA-1 mice, which are prone to CIA, have more Th17 and less Th1 cells compared to the resistant B6 mouse strain, indicating that the Th1/Th17 balance could be a crucial factor in the susceptibility for arthritis. However, the suppressive effect of IFN-γ on IL-17 production does not mean that IFN-γ and Th1 are not contributing to arthritis. On the contrary, in PG-induced arthritis, severe arthritis is dependent on the production of IFN-γ, and is independent of IL-17 [21]. Both Th1 and Th17 therefore contribute to the arthritic process, and the cytokine environment seems to have a great influence on the balance of Th cell subsets that will be found at the site of joint pathology.

IL-4: Directly or indirectly suppressing IL-17 secretion

Not only the Th1-related cytokines IL-12 and IFN-γ, but also the Th2 cytokine IL-4 influences IL-17 expression during arthritis. During CIA severe bone erosions was prevented by IL-4 gene therapy [22]. Local IL-4 overexpression suppressed IL-17 and RANKL expression in the synovium and inhibited the formation of osteoclast-like cells, and also prevented collagen type I breakdown in human bone explants directly. Targeting IL-17 *via* genetically modified murine dendritic cells (DCs) that secrete IL-4 led to similar findings [23]. IL-4-transduced DCs expressed higher levels of IL-12p35 mRNA, but lower levels of IL-23p19 and the common subunit p40.

These IL-4-DCs also secreted increased quantities of IFN-γ. Since blocking of IL-12, IL-23, or IFN-γ in co-cultures of IL-4-DCs plus T cells caused only modest changes in IL-17 production, IL-4 itself might also play an important role in directly suppressing IL-17 production.

IL-6, a pleiotropic cytokine influencing Th17 development

IL-6 is a pleiotropic pro-inflammatory cytokine involved in many processes like acute-phase response, fever, and osteoclast formation. Recently it was demonstrated by multiple groups that IL-6 is also involved in murine Th17 differentiation *in vitro*. IL-6 and TGF-β together induced the differentiation of Th17 cells from naive T cells (Fig. 2), thereby inhibiting the generation of TGF-β-induced regulatory T cells. Although the exact role of IL-6/TGF-β is still subject of debate, a role for IL-6 in IL-17 production has been demonstrated in experimental arthritis models. SKG mice spontaneously develop autoimmune arthritis due to a ZAP70 mutation that results in high numbers of self-reactive T cells. In this Th17-driven model, IL-17 deficiency completely abrogated this model, whereas IFN-γ deficiency exacerbated arthritis [24]. IL-6-deficient SKG mice completely lacked IL-17$^+$ T cells and did not develop arthritis, which indicates that IL-6 is crucial for *in vivo* development of Th17 cells. Also, in AIA, IL-6 deficiency resulted in less IL-17 production, fewer osteoclasts, and reduced arthritis severity [25]. In clinical trials, the anti-IL-6 receptor monoclonal antibody has been demonstrated to ameliorate the signs and symptoms of RA. Although improvement was found in lots of clinical and biological parameters, it is not yet known whether this clinical efficacy of blocking IL-6 signaling can also partially be attributed to inhibiting Th17 development.

Enhanced T cell activation by IL-15

As with IL-6, the first clinical trials for IL-15 using a neutralizing antibody have shown promising results in the treatment of RA. IL-15 regulates T and natural killer cell activation and proliferation, and also directly stimulates IL-17 production by T cells. Blocking of IL-15 during CIA suppressed incidence and severity of arthritis [26, 27]. IL-15 deficiency slightly decreased incidence and severity of arthritis, while IL-15 transgenic mice showed increased arthritis [28]. Both IFN-γ and IL-17 levels were reduced by inhibition of IL-15 signaling, whereas the levels of antigen-specific IFN-γ and IL-17 production were significantly higher in IL-15 transgenic mice compared to wild-type mice. These data suggest that IL-15 not only contributes to the progression of experimental arthritis by affecting Th17, but more generally by enhancing T cell activation and/or proliferation, including IFN-γ-producing Th1.

New kids on the block: IL-21, IL-22, and IL-27

One of the effects of IL-15 is the induction of IL-21. IL-21 is a T cell-derived cytokine that modulates T cell, B cell, and natural killer cell responses. Blocking of IL-21 during CIA and rat adjuvant arthritis reduced the clinical and histological signs of arthritis [29]. Interestingly, treatment with this murine IL-21 receptor Fc fusion protein resulted in enhanced expression of IFN-γ in the paws and lymph node cultures, whereas antigen-specific IL-17 production was suppressed. In that report, the role of IL-21 in Th balances was not yet suggested, but recently IL-21 was described as an autocrine Th17 factor that promotes or sustains Th17 lineage commitment (Fig. 2). Blocking of IL-21 therefore leads to abrogated Th17 differentiation, suggesting an interesting therapeutic approach for RA targeting upstream of IL-17.

IL-22 is a novel cytokine of the IL-10 family that is preferentially produced by Th17 cells, and IL-23 or IL-6 can directly induce the production of IL-22 from naive T cells. The role of IL-22 in (experimental) arthritis is still unknown, but in psoriasis-like skin inflammation was demonstrated that blocking of IL-22 suppressed development of disease and reduced Th17 cytokine expression [30]. However, the role of IL-22 in autoimmune diseases is still subject of debate, since IL-22 deficiency during experimental autoimmune encephalomyelitis (EAE) showed that IL-22 was not involved in the pathogenesis of EAE [31].

IL-27, the latest member of the IL-12 family, is produced by antigen-presenting cells in response to IFN-γ. This IL-12 family member seems to have a conflicting role in immunity, having both pro-inflammatory and anti-inflammatory properties. IL-27 contributes to Th1 cell development by increasing the IL-12 responsiveness of naive T cells, up-regulation of the Th1 transcription factor T-bet, and down-regulation of the Th2 transcription factor GATA-3 (Fig. 2). On the other hand, IL-27 has anti-inflammatory properties, since it suppresses the development of IL-17-producing T cells (Fig. 2) and induces the production of IL-10 by effector T cells *in vitro*. The role of IL-27 has also been investigated in various arthritis models. In rat adjuvant arthritis, anti-IL-27 antibodies provided protection against severe arthritis accompanied by suppressed IFN-γ production [32]. In the IFN-γ-dependent PG-induced arthritis, IL-27R deficiency resulted in delayed development of arthritis and suppressed severity [33]. The knee joints of these IL-27R$^{-/-}$ mice have not only reduced expression of IFN-γ, but also suppressed expression of IL-17. These *in vivo* data only partially confirm the previous *in vitro* findings: blocking of IL-27 indeed suppressed the Th1 response, but did not lead to the expected enhancement of Th17 development.

Although not all Th17-related cytokines have been studied extensively in arthritis models, other autoimmune models like EAE have shown great predictive value in the past with respect to involvement of IL-17, IL-12, and IL-23 in experimental arthritis. The balance of all these T cell-modulating cytokines seems to be critical in

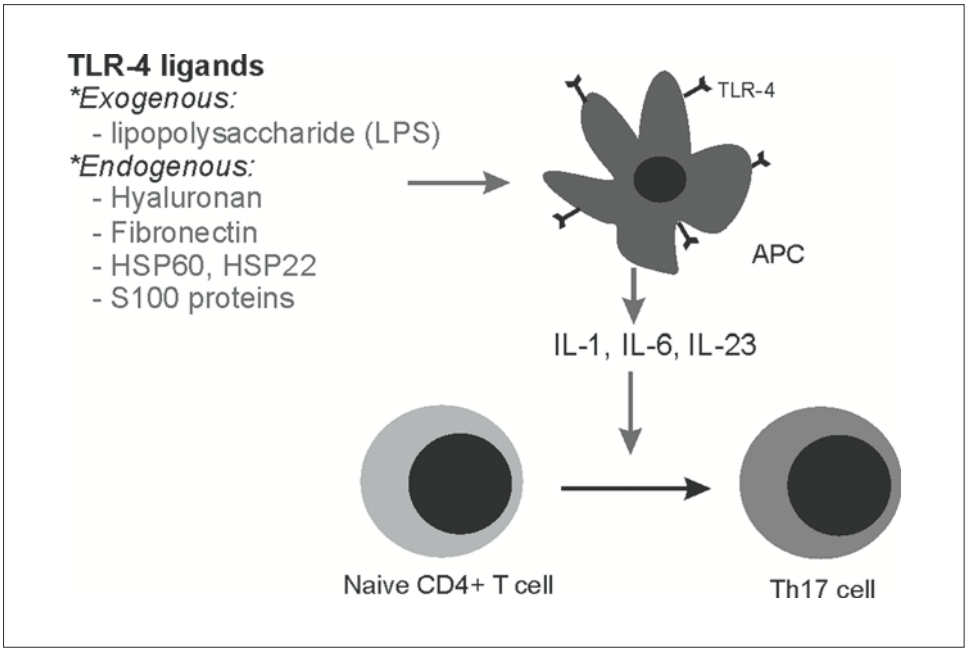

Figure 3
TLR-4 activation contributing to arthritis progression via *Th17 activation.*

determining the differentiation of naive T helper cells into the various T cell subsets. Disturbance of this balance could be the trigger to development of arthritis, but can also be the driving force for the progression of arthritis. A recent study in IL-1Ra$^{-/-}$ ×TLR-4-deficient mice showed that despite similar disease incidence, TLR-4 deficiency resulted in suppressed arthritis severity in the chronic phase of arthritis [34]. TLR-4 ligands, probably endogenous factors generated by inflammation and tissue damage, promoted the progressive joint pathology by driving increased numbers and activation of Th17 cells through induction of IL-1 and IL-23 (Fig. 3). Therefore, not only T cell-modulating cytokines but also TLRs could be interesting targets to inhibit the contribution of the IL-17-producing Th17 cells in arthritis.

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Dual role of IL-17 in allergic asthma

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Abstract

A proinflammatory role of IL-17 in autoimmune disorders has been favored, although there is evidence that IL-17 has a dual role as negative regulator. Here we review the concept of dual IL-17 functions in the light of recent strategies to use IL-17 neutralization as potential alternative to neutralizing TNF and IL-1 treatments in chronic inflammatory disorders. Expectedly, in allergic lung inflammation, neutralization of IL-17 inhibited neutrophil recruitment. However, this IL-17 antibody treatment concomitantly increased eosinophil recruitment by neutralizing IL-17's dual role as negative regulator. IL-17 negatively regulated dendritic cell function and activation of T helper cell (Th)2 cytokine production. Furthermore, IL-17 inhibited Th2-characteristic chemokine and adhesion molecule expression. On a mechanistic level, IL-17 acted on IκB-β by preventing degradation and in turn leading to reduced NF-κB activation or IL-17 inhibited transcription factor IRF-1. Therefore, anti-IL-17 therapy, although presenting a promising lead in chronic inflammatory disorders, bears a potential risk of exacerbating allergic asthma.

IL-17 production by antigen-specific Th17 cells inhibits Th2 response

Interleukin-17 (IL-17, also know as IL-17A) is the founding member of a multimember cytokine family consisting of IL-17A to IL-17F [1]. It forms homodimers containing five highly conserved cysteine residues forming a cysteine knot. Receptor IL-17R (also know as IL-17RA) ligates IL-17 and is involved in IL-17F binding. It binds none of the other IL-17 family members [2].

IL-17 is produced by the memory T cells termed Th17, a T helper cell lineage distinct from Th1 and Th2 cells, which is negatively regulated by IFN-γ and IL-4 [3, 4]. Unchecked activation of Th17 cells by IL-23 is linked to chronic inflammation in experimental autoimmune encephalomyelitis (EAE) and type II collagen-induced arthritis, two prototypical 'Th1' disease models [5, 6]. Recently, IL-12, and thus Th1 as effector cells, were shown to reduce disease development and IL-17 contents [7]. These findings favored the definition of IL-17-producing T cells, Th17, as a lineage distinct from Th1.

Th 17 Cells: Role in Inflammation and Autoimmune Disease,
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These findings also raised the question of a comparison of Th17 and Th2 responses beyond the finding that Th2 cytokine IL-4 down-regulates IL-17 production. It has recently been shown that IL-17 was required during antigen sensitization to develop a Th2 response in allergic asthma, as shown in IL-17R-deficient mice [8]. In this model, IL-17 expression increased further upon pulmonary antigen challenge, therefore its function in the effector phase of allergic asthma was addressed. Most strikingly, neutralization of IL-17 augmented the allergic response, while recombinant IL-17 administration reduced pulmonary eosinophil recruitment and bronchial hyperreactivity (Table 1). Furthermore, recombinant IL-17 reduced eosinophil-chemokine eotaxin (CCL11) and thymus- and activation-regulated chemokine (TARC/CCL17) in lungs *in vivo*; antigen uptake by dendritic cells, and IL-5 and IL-13 production in regional lymph nodes were also reduced by recombinant IL-17. These findings demonstrated a novel negative regulatory role of IL-17. Therefore, endogenous IL-17 has a dual role. While it is essential during antigen sensitization to establish allergic Th2 response, in sensitized mice IL-17 attenuates the Th2 response.

Increasing evidence supports this novel role of IL-17 as downmodulator of a committed immune response

It has in the past abundantly been described that full acquisition of pathogenic function in EAE by effector Th17 cells is mediated by IL-23. However, as shown most recently, stimulation of the myelin-reactive T cells with TGF-β plus IL-6, instead of IL-23, completely abrogated their pathogenic function despite up-regulation of IL-17 production [9]. These regulatory Th17 cells failed to up-regulate the proinflammatory chemokines crucial for central nervous system inflammation. In contrast, the regulatory Th17 cells produced IL-10, which had potent anti-inflammatory activities. This study by Cua's group [9] did not show whether IL-17 directly conveyed negative regulation of inflammation, which was due rather to co-expressed IL-10 in EAE. Several experimental approaches listed below have indeed demonstrated direct inhibitory functions of recombinant and endogenously produced IL-17 *in vitro* and *in vivo*. Furthermore, an IL-17-induced expression of the anti-inflammatory IL-10 has been demonstrated in macrophages; yet IL-17 has moderate effects on monocytes and macrophages [10].

First, expression of recombinant murine IL-17 in Vaccinia virus increased viral virulence significantly in mice [11], suggesting that IL-17 negatively regulated the anti-viral host defense. Second, administration of recombinant IL-17 ameliorated and negatively regulated the late phase of experimental autoimmune neuritis (EAN), a model of peripheral nerve demyelination [12].

Third, *in vitro* studies provided possible mechanisms of how IL-17 acts in a suppressive manner. IL-17 inhibited the chemokines RANTES (CCL5), Fractalkine (CX3CL1), and CTACK (CCL27) [13–15] and the mononuclear leukocyte adhesion

Table 1. Recombinant IL-17 inhibits allergic asthma in C57BL/6 and BALB/c mice

	BALB/c		**C57BL/6**	
	OVA	OVA/IL-17	OVA	OVA/IL-17
BHR (arbitrary units)	531 ± 168 (100%)	365 ± 151 (59%, $P = 0.045$)	154 ± 30 (100%)	97 ± 51 (41%, $P = 0.034$)
Total cells ($\times 10^4$)	173 ± 63 (100%)	114 ± 62 (63%, $P = 0.01$)	354 ± 155 (100%)	47 ± 23 (9%, $P = 0.004$)
Eosinophils ($\times 10^4$)	108 ± 41 (100%)	50 ± 38 (45%, $P < 0.001$)	282 ± 125 (100%)	14 ± 17 (5%, $P = 0.003$)
Lymphocytes ($\times 10^4$)	22 ± 14 (100%)	14 ± 9 (62%, $P = 0.037$)	30 ± 14 (100%)	5 ± 5 (16%, $P = 0.003$)
Neutrophils ($\times 10^4$)	0.7 ± 1.4 (100%)	0.5 ± 0.7 (68%, $P < 0.001$)	n.d.	n.d.
Macrophages ($\times 10^4$)	35 ± 21 (100%)	50 ± 26 (164%, $P = 0.079$)	30 ± 12 (100%)	20 ± 4 (39%, $P = 0.067$)
EPO ($OD_{490\ nm}$)	0.699 ± 0.028 (100%)	0.577 ± 0.112 (77%, $P = 0.03$)	0.249 ± 0.083 (100%)	0.091 ± 0.033 (10%, $P = 0.025$)
IL-5 BAL (pg/ml)	54.3 ± 29.8 (100%)	0 ± 0 (0% $P = 0.005$)	n.d.	n.d.
IL-4 BAL (pg/ml)	8.9 ± 7.1 (100%)	0.4 ± 0.5 (5%, $P = 0.03$)	13.3 ± 3.8 (100%)	0.3 ± 0.5 (2.3%, $P = 0.04$)

Mice were s.c. immunized and i.n. challenged either with OVA or OVA plus IL-17, as described previously [8]. Absolute values are given for the allergen-induced airway dysfunction (BHR), differential cell counts in the bronchoalveolar lavage (BAL), eosinophil peroxidase activity (EPO) in lung tissues, and IL-5, IL-4 concentrations in BAL. Relative values (in parentheses) are calculated as follows: for i.n. OVA challenge 100% represents values after subtraction of i.n. NaCl treatment. The relative values obtained by the OVA/IL-17 vs. OVA stimulation are given in percentage. Differences between OVA and OVA/IL-17 groups are statistically significant at $P < 0.05$. *The data represent the mean ± SD of eight animals for C57BL/6 and ten animals for BALB/c mice (n.d.: not detected).*

molecule VCAM-1 in TNF-activated mesenchymal cells [16]. Fractalkine, CTACK, RANTES, and VCAM-1 are involved in inflammatory responses of both Th1 and Th2 types. RANTES and VCAM-1 are essential in the recruitment of mononuclear cells and VCAM-1 is involved in the formation of germinal centers (present in autoimmunity). Therefore, existence of the novel negative regulatory role of IL-17 needs to be revisited in multiple inflammatory and immune disorders.

Revisiting the role of IL-17 in multiple immune responses would help address the question as to whether IL-17 acts like a regulatory T cell (Treg) cytokine such as TGF-β or IL-10, which reduce allergic pulmonary challenges as well as vast T cell responses [17–19]. Indeed, in initial experiments IL-17 was described as a Treg cytokine in cell cultures, inhibiting vast T cell responses [17, 20]. Furthermore, Treg cells and TGF-β promote under proinflammatory conditions the development of Th17 cells and production of IL-17 [21]. Therefore, IL-17, as a downmodulator of the response of dendritic cells and Th2, provides evidence for a novel feedback mechanism by which Treg cells may control a Th2 response in the effector phase of allergic asthma.

Excess endogenous IL-17 production in the absence of IL-4 signals *in vitro* and *in vivo*

IL-17 production is induced by TGF-β in a proinflammatory milieu, including the presence of IL-6, IL-1, or TNF, and its production is sustained by IL-23. In contrast, IL-17 production is inhibited by IL-4, IFN-γ, IL-25 (IL-17E), or IL-27. In lungs, IL-17 has been shown to originate from antigen-specific Th17 cells as well as from an iNKT subpopulation, which is NK1.1 negative [22]. In an allergic lung response IL-17 production was induced by IL-23 and controlled by IL-4 receptor signaling [8]. In mice lacking IL-4 responsiveness, IL-17 was overproduced correlating with reduced effector functions of allergic asthma. The inhibition of the Th2 response was indeed ascribed to endogenous IL-17, as assessed using IL-17 neutralizing antibody treatment *in vivo*.

Therefore, a novel mechanism of how IL-4 promotes a Th2 response was proposed, i.e., by suppression of the novel suppressor molecule IL-17. This added a novel function of IL-4 to the list of its proallergic effects, including differentiation of Th2 lymphocytes, inhibition of T lymphocyte apoptosis, induction of IgE production, promotion of eosinophil transmigration into the lungs, mucus hypersecretion, and bronchoconstriction [23–26].

Molecular mechanism of negative regulatory IL-17 effect

On a mechanistic level, IL-17 elicits dual effects and reportedly promotes expression of proinflammatory (hemopoietic, CXC-chemokines, acute phase) factors [27, 28], whereas it inhibits the production of mononuclear cell recruiting molecules like TNF-induced VCAM-1 and CC-chemokine RANTES [16]. This dual effect of IL-17 in human cell cultures predicted a reduced mechanism of mononuclear cell recruitment *in vivo*. CC-type chemokines RANTES (CCL5), TARC (CCL17) and eotaxin (CCL11) were induced by antigen OVA *in vivo*. TARC primarily attracts

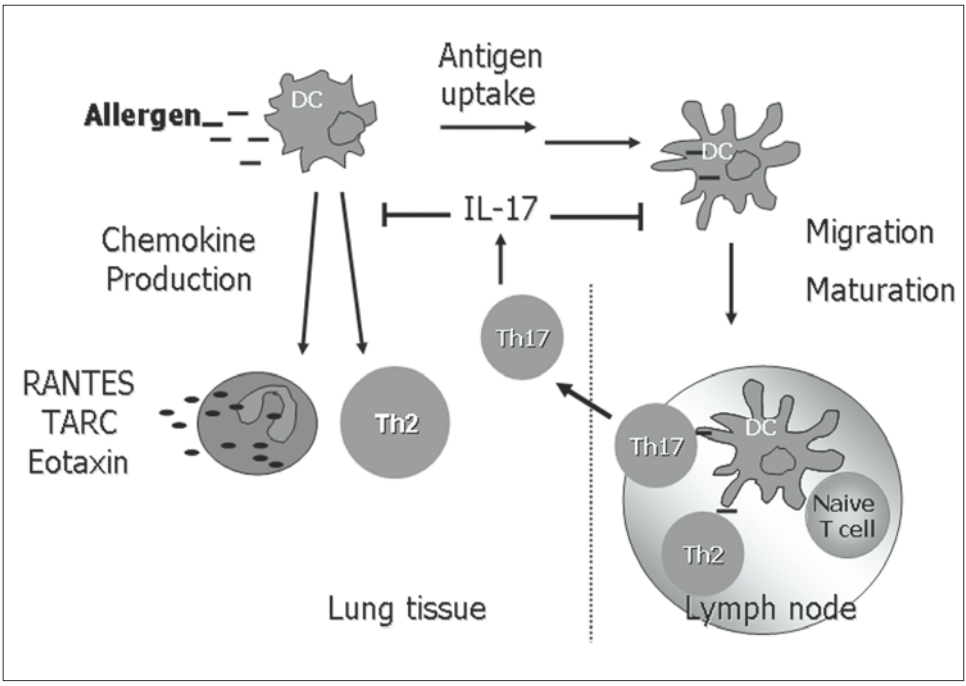

Figure 1
Schematic presentation showing that diminished cell attraction seems to be the pivotal mechanism of how IL-17 attenuates the allergic response.

CCR4-positive Th2 cells. IL-17 reduced TARC production, which correlated with reduced lymphocyte counts and Th2-derived IL-5 concentrations in lung tissues. The expression of the major eosinophil attractant, eotaxin, were also reduced by IL-17 and accompanied by reduced eosinophil infiltration in the airways. Indeed, reductions or absence of these CC-chemokines reportedly ablate allergic asthma [29–31]. Therefore, diminished cell attraction seems to be the pivotal mechanism of how IL-17 attenuates the allergic inflammation (Fig. 1).

Further IL-17 effects like acute-phase IL-6 and prostaglandin (PG)E2 elevations may also have corroborated to locally reduce the allergic inflammation in the lungs. IL-6 elevations inhibited aeroallergen-induced Th2 inflammation [32, 33]. PGE2 elevations reduced pulmonary allergy specifically *via* the E3 receptor [34]. Therefore, while IL-17 may up-regulate negative-regulators IL-6 and PGE2, it has a direct inhibitory effect on the local production of Th2 cytokines IL-4, IL-13, and IL-5 in the lung and regional lymph nodes [8]. Mechanistically, IL-17 inhibits dendritic cell activation and antigen uptake, which leads to reduced activation of T cells and reduced IL-4, IL-13, and IL-5 production, resulting in reduced allergic response.

However, the inhibition of the Th2 response by IL-17 represented a reduction rather than a complete blockade. Intact anti-allergen IgE concentrations in the circulation may explain why IL-17 did not completely block, but rather reduced, pulmonary allergy and asthma. Elevated IgE concentrations reportedly correlate with and contribute to allergic reactions [35], although it is not sufficient for the development of allergy. Therefore, IL-17 acts as negative regulator of established Th2 response locally in lungs.

High efficiency of inhibitory function of IL-17 *in vivo* and *in vitro*

Intranasal administration of recombinant IL-17 reduced eosinophil recruitment and a Th2 response, while neutrophil recruitment was not induced when IL-17 was applied locally at low doses of 2.5 µg/kg to allergen-treated mice [8]. These findings were supported by the following cell culture data [16]. IL-17 inhibited TNF-induced chemokine RANTES expression in human synovial fibroblasts and mouse lung fibroblasts. This inhibitory activity of IL-17 was sixfold more potent than its stimulatory activity on TNF-α-induced IL-6 or IL-8 secretion (IC_{50} = 0.2 ng/ml *versus* ED_{50} = 1.2 ng/ml), measured in the same cells. Furthermore, neutralization of the human IL-17R by antibodies competitively reversed the IL-17-induced IL-6 up-regulation. However, anti-IL-17R antibody only partially neutralized the inhibitions of RANTES production by IL-17. Yet, IL-17R was essential for the RANTES inhibition, as assessed in IL-17R-deficient cells. Therefore, inhibitory and stimulatory functions of IL-17 involve IL-17R but show distinct dose responses, and in turn different sensitivities to an IL-17R antagonizing antibody. These findings suggest a higher efficiency of the inhibitory over the stimulatory IL-17 functions and may explain why a net negative regulatory effect of IL-17 manifests in chronic inflammation *in vivo* where IL-17 production is low.

Molecular signaling of inhibitory IL-17 effects

IL-17 interferes at TNF-activated NF-κB signaling in human synoviocytes [16]. This inhibition is immediate, within 20 min, and proposes a direct effect of IL-17 rather than *via* expression of secondary mediators. The reduced degradation of specifically IκB-β, but not IκB-α, provides a late inflammatory phase control mechanism by IL-17 for the following reason. It has been shown that inhibitor IκB-α is of importance for the transient inactivation of NF-κB, whereas, IκB-β as part of a multimeric complex is involved in the persistent inactivation of NF-κB [36, 37]. The fact that IκB-β but not IκB-α is affected by IL-17, further supports the possibility that IL-17 is implicated in the regulation of the chronic phase of inflammation and immunity.

However, interferences of IL-17 on TNF-induced NF-κB activity virtually depend on the cell type and promoter targeted by TNF. For example, unlike the synoviocytes described above [16], TNF induced NF-κB binding was only moderately and not statistically significantly reduced by IL-17 in colonic myofibroblasts [13]. In those cells it was proposed that IL-17 interfered at the TNF-induced RANTES production mainly through inhibition of IRF-1. This in turn prevented the cooperation of IRF-1 with the NF-κB activity.

Furthermore, in macrophages it has been shown that IL-17 inhibited TNF expression transiently, the effect of IL-17 being biphasic with an early decrease of TNF release (at less than 30 min) and a marked stimulation later on (by 6 h) [10]. By 60 min and later, IL-17 also inhibited cAMP production, and the transcription factor activities of CREB, AP-1, and NF-κB in the macrophages [10].

Inhibitory IL-17 function, a possible risk in an IL-17 antibody therapy in arthritis?

The pathological role of IL-17 in autoimmune disorders has been convincingly documented and, hence, so far favored, even though there is emerging evidence that IL-17 also has negative modulatory function in antigen-specific immune processes and allergic asthma [8, 9, 12].

Increased IL-17 concentrations in allergic asthma, chronic bronchitis, chronic obstructive pulmonary disease, cystic fibrosis, acute respiratory distress syndrome (for review see [38]) and also rheumatoid arthritis have been linked to the pathology of the diseases [39]. IL-17 neutralization inhibits experimental murine arthritis [39] and is a potential alternative therapy to TNF neutralization in rheumatoid arthritis. The novel negative regulatory function of IL-17 indicates, however, that such a therapy bears the potential risk of exacerbating allergic asthma. Therefore, an anti-IL-17 treatment in chronic inflammatory disorders seems very promising, inhibiting neutrophil recruitment in inflamed lungs and joints, while the exacerbating Th2 response in experimental allergic response by anti-IL-17 antibodies may exclude respective groups at risk from such a therapeutic anti-IL-17 treatment.

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Contribution of IL-17 to the pulmonary inflammatory response

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Abstract

Airway exposure to endotoxin and other microbial Toll-like receptor (TLR) agonists induces a rapid production of mediators including IL-1, neutrophil recruitment and bronchoconstriction, which are abrogated in mice deficient for distinct TLRs or the common adaptor molecule myeloid differentiation factor 88 (MyD88). Intranasal IL-17 administration causes acute neutrophilic lung inflammation in a proinflammatory environment. Recent investigations revealed that IL-17 is up-regulated upon endotoxin aerosol exposure and neutralization of IL-17 diminished endotoxin-induced inflammation, suggesting a role of endogenous IL-17 in endotoxin-induced lung inflammation. Furthermore, administration of IL-1β mobilizes neutrophils and induces IL-17 production in the lung. Therefore, IL-17 might participates in IL-1β-induced lung inflammation. Importantly, lung injury leads to NALP3 inflammasome activation, leading to IL-1β-dependent acute inflammation. The participation of IL-17 in this response is discussed. In conclusion, TLR-agonist and injury-induced lung inflammation depend in part on IL-1β and IL-17. The role of inflammasome activation cleaving pro-IL-1β leading to mature IL-1β and IL-1β-dependent IL-17 production and inflammation need to be explored further.

Toll-like receptor (TLR)-dependent neutrophil recruitment

Microbial TLR agonists cause neutrophil recruitment

The innate immune system senses microbial products through TLR, which are broadly distributed in the lung and the body [1, 2]. TLR engagement by microbial products causes cell activation, which often results in a proinflammatory response. Microbial agonists may be of bacterial, viral, fungal and parasitic origin and activate discrete TLR.

Pulmonary exposure to bacterial and fungal products, including endotoxin (lipopolysaccharide, LPS), plays an important role in the development and progres-

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sion of chronic respiratory diseases such as asthma [3–5]. In an experimental situation, intranasal endotoxin from Gram-negative bacteria provokes acute pulmonary inflammation with neutrophil recruitment into the alveolar space and lung, epithelial damage, alveolar-capillary leak and bronchoconstriction in normal C57BL/6 mice [6, 7], which are TLR4 expression dependent. Endotoxin-induced bronchoconstriction and neutrophil recruitment into the lung are abrogated in mice deficient for TLR4 or CD14. In addition, production of TNF, IL-12p40, and keratinocyte-derived chemokine (KC), and protein leakage are also dramatically reduced [8].

Other bacterial products such as macrophage-activating lipopeptide-2 (MALP-2) or bacterial lipoprotein (BLP) activate the TLR2 receptor leading to acute lung inflammation, while bacterial flagellin is a proinflammatory TLR5 agonist [9, 10]. The inflammatory response is abrogated in mice deficient for TLR2 or TLR5. Similarly, viral single-stranded RNA have TLR3 agonistic properties. Poly IC is a synthetic TLR3 agonist that causes acute neutrophil recruitment into the bronchoalveolar lavage (BAL) and lung inflammation, which is absent in TLR3-deficient mice. This is not an exhaustive list of agents activating TLR and other pattern recognition receptors that lead to inflammation.

The lung is continuously exposed to environmental agents, and the inflammatory response is believed to be different from those present in less exposed, accessible sites [11, 12]. We have recently shown that the TLR adaptor myeloid differentiation factor 88 (MyD88) is critical for the airway inflammatory response to endotoxins [13]. MyD88 is at the crossroad of multiple TLR-dependent and TLR-independent signaling pathways, including IL-1R, IL-18R and IL-33, or the focal adhesion kinase, FAK [14]. However, in certain infection models the extreme sensitivity of MyD88-deficient mice may be ascribed, at least in part, to deficient IL-1R/IL-18R signaling, as shown recently for cutaneous *Staphylococcus aureus* infection [15] and our own unpublished results on mycobacterial infections (Fremond et al., 2007).

Endogenous TLR ligands may activate TLR and induce lung inflammation

In addition to the microbe-derived TLR agonists, endogenous products that engage the TLR or Nod-like receptors have been identified. Danger signals or "alarmins" including uric acid, ATP, heat shock proteins (HSPs), HMGB1 and IL-1α are molecules generated by stressed, damaged or dying cells [16] and are released upon cell death. These endogenous mediators activate the innate immune system by specific receptors and induce inflammation. They allow maturation of dendritic cells and induction of the adaptive immune response, which may cause inflammation. Uric acid was identified as the major danger signal released by damaged cells and as a strong endogenous adjuvant [17]. Recent investigations demonstrate that uric acid crystals induce activation of the NALP3 inflammasome, leading to IL-1β and inflammation peritonitis [18]. These data suggest that injury or stress resulting in

the release of endogenous mediators activates the innate immune system and causes inflammation.

IL-17 contributes to the inflammatory response in the lung

Recent investigations suggested that the T cell derived cytokine IL-17 participates in the acute pulmonary inflammatory response [19–21].

Recombinant human (rh) IL-17 was shown to induce the production of IL-8 by human bronchial epithelial and endothelial cells, and the intratracheal instillation of IL-17 in rats elicited neutrophil recruitment and macrophage inflammatory protein-2 (MIP-2) production in the BAL, both of which were inhibited by neutralizing IL-17 antibodies [22].

To link the proinflammatory effects of endotoxin with that of IL-17, endotoxin was instilled intratracheally in mice. Endotoxin elicited the production of IL-17 within 24 h, together with IL-6 and MIP-2 release in the airways. Neutralization of IL-17 abrogated neutrophil recruitment [23]. Further, neutralization of IL-6 and MIP-2 inhibited the neutrophil accumulation. Ferretti and colleagues [24] reported that endotoxin causes a first peak at 24 h, followed by a second peak of neutrophil recruitment in the airways, the latter coincided with IL-17 production in the BAL fluid and lung. Neutralization of IL-17 diminished neutrophils at 48 h as well as the production of the chemokines MIP-2 and KC in the BAL [24]. Investigations in SCID mice and transcriptional analyses of purified cells from BAL suggested that in addition to T cells, neutrophils, but not macrophages expressed IL-17 [24]. The finding that non-T-cells produce IL-17 is interesting and needs additional confirmation.

Recent evidence further suggests that a subpopulation invariant natural killer T (iNKT) cells that lack the NK1.1 marker (NK1.1) secrete high amounts of interleukin IL-17 and low levels of IFN-γ and IL-4 [25]. NK1.1$^-$ iNKT cells produce IL-17 upon synthetic [α-galactosylceramide (α-GalCer) or PBS-57], as well as natural (LPS or glycolipids derived from *Sphingomonas wittichii* and *Borrelia burgdorferi*) stimulation. NK1.1– iNKT cells are found more frequently in the lung, which is consistent with a role in natural immunity to inhaled antigens. Airway neutrophilia induced by α-GalCer or LPS instillation was significantly reduced in iNKT cell-deficient Jα18 mice, and was abolished by neutralizing IL-17 monoclonal antibody. Therefore, NK1.1$^-$ iNKT lymphocytes represent a new population of IL-17-producing cells that can contribute to neutrophil recruitment through preferential IL-17 secretion.

Infection with *Klebsiella pneumoniae* has been used as another way to test the role of IL-17 in neutrophil recruitment and host resistance [26]. IL-17 receptor-deficient mice succumbed rapidly to intranasal *K. pneumoniae* challenge with uncontrolled infection, delayed and diminished neutrophil recruitment in the alveolar space and reduced MIP-2 and G-CSF production in the lung [26]. Therefore, for an acute inflammatory response and to clear the bacilli IL-17R signaling is critical.

IL-17 has been found in sera and BAL fluids from asthmatic patients, suggesting a role in allergic reactions [27]. Experimentally, IL-17 has been shown to be necessary for antigen-induced allergic inflammation of the airways and T cell activation, since the response was ablated in IL-17-deficient mice [28]. We have recently shown that IL-17 receptor signaling is dependent IL-23 production and elevated levels of IL-17 in the lung of allergic mice [29]. However, the administration of neutralizing IL-17 antibody augmented the allergic response, which suggested for the first time that IL-17 has a secondary role in established asthma, which may consist in down-regulating the hypersensitive response [29] (see also accompanying contribution by Schnyder et al.).

Lastly, we revisited the role of IL-1β in the allergic response and confirmed a requirement of IL-1 receptor signaling [30]. This indeed suggests that IL-1β may be required for the inflammatory response mediated by IL-17, and work is in progress to define this pathway. We found that rmIL-1β induces acute inflammation [31] and we are presently exploring the role of IL-17 in IL-1β induced neutrophilia.

In conclusion, the available data with the TLR4 and NKT ligands demonstrate that IL-17 is induced and plays a role in inflammation. The observation that IL-1β up-regulates IL-17 raises the questions to what extent IL-1β-induced inflammation depends on endogenous IL-17.

Critical role of IL-1 in lung inflammation

Recent investigations in several inflammatory models suggested that IL-1β indeed may be critical in inflammation. First, in acute cigarette smoke-induced inflammation in mice, it was found that neutrophil recruitment in the alveolar space depended on TLR4 and IL-1R signaling as the response was ablated in gene-deficient mice [32]. Cigarette smoke induced IL-1 and HSP70, which is a known agonist of TLR4, explaining the absence of inflammation in TLR4-deficient mice [32]. However, the role of IL-1 in chronic model of cigarette exposure mimicking chronic obstructive pulmonary disease has not been investigated.

Second, in an acute lung injury model induced by a single intranasal administration of bleomycin, we found that neutrophil and later lymphocyte recruitment followed by lung fibrosis depended on IL-1β [31]. Indeed, IL-1R-deficient mice or neutralization of IL-1β abrogated bleomycin-induced inflammation and fibrosis [31].

Third, the inflammatory response to uric acid crystals as observed in clinical gout has been demonstrated to be IL-1β dependent [18, 33]. We then investigated the inflammatory response to intranasal mycobacteria administration. We found that a protective host response required functional IL-1β, as IL-1R-deficient mice succumbed to uncontrolled infection [34]. Lastly, rmIL-1β administered by the intranasal route induced lung inflammation [31].

Therefore, the different models suggest a critical role of IL-1 in the inflammatory response, and recent studies shed some lights on the mechanisms of IL-1β maturation.

Inflammasome activation and IL-1β maturation

The newly discovered family of cytoplasmic proteins, the NALPs, has been implicated in the activation of caspase-1 by the TLRs during the cell's response to microbial infection. Like the structurally related apoptotic protease-activating factor-1 (APAF-1), which is responsible for the activation of caspase-9, the NALP1 protein forms a large, signal-induced multiprotein complex, the inflammasome, resulting in the activation of proinflammatory caspases [35].

Uric acid crystals occurring in gout have recently been shown to activate the NALP3 inflammasome complex [18]. Uric acid crystals engage the caspase-1-activating NALP3 (or cryopyrin) inflammasome, resulting in the production of active IL-1β and IL-18. Macrophages from mice deficient in various components of the inflammasome such as caspase-1, ASC (apoptosis-associated speck-like protein containing a caspase recruitment domain) and NALP3 are defective in crystal-induced IL-1β activation. Moreover, neutrophil influx is impaired in crystal-induced peritonitis in inflammasome and IL-1R-deficient mice. These findings provide insight into the molecular mechanism of the inflammatory conditions of gout and support a pivotal role of the inflammasome in inflammatory diseases.

Lung injury-induced inflammation provoked by bleomycin is also reduced in ASC-deficient mice, which is a critical adapter of the NALP3 inflammasome complex [31]. Therefore, the data suggest that lung injury activates the NALP3 inflammasome complex to produce mature IL-1β, resulting in inflammation.

Mediators or endogenous danger signals activating the inflammasome complex represent a topic of broad interest. Lowering the intracellular K^+ level may be a mechanism of NALP3 activation [36, 37]. Among several hypotheses, uric acid was identified in dying cells [17]. Dying cells stimulate inflammation, which is MyD88 and IL-1R dependent. This inflammatory response requires IL-1α, and IL-1R function is essential on non-bone marrow-derived cells. The data suggest that inhibiting the IL-1R-Myd88 pathway *in vivo* could block the damage from acute inflammation that occurs in response to sterile cell death [38]. However, no direct link could be made between cell death and uric acid levels [39].

Lysophosphatidic acid has been identified following lung bleomycin injury and may represent another mediator leading to lung fibrosis. Lysophosphatidic acid levels were increased in BAL fluid following bleomycin injury, and mice lacking one of its receptors, LPA1, are markedly protected from fibrosis [40]. Fibroblast recruitment and vascular leakage, two responses that may be excessive when injury leads to fibrosis rather than to repair were reduced in the absence of LPA1, whereas leu-

kocyte recruitment was unchanged. In patients with idiopathic pulmonary fibrosis, lysophosphatidic acid levels in BAL fluid were also increased [40]. LPA1 therefore represents a new therapeutic target for diseases in which aberrant responses to injury contribute to fibrosis, such as idiopathic pulmonary fibrosis.

In conclusion, present knowledge about injury-induced inflammasome activation, maturation of IL-1β and IL-18 is emerging and the role of IL-1α and other mediators is incomplete. There is evidence suggesting that lung injury leads to inflammasome complex formation and caspase-1 activation, which results in mature IL-1β that might elicit IL-17 production. However, the link between IL-1β and IL-17 and the role of IL-17 as a critical inflammatory effector cytokine in lung inflammation needs to be explored further.

Obviously, the biochemical identification of these pathways has therapeutic implications, and may lead to novel therapeutic targets and drugs.

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The role of IL-17 in experimental autoimmune myocarditis

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Abstract

Experimental autoimmune myocarditis (EAM) represents a $CD4^+$ T cell-mediated mouse model of inflammatory heart disease. Induction of autoreactive, heart-specific $CD4^+$ T cells depends on Toll-like receptor-mediated activation of self-antigen-loaded antigen-presenting cells beyond a genetically determined threshold. Recent findings suggest that the expansion of a specific subset of heart-pathogenic $CD4^+$ cells characterized by IL-17 production is required for disease development. Accordingly, cytokines promoting Th17 $CD4^+$ expansion, such as IL-6, IL-23, and IL-1 are key players in EAM. Understanding the specific role of distinct cytokines during induction and progression of EAM expands our knowledge on the mechanisms of inflammatory heart diseases and contributes to the development of novel treatment strategies in the future.

Autoimmunity in inflammatory heart disease

Dilated cardiomyopathy represents the most common cause of heart failure in young patients and often evolves from myocarditis [1–3]. Worldwide, infections with the parasitic protozoan *Trypanosoma cruzi* (Chagas disease), which is endemic in Southern America, are the leading cause of myocarditis. In developed countries, however, viral infections are common triggers of cardiac inflammation [1–5]. Besides enteroviruses or adenoviruses, parvovirus, hepatitis C virus, human immunodeficiency virus, human herpesvirus (HHV)-6, HHV-7, cytomegalovirus, and Epstein-Barr virus have been detected in hearts of patients with inflammatory dilated cardiomyopathy [2, 3]. Virus infections directly contribute to cardiac tissue destruction by cleaving the cytoskeletal protein dystrophin, leading to a disruption of the dystrophin-glycoprotein complex [6]. Moreover, clinical observations and insights from animal models provide evidence that autoimmunity plays a relevant pathogenetic role in most cases of human myocarditis and in many patients with dilated cardiomyopathy [7]. Notably, many patients with dilated cardiomyopathy develop heart-specific autoantibody responses [5, 6]. In addition, immunosup-

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pressive therapy can improve heart function in subgroups of patients, particularly in individuals without evidence for persistence of viral genomes in heart biopsies [8, 9].

In several susceptible mouse strains, infections with the human pathogen Coxsackie virus type B3 (CVB3) result in chronic myocarditis progressing to heart failure, even after clearance of the virus [10, 11]. In mice, chronic myocarditis following CVB3 infection is T cell mediated because adoptive transfer of T cells, but not serum from diseased mice, transfers disease in severe combined immunodeficient (SCID) recipients genetically lacking B and T cells [12]. Furthermore, peripheral blood lymphocytes from patients with dilated cardiomyopathy could also transfer disease to SCID mice [13]. Inflammatory infiltrates in affected mouse hearts are characterized by the interstitial and patchy accumulation of mononuclear cells and mirror the histological pattern observed in human myocarditis. The marked reduction of inflammation in infected mice after depletion of $CD4^+$ T cells, and the partial reduction of disease severity after depletion of $CD8^+$ T cells, suggest that T cells play an important role in the progression of myocarditis in CVB3-infected mice [12, 14, 15]. Taken together, these findings indicate that heart-specific autoimmunity plays an important role in post-viral myocarditis and argue for a critical role of T helper cells in disease development.

The experimental autoimmune myocarditis model

Immunization models offer the advantage to study and disease pathogenesis *in vivo* in the absence of infection. Indeed, immunization with α-myosin or specific α-myosin peptides together with strong immunostimulants, such as complete Freund's adjuvant (CFA), induces heart-specific inflammation (experimental autoimmune myocarditis, EAM) in susceptible mouse strains, such as BALB/c ($H\text{-}2^d$) and A/J ($H\text{-}2^a$) mice [16, 17]. The most pathogenic epitope for BALB/c mice has been identified on the α-myosin heavy chain (MyHC-α) protein [17]. Activation of antigen-presenting cells (APC) that take up and process self antigen is a prerequisite for T cell priming. Toll-like receptors (TLRs) represent a family of transmembrane receptors that are essential for innate immune responses to pathogens. The different TLRs exhibit different specificities for microbial patterns, such as LPS or double-stranded RNA, as well as for some endogenous products such as stimulatory signals released by dying cells. Recently, it was shown that α-myosin-loaded dendritic cells activated through TLR ligands, such as LPS or double-stranded RNA, can induce autoimmune myocarditis in BALB/c mice [18]. Disease induction by vaccination with self-antigen-loaded dendritic cells offers an attractive approach to study the APC-effector cell interactions and priming mechanisms of autoimmune T cells *in vivo*.

In BALB/c mice, myocarditis severity peaks 21 days after the first myosin-peptide/CFA immunization or 12–15 days after the first injection of activated, peptide-

loaded dendritic cells. In affected mice, impaired cardiac contractility correlates with the percentage of $CD4^+$ T cells among heart-infiltrating $CD45^+$ cells. In fact, depletion of $CD4^+$ T cells in A/J ($H\text{-}2^a$) mice led to protection from myocarditis after subsequent myosin immunization, whereas depletion of $CD8^+$ T cells reduced severity but not prevalence of disease [12, 14]. The decisive role of $CD4^+$ T cells in autoimmune myocarditis induction, however, was shown in adoptive transfer experiments. Injection of *in vitro* re-stimulated $CD4^+$ T cells isolated from MyHC-α-immunized diseased mice induced myocarditis, albeit at a low severity grade, in SCID mice or LPS-pretreated wild-type mice [12]. Importantly, however, adoptive transfer experiments do not work in naïve wild-type mice.

IL-17-producing $CD4^+$ T cells mediate autoimmune myocarditis

EAM represents a $CD4^+$ T cell-mediated disease. Historically, two major $CD4^+$ T cell subsets have been defined according to their cytokine production pattern: IFN-γ-producing Th1 cells, and Th2 cells releasing IL-4. The majority of the heart-infiltrating cells share a monocyte/macrophage phenotype, suggesting that Th1 signals predominate. In addition, signaling through the receptor of the key Th2 cytokine IL-4 is dispensable for EAM development [19, 20]. These findings would point to a pathogenic role of Th1 differentiation in EAM. However, loss of either the Th1 effector cytokine IFN-γ or its receptor results in markedly increased disease severity, implying that the key Th1 cytokine IFN-γ is a negative regulator of EAM [19–22]. In addition, mice lacking T-bet, a T-box transcription factor essential for Th1 lineage differentiation, show enhanced myocarditis severity [20].

These apparently contradictory findings were finally explained by analysis of gene-targeted mice with mutations in the Th1-driving cytokine IL-12 and its receptor. IL-12p70 binding to the IL-12 receptor represents a heterodimer consisting of an IL-12p40 and an IL-12p35 subunit. The IL-12p70 receptor, on the other hand, consists of two subunits, IL-12Rβ1 and IL-12Rβ2, and induces the recruitment of JAK2 and activation of STAT4 upon activation. Experiments with knockout mice revealed that both the IL-12β1 and the IL-12p40 subunit were essential for myocarditis induction [20–22]. In contrast, IL-12p35-deficient mice developed more severe autoimmune myocarditis compared to wild-type littermate controls [21]. These apparent paradoxical findings were finally explained by the discovery that IL-12p40 represents a shared subunit between IL-12p70 and IL-23, another cytokine of the IL-12 family. IL-23 is composed of IL-12p40 and a distinct p19 subunit. Moreover, the IL-23 receptor shares the β1 chain with the IL-12p70 receptor. IL-23 has recently been recognized as a key mediator promoting the expansion and survival of another subset of $CD4^+$ T cells characterized by the production of the cytokine IL-17. Several lines of evidence clearly suggest that IL-17-producing $CD4^+$ T cells, Th17 cells, represent a distinct subset of $CD4^+$ T cells, developing as a Th1

and Th2 cell-independent lineage [23–26]. Th17 cells play a key role in the development of various models of chronic and autoimmune inflammation, such as experimental autoimmune encephalomyelitis [26], graft-*versus*-host disease [27], and allergic asthma [28]. Recent data provided evidence that autoimmune myocarditis development critically depends on the IL-23–STAT4 axis [22] and on the expansion of heart-specific Th17 $CD4^+$ T cells [20, 29–31]. Notably, the direct pathogenic role of Th17 cells was illustrated in adoptive transfer experiments. Immunization of mice with CFA/myosin peptide results in relatively low numbers of heart-specific Th17 cells in peripheral lymphatic organs. This observation explains why adoptive transfer with *in vitro* re-stimulated $CD4^+$ T cells isolated from draining lymph nodes or spleens of diseased mice only result in minimal cardiac inflammation. Selective *in vitro* expansion of MyHC-α-specific IL-17-producing $CD4^+$ T cells in the presence of recombinant IL-23 and anti-IL-12p70 antibodies, however, yields heart-specific Th17 T cell lines that are highly pathogenic after transfer in wild-type mice [31]. Based on these findings, the question arises whether IL-17 is rather a surrogate marker of a pathogenic T helper cell subset or a disease promoting cytokine by itself. The observations that systematic depletion of IL-17 in MyHC-α/CFA immunized mice by an anti-IL-17 antibody [31] or by active vaccination [29] both ameliorate disease severity and the recruitment of inflammatory cells suggest a direct pathogenic role of IL-17 in EAM.

Self-antigen-loaded dendritic cells play a critical role in EAM induction

The efficient priming of heart-specific, autoreactive T cells requires co-operation between the innate and adaptive activation signals beyond an individual and genetically defined threshold level. Dendritic cells are sentinels of the immune system scavenging of foreign pathogens as well as cell debris and necrotic tissues. Nonspecific activation of dendritic cells through TLRs is supposed to be critical for autoreactive T cell priming. Once activated, CD40 ligand expressing autoreactive T cells further increase the priming efficacy of the dendritic cells by a positive feedback loop that promotes production of pro-inflammatory cytokines and survival of activated dendritic cells *via* CD40–CD40L interaction [32].

Several lines of evidence point towards a critical role of dendritic cells in inflammatory heart diseases. Clinically, total peripheral blood dendritic cells are elevated in patients with end-stage heart failure [33]. In mice, a specific subset of interstitial cells expressing the dendritic cell marker CD11c had been identified in perivascular heart tissue. Interestingly, these cells constitutively express cardiac self antigens on MHC class II molecules even in the healthy heart [34]. The number of these cells and up-regulation of their MHC class II expression parallels the development of cardiac inflammation [35, 36]. The precise role of these heart resident dendritic cells is not clear.

As mentioned above, dendritic cell activation is largely mediated by TLRs. TLRs represent a family of evolutionarily conserved transmembrane receptors [37]. Importantly, different TLR exhibit different specificities for microbial patterns such as LPS or double-stranded RNA, as well as for some endogenous products such as heat-shock proteins and other stimulatory signals released by dying cells [37]. Because antigen-presenting dendritic cells process not only foreign antigens but also damaged self tissue, we expect that activation of self-antigen-loaded dendritic cells beyond a certain threshold would overcome counterregulatory mechanisms of peripheral tolerance and result in the expansion of autoreactive, self-aggressive T cells. This has been termed the "adjuvant effect" of infection in the pathogenesis of autoimmunity [38]. Accordingly, injection of mice with MyHC-α-loaded dendritic cells activated through TLR ligands such as LPS or CpG triggers autoimmune myocarditis and heart failure in susceptible BALB/c mice [32]. Furthermore, mice lacking the common adaptor molecule MyD88 for different TLRs are protected from autoimmune myocarditis [39].

Importantly, the idea that innate activation of self-antigen-loaded dendritic cells is sufficient to induce pathogenic autoreactive T cells does not exclude antigenic mimicry in putting the organism at risk for autoimmune heart disease; it is conceivable that an immune system that was exposed to symptomatic or subclinical infections with any microorganisms containing self-antigen-like structures is more susceptible to boost an autoreactive T cell response after a second hit that releases self antigen on the background of a nonspecific inflammatory response [1]. This concept nicely fits well-known clinical observations. First, it might explain why some patients develop heart-specific autoimmunity after non-infectious tissue damage, i.e., after cardiac surgery or myocardial infarction [40, 41]. We believe that tissue damage of any cause results in uptake of self antigens by dendritic cells. If these self-antigen-loaded dendritic cells become activated, this might be sufficient for the initiation of an autoimmune response depending on the genetic susceptibility of the affected individual [42, 43]. Second, bacterial and viral infections often worsen the condition of patients with dilated cardiomyopathy, i.e., result in sustained impairment of cardiac contractility. Here we expect that the activation of self-antigen-loaded dendritic cells boost autoreactive T cells aggravating cardiac inflammation.

The role of the Th17 inducing cytokines IL-6 and IL-1 in autoimmune myocarditis

Activation of antigen-presenting dendritic cells through TLRs results in up-regulation of MHC class II as well as co-stimulatory molecules. Activated dendritic cells produce high amounts of pro-inflammatory cytokines, which exert auto- and paracrine effects on both APCs and T cells (Fig. 1). Major pro-inflammatory cytokines released by TLR-activated dendritic cells include IL-12p40, IL-1β, IL-6, and

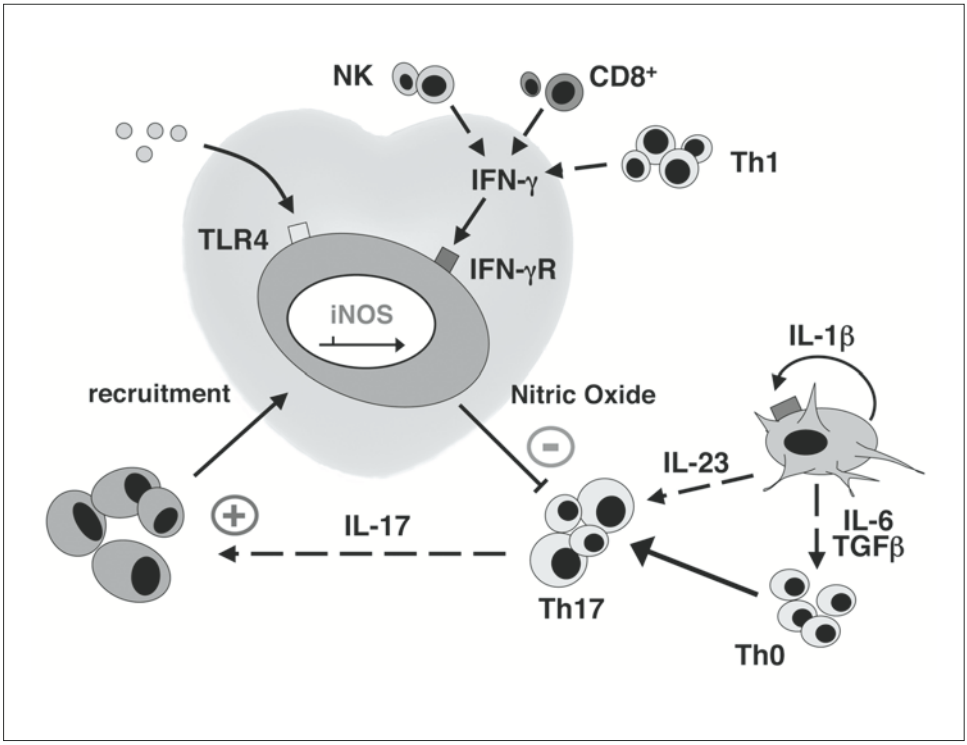

Figure 1
Activated dendritic cells expressing cardiac self-antigen prime autoreactive, IL-17 releasing $CD4^+$ T cells in an IL-6 and IL-1 dependent manner. IL-23 promotes the expansion of autoreactive IL-17 releasing T cells, which promote the recruitment of monocytes to the heart. Heart-infiltrating monocytes, on the other hand, represent a key element in a negative, interferon gamma-dependent and nitric oxide-mediated feedback mechanism confining the autoreactive T cell response.

TNF-α, which are all critical for EAM induction and progression [18, 20, 39, 44]. Gene-targeted mice lacking the TNFRp55receptor, for example, are protected from disease, and it had been hypothesized that TNF-α mediates the activation of heart-resident dendritic cells as well as adhesion molecules on the vascular endothelium [33].

As mentioned before, IL-12p40 represents a subunit of IL-23 that has been recognized as a critical mediator for the expansion and survival for the pathogenic heart-specific Th17 T cell subset [23–25]. On the other hand, IL-1 and IL-6, together with TGF-β, are required for the priming of autoreactive Th17 T cells [26, 45]. From this point of view, it is not surprising that mice lacking IL-6 as well as IL-1

receptor (IL-1R) type I-deficient mice are protected from EAM [18, 44]. Indeed, $CD4^+$ T cells from both MyHC-α/CFA immunized IL-6-deficient and IL-1R type I-deficient mice proliferate poorly after *in vitro* re-stimulation with cardiac antigen and produce only irrelevant levels of IL-17 compared to T cells isolated from immunized wild-type mice (Valaperti and Eriksson, unpublished results). Interestingly, however, transfer of myosin peptide-pulsed bone marrow-derived non-activated wild-type dendritic cells to IL-1R type I-deficient mice restored disease susceptibility [18] and Th17 T cell responses (Valaperti & Eriksson, unpublished observation) in myosin-peptide/CFA-immunized mice. These findings suggest that disease resistance of IL-1R type I-deficient mice cannot be explained by its effects in the priming of autoreactive Th17 cells alone. In fact, IL-1 is also required to promote the auto/paracrine activation of self-antigen-loaded dendritic cells [18]. Furthermore, these data imply that in the presence of antigen-loaded and activated wild-type dendritic cells, Th17 cells can develop in the absence of IL-1R type I signaling in the T cell compartment. Further experiments are needed to identify cytokines and mediators compensating for the absence of IL-1 signaling in Th17 priming.

Opposing roles for IFN-γ and Th17 cells in EAM

Immunization of BALB/c mice with MyHC-α-peptide/CFA results in a mixed T cell response. In fact, *in vitro* re-stimulation of $CD4^+$ T cells from peripheral, draining lymph nodes of diseased mice mainly results in the production of IFN-γ and IL-17, indicating expansion of both the Th1 and Th17 subsets. In diseased hearts, however, Th17 cells predominate and heart-infiltrating $CD4^+$ T cells do not release IFN-γ [21]. Nevertheless, the Th1 cytokine IFN-γ released from other heart-infiltrating cells, such as nonspecific $CD8^+$ T cells, negatively regulates autoimmune myocarditis [21]. Accordingly, mice lacking IFN-γ or the IFN-γ receptor develop more severe, progressive disease [19–22]. These data are in line with observations from the experimental autoimmune encephalomyelitis model, were IFN-γ confines the expansion of autoreactive T cells. IFN-γ might impair the generation and expansion of autoreactive T cells on many levels. *In vitro*, IFN-γ directly suppresses the generation of Th17 cells [23–25]. In EAM, IFN-γ impairs the apoptosis of $CD25^+CD4^+$ regulatory T cells [46] and promotes nitric oxide (NO)-mediated T cell growth arrest [19, 31].

Mac-1 (CD11b)-expressing monocytes represent the major fraction of heart-infiltrating cells in EAM [31]. *In vivo* experiments with IL-17-depleting antibodies demonstrated that IL-17 directly mediates the recruitment of CD11b-expressing monocytes to the heart. So far, monocytes have been considered an active, disease-promoting substrate of inflammation only. Recent findings, however, suggest that $CD11b^+$ cells also represent a key element in the IFN-γ-dependent negative feedback mechanism confining autoreactive T cell expansion and cardiac inflammation (Fig. 1). Mechanistically, IFN-γ induces NO synthase 2 (NOS2) up-regulation in

monocytes. NOS2 up-regulation promotes local release of NO, which has been recognized as a short living mediator of reversible T cell growth arrest at physiological levels.

This negative feedback mechanism makes sense as it allows the immune system to promote the quick IL-17-dependent recruitment of monocytes to a potentially infected site. IFN-γ, on the other hand, is required for the optimal activation of monocytes and macrophages dealing with an infective agent. At the same time, IFN-γ limits the expansion of activated T cells and protects from exaggerated or even auto-aggressive T cell responses. Further studies are needed to address the question whether IFN-γ treatments or even vaccination strategies promoting Th1 T cell responses might protect mice from EAM.

Conclusions

EAM is a helpful *in vivo* model to study the pathogenesis of human inflammatory heart diseases. It illustrates the co-operation between innate and adaptive immunity as the critical event triggering heart-specific autoimmune inflammation. Experiments with gene-targeted mice lacking specific cytokines and cytokine receptors led to the identification of the IL-17-producing subset of heart-specific $CD4^+$ T cells as the pathogenic substrate promoting the recruitment of inflammatory cells to the heart. Furthermore, current levels of evidence suggest a negative regulatory role of IFN-γ and Th1 differentiation in autoimmune heart disease. It is not clear, however, to what extent these observations can be transferred to the human system. Additional studies are needed to identify self-pathogenic T cell subsets in patients with active myocarditis. Nevertheless, insights from the autoimmune myocarditis model have advanced our understanding of inflammatory heart disease and this model offers an attractive tool to develop innovative and novel treatment strategies.

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Th17 cells in organ transplantation

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Abstract

A newly discovered subset of T helper cells, Th17 cells, has been implicated in a number of models of autoimmunity. However, their role in transplantation remains undefined. This is despite studies showing a link between IL-17, a cytokine secreted by Th17 cells, and acute allograft rejection. This chapter summarises current evidence for a role of Th17 cells in transplant rejection, tolerance and ischaemia reperfusion injury.

Th17 cells are a recently discovered 'arm' of T helper cells that can differentiate from naïve T helper cells following their activation. Although Th17 cells have been cast as major instigators of inflammatory damage in a number of models of autoimmunity, including collagen-induced arthritis (CIA) [1–3], experimental autoimmune encephalomyelitis (EAE) [4–7] and dermal inflammation [8], their role in transplantation immunity is ambiguous (Fig. 1). A recent study suggests that donor-derived Th17 and Th1 cells can mediate chronic graft-*versus*-host-disease *via* enhanced autoreactivity [9]. In solid-organ rejection models, some studies have demonstrated a link between the Th17-secreted cytokine IL-17 and acute allograft rejection.

IL-17 is a proinflammatory cytokine that has multiple targets and triggers the release of further mediators downstream. Predominantly, it functions to stimulate granulopoiesis and the migration of neutrophils to the site of inflammation [10]. In one study designed to document the kinetics of IL-17 mRNA expression during renal allograft rejection in a rat model, IL-17 mRNA was detectable by postoperative day 2, peaked at day 5 before declining, becoming undetectable by day 9 [11]. Neutralization of IL-17 by administration of an IL-17R:IgG-Fc fusion protein was found able to prevent the acute rejection of aortic allografts [12] and to prolong the survival of cardiac allografts [13, 14]. In humans, increased IL-17 protein levels have been detected in rejecting renal allografts and in bronchoalveolar lavage during acute lung allograft rejection [15, 16]. Further studies are needed to link IL-17

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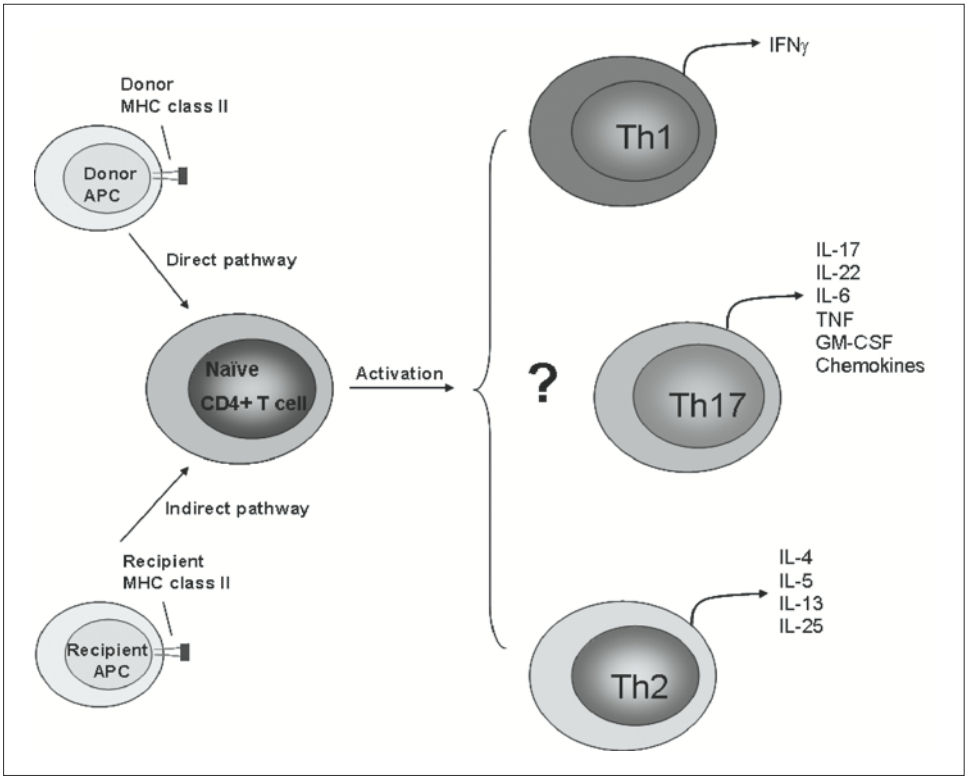

Figure 1
Th17 cells – Potential players in transplant rejection.

production to Th17 cells as other cells such as natural killer, natural killer T and γδ T cells are also producers of IL-17 [17].

The possible involvement of IL-17, which is not produced by Th1 or Th2 cells, in graft rejection further highlights the deficiencies of the Th1/Th2 paradigm in transplant immunology. Th1 cells were traditionally thought to be key perpetrators of inflammatory damage in allograft rejection but surprisingly, a deficiency of IFN-γ, the archetypal Th1 cytokine, led to the accelerated rejection of full MHC-mismatched cardiac allografts [18]. One possible mechanism to explain this observation could be a reduction in nitric oxide synthase and indoleamine-2,3-dioxygenase production normally mediated by IFN-γ [19]. However, alternative possibilities are that other types of T cell can play a role in allograft rejection. There is evidence for extensive cross-regulation between the Th1, Th2 and Th17 pathways (Fig. 2). For example, Th2-produced IL-4 and IL-25 can promote Th2 responses but suppress Th17 function [20, 21]. Similarly, IFN-γ antagonizes TGF-β-induced

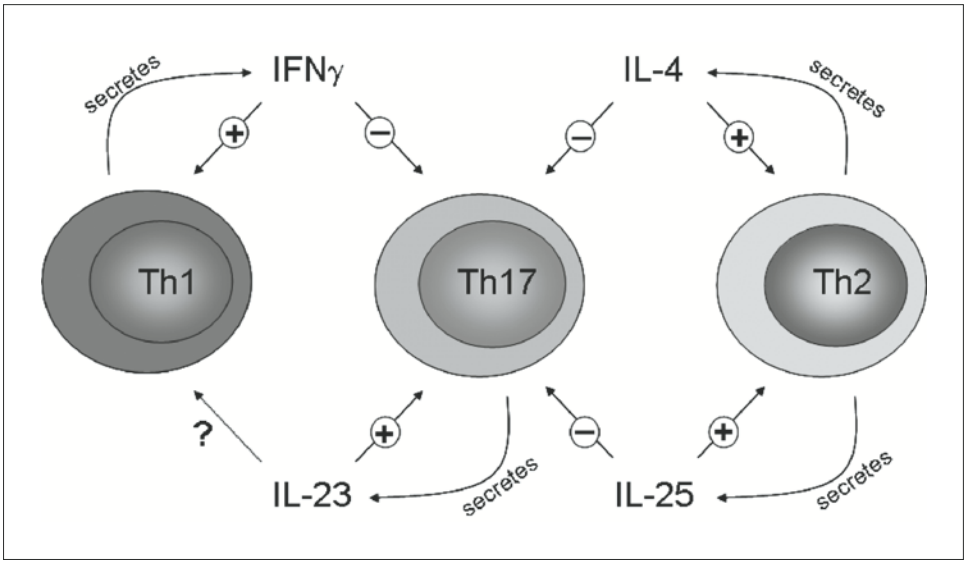

Figure 2
Cross-regulation between Th1, Th2 and Th17 pathways.

Th17 development and inhibits IL-17 and IL-23 production [20]. Allograft pathology in IFN-γ-deficient animals showed reduced mononuclear infiltration and more intense neutrophil infiltration [18]. This may arise as a consequence of the lifting of the inhibitory signal on Th17 differentiation by the absence of IFN-γ, leading to increased IL-17 production and neutrophil recruitment.

Th17 cells may also share a complex link with regulatory T cells (Tregs), with consequent ramifications for the potential use of Tregs to induce transplant tolerance [22]. When exposed to TGF-β, naïve mouse $CD4^+$ T cells (nTh) can differentiate into Tregs [23]. However, nTh can develop into Th17 cells when exposed to TGF-β *and* IL-6 [24]. Therefore, in this setting, IL-6 appears to be a 'switch' factor that determines whether nTh acquires a regulatory or a proinflammatory phenotype.

In vitro, IL-6 and TGF-β-producing Tregs can generate IL-17-producing T cells from nTh [25, 26]. Furthermore, a recent study showed that Tregs, in the presence of IL-6 or activated dendritic cells, can themselves differentiate into IL-17-producing Th17 cells [26]. In a T cell-mediated autoimmunity model, adoptively transferred Tregs increased IL-17 production by effector T cells and failed to prevent disease established by these cells [27]. Kuchroo and colleagues [7] also showed that, in EAE, Tregs were unable to abrogate IL-17 production by T cells that produced high amounts of IL-6 and TNF-α. In an inflammatory milieu, IL-6 production may tip the balance against Treg regulation towards Th17 induction. In transplantation,

such an inflammatory milieu may be established by ischaemia reperfusion injury (IRI) of the transplanted graft.

IRI in the transplant setting is defined as the period from when the graft is retrieved from the donor (ischaemia) to sometime after its revascularisation in the recipient (reperfusion) [28]. The ischaemic phase may include a 'warm ischaemic interval' before or during removal from the donor, and a 'cold ischemic interval' associated with preservation and storage of the graft. IRI contributes to acute graft failure, delayed graft function and increased alloimmunity and graft rejection [29]).

IL-6, an essential differentiation factor for Th17 cells, is implicated in IRI. IL-6 is expressed within 1 h of reperfusion following renal ischaemia [30]. Urinary IL-6 levels also appear to correlate with the severity of renal injury [31]. Both mice that have been treated with anti-IL-6 antibody or IL-6-deficient mice show attenuated renal dysfunction arising from IRI and have reduced neutrophil infiltration and expression of adhesion molecules [32]. In renal IRI, macrophages may be a source of IL-6. The transfer of IL-6-sufficient macrophages into IL-6-deficient mice restores susceptibility to IRI [33]. Increased IL-6 expression in IRI may lead to the induction of Th17 cells. Recently, Yoshida and colleagues [34] have shown that lung ischaemia reperfusion injury could induce type V collagen-specific T cells that secreted IL-17.

The role of Th17 cells is still unclear in transplantation immunology. Along with Th1 cells, Th17 cells may mediate allograft rejection and their role may be more important when Th1 responses are suppressed. Their relationship with Tregs in the transplantation setting warrants further investigation as it may mean anti-IL-6 agents are needed to block Th17 differentiation to enable effective Treg function in establishing transplant tolerance. Finally, IL-6 induced by IRI may promote IL-17 production and neutrophil infiltration, thus contributing to acute graft failure.

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Role of IL-23/IL-17 in infection and tumor control

Is IL-17 required to control tuberculosis?

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Abstract

We review the state of knowledge regarding the role of IL-17 in tuberculosis (TB). IL-17 is clearly induced following exposure to mycobacteria in mice and humans and therefore its role in both protection and the immunopathological consequences of infection must be fully defined. IL-17-producing T cells can be seen in both mice and humans and these cytokine-producing cells are dependent to a large degree upon IL-23. Based on what we know of the function of IL-17 and the nature of TB, it would be surprising if this were a disease where IL-17 would have a dramatic impact; indeed the experimental data suggests that it is not required for control of bacterial growth. However, while it is clear that IL-17 is present during TB, its function(s) is not yet known. Key questions that will help elucidate function include – the role the mycobacteria plays in induction and regulation of the IL-17 response and the role IL-17 plays in modulating the inflammatory response during chronic disease.

Introduction

There are two main reasons for studying IL-17 and tuberculosis (TB). The first is to determine whether it IL-17 is important in disease. The second, more esoteric reason is that the dynamic interaction between host and pathogen may allow us to identify as yet unknown functions for this cytokine. Therefore, when considering the role of IL-17 in TB, the ability of this cytokine to mediate and regulate anti-bacterial and inflammatory activities should be determined. In addition, the factors that mediate induction and regulation of this cytokine should also be addressed.

TB is a disease resulting from the interaction of a chronically persisting intracellular bacteria and a responsive but regulated immune response [1, 2]. While cellular immunity is essential to control bacterial growth, the development of inflammation can lead to tissue damage. The acquired cellular response consists of antigen-specific effector T cells and B cells and, while the requirement for T cells in control of infection is clear, the role of B cells and immunoglobulin is less so. From both animal

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studies and patient data it appears that CD4 T cells are the primary mediators of protection and that the IL-12/IFN-γ axis is important in controlling disease [1, 2]. CD8 T cells have the ability to protect the host from disease and have unique anti-bacterial activity but the absence of these cells can be overcome by the presence of CD4 T cells [1, 2]. There is active regulation of the acquired immune response and it is likely that this serves to limit not only anti-bacterial activity but also tissue damage during chronic disease [3]. Taking into account the known functions of IL-17, this cytokine could impact the innate, acquired and inflammatory response during TB.

Evidence for a role of IL-17 in TB

Support for a role of IL-17 in disease is provided by the fact that mRNA and cellular analyses demonstrate that IL-17 is induced following infection of mice with *Mycobacterium tuberculosis* (Mtb) [4]. More importantly, antigen-specific IL-17-producing cells can be detected in both exposed individuals and TB patients, thus providing substantial impetus for investigation of this cytokine [5]. In mice, IL-17 production occurs rapidly upon a high-dose intratracheal challenge with *Mycobacterium bovis* BCG (BCG) and this response is derived from the γδ T cell population [6]. This population is also a major producer of IL-17 in response to Mtb infection in the lung [7]. In the absence of the early γδ T cell-derived IL-17 response to BCG, there is a reduced early neutrophil and reduced later mononuclear response in the lung [6] (Fig. 1A). In Mtb infection of IL-23-deficient mice, there is very little IL-17 mRNA induction in the lung and in this model the granulomatous response is altered [4]. In this regard, it is tempting to think that the disrupted granulomatous response seen in Mtb-infected γδ T cell-deficient mice [8] may relate to the loss of an early IL-17 response. The presence of IL-17-producing γδ T cells is particularly interesting in light of the recent demonstration that unrestrained IL-17-producing γδ T cells can lead to tissue damage and death in a fungal model of infection [9]. Indeed, regulation of these IL-17-producing γδ T cells is superoxide dependent [9] and in the absence of superoxide there is an increase in neutrophil accumulation in the TB granuloma in mice [10], suggesting that the ability of IL-17-producing γδ T cells to recruit neutrophils may also be regulated by superoxide, this should be investigated. There are other innate sources of IL-17 in the lung such as invariant NK T cells [11] but the role of this response in TB has not been addressed.

Evidence for an antigen-specific IL-17 response in TB

Recent thinking posits that IL-17 is an ancient cytokine that acts to bridge the gap between innate and acquired immunity [12, 13]. In TB it appears that IL-17 is pres-

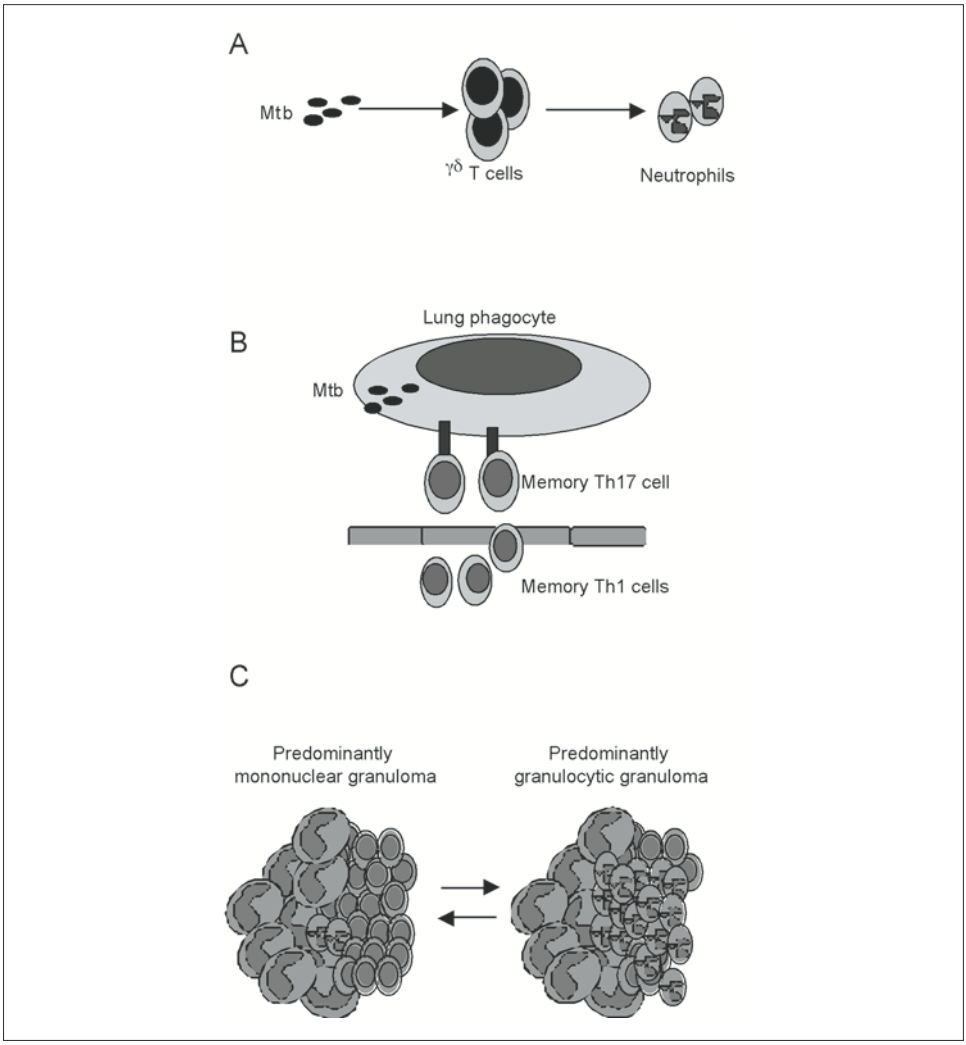

Figure 1.
Functions of IL-17 during Mtb infection. (A) Upon mycobacterial infection in the lung, γδ T cells respond to the bacteria, generate IL-17 and mediate recruitment of neutrophils to the lung. Absence of this response limits early neutrophil recruitment and alters later mononuclear accumulation. (B) Vaccine-induced memory T cells, expressing the tissue-homing chemokine receptor CCR4, recognize infected lung phagocytes and respond by producing or inducing CXCR9, CXCR10 and CXCR11 in the lung. These chemokines then recruit memory Th1 cells that mediate accelerated control of bacterial growth. (C) The dynamic development of the granuloma is modestly altered in the absence of IL-23 and IL-17. One question is whether a larger or longer challenge with mycobacteria may drive a greater role for IL-23/IL-17 such as is seen in fungal infections.

ent both during the innate and acquired response. This has been shown in mice [4, 7, 14] and more recently in humans [5]. Whether the acquired IL-17 response depends upon the innate IL-17 response is not yet clear. The antigen-specific IL-17-producing T cell response is induced with the same kinetics in the mouse as the IFN-γ-producing antigen-specific response but at about a five- to tenfold lower number of cells [4, 14]. Interestingly, despite the reports of IFN-γ and IL-17 responses counter-regulating each other and the fact that the IL-17 response is regulated by IFN-γ following BCG infection [15], the antigen-specific IL-17 response is maintained in the lung during Mtb infection despite the presence of a strong IFN-γ response [4]. IL-17-producing T cells (Th17) require TGF-β to become polarized in both mice and humans [13, 16] and inflammatory cytokines such as IL-6 act to direct the cells to a Th17 rather than regulatory, FoxP3-expressing phenotype (Treg) [17]. IL-23 then acts to promote the release of IL-17 by polarized cells [18] (Fig. 2). IFN-γ limits the amount of IL-23 made by dendritic cells infected with mycobacteria [15], and this may be one way the IFN-γ-producing T cells (Th1) regulate the Th17 response. It is likely that the nature of dendritic cell activation and the local cytokine environment will determine the polarization of naïve T cells during Mtb infection. These conditions will be defined to some degree by the impact of mycobacterial molecules on dendritic cell pattern recognition receptors. The importance of these early conditions in defining polarization is highlighted by the fact that the requirement for induction of the *il12rb1* gene (a component of both the IL-12 and IL-23 receptor) in naïve T cells is lower for the induction of IL-17-producing cells than it is for IFN-γ-producing cells [19]. Comparing the ability of different mycobacteria (BCG *versus* Mtb) to induce and maintain Th1 and Th17 populations may provide insight into how these populations regulate each other *in vivo*.

Evidence for acquired IL-17 immunity in humans

Following Mtb exposure, humans generate populations of cells that secrete several cytokines. Recent work demonstrates that the antigen-specific IL-17-producing cells from purified protein derivative of Mtb (PPD)-positive individuals are CD4 T cells and that these cells are largely distinct from IFN-γ- and IL-22-producing cells [5]. These populations of cytokine-producing cells can also be detected in the peripheral blood of patients with active TB but, while IL-22-producing cells are present also in the bronchoalveolar lavage, the IL-17-producing cells cannot be detected in this location [5]. The reasons for this apparent compartmentalization are intriguing and should be investigated. In this regard in the mouse model, the IL-17 response in the lung is less restrained when the essential Th1 transcription factor T-bet or the Th17 regulating cytokine IL-27 is absent (A.M.C., unpublished observation) (Fig. 2), suggesting that, while the IL-17 response does occur in the lungs of Mtb-infected mice, the Th1 response limits it. That this may be occurring in the human lung is

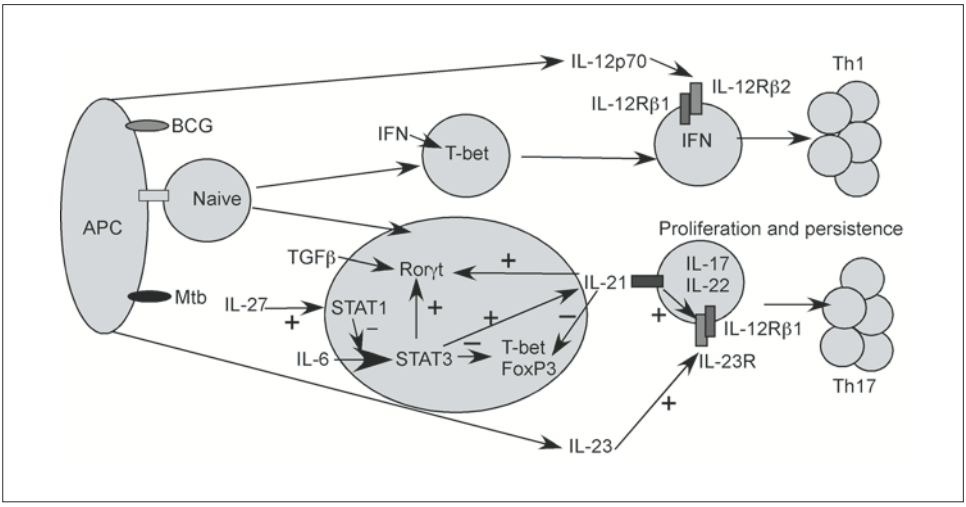

Figure 2
Polarization of Th17 cells during mycobacterial infection. TGF-β drives induction of the transcription factor RORγt; if no inflammation is present, cells will tend towards a regulatory phenotype. If mycobacteria are present, however, IL-6 will induce IL-21, which in turn promotes more RORγt and expression of the IL-23R and thereby responsiveness to IL-23. Naïve cells polarized with these cytokines can then produce both IL-17 and IL-22. Induction of the Th1 transcription factor T-bet in naïve T cells conversely promotes expression of the IL-12Rβ2 molecule, which allows the cells to respond to IL-12p70 and produce IFN-γ. The IL-12Rβ1 component of both IL-12R and IL-23R requires different levels of stimulus to be expressed. If IL-27 is present then STAT1 is activated, and this reduces the impact of IL-6 on the polarizing T cells and reduces Th17 development. Both IL-12 and IL-23 appear to be required for long-term persistence of Th1 and Th17 cells, respectively, during Mtb infection. BCG and Mtb interact with the antigen-presenting cell to define the conditions that pertain during activation of the naïve T cells.

suggested by the fact that IFN-γ limits the IL-17 response in *in vitro* BCG-driven cultures of cells from PPD-positive individuals [5]. Recent work has also characterized the cell surface phenotype of human T cells responsive to Mtb antigens. In one study of a Swiss population, IL-17 was not detected strongly in response to PPD of Mtb; cells that did respond were $CD45RA^{-}CD25^{-}CCR6^{+}CXCR3^{+}$ and produced mainly IFN-γ [20]. In contrast, in a South African population, the BCG-reactive cells were $CD45RA^{-}$ cells and those making IL-17 or IL-22 were predominantly $CCR7^{+}CD27^{+/-}$, whereas those making IFN-γ were predominantly $CCR7^{-}CD27^{-}$ [5]. These differences may represent the fact that the South African population is more exposed; this is an area for further investigation.

Can IL-17 mediate protection?

IL-17 acts to regulate granulocyte homeostasis [21], chemokine expression and cell recruitment [22, 23] and to orchestrate germinal center formation [24]. IL-17 is required for protection against *Klebsiella pneumonia* [22], *Citrobacter rodentium* [25], *Candida albicans* [26] and *Mycoplasma pulmonis* [27]. Finally, IL-17 has been extensively implicated as a mediator of autoimmune-mediated disease (reviewed in [18]). These myriad functions make it difficult to immediately identify where within the complex interaction between host and bacteria IL-17 is likely to act. What is clear from primary infection studies is that IL-17 does not act to reduce bacterial burden in the lung. Following low dose aerosol infection of IL-23-deficient mice with Mtb, there was no difference in the level of bacterial burden within the lung, this despite the fact that there was very little IL-17 mRNA induced or a Th17 response [4]. Similarly, in a BCG model, absence of IL-23 or IL-17 did not alter the bacterial burden [28]. Interestingly, in both these studies IL-23 could mediate protection in the absence of IL-12 but this was associated with the ability of IL-23 to promote IFN-γ responses [4, 28]. Despite this absence of protective activity it was possible to improve bacterial control during pulmonary Mtb infection if IL-23 was delivered prior to infection [29], suggesting that the natural IL-23/IL-17 response may be improved upon.

Whether IL-23 and IL-17 play a protective role in human disease has not yet been clarified. It is apparent, however, that patients with defective *IL12Rb1* gene function are susceptible to mycobacterial disease, that their T cells are defective in their response to both IL-12 and IL-23 [30, 31] and that IL-23 drives efficient IL-17 responses in human T cells [32]. In view of the murine data it is likely that the role of IL-17 in protection against TB will be subtle and therefore very difficult to define in humans; however, this should not deter investigation. In this regard, the association between polymorphisms in the *IL23R* gene and damaging inflammation [33, 34] provides impetus and potentially useful subjects for analysis.

How does IL-17 impact vaccine-induced protection?

Despite the absence of a primary role in controlling bacterial burden, the absence of IL-23, and thereby IL-17, does result in reduced protection in a defined model of immunological memory in mice (Fig. 1B). In this model, the accelerated memory Th1 response induced by a subunit vaccine was lost in the absence of IL-23 and this was associated with the absence of an IL-17-producing $CD4^+$ memory population in the lungs of vaccinated IL-23-deficient mice [14]. The expression of IL-17 mRNA in vaccinated lungs correlated with the expression of the chemokines CXCL9, CXCL10 and CXCL11, and in the absence of this response the accelerated accumulation of CXCR3-expressing IFN-γ-producing memory cells failed to

occur and thus vaccine-induced protection was lost [14]. The vaccine-induced IL-17-producing cells were also shown to be positive for the tissue-homing chemokine receptor CCR4 and capable of populating normal lung tissue. This suggested that memory cells induced by vaccination, could populate non-inflamed tissue and provide a surveillance function [14].

When IL-23 was added to a DNA vaccine regimen it was capable of increasing both IFN-γ and IL-17 responses and protection to a degree equivalent to IL-12 [35, 36]. In view of the down-regulatory activity of IL-27 with regard to IL-17 cellular responses [37, 38] it is not surprising that this cytokine fails to improve vaccine-induced protection [35]. In contrast to these studies, BCG vaccination did not require IL-23 to be protective against a subsequent BCG challenge [28]; however, as this was a systemic challenge the need for a surveillance cell would be less evident. It is also the case, however, that the cross-regulation of Th17 and Th1 cells is more apparent following BCG infection; thus a substantial Th17 response cannot be detected following subcutaneous or systemic delivery of BCG unless the IL-12/IFN-γ axis is absent [15, 36]. While this reductionist approach has improved our understanding of how memory T cell responses are expressed in the lung, we need to more fully understand how BCG vaccination works in order to improve upon this variably effective vaccine.

How could IL-17 impact inflammation?

Dissecting the role of such a pluripotent entity as IL-17 in a chronic disease such as TB is difficult. A major confounding factor is that the granulomatous structure, even within the tractable mouse model, is a constantly developing entity [39]. The ability of IL-17 to impact granulocyte homeostasis and accumulation could be a major factor in granuloma formation. IL-17 is likely a mediator of stress-induced granulopoiesis acting *via* G-CSF [40]; however, in the absence of IL-23 and IL-17 there is only a modest reduction in the extent of the granulomatous response in mice and the granulocytic response is equivalent [4] (Fig. 1C). This is in contrast to the impact of IL-17 and IL-23 on the inflammatory response to fungal infection *via* the mucosal route, where IL-23 and IL-17 down-regulate the protective Th1 response and increase the pyogranulomatous nature of the inflammation [41]. Further, IL-23 and IL-17 improve survival and reduce the killing activity of neutrophils in this fungal model [41]. The reported potential for neutrophils to impact granuloma formation during Mtb infection [42] and the causal connection between early and enhanced neutrophil recruitment with susceptibility to TB [43] make it essential that we determine the role of IL-17 in neutrophil function during Mtb infection. It is possible that low-dose aerosol infection with Mtb does not stress the system as much as the fungal models, and it will be important to determine whether increased duration of infection or increased dose serve to identify a role for IL-17 in the function of neutrophils in Mtb.

An important aspect of the Mtb granuloma that is often dismissed is that a large component of the granulomatous response consists of B cells that accumulate in secondary lymphoid structures in both mice and humans [44–46]. Further, an altered granulomatous response [47] and reduced protection can be seen following Mtb infection of B cell-deficient mice [48]. The ability of IL-17 to mediate germinal centers [24] may impact the B cell response to TB and this potential impact should not be ignored.

How does IL-23 impact the IL-17 response?

IL-23 is a key mediator of IL-17 secretion in memory T cells [49] and γδ T cells [7], and in its absence there is very little IL-17 produced in response to Mtb infection [4]. Mycobacteria can induce IL-23 in dendritic cells [7, 15] and this induction can be regulated by IFN-γ, at least for BCG [15]. Recent studies have implicated dectin-1 in the induction of IL-23 and as a promoter of IL-17-producing CD4 T cells during fungal infection [50]. During mycobacterial infection, dectin-1 has been implicated as a mediator of macrophage [51] and dendritic cell [52] activation with a specific role for dectin-1 in induction of IL-12p40, a subunit of IL-23. The role of dectin-1 in stimulating the phagocytic response to fungal bodies is dependent on developmentally regulated display of β-glucan moieties [53, 54]. It is possible therefore that the expression of different dectin-1 ligands on different mycobacteria species will regulate the amount of IL-23 and therefore the amount of IL-17 they induce. Thus one could hypothesize that while the Th1 response can regulate the Th17 in BCG infection it is possible that a greater induction of IL-23 by Mtb allows the Th17 response in TB overcome Th1 regulation [4, 15].

Although little has been done to characterize the very early cellular response to mycobacterial infection directly *in vivo*, the response of CD4 T cells and dendritic cells to vaccination with trehalose dicorynomycolate (TDM, or cord factor) has been studied. TDM is the primary inflammatory mediator of mycobacteria and alterations in its structure affect the granulomatous response in the lung [55]. In these vaccine studies it was shown that delivery of an IA^b-restricted peptide in the presence of TDM and the detoxified monophosphoryl lipid A (MPL) resulted in early expression of IL-6, TGF-β and IL-23 in dendritic cells and IL-17 but not IFN-γ in the CD4 T cells [14]. This early IL-17 corresponded with early expression of the IL-23 receptor but not the IL-12 receptor in the CD4 cells [14]. These data suggest that Th17 cells, while not requiring IL-23 to become polarized [16], were able to respond to IL-23 very early during activation. In the absence of IL-23 there is a small but reproducible reduction in very early proliferation of CD4 T cells [14]. The initiation of Th17 cells during Mtb infection is less well defined, although the absence of IL-23 does ablate the IL-17 response. It is likely that the balance between IL-6 and TGF-β during activation of naïve T cells will define the relative induction

of a Th17 or Treg population [17] and the development of this balance during Mtb infection is an important area of study. This is particularly true in light of the observed function of Treg cells in limiting bacterial control [3, 56].

Conclusions

This review does not answer the question raised in the title, as we do not yet know whether IL-17 is a positive or negative force in the TB disease process; indeed it may simply be a bystander. We know however that it is induced both in innate and acquired cells in mice and humans and that the expression of this response is dependent upon IL-23. Induction of the IL-17 response is likely to be dependent upon the counter regulation of IL-12 and IL-23 at the dendritic cell level and this may be related to the level of IL-23 inducing moieties (such as β-glucans) on the mycobacterial surface. There is some evidence that the IL-23-dependent IL-17 response impacts the granuloma structure in mycobacterial infection and this may be more easily detected if the antigenic challenge is larger or the if time of infection prolonged. In conclusion, therefore, while the evidence is strong for the induction and expression of IL-17 during tuberculosis in both mice and man, the evidence for a significant impact on disease is less strong. There are several areas that should be investigated to clarify the role of IL-17 in TB. These are: the impact of IL-17 on inflammation and tissue damage, the induction and regulation of the IL-17 response, the role of surface molecules of various mycobacterial species in determining the IL-17 response, the impact of IL-23R polymorphisms on TB in humans, the ability of IL-17-producing memory cells to improve vaccine-induced protection, and the impact of IL-17 and IL-23 on neutrophil and B cell function during TB.

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IL-17 and mucosal host defense

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Abstract

IL-17, a cytokine initially cloned from memory CD^+ T cells is produced by Th17 cells, a new lineage of T cells that are controlled by the transcription factor RORγt, as well as γδ T cells and NK T cells. IL-17A and IL-17F use both IL-17RA and IL-17RC for signaling. IL-17RA is widely expressed in myeloid cells, fibroblasts, and epithelium. IL-17RA signaling is critical for mucosal immunity in the lung against extracellular bacterial infection through the regulation of granulopoietic growth factors and CXC chemokines required for neutrophil recruitment, as well as anti-microbial protein expression in epithelium. IL-17RA has a limited role in controlling the primary response to intracellular pathogens such as *Listeria monocytogenes* or *Mycobacterium tuberculosis*, which require Th1 immunity. However, in the setting of vaccine-induced immunity, IL-17 regulates the recruitment of Th1 cells and is required for optimal vaccine responses for both extracellular and intracellular pathogens.

Introduction

The critical role of $CD4^+$ T cells in host defense against a variety of pathogens became clearly evident through the acquired immunodeficiency syndrome (AIDS) epidemic. It has been demonstrated that the risk of AIDS defining illnesses such as *Mycobacterium tuberculosis*, *Pneumocystis*, and *Toxoplasma gondii* show clear inverse relationships between $CD4^+$ T cell count in peripheral blood and the risk of infection [1, 2]. With the discovery of the T cell subsets Th1 defined by IFN-γ production and Th2 cells defined by IL-4, IL-5, and IL-13 production and the use of selective gene deletions within these effector molecules, it became possible to ascribe specific infections with defects along these T cell lineages. Impaired production of the Th1 effector IFN-γ results in susceptibility to *M. tuberculosis* infection or an increase in susceptibility to *Toxoplasma* infection [3–6]. Alternatively defective Th2 response predispose to infection with helminths [7].

Although the Th1 and Th2 paradigm explained much of the risk of opportunistic infections associated with HIV infection, not all AIDS-associated infections fell into

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intracellular or helminthic pathogens. Specifically, the mechanism by which CD4 deficiency predisposed patients to extracellular pathogens such as bacterial pneumonia remained unclear. This became particularly evident as prophylaxis against the opportunistic pathogen *Pneumocystis* became widespread in the late 1980s to the early 1990s and bacterial pneumonia increased as a major pulmonary complication of AIDS [8]. Just like infections ascribed to Th1 or Th2 immunity, epidemiological data also suggested that lung infections with extracellular bacteria were also inversely associated with $CD4^+$ T cells in peripheral blood [8]. However whether this susceptibility was due to a defect in Th1 or Th2 immunity was unclear. It was hypothesized that the susceptibility of HIV-infected patients with low $CD4^+$ T cell counts could be due to lack of T cell helper function and the generation of neutralizing or opsonic activity against pathogens such as *Streptococcus pneumoniae*, *Klebsiella pneumoniae* or *Pseudomonas aeruginosa*. However, this immune defect would not necessary explain susceptibility to primary infection.

IL-17 was cloned in 1993 and it expression was largely restricted to $CD4^+$ memory T cells [9]. IL-17 receptor was cloned in 1995 [10] and is a Type I transmembrane protein. The initial biological activity attributed to IL-17 from $CD4^+$ T cells was that it supported the differentiation of granulocyte progenitors by stimulating granulocyte-colony stimulating factor (G-CSF) and IL-3 secretion from bone marrow stromal cells [11]. Thus, we hypothesized that IL-17 and IL-17R signaling may regulate granulopoiesis *in vivo* and that this differentiation and recruitment of neutrophils by IL-17-producing T cells may in part explain the susceptibility of $CD4^+$ T cell-deficient host to extracellular pathogens.

IL-17, granulopoiesis, and host defense

Overexpression of IL-17 in mice showed that IL-17 markedly expands both neutrophil progenitors in bone marrow and spleen as well as mature neutrophils in peripheral blood. The expansion of neutrophil progenitors by IL-17 is mediated by the induction of G-CSF and the transmembrane form of stem cell factor (scf) [12]. Antibodies to G-CSF and studies in scf mutant mice markedly attenuate the response to IL-17. IL-17R KO mice have near normal circulating numbers of neutrophils in peripheral blood but are much more susceptible to sublethal gamma irradiation and show reduced neutrophil recovery [13]. These data suggest that stress-induced granulopoiesis requires IL-17RA signaling. Moreover, IL-17R KO mice challenged with the gram-negative pathogen *K. pneumoniae* also show reduced neutrophil progenitor mobilization compared to wild-type mice [14]. Wild-type mice show a nearly 100% increase in granulocyte/macrophage-colony-forming units (GM-CFU) and over a 200% increase in high proliferative potential colonies containing 50 or more cells. However, this response is nearly absent in IL-17RA KO mice despite higher levels of IL-17A ligand in the lungs of these mice [14], demonstrating that IL-17RA

signaling is critical for this response. This defect is likely due defective production of G-CSF, IL-6, and scf (Fig. 1) [12, 14]. Mice and humans with leukocyte adhesion defects such as CD18 deficiency show elevated levels of G-CSF and circulating neutrophils. Ley and colleagues [15] have shown that this expansion of neutrophils is regulated by IL-17 and G-CSF. This G-CSF response is ultimately regulated by gut-derived IL-23 [16], which regulates IL-17 production by T cells. Stark et al. [16] have proposed a model by which the normal uptake of apoptotic neutrophils in the lamina propria of the gut antagonizes IL-23p19 expression, thereby negatively regulating IL-17 production by gut T cells, which allows precise physiological regulation of circulating neutrophil numbers. In this model approximately 60% of the IL-17-producing T cells are γδ T cells, 25% NKT-like cells, and only approximately 15% are $CD4^+$ T cells.

In addition to regulation of granulopoiesis, IL-17RA show reduced PMN emigration in to the lung after bacterial challenge with *K. pneumoniae*, which is associated with reduced levels of CXCL2 [14]. Indeed, IL-17 has been shown to regulate the production of a number of chemokine ligands of CXCR1/CXCR2 in fibroblasts and epithelial cells, along with emigration of neutrophils into mucosal sites [17]. IL-17A stimulation of human bronchial epithelial, venous endothelial cells, and human synoviocytes results in the production and release of CXCL8 (IL-8) [17]. IL-17A also induces CXCL6 in human bronchial epithelial cells [17] and CXCL5 in murine in osteoblasts *in vitro*. IL-17A and IL-17F induce both G-CSF and CXCL1 in human bronchial epithelial response and the response to both ligands is antagonized by an anti-IL-17R monoclonal antibody, suggesting that both IL-17A and IL-17F require IL-17R for a functional signaling receptor complex [18]. Induction of CXCL8 by IL-17A in human synoviocytes *in vitro* requires NF-κB and the PI-3 kinase/Akt pathway [19]. In addition, p38 mitogen activated protein (MAP) kinases and extracellular signal regulated kinase (ERK), are required for the production of CXC chemokines to IL-17A in HBE cells [20]. These CXC chemokine responses to both IL-17A and IL-17F show marked synergy with tumor necrosis factor (TNF)-α [17]. Recently it has been demonstrated that in mouse embryonic fibroblasts the induction of CXCL1 by IL-17A and IL-17F requires the downstream adaptor Act1 and the induction of CXCL1 is only partly dependent on NF-κB [21]. Another potential transcription factor responsible for IL-17-induced CXCL1 gene expression is CEBPδ, which has been shown to mediate some of the synergy of gene expression observed with IL-17A and TNF-α dual stimulation [22].

In addition to defective recruitment of neutrophils in response to gram-negative bacterial infection [14], the local administration of IL-17A into the lung or synovial space of experimental animals results in a significant accumulation of neutrophils into these specific tissue compartments *in vivo* [17]. This neutrophil emigration in the context of the lung is blocked by a neutralizing and specific anti-IL-17 antibody or pretreatment with glucocorticoids [17]. This effect is also observed with IL-17C, IL-17E, and IL-17F; however, IL-17E also results in significant emigration of eosino-

phils [17] consistent with its Th2 cytokine profile. In addition to eliciting the ligands for CXCR1 and CXCR2 and neutrophil emigration, local administration of IL-17A into the lungs of experimental animals results in an increase in neutrophil elastase and myeloperoxidase (MPO) activity in rats *in vivo* [17].

It has been recently show that IL-17A and IL-17F is produced by a specific subset of $CD4^+$ T cells, Th17 cells, in addition to γδ T cells and NKT cells [23]. This subset of T cells also produce IL-22 *in vitro* and in mice *in vivo* [24] and in humans both IL-22 and IL-26 [25]. IL-17R KO mice are not susceptible to *Listeria monocytogenes* or *M. tuberculosis* infection [26]. Moreover, Th1 immunity as assessed by IFN-γ-recall responses to *L. monocytogenes* is not impaired in IL-17R KO mice. Thus, it appears the lack of IL-17R signaling results in selective defects to extracellular bacteria [14, 26]. In support of this, mice deficient in IL-23, a key survival factor for Th17 cells, also show reduced survival and reduced IL-17 levels in response to *K. pneumoniae*. Reconstitution of local IL-17 levels in the lung in IL-23 KO mice restores bacterial killing as well as the local levels of G-CSF, CXCXL1 and CXCL5 [27]. In contrast, IL-23 plays a limited role in primary *M. tuberculosis* infection [28]. Further evidence supporting a role of IL-17 in host defense against extracellular pathogens is provided by data from Chung et al. [29] that demonstrates that intra-abdominal abscess formation due to the anaerobic bacterium *Bacteroides fragilis* requires IL-17A. IL-23 has also been shown to be critical for mucosal host defense in the gut in response to infection with *Citrobacter rodentium*, which is a naturally occurring pathogen in mice [30]. Interestingly, in this model, IL-17A was produced in both IL-23p19-deficient and wild-type mice, suggesting that the susceptibility of IL-23p19$^{-/-}$ mice is independent of IL-17A [30]. In addition to extracellular bacteria, Huang et al. [31] have shown that IL-17RA signaling is also required for optimal host defense against *Candia albicans*. Antibodies against IL-23p19 antagonize pulmonary IL-17 production, neutrophil emigration, and host defense against *Mycoplasma pulmonis* [32]. In addition to regulating CXCR1/2 ligands and G-CSF, IL-17 regulates anti-microbial peptide production of β defensins [33] and calgranulins in human bronchial epithelial cells and keratinocytes. Recently, it has been shown that IL-22, a product of Th17 cells, synergizes with IL-17 in inducing these anti-microbial proteins in human keratinocytes, which lends further support of a role of the T cell lineage in mucosal immunity (Fig. 1) [24]. Due to the fact that there is redundancy in the β defensin and calgranulins gene family, further experimentation is required to determine the individual contributions of these proteins in ThIL-17 (now termed Th17)-mediated mucosal immunity.

Cellular sources of IL-17 to infection *in vivo*

Although the factors that regulate the differentiation of naive αβ T cells into IL-17-producing T cells is becoming much clearer, with contributions of TGF-β, IL-6 and

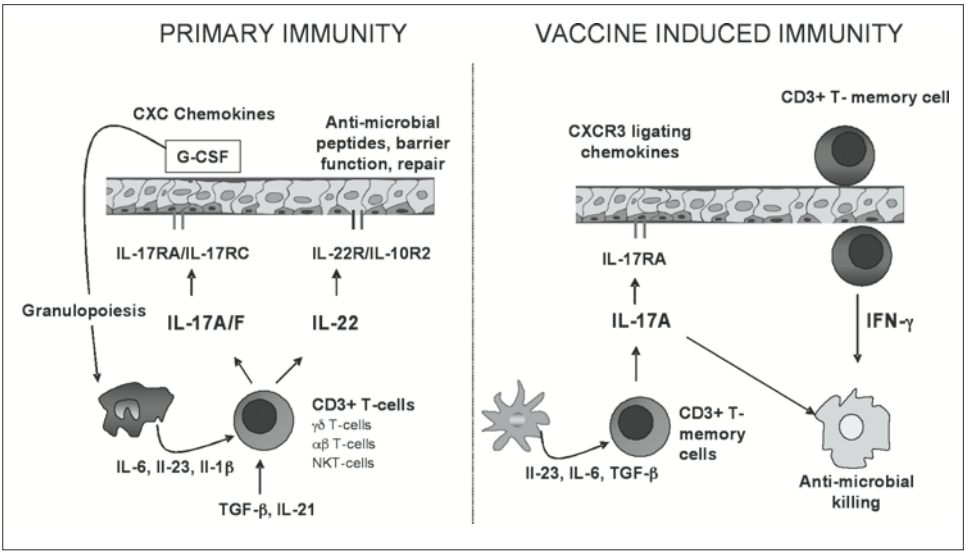

Figure 1
Model of IL-17-producing cells in primary and vaccine induced immunity. In primary immunity, IL-17 and Il-22 can be synthesized by several T cell subsets. A likely critical target of IL-17 and IL-22 is mucosal epithelium where IL-17 augments G-CSF and CXC chemokine production, resulting in recruitment of neutrophils. IL-22 along with IL-17 also augments anti-microbial peptides important for control of extracellular pathogens. In vaccine-induced immunity there is an expansion of memory T cells that can also up-regulate CXCR3 ligands through an IL-17-dependent mechanism, resulting in augmented recruitment of Th1 effectors leading to control of both extracellular and intracellular pathogens.

IL-23 [23], the regulation of IL-17 by T cells *in vivo* is more complex. In response to stimulation by lipopeptides from *Borrelia burgdorferi*, IL-17A localizes to a subset of T lymphocytes expressing TNF-α and GM-CSF [34]. Moreover, $CD8^+$ T cells have been shown to produce IL-17 in response to *K. pneumoniae in vitro* and depletion of $CD8^+$ cells partially abrogates lung IL-17 responses *in vivo* to the same infection [17]. Further evidence for a predominant role of T cells is the fact that SCID mice show over a 90% reduction in IL-17A concentration within the lung after endotoxin exposure [17]. Recently, Michel et al. [35] have demonstrated that a critical source of IL-17 in the airway is a novel NK1.1-negative, α-GalCer-positive invariant NKT cell population and this population is critical for airway neutrophilia in response to endotoxin (Fig. 1). Lockhart et al. [36] have shown that γδ T cells are the predominant source of IL-17 in *M. tuberculosis* infection in mice. In response to the extracellular pathogen *E. coli*, Shibata et al. [37] have shown that the Vγ1 subset of γδ T cells are critical for IL-17 production in an intraperitoneal model of infection.

Role of IL-17 in vaccine-induced mucosal immunity

Although there are convincing data showing a protective role for IL-17 in immunity to primary infections, the role of IL-17 in memory immune responses to infections is less well studied and understood. The fact that IL-23 acts on memory or activated T cells that express IL-23R and produce IL-17 [38] suggests that these cells may have a role to play in vaccine-induced immunity. Several recent studies using peptide or DNA vaccines encoding mycobacterial antigens show that vaccine-induced CD4 T cell responses result in induction of both IFN-γ as well as IL-17 production [39–41]. Following challenge with *M. tuberculosis*, protection is clearly associated with the recruitment of IFN-γ-producing cells to the lung, resulting in activation of macrophages and mycobacterial killing [42]. In the vaccine-induced protection model (Fig. 1), IL-17 memory cells have been reported to play a critical role in accelerating this recruitment of protective IFN-γ CD4 T cells to the lung by up-regulating CXCR3 ligating chemokines (CXCL9, CXCL10 and CXCL11) [39]. The generation of the IL-17 vaccine-induced response is dependent on IL-23, since the vaccine-induced protection is lost in IL-23 KO mice [39]. Further data from peripheral blood of mycobacteria-exposed healthy adults show that about 20% of cytokine-producing CD4 T cells express IL-17 or the related cytokine IL-22 and display central memory phenotype [43]. In patients with tuberculosis, the frequency of IL-17- and IL-22-producing CD4 T cells were reduced, suggesting that the cytokine-producing cells may have migrated to the lung, i.e., to the site of active infection [43]. The only available vaccine against tuberculosis, *M. bovis* BCG is not very effective at protecting against pulmonary tuberculosis [44] and has prompted the search for more effective vaccines against tuberculosis. This information on the generation and function of protective cytokine-producing CD4 T cell memory responses will play a critical role in design of new vaccines for tuberculosis.

Vaccine-induced protection against *Bordetella pertussis* has also been shown to be mediated by IL-17 [45]. IL-17-producing cells are induced by vaccination with whole cell pertussis vaccines, and IL-17 neutralization reduces protection following a pulmonary challenge with *B. pertussis*. However, in this model, IL-17 was suggested to have a protective role *via* direct activation of macrophages and *B. pertussis* killing [45]. IL-17A was shown to be critical in a CD4 T cell-dependent, antibody-independent model of vaccine-induced protection following *S. pneumoniae* challenge [46]. Although the specific mechanism was not defined, treatment with antiserum to IL-17A resulted in reduced immunity to pneumococcal colonization compared to the control serum-treated mice. These data suggest that there is an important role for IL-17 in vaccine-induced responses against bacterial infections.

IL-17 has also recently been associated with protection against murine rotavirus in a vaccine-induced model. Mucosal immunization of mice with rotavirus V6 protein reduces rotavirus fecal shedding and was associated with the presence of

memory cells producing IFN-γ and IL-17 in the intestine [47–49]. In this model, it is thought that CD4 T cell-derived IFN-γ is the major protective mechanism [50]. However, the IL-17 CD4 T memory response may have a more indirect role to play in protection. These data clearly suggest a protective role for IL-17 in vaccine-induced immune responses in bacterial and viral infections.

Conclusions

Evidence clearly supports the notion that Th17 cells are a distinct lineage that may have evolved to mediate mucosal host defense against extracellular pathogens. Moreover, there is emerging evidence that these cells are critical in mediating vaccine-induced protection to a variety of pathogens (Fig. 1). Part of this effect is likely mediated by regulation of chemokines. In the setting of IL-17 neutralization, there may be a slightly increased risk of infection as well as a compromised response to some vaccines. However, the risk-benefit of IL-17 neutralization has to be considered against existing immunosuppression, which may carry a similar if not greater risk due to the relative lack of specificity of many of these immunosuppressive drugs, such as steroids and calcineurin inhibitors.

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IL-23 orchestrates the switch from tumor immune surveillance to tumor-promoting inflammation

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Abstract

Human tumor cells acquire and accumulate mutations and transcriptional changes that provide growth and survival signals and a tumor-promoting microenvironment. Over the last few decades it has become clear that the mammalian immune system is able to recognize these genetic and epigenetic changes, and that T cells specific to oncogenes and oncofetal antigens are present in human cancer patients and their tumors. Immune-mediated inflammation, however, increases tumor incidence and progression. Epidemiologically, inflammatory disease-inducing cytokines have also been linked to tumor progression. However, the nature of the pro-inflammatory T cells that control the chronic inflammatory response, and their regulation by cytokines like IL-23, only became known recently. This review attempts to summarize our knowledge of pro-inflammatory T cells in cancer, and the cytokines that contribute to the deregulation of tumor-promoting inflammation and its inhibitory consequences on the tumor cell elimination by cytotoxic T cells.

Introduction

The involvement of the immune response in the development of human cancer has two opposing faces.

Many of the mutational changes occurring in a cancer cell activate the antigen-specific recognition machinery of the adaptive immune system and lead to the suppression of tumor incidence and tumor progression. The evidence supporting this immune-surveillance theory comes mainly from tumor models in immunodeficient and genetically modified mice [1]. The increased tumor incidence in immune-suppressed individuals such as transplant recipients or acquired immune deficiencies lends additional support to this concept in humans [2]. The mechanisms essential for immune surveillance involves cytotoxic $CD8^+$ T cells, which infiltrate the tumor and eliminate tumor cells.

Surprisingly, however, immune-mediated inflammatory processes also fuel tumor incidence, tumor growth and tumor progression. Patients with chronic infections

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such as *Helicobacter pylori* infections of the stomach, develop gastric cancer with a significantly increased frequency. Mouse models with deficiencies in inflammatory responses show an increased resistance against chemically or transgenically induced tumors in mice. In particular, cytokines involved in the development and activity of inflammatory T cells appear to be essential for tumor development in mice. Clinical and experimental findings demonstrated the up-regulation of pro-inflammatory molecules in tumor progression, particularly during latest stages and during tumor cachexia [3]. Several of the cytokines linked to tumor-promoting inflammation such as TNF-α, TGF-β, IL-6 and IL-23 are functionally linked to the newly discovered Th17 $CD4^+$ helper cell lineage.

Inflammatory T cells named "Th17" after their signature cytokine IL-17 stimulate an effector cell population quite different from the cells involved in immune surveillance. Monocytes, macrophages granulocytes and endothelial cells are amongst the primary effectors induced by Th17 T cells. Both those innate inflammatory effector cells and the cytokines inducing Th17 induction are clinically associated with faster tumor progression and an unfavorable prognosis.

Dependent upon its regulatory elements, the immune system appears to either prevent or promote tumor development and growth. The decision between these opposite fates might come after or despite the fact that the antigenic properties of the tumor have been recognized. The selection pressure on the growing tumor will ultimately eliminate immune deviation toward an inflammatory but non-cytotoxic cytokine milieu. This review attempts to summarize our knowledge about the cytokine-mediated imbalance of immune mediators in cancer.

The ups and downs of the tumor immune interface

The initial observations that antigen-specific CD8 T cells isolated from human cancer patients recognize human tumors were made more than 20 years ago. The cloning of the first definitive tumor antigens followed a few years later [4, 5]. Since then, it has become clear that the human immune system recognizes the antigenic profile of tumors relatively well [6, 7]. Many of the described tumor antigens are, however, not new antigens to the immune system. Cancer testis antigens for example are normally expressed in the gonads but not sufficiently presented to the immune system to elicit immune tolerance. Only the inappropriate or high expression in the dedifferentiating tumor cell or possibly even the immune stimulatory context of the tumor leads to the expansion of a tumor-specific T cell response. The recent sequence analysis of the genomes of human tumors revealed now a substantial number of mutations accumulated in a typical human tumor cell [8]. Predictions of antigenic peptides from the tumor genome analyses suggest the presentation of a sufficiently high number of truly novel tumor antigens to enable immunological discrimination between the immunological self and the tumor [9].

The infiltration of cytotoxic T cells into tumor tissue is limited, but T cell presence into human tumors correlates with an improved prognosis for the patient [10, 11]. To solve this dilemma, therapies have been attempted to expand the repertoire of tumor-specific T cells in patients, either by antigen-specific vaccines or adoptive transfer of *in vitro*-expanded cytotoxic T cells. However, even significant expansions of the numbers of circulating tumor-specific T cells do not often translate into long-term therapeutic benefits [12], even when the antigens recognized by the expanded and activated T cells are still expressed in the tumor [13]. It is important to note that the induced or transferred T cells are sufficiently specific and sensitive to eliminate malignant cells *in vitro* but frequently fail to do so in the patient. In experimental mouse models, appropriately stimulation or resurrection of nascent immune surveillance appears capable of eliminating not only the tumor cells but also tumor-promoting stroma [14, 15]. The latter successes are mostly observed in small and early tumors or tumors of mesenchymal origin, while well established and clinically relevant stages of epithelial cell-derived malignancies of mouse tumors escape the same immune therapy. It is those late-stage carcinomas that typically pose the therapeutic challenges in the clinic.

The success of tumor immune surveillance and tumor-directed immune therapy depends not only on the presence of an appropriately activated, tumor-specific T cell but also on the expression of major histocompatibility complex type I (MHCI) on the tumor cell. Expression of MHCI molecules is, however, frequently lost in human tumors and tumor-derived cell lines [16]. A variety of tumor cell lines have lost one copy of the genomic region containing the MHCI molecules; the expression of MHCI protein in particular on the surface expression of MHCI of the cell is often barely detectable. Induction by IFN-γ can, however, restore self presentation in a variety of tumor cells, indicating that the absence of IFN-γ and an epigenetic silencing mechanism might frequently suppress MHCI expression.

IL-23 and IL-12: Inflammation or cytotoxicity

Recent findings in mice deficient for the cytokines IL-12 or IL-23 indicated that the balance between those two heterodimeric, immune stimulatory cytokines control polarization of the immune response to transformed tissues.

IL-23 and IL-12 share not only a common subunit but they have the same cellular sources and the same cellular targets. Both are composed of the shared subunit p40, and a defining subunit, IL-23p19 or IL-12p35 [17]. IL-12 uses the heterodimeric receptor IL-12Rb1 and IL-12Rb2, while IL-23 activates IL-12Rb-1/IL-23R dimers [18]. IL-12 and IL-23 utilize similar intracellular signaling pathways, the signal transducers and activators of transcription, STAT1, 3, 4 and 5 [18, 19]. Due to the high expression of IL-12 and IL-23 receptor mRNA expression in T cells, NK cells, and NKT cells, lymphocytes are considered to be the primary target cells for those

cytokines. Lower receptor mRNA levels are also present in myeloid cells and other cells, possibly indicating a direct signaling pathway in innate immune cells. Both cytokines are produced primarily by activated antigen-presenting cells in response to the activation of pattern recognition receptors such as Toll-like receptors [20]. Underscoring the physiological relevance of the cytokine family, IL-12p40-deficient mice, lacking IL-12 and IL-23, are highly susceptible to numerous bacterial, fungal and parasite infections including *Salmonella*, *Citrobacter*, *Cryptococcus* and *Leishmania* species [21]. During the response against most of those pathogens, IL-12-mediated processes are essential, while the IL-23 contribution is often only detected in the simultaneous absence of IL-12 [17]. Intranasal infection with *Citrobacter* or *Klebsiella*, however, required IL-23-mediated host responses in mice [22, 23].

IL-12p40- or IL12Rb1-deficient humans suffer exclusively from mycobacterial and salmonella infection but show normal resistance to most other pathogens, including viruses [24]. This difference could be due species differences or, more likely, due to the relatively large inoculum size and the per-acute disease course chosen in most mouse models of infectious diseases. A natural infection initiated by the ingestion of a small number of infectious virulent particles might test different qualities of the immune system than those necessary to survive an overwhelming flood of pathogens.

The inflammatory Th17 T cell lineage

Despite their similarities, IL-12 and IL-23 drive divergent immunological pathways (reviewed in [25]). IL-12 primes and maintains the development of the IFN-γ-producing Th1 cells and enhances antimicrobial and cytotoxic responses. IL-23 is essential in the pro-inflammatory function of a memory T cell subset characterized by the production of the cytokine IL-17, named therefore Th17 [26]. Th17 cells develop from naïve T cells under the influence of TGF-β and IL-6 [23, 27]. Interestingly, both cytokines are also known to be present at high levels in the tumor environment and are typically associated with an unfavorable prognosis for the patient. IL-23R is up-regulated upon antigen stimulation. Although the role of IL-23 in the generation of Th17 cells *in vitro* and *in vivo* is still being investigated, IL-23 appears to be crucial for the function, survival and propagation of this important T cell population in the inflamed environment. One important role for IL-23 was recently discovered in the IL-23-mediated suppression of the TGF-β induction of the anti-inflammatory cytokine IL-10 [28]. TGF-β stimulation alone increases the up-regulation of the foxp3 transcription factor, allowing differentiation into regulatory T cells, simultaneous stimulation with IL-6 diverts the T cell toward the pro-inflammatory Th17 cell [29]. IL-17 engages its receptor – commonly found on stromal, epithelial, endothelial cells and monocytes – resulting in the release of additional inflammatory factors (such as IL-1, IL-6, IL-8, TNF-α, prostaglandin

E2, ICAM and several chemokines) to further the inflammatory cascade [30, 31]. Indeed, the IL-23/IL-17 inflammatory pathway, rather than the IL-12/IFN-γ pathway, has been highlighted by various recent reports as being central to inflammatory conditions exhibited in psoriasis, ischemic injury, inflammatory bowel disease [32], and autoimmune inflammation of the joint and brain [25]. In mouse models of inflammatory diseases Th17 cells have been identified as the major pathogenic population [26]. While most of the attention has been concentrated on IL-17 production in CD4 T cells, there is increasing evidence that NK, γδ T cells, and CD8 T cells can express IL-17 and are even, in some instances, the dominant source of this pro-inflammatory cytokine [33, 34].

The inflammatory control at the tumor site

IL-12 and IL-23 also appear to control the bifurcation of tumor-infiltrating immune cells into tumor immune surveillance or tumor-associated inflammation, respectively. In the local tumor microenvironment, IL-23 not only induces the hallmarks of chronic inflammation such as metalloproteases, angiogenesis and macrophage infiltration, it also reduces anti-tumor immunosurveillance by locally suppressing the presence of CD8 T cells. The absence of IL-12 leads to an exacerbation of the myeloid-driven inflammation with a coincident lack of CD8 T cells [35]. Increased CD8 T cell infiltration and enhanced tumor immune surveillance was observed in the IL-23-deficient animals or upon injection of IL-12.

IL-12 treatment in preclinical tumor models promotes immune surveillance against transplanted syngeneic tumors. IL-12 promotes the activity of IFN-γ-producing Th1 cells and the proliferation and cytotoxic activity of $CD8^+$ T cells and NK cells. IFN-γ is not only rate limiting for T cell activity but also induces the expression of MHCI in tumor cells and thereby enables the recognition of tumor antigens [36]. In many human tumors, MHCI is expressed only at very low levels, unless induced by IFN-γ [16]. Tumor immune surveillance in mouse models is also largely dependent on IFN-γ-expressing T cells [37]. In the absence of IL-12, or in a cytokine milieu preventing Th1 differentiation and IFN-γ expression, MHCI expression might be limited and the recognition of the tumor-specific epitopes by $CD8^+$ T cells might be relatively less efficient.

Therapeutic options

Following on the heels of the successes with anti-tumor therapies using IL-12 in mice, cancer patients have been treated with recombinant IL-12 in several clinical studies [38]. Unfortunately, systemic toxicities became apparent before clinical responses against the tumor had been achieved. The toxicities appeared to be

associated with a systemic induction of IFN-γ and were most likely manifestations of a systemic autoaggressive immune response. Subsequent attempts to focus the response to the antigenic profile of the tumor by combining IL-12 therapy with melanoma-specific peptide vaccines have so far not revealed enhanced clinical benefits in the IL-12 treatment arms [39]. The reason for the disappointing difference in treatment outcome might again lie in the differences between the model and the original. By the time treatment is given, most human cancers have been exposed to the patient's immune system for several years. Not only are the human T cells specific to the tumor antigens typically described as tolerant, anergic or exhausted, the expression pattern of the tumor might have been shaped to enable immune evasion of the tumor. While systemic delivery of IL-12 might stimulate the *de novo* formation of a strong Th1 response, chronic immune tolerance might respond better to the removal of an endogenous inhibitor to Th1 differentiation. One of these inhibitors, IL-23, is significantly overexpressed in the large majority of human tumor types [35].

Experimental contradictions

Experiments in mice using tumor cells expressing IL-23 or IL-12 were similarly efficient in inducing rejections of the syngeneic transplanted tumors [40]. The adoptive transfer of Th17 polarized, tumor-specific $CD4^+$ T cells led, in conjunction with irradiation, to the elimination of surprisingly large B16 melanoma tumors in mice [41]. When compared to Th1 cells, the transferred Th17 polarized cells had a survival benefit in the tumor, the lymph node and the spleen of the tumor-bearing animal. Ultimately, however, the therapeutic efficacy was dependent upon the IFN-γ sensitivity of the host, and not the presence of IL-23 or IL-17, possibly indicating *in vivo* differentiation of the transferred population into IFN-γ-producing Th1 cells.

At first sight, these data contradict the interpretation drawn from autochthonous tumor models in IL-23-deficient mice and syngeneic transplantations into IL-23 receptor knockouts. There, loss of either IL-23 ligand or receptor strengthened the immune surveillance, increased CD8 activity and decreased tumor-promoting inflammation [35].

Transplantation of a large bolus of antigenic tumor cells challenges primarily the expansion rate of tumor-reactive T cells, while the control of effector function appears to be of greater importance in the equilibrium of a stably established tumor mass. Immune recognition of human tumors might, however, follow quite different kinetics with only limited antigen exposure at first but the presence of an antigenic challenge for years. The failure of tumor immunity in humans is therefore more likely the consequence of a modulation of long-term immunological memory and might even affect *bona fide* immune tolerance. A summary of the IL-23-driven switch from immune surveillance to tumor-promoting inflammation is given in Figure 1.

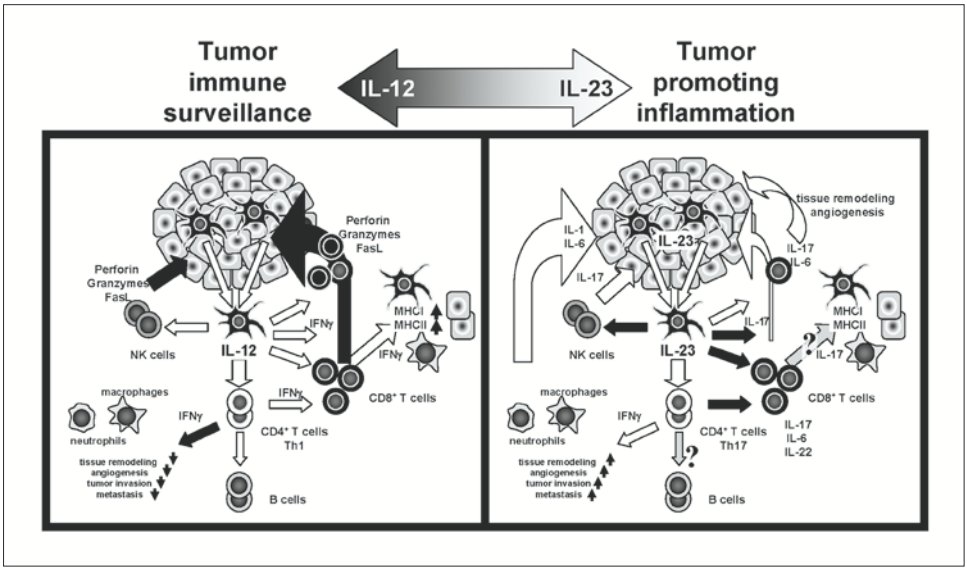

Figure 1
The IL-23-driven switch from immune surveillance to tumor-promoting inflammation. Tumor-derived antigens, induced by mutations of proteins, defective ribosomal products or inappropriate overexpression endogenous proteins stimulate antigen-specific immune responses in the inflammatory milieu of the tumor. The composition of the cytokine milieu, however, regulates which effector molecules and cells are deemed appropriate to eliminate the challenge, like a nascent or metastasizing tumor. IL-12 induced IFN-γ induces a number of key processes that contribute to immune surveillance and the inhibition of tumor-promoting inflammation. The switch IL-12 to IL-23 and from IFN-γ to IL-17 activates an immune defense, largely inefficient in eliminating tumor cells. In contrast, IL-23-driven myeloid inflammation leads to immune-mediated tumor promotion, with strong similarities to chronic auto-inflammatory diseases (white arrows: stimulatory interaction; black arrows: inhibitory interaction).

The detrimental effectors of the Th17 T cell lineage

In mouse models of autoimmune diseases, IL-23 induces chronic inflammation in part through the stimulation of innate myeloid effector cells and stromal activation and many aspects of IL-23-dependent tissue inflammation can be recapitulated in the absence of T cells [42]. However, IL-23 also controls the activity of Th17 T cells. While Th17 develop from naïve T cells under the influence of TGF-β and IL-6, they subsequently require IL-23 to suppress endogenous IL-10 and become proficient in their pro-inflammatory function [28]. This function of the adap-

tive immune system orchestrates inflammatory tissue destruction often observed not only in chronic inflammatory lesions but in particular in cancer tissues and systemically in the late stages of cancer. IL-17, induced by IL-23 in tumors, is an attractive candidate for causing tumor-promoting inflammation, because IL-17 promotes angiogenesis in a variety of models and induces matrix metalloproteinases (MMPs), two events that potentiates tumor growth [43]. In addition, IL-17 controls neutrophil chemotaxis, proliferation and maturation, further fueling the innate immune activation [30]. Moreover, IL-17-producing CD8 and $CD4^+$ T cells have recently been reported to be widely present in human and mouse tumor microenvironments [44]. It has also been suggested that CD8 T cells expressing IL-17 largely lack cytotoxic capacity [45]. In the mouse epidermis, IL-23 can induce, independent of IL-17, angiogenic erythema, inflammation and keratinocyte hyperproliferation, phenocopying aspects of human psoriatic lesions [46]. The IL-23-induced effects on the proliferation of keratinocytes and mesenchymal cells are not strictly dependent upon IL-17 or IL-22, since both IL-17 and IL-22 deficient mice show only a reduced susceptibility to the pro-inflammatory effects of IL-23 [47]. Redundant effects of either IL-17A and IL-17C or of cytokines activating the IL-20 receptor type 2 [46] possibly rescue the single cytokine deficiency. IL-17 and IL-22 family members directly stimulate endothelial, mesenchymal and epithelial cells. Both cytokines coordinately induce an increase in the expression of antimicrobial proteins in the skin keratinocytes [48]. IL-17 and IL-22 are also responsible for the up-regulation of chemokines attracting macrophages, endothelial progenitors and mesenchymal stroma [30]. Most tumor cells express, similar to their normal progenitors, receptors for IL-17 and IL-22 (unpublished). It is not yet known whether the indirect function on the tumor stroma or a direct function on the tumor cells is rate limiting.

Conclusions

For decades chronic inflammatory conditions have been observed in association with tumor incidence, tumor progression and detrimental prognosis in human cancer patients. The understanding of these chronic inflammatory processes in immune-mediated inflammatory diseases has seen major progress in recent years. The discovery of a pro-inflammatory T cell lineage, Th17, and the regulation of inflammation by the IL-23–IL-17 axis of cytokines have led to dramatically new concepts for the treatment of inflammatory diseases. Similar pathways and similar effector cells are also suggested to fuel the inflammation promoting cancer angiogenesis, invasion and metastasis. The finding that the regulators of cytotoxicity and immune-mediated tumor cell elimination just represent the flipside of those inflammatory pathways, opens avenues of therapeutic interventions.

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IL-17 in clinical autoimmune disease

IL-17 and Th17 cells in rheumatoid arthritis

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Abstract

IL-17 was identified in 1995/96 as a T cell-derived cytokine with effects on inflammation and neutrophil activation. Rheumatoid arthritis has emerged as the most studied situation to justify the selection of IL-17 as a therapeutic target. By interacting with other proinflammatory cytokines, IL-17 was found to induce bone and cartilage destruction. In 2006, the precise cell source of IL-17 was identified in the mouse. These cells were named Th17 and their key role was demonstrated in various situations associated with inflammation and matrix destruction. These new findings confirmed and extended the results previously obtained following the identification of IL-17 as a T cell-derived cytokine. At the same time, additional information was obtained on the other members of the IL-17 family and on the structure of the IL-17 receptor complex. Such knowledge has further extended the choice of possible modalities to control IL-17.

Introduction

The focus on IL-17 increased sharply when, in 2006, the precise cell source of IL-17 was identified in the mouse. These cells were named Th17 cells and were associated with a key role in various situations associated with inflammation and matrix destruction. These new findings confirmed and extended the results previously obtained following the identification of IL-17 as a T cell-derived cytokine 10 years earlier.

Demonstration of the role of IL-17 in many inflammatory conditions further supported the concept of IL-17 targeting for treatment. We review these new findings in light of the previous knowledge [1]. We focus on rheumatoid arthritis (RA), which has emerged as the most studied situation to justify the selection of IL-17 as a therapeutic target. We will not discuss the situation in mouse models of arthritis, which is discussed in another chapter.

Th 17 Cells: Role in Inflammation and Autoimmune Disease,
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First links between IL-17 and RA

IL-17 was described in 1995/96 as a proinflammatory cytokine produced by T cells. The link with RA was established from the very first reports [2]. The key experiment was the demonstration that addition of IL-17 to synoviocytes from RA patients was able to increase the production of two key cytokines associated with inflammation: IL-6 and IL-8. IL-6 acts on the liver during the systemic phase of inflammation, to release acute-phase proteins such as C-reactive protein. IL-8 is a chemokine inducing the migration of polymorphonuclear neutrophils (PMN). To further support the role of IL-17 in PMN biology, these early reports showed that IL-17 induces the release from synoviocytes and fibroblasts of hematopoietic growth factors associated with PMN differentiation and function [2], an early indication of its role in host defense.

The first results on the production of IL-17 in arthritis were obtained when an ELISA was made available. Levels of IL-17 were found to be higher in RA synovial fluids when compared to osteoarthritis (OA) fluids [3]. It was critical to demonstrate that the measured IL-17 in synovial fluid was functional and produced by the synovium itself. The first step was to demonstrate the production of functional IL-17 by RA synovium [4]. Quantification of IL-17 was performed using a specific biological assay looking at the production of IL-6 by synoviocytes stimulated with RA synovium supernatants first preincubated with a blocking anti-IL-17 antibody. Functional IL-17 was spontaneously produced by many RA samples at high levels, a few OA at very low levels and none of normal synovium explant cultures. At the same time, IL-17 mRNA expression was demonstrated in RA but not in OA synovium samples.

New IL-17 family members and structure of the IL-17 receptor

The published results mainly refer in fact to IL-17A, the founding member of the IL-17 family, which includes IL-17A–F. IL-17F has a 50% sequence homology with IL-17A. When used alone on synoviocytes, IL-17 F has similar effects as IL-17A but to a lower extend [5]. Sometimes even IL-17F has minimal or even no effect when used alone. However, when combined with TNF-α, a synergistic effect is observed, almost as potent as with IL-17A.

The structure of the IL-17 receptor (IL-17R) has been recently clarified. The first described IL-17R had a rather low affinity, suggesting the presence of additional chains [6]. At least two members are now taken into account [7]. The first receptor is the original IL-R renamed IL-17RA. The second is IL-17RC. The physical association of the two receptors has been shown, although it is still unclear if these are two chains of a single receptor or two different receptors. On RA synoviocytes, it appears that the two receptors can bind either IL-17A or IL-17F, possibly with

different affinities. These findings result from studies using small interference RNA, and show the inhibition of the cell surface expression of one or the two receptors. Inhibition of the expression of one of the two receptors is sufficient to inhibit the response to IL-17 when used alone in synoviocytes [5]. However, the inhibition of the two receptors is needed to reduce the response to the combination of IL-17 with TNF in synoviocytes. Similar results were obtained with inhibitory antibodies against the two receptors.

In contrast with the limitation of IL-17 production to T cells, its receptor complex is widely expressed by many tissue cell types. Following receptor interaction, IL-17 shares transcriptional pathways with IL-1 and TNF-α. In particular, the MAP kinase p38 and NF-κB are the key transcriptional factors for IL-17 function [8]. These pathways have been identified in synoviocytes, chondrocytes, and osteoblasts. In RA blood, mRNA levels of IL-17RA and RC are increased [5].

Action of IL-17 on RA joint targets

Following the discovery of IL-17, a number of human diseases have been associated with IL-17 overexpression and production. Although the list of diseases will certainly increase, RA emerged as the most studied situation, making IL-17 a therapeutic target. In addition, expression of IL-17 by synovium biopsies is associated with increase disease severity [9]. Although we focus here on RA, most of these results can probably be transferred to the long list of conditions where chronic inflammation is associated with matrix destruction. Here we consider the different cell types present in the RA joint and we summarize the key properties of IL-17 on these targets.

On synoviocytes, IL-1β, TNF-α and IL-17 induce cytokine and chemokine production, IL-1 being much more potent than IL-17 or TNF-α [10]. Using low concentrations of IL-17 and IL-1β in combination, a synergistic effect is observed. As an example, such synergy was observed between IL-1, TNF-α and IL-17 for CCL20 production, a chemokine involved in the migration of immature dendritic cells and of memory T cells, specifically the IL-17-producing T cells [11, 12]. These findings indicate that low levels of cytokines produced by monocytes (IL-1 and TNF-α) and T cells (IL-17) can act together on synoviocytes.

The sequential analysis indicates that synergy is observed only when synoviocytes are first exposed to IL-17 and then to TNF. Synergy is not observed when cells are exposed first to TNF then to IL-17. This is in line with the role of these Th17 cells in the amplification of the initial response associated with TNF and IL-1 secretion. This synergistic effect is related to an increased mRNA stabilization with IL-17 [13].

Although synergistic interactions between IL-17 and TNF are common, inhibitory effects are sometimes observed. Regarding the production of chemokines, there is a synergistic interaction for the production of CXC chemokines, the structure of

which includes the ELR (Glu-Leu-Arg) motif. A specific up-regulation of ELR^+ CXC chemokines such as IL-8 is observed in IL-17A-treated synoviocytes [5].

On human chondrocytes, IL-17 induces prostaglandin E2 and nitric oxide production by cartilage explants in an IL-1-independent way [14, 15]. IL-17 increases collagenase production leading to matrix destruction and defective synthesis. On osteoblasts, IL-17 induces IL-6 and enzyme production leading to the inhibition of bone formation. During interactions between osteoblasts and osteoclast precursors, the presence of IL-17 induces osteoclastogenesis [3]. IL-17 and other cytokines stimulating osteoclastogenesis, such as IL-1β and TNF-α, increase the expression of RANKL with a decrease of osteoprotegerin expression in osteoblasts/stromal cells [16]. IL-17-producing T cells express the membrane form and secrete the soluble form of RANKL [17]. These functions make IL-17 a new cytokine involved in bone resorption [18]. In the context of RA, T cells in juxta-articular bone are the source of IL-17, which then acts locally leading to destruction [19]. In these conditions, addition of IL-1 increases the effect of IL-17. Blocking of bone-derived endogenous IL-17 with specific inhibitors results in a protective inhibition of bone destruction in RA juxta-articular bone. In conclusion, the demonstration of the contribution of IL-17 derived from synovium and bone marrow T cells suggests the control of IL-17 for the treatment of RA by blocking its effect on bone destruction.

These studies showed an increased production of functional IL-17 by RA synovium but also by bone explants, indicating the role of T cells in juxta-articular bone destruction [4, 20]. As expected this effect was associated with RANK ligand expression by these T cells, interacting with RANK-expressing cells, certainly osteoclasts but also mature dendritic cells [20, 21].

Th17 cells, the cellular source of IL-17

The most critical and recent step has been the identification of the cell source of IL-17 in 2006 [22]. IFN-γ is characteristic of Th1 cells and IL-4 of Th2 cells. The source of IL-17 was found to be different and these cells were named ThIL-17 or Th17 for short. The new finding was the inhibition of IL-17 by IFN-γ. The next step was the discovery of IL-23, another monocyte product shown to be a key cytokine for the Th17 pathway [23].

Cytokine receptors have been as markers of T cell subsets, using the IL-12-specific IL-12R β2 chain, as a marker of Th1 cells, and the IL-23-specific IL-23R chain, as a marker of Th17 cells. The IL-12R β1 chain is common to both the IL-12 and IL-23 receptors. The inhibitory effect of IL-17 on the Th1 pathway results from the IL-17-induced inhibition of IL-12R β2 expression making cells not responsive to IL-12 effects on IFN-γ production.

In synovium, IL-17-producing cells represent a minor subset of lymphocytes found in lymphocytic infiltrates around vessels [24]. There, these T cells are in

contact with synoviocytes, monocytes, and dendritic cells. In bone, the same interactions also occur, although chondrocytes, protected by the extracellular matrix of cartilage, are rather the target of soluble factors. In the presence of anti-IL-17-blocking antibody, MMP-1 production and collagenase activity by RA synovium is reduced and associated with a reduction of type I collagen fragments released in supernatants, demonstrating the direct contribution of IL-17 to destruction [25]. From these results, IL-17 and its producing T cells appear to contribute to the RA inflammatory destructive process.

Cell interactions as in the RA synovium can be reproduced *in vitro* by incubating fixed T cell lines and clones derived from RA synovium with cultured synoviocytes [26]. Under these conditions following contact between fixed T cells with RA synoviocytes, IL-17-producing cells are the most potent inducers of IL-6 production by synoviocytes [27]. Moreover, they inhibit synoviocyte collagen synthesis. Opposite results are obtained with Th2 clones. These results indicate that IL-17-producing Th1 cells contribute to the defective repair activity in joint inflammation.

In these RA clones, IL-17 is often produced in association with IFN-γ but never with IL-4 [26]. These double-positive cells are also isolated from normal blood [28]. However, *in situ* immunostaining of the RA synovium shows two isolated populations of T cells producing either IFN-γ or IL-17. Double-positive cells are rarely seen. It thus remains to be demonstrated whether these secreting patterns are still undergoing maturation or represent the end stage of cell differentiation. In this context, these cytokine secreting T cells have a particular morphology with a plasma cell appearance, a pattern that can be induced *in vitro* and is associated with the loss of TCR and CD3 but not of CD4 [24]. As for B cells, the plasma cell morphology of the IFN-γ- and IL-17-producing cells strongly suggests that this is a fixed pattern related to a final stage of differentiation.

Th17/regulatory T cell balance

A key issue is the interaction between Th17 and regulatory T cells [29]. Regulatory T cells are in charge of the control of the immune response. At baseline in the absence of any particular stress, regulatory T cells are active and limit the intensity of the baseline response. In the context of stress such as seen during infection or inflammation, this control is turned off to let the defense mechanisms be activated.

Chronic inflammation activates the Th17 pathway. At the same time the regulatory T cell pathway is inhibited through the production of proinflammatory cytokines such IL-6 and IL-1. In chronic inflammatory disease such as RA, this situation is chronically maintained, with induction of disease-associated mechanisms and inhibition of protective mechanisms. Although regulatory T cells can be seen at the site of inflammation, their function is defective contributing to chronicity [10]. Accordingly, induction of functional regulatory T cells has been observed in patients

responding to TNF inhibition. Such mechanism may well be critical to explain the mode of action of TNF inhibition.

IL-17 targeting for treatment

The demonstration of the possible role of Th17 cytokines in RA could justify the control of this pathway with direct and indirect methods. The most direct way is the use of IL-17-specific inhibitors. Several molecular inhibitors of IL-17 are already available such as monoclonal antibodies against IL-17 and soluble IL-17 receptors. Preclinical studies have demonstrated their efficacy in mouse and human models. As a rule, the combination of IL-17 and TNF-α inhibitors is more efficacious in these models [30]. Clinical trials with some of these inhibitors are planned or just starting. More recent issues are the need to target other IL-17 family members, such as IL-17F, or the two IL-17 receptor chains.

An indirect way to control the Th17 pathway is the inhibition of IL-23. Treatment with a monoclonal antibody against the p40 subunit, which is common to IL-12 and IL-23, has already shown clear efficacy in psoriasis and other diseases [31]. Specific inhibitors of IL-23 have not been tested in these conditions and it is unclear how their risk-benefit ratio will compare with that of p40 inhibitors. This should clarify the specificity of each of these cytokines.

Some cytokines have anti-IL-17 properties and as such could be used as therapeutic tools. IL-4 inhibits the production and functions of IL-17 on RA targets [10, 11]. IL-25 also named IL-17E, a member of the IL-17 family, down-regulates Th17 function in autoimmune inflammation [32]. As opposed to IL-17A or F, it inhibits the production of IL-1 and IL-23, thus having an anti-inflammatory effect. In addition, by inhibiting IL-23, it reduces the induction of Th17 cells.

New modalities of treatment have to be developed that will include the effects of Th17 cells in the induction of other proinflammatory cytokines and also their effects on the function of regulatory T cells [29]. Since IL-17 can induce tissue cells to produce TNF-α, IL-6 and IL-1, these cytokines may not only promote inflammation but also inhibit regulatory T functions at the tissue sites. Currently, disease recurs when treatment with inhibitors of TNF-α is stopped. Combination with IL-17 inhibitors could be a way to better control not only the inflammation itself, but also the regulatory T cell functions. This effect is already induced in part by TNF-α inhibitors [33]. In active RA, regulatory T cells preferentially accumulate in the affected joints but are not able to regulate tissue inflammation. TNF-α inhibitors may control disease not only by inhibiting effector TNF-α function and reducing inflammation, but also by correcting at least in part, the defect in regulatory T cell functions [33]. Similar concepts may apply to IL-6 or IL-1 inhibitors.

This suggests that interfering with the Th17 pathway might produce novel targets for immune-mediated diseases. At the same time, the safety issues related to the

critical role of IL-23 and IL-17 in host defense have to be considered. This includes not only infections with extracellular bacteria but also fungal and opportunistic infections through the interactions between the Th1 and Th17 pathways.

The position of IL-17 inhibition in the treatment of RA remains to be defined. Coming back to the synergistic interactions, an enhanced inhibitory activity is observed with the combination of TNF and IL-17 inhibitors using *ex vivo* samples of RA synovium and bone [30]. Accordingly, primary or secondary lack of response to TNF inhibitors may represent a possible indication. Anti-TNF non-responders may have an IL-17-driven disease or secondary loss of response to TNF inhibition may result from the induction or use of other pathways, possibly involving IL-17, and taking over the initial predominant TNF contribution. It is possible that the combined inhibition of TNF and IL-17 may have the advantage of targeting two different cell types, monocytes and T cells. This would also control the enhancing effects of IL-17 on TNF production by monocytes.

Conclusion

The story of IL-17 has started 10 years ago and this is the time it took to become a cytokine in fashion [34]. The identification of the Th17 subset indicates that some T cells are involved in and amplify the link between chronic inflammation and extracellular matrix destruction. Similar concepts apply to other complex diseases, with inflammation-induced destruction. Tools are now getting ready to verify if these concepts are indeed correct.

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Targeting Th17 cells in CNS immune pathology

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Abstract

Multiple sclerosis (MS) is a demyelinating disease of the central nervous system (CNS) mediated by autoreactive T lymphocytes. A new class of $CD4^+$ T cells, the Th17 lineage, has recently been described and has been implicated in initiating immune responses against CNS autoantigens. Findings in experimental autoimmune encephalomyelitis (EAE, the animal model for MS) suggest that targeting Th17 cells may have a beneficial outcome for patients suffering from MS. Several existing and emerging therapeutic strategies are discussed based on the manner in which they target Th17-mediated autoimmunity: lymphocyte depletion, prevention of Th17 development, and prevention of Th17 function. T cell-ablating agents are not Th17 specific and are associated with toxicity and opportunistic infections. The prevention of Th17 differentiation can be achieved experimentally by neutralizing cytokines required for Th17 development and by the administration of cytokines or other chemicals that interfere with differentiation; however, these strategies may also lead to disease. Prevention of functional Th17 responses can be accomplished by inhibiting leukocyte trafficking or by neutralizing IL-17. While several promising therapeutic candidates have been identified using EAE and clinical experimentation, both the risks of immunomodulation as well as the efficacy of such candidates in human patients need to be completely characterized and carefully considered.

Introduction

Multiple sclerosis (MS) is a disorder of the central nervous system (CNS) in which the immune system targets CNS myelin peptides and mediates the destruction of CNS tissue, resulting in demyelination, neuronal damage and subsequent disability. It has been known for some time that $CD4^+$ T lymphocytes, or T helper (Th) cells play a causative role in the disease, although precise details of the immune response raised against CNS antigens are still being clarified. Upon encountering antigen, Th lymphocytes differentiate from their naïve phenotype into Th1, Th2, Th17, or regulatory T cell (Treg) lineages, depending on the conditions under which they first become activated. It is popularly believed that the Th17 lineage, with further

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contribution from Th1 cells, is responsible for much of the autoimmune damage to the CNS in MS [1, 2].

The various lineages of Th cells are defined by their biological function, commonly expressed in terms of their profile of secreted cytokines. Th1 cells mediate inflammation, and are most noted for secreting IFN-γ, while the Th2 lineage directs humoral responses *via* the secretion of IL-4, IL-5, IL-10, and IL-13 [3]. For several years, the Th1/Th2 paradigm dominated the landscape of immunology. However, these two subtypes of activated Th cells are now joined by the generally immunosuppressive Tregs and the potently pro-inflammatory Th17 lineage [4, 5]. As described in more detail in preceding chapters, Th17 cells secrete IL-17 and represent a lineage of Th cells distinct from the Th1 and Th2 populations. Th17 cells derive from naïve $CD4^+$ precursors in a process dependent on the cytokines TGF-β, IL-6, and IL-23; and their differentiation is inhibited by cytokines associated with the other T effector lineages: IFN-γ and IL-4 as well as IL-2 (reviewed in [5]). The transcription factor RORγt is widely accepted to be the master regulator of Th17 differentiation, directing lineage-pertinent functions analogous to T-bet, GATA-3, and Foxp3 in Th1, Th2, and regulatory T lineage differentiation, respectively [5]. The PI3K/Akt/NF-κB pathway seems to play an obligatory role in Th17 development and functions by linking cytokine receptor signaling to the expression of RORγt and IL-17. For autoreactive Th17 cells to cause disease, they must differentiate from naïve $CD4^+$ cells and migrate to the pertinent tissue (the CNS for the purposes of this review), and secrete IL-17 *in situ*, which elicits inflammation by recruiting neutrophils and otherwise mediating tissue destruction [2].

A pathological role for $CD4^+$ T cells has been clearly demonstrated in several human autoimmune diseases and their respective animal models, and recently, the Th17 lineage has garnered much attention in basic and clinical research as the major immunopathological population [2, 4–6]. Experimental autoimmune encephalomyelitis (EAE) is the animal model for MS, and has contributed profoundly to the understanding of Th17 cells in CNS autoimmunity, although EAE/MS was long considered to be mediated by a Th1 response [1–3, 5]. In support of this hypothesis, elevated levels of IFN-γ, IL-12 (required for the development of Th1) in the serum or cerebrospinal fluid, or the infiltration of IFN-γ-producing Th1 cells into the CNS correlated positively with disease severity. Moreover, disease can be induced experimentally by the transfer of Th1 cells (reviewed in [3, 5]). Correspondingly, genetic targeting of T-bet, the master Th1 transcription factor, renders mice resistant to EAE induction [7]. Despite this evidence, several observations confounded the Th1-centric theory of autoimmunity and were instrumental in the identification of the Th17 lineage. Briefly, illuminating experiments by Langrish et al. [8] and Cua et al. [9] demonstrated that the Th1-associated cytokine IL-12 and the Th17-associated IL-23 share a common

subunit – p40. Therefore, the neutralization of IL-12p40 (either by gene targeting or antibody neutralization), which caused EAE resistance, was not due to a defect in Th1 differentiation, but was instead attributed to an interference with the development of a previously undescribed Th17 subset [8, 9]. The presence of copious IL-17 in the cerebrospinal fluid of MS patients and large numbers of IL-17$^+$ T cells in the CNS of EAE-primed mice confirmed that a third population of CD4$^+$ effectors was associated with CNS immune pathology. In light of these findings, the model of disease induction in EAE was revised to feature Th17 cells as the primary initiators of CNS inflammation and a similar paradigm shift is taking place in MS where links between IL-12 and disease are being re-evaluated due to this subunit commonality [1, 3, 5, 10]. While Th17 cells unquestionably contribute to CNS immune pathology, the Th1 lineage likely also contributes importantly to CNS autoimmunity. It is not yet clear if Th17 cells mediate pathology independently or in concert with Th1 cells. Investigations addressing this issue are currently ongoing.

Many of the emerging approaches to target Th17 have been developed using the EAE model of MS. While multiple differences in disease expression exist between EAE and MS, it is widely believed that EAE represents a useful tool for developing MS treatment strategies [1, 11]. MS has been a challenging disease to study due to its complexity and multiple forms. It is attractive, therefore, that EAE exhibits relapsing-remitting and chronic-progressive disease courses, depending on the mouse strain and encephalitogenic antigen employed [11]. As discussed above, studies of EAE have definitively demonstrated the causative role of Th17 lymphocytes in disease, and the current literature espouses three main strategies to prevent this inflammatory population of lymphocytes from exerting immunopathological effects in the CNS. The first to be discussed is global immune suppression achieved by cytotoxic pharmacological agents (Mitoxantrone, cyclophosphamide, Cladribine, and cyclosporine) or by monoclonal antibodies that target lymphocytes for lytic destruction (anti-CD20 and anti-CD52). The second method to be discussed is the blockade of Th17 differentiation or immune deviation through the use of cytokines, anti-cytokine antibodies, and other drugs. The final section of this review discusses limiting Th17 autoimmune response by targeting effector cytokines and lymphocyte trafficking. These strategies are represented graphically in Figure 1. It should be noted that perturbation of the Th17 compartment may result in an increased risk of infections. IL-17 is known to be important for the recruitment of neutrophils, and IL-17$^{-/-}$ animals suffer from *Klebsiella pneumoniae* and *Citrobacter rodentium* infections due to a failed phagocytic response (reviewed in [2]). Likewise, humans with mutations to the IL-23/IL-17 axis are at higher risk of contracting respiratory tract infections. However, as discussed below, this risk is generally manageable and should not offset the potential benefit of Th17 targeting in CNS autoimmunity.

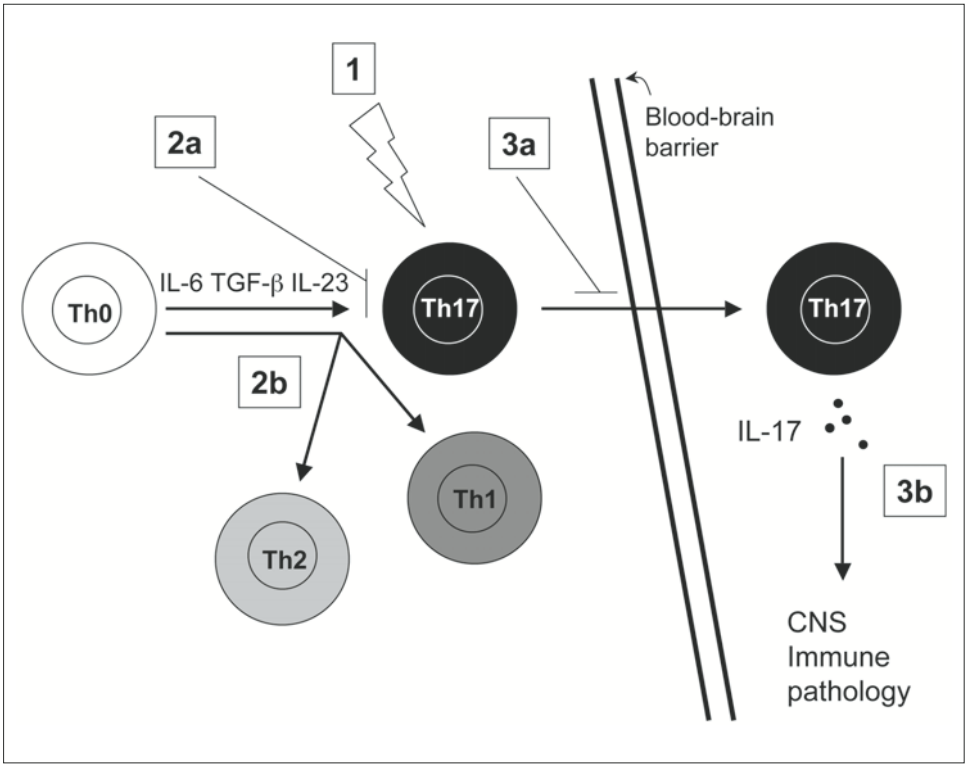

Figure 1
Current and emerging strategies for preventing CNS autoimmunity. Naïve, autoreactive T cell clones responding to self antigens in the presence of IL-6, IL-23, and TGF-β develop into inflammatory Th17 cells, cross the blood-brain barrier into the CNS, and exert their effector function. The major approaches to curtailing the Th17 response in MS/EAE are: (1) T cell depletion (Alemtuzumab, mitoxantrone, Cladribine, cyclophosphamide, and perhaps cyclosporine A). (2) Interference with Th17 development (anti-IL-23, exogenous IL-25, IL-27, or IFN-β). (3) Prevention of Th17 function (sequestration with Fingolimod, Natalizumab, or IL-17 antibody neutralization). Some current and proposed therapeutic agents target multiple processes simultaneously.

Global immune suppression

Although global immune suppression by lymphocyte depletion does not specifically target the Th17 response, it deserves mention because this approach's success often coincides with a decrease in IL-17 production. The primary advantage to global immune suppression is that it is generally very effective. Additionally, many

immunosuppressive agents are currently in use or in advanced (i.e., Phase III or IV) clinical trials. Lymphocyte depletion results in a loss of immune surveillance, and is therefore associated with an increased risk of infection or leukemia. Thus, specific targeting of Th17 cells, or preferably only the autoreactive T cell clones, without destroying the host's entire immune competence would be favored. The agents discussed below represent strategy 1 in Figure 1.

Monoclonal antibody depletion

There are two monoclonal antibodies currently used to deplete lymphocytes in clinical trials. Rituximab is specific for the B lineage marker CD20 and is therefore beyond the scope of this review. Anti-CD52 (Alemtuzumab, or Campath-1H) targets the T cell compartment, although it should be noted that B cells also express CD52 and are likewise depleted by Campath-1H (reviewed in [1, 12]). In addition to being expressed on better than 95% of T and B cells, CD52 is also expressed on monocyte/macrophage lineage cells. Originally developed to treat chronic lymphocytic leukemia, CD4 cells do not recover for 61 months and CD8 numbers remain low for 30 months following Alemtuzumab treatment. This treatment is effective in the short term, as patients with various forms of MS demonstrate an approximate 90% reduction in lesions and suffer from much less frequent relapses (2.2 to 0.14 relapses/year). Long-term data (average 7.5 year follow-up) are less remarkable, as few patients remain stable. Roughly 90% worsen over the long term. Additionally, a significant proportion of patients receiving Campath-1H suffer from bacterial sepsis and cytomegalovirus re-activation. An unexpected side effect to Campath-1H administration is the development of autoimmune hyperthyroidism (Graves' disease) in 27% of severe-progressive MS patients. As a result of these complications, Phase III clinical trials have been suspended [12, 13].

Inhibitors of proliferation/cytotoxic agents

Cytotoxic pharmacological agents offer an alternative to monoclonal antibody depletion for treating CNS autoimmunity, and many have either already received FDA approval, or are in advanced trials. Currently, two DNA synthesis inhibitors: mitoxantrone (Phase IV/approved) and cyclophosphamide (FDA approved, but not licensed for MS treatment) are used for aggressive forms of MS [13, 14]. In addition to lymhpocytoxicity, these two drugs may also function as immune deviators; that is, they skew the overall immune response toward a Th1 or Th2 type response at the cost of a Th17-type response. Mitoxantrone treatment is associated with a decrease in IFN-γ, but there is also a decrease in IL-6 and matrix metalloproteinase 9

(MMP9), which suggests that Th1 and Th17 responses are inhibited (IL-6 is required for Th17 differentiation, while MMP activity is known to be up-regulated by IL-17) [2, 14]. Furthermore, increases in either IL-10$^+$ T cells or IL-10 itself in conjunction with IL-4 have been reported in patients treated with mitoxantrone or cyclophosphamide, indicating anti-inflammatory properties. Cladribine is a third cytotoxic agent used to treat MS, and it functions by inhibiting adenosine deaminase and causing a toxic buildup of metabolites. It exerts powerful anti-inflammatory effects and Phase III trials are ongoing [13]. As is to be expected following a large-scale loss of lymphocytes, these three drugs carry risks of opportunistic infections and leukemia. Anti-microbial prophylaxis is commonly required for patients receiving Cladribine or cyclophosphamide. There is also an increased incidence of leukemia (usually acute promyelocytic or acute myelocytic leukemia) and cardiotoxicity associated with mitoxantrone use [14]. These adverse effects make these drugs suitable only for treatment of aggressive forms of MS if non-ablative forms of immunomodulation are ineffective.

The well-known immunosuppressive agent cyclosporine A (CSA), which has been used for decades to control allograft rejection, has recently been shown to down-regulate the Th17 response. Although these experiments were carried out using CD4$^+$ T cells from individuals with rheumatoid arthritis (RA), it is attractive to think the findings would translate well into treatment of MS or EAE. Cho and colleagues [15] demonstrated that T cells treated with CSA secreted reduced levels of IL-17. Observed defects in cytokine production were attributed to down-regulation of PI3K/Akt signals, a decrease in the DNA-binding capacity of NF-κB, and a consequential decrease in IL-17 mRNA. Owing perhaps to a fundamental difference in the autoimmunity of RA *versus* MS, or perhaps to probable toxic effects, it is unclear why a potent inhibitor of IL-17 production, such as CSA is not more effective in MS treatment.

Prevention of Th17 differentiation

Despite the efficacy and availability of immunoablative agents, there are serious risks associated with such drastic approaches. A more attractive strategy would eliminate Th17 cells while sparing the patient's immune competence. One method by which Th17 cells may be specifically targeted in CNS autoimmunity is to prevent their differentiation from naïve precursors. The developmental requirements of the Th17 lineage have been the focus of intense investigation over last 3–4 years, and several findings provide optimism that targeting lineage-specific cytokines, transcription factors, or signaling pathways is of potential value in a clinical setting. Targeting IL-23 and *t-bet*, as well as Th17 lineage antagonism by IL-25, IL-27, and IFN-β are discussed here. These molecular targets comprise strategies 2a and 2b in Figure 1.

IL-23 neutralization

Of the three cytokines required for mounting a proper Th17 response, IL-23 may be the most suitable target because IL-23 does not exhibit pleiotropic activity to the same degree as TGF-β or IL-6. Following the rationale that IL-23p19-deficient mice are resistant to EAE [8, 9], Chen et al. [16] showed that administration of a monoclonal anti-IL-23p19 antibody significantly improves the clinical outcome of EAE. Specifically, IL-23 neutralization delayed onset time, and reduced clinical scores, incidence, and mononuclear (lymphocytic as well as monocytic) infiltration of the CNS, all of which were associated with a decrease in Th17 numbers and IL-17 serum levels. Excitingly, antibody neutralization of IL-23 prevented acute disease if administered prior to onset, but also acted therapeutically when administered at the peak of disease in relapsing-remitting EAE [16]. Some preliminary *in vitro* studies have been performed using small interfering RNA (siRNA) to disrupt IL-23 synthesis by antigen-presenting cells [10]. Briefly, dendritic cells (DCs) collected from MS patients, which are reported to secrete higher levels of IL-23 than those from healthy donors, secreted reduced amounts of IL-23 following treatment with a specific siRNA. Following IL-23 interference, DCs from affected donors become poor T cell stimulators in mixed leukocyte reactions despite a lack of observed changes in MHC or costimulatory molecule expression. Furthermore, TNF-α secretion is decreased while IL-10 secretion in increased, suggesting that IL-23-deficient DCs skew immune responses toward the Th2 and away from Th17 phenotype. While these findings demonstrate the potential of siRNA targeting of IL-23, a disease-appropriate demonstration of reduced autoimmune function is still lacking.

Lineage antagonism by exogenous cytokine administration

Neutralization of cytokines required for Th17 development has yielded encouraging results in preliminary studies, and in complementary fashion, the administration of cytokines refractive to Th17 development has been shown to be effective against animal and human CNS autoimmunity. Following the observation that IL-27R$^{-/-}$ mice are extremely sensitive to EAE caused by a Th17 hyperresponse, Fitzgerald and colleagues [17] showed that T cells activated in the presence of IL-27 generally fail to transfer disease. While they were able to demonstrate a profound reduction in disease incidence, CNS pathology, leukocyte infiltration into the CNS and IL-17 secretion in the IL-27-treated group, the mechanism remained unclear, although it was postulated that IL-27 functions to attenuate the effector phase of disease. While these findings suggest a therapeutic role for IL-27 in ongoing disease, it is also plausible that IL-27 prevented disease by blocking Th17 development in activation cultures prior to adoptive transfer. A more complete understanding of when IL-27 inhibits Th17-related pathology would be required for effective treatment.

A second cytokine recently shown to antagonize Th17 development is IL-25 (also known as IL-17E). IL-25-deficient mice were shown to be highly susceptible to EAE, which is attributed to an increase in IL-23 in the periphery and greater numbers of Th17 cells in the CNS [18]. The abnormally strong Th17 response coupled with an observed decrease in Th2 cytokines in IL-25$^{-/-}$ mice suggests that IL-25 could perhaps be used to deviate the immune response away from pathological autoimmune inflammation. Indeed, exogenous IL-25 protected wild-type SJL mice from disease by diminishing the Th17 response and increasing the amount of secreted IL-4, IL-5, IL-10, and IL-13. Despite the increase in Th2 cytokines, seemingly at the expense of IL-17, the immune deviation was found to be dependent not on IL-4, but on IL-13. The authors suggest that this previously undescribed action of IL-13 may involve DC regulation. Further research on this subject would be of immense interest, due to the profound influence CNS DCs exert on MS/EAE [1, 19]. Perhaps the most attractive quality of IL-25 administration is that it acted both to prevent the onset of EAE and to function therapeutically to diminish ongoing disease [18].

Of all of the molecules administered or targeted for disruption of Th17 cells in CNS autoimmunity, only IFN-β has been approved for use in a clinical setting [1, 13]. Mixed results have been reported with regard to the efficacy of IFN-β in the treatment of EAE. In fact, even in MS patients, IFN-β is only partially curative, and best results are observed when IFN-β is used in conjunction with another form of therapy [13]. Nonetheless, it is commonly used and in some patients significantly slows the relapse rate and reduces the incidence of CNS lesions. Owing to the diversity in functions of type I interferons, IFN-β appears to regulate MS in two separate ways [20]. The first is by altering the DNA-binding capacity of Th17 lineage-specific transcription factors. The binding capacity of NF-κB is diminished in the presence of IFN-β, and this is accompanied by a decrease in IL-17 transcription. This finding is consistent with the clinical observation that MMPs are decreased in patients receiving IFN-β, as the MMP activity is a known mechanism of IL-17-mediated tissue destruction [1]. Conversely, the ability of the Th2 factor STAT-6 to bind DNA is enhanced in the presence of IFN-β, and consequent increases in IL-4 message level have been reported [20]. In addition to immune deviation, IFN-β may also act to prevent leukocyte migration *via* an increase in soluble cell adhesion molecules.

Despite recent advances in induction of immune deviation *via* exogenous cytokine administration, clinical trials should be approached with caution due to the worsening of MS following IFN-γ treatment. IFN-γ is known to inhibit Th17 differentiation *in vitro*, and it was therefore expected to curtail CNS autoimmunity by shifting the immune response away from Th17 in favor of Th1 [3]. The disappointing performance of IFN-γ in clinical studies could be ascribed to the incomplete understanding of the Th1 contributions to MS/EAE, or to the plasticity of early Th1 and Th17 precursors. Furthermore, several of the studies described here have noted the presence of CD4^{+} T cells in the CNS that produce both IFN-γ

and IL-17, and that these double-positive T cells were associated with potent CNS inflammation [16]. The notion that the Th1 and Th17 lineages are closely related was recently strengthened by showing that co-regulation of Th1 and Th17 immunity is accomplished, at least in part, by the canonical Th1 transcription factor t-bet [7]. Due to the incompletely understood relationship between Th1 and Th17 cells in disease, it seems prudent to avoid Th1 skewing when targeting the Th17 lineage in MS/EAE. One experimental strategy that fits this criterion is the targeting of t-bet with siRNA. As would be expected, given that T-bet$^{-/-}$ mice are highly resistant to EAE, knocking down T-bet in EAE-primed mice resulted in a significant improvement in disease course as well as a decrease in both IFN-γ and IL-17. The IL-17 defect was attributed to a decrease in IL-23R expression. While this approach yielded exciting results, the use of siRNA to treat diseases is hampered by its own challenges. Improvements in this technology would create more options for MS treatment by allowing the expression of logical target genes (e.g. IL-23 and t-bet) to be specifically decreased.

Prevention of Th17 response/function

In addition to induction of lymphopenia and the disruption of Th17 differentiation, the targeting of the Th17 response by either neutralizing the effector cytokine itself, or by lymphocyte sequestration has proved to be of clinical benefit. This section discusses the antibody neutralization of IL-17, as well as the sequestration of leukocytes by sphingosine 1-phosphate receptor ($S1P_1$) agonists and the monoclonal antibody Natalizumab (anti-VLA4). These approaches are designated 3a and 3b in Figure 1.

Studies by Hofstetter and colleagues [21], in which the causative role of IL-17 in EAE was demonstrated, complemented the finding that IL-23 and not IL-12 was responsible for disease development. Furthermore, this study illustrated the feasibility of IL-17 neutralization as a method to reduce CNS immune pathology. Although significant reductions in clinical score were observed in anti-IL-17-treated animals, symptoms were still observed. A subsequent comparison of anti-IL-17 and anti-IL-23 treatment by Chen et al. [16] revealed that neutralizing IL-23 is more effective at reducing EAE symptoms and incidence than targeting IL-17 directly. Despite the suggested inferiority of anti-IL-17, an exciting series of papers appearing in the European Journal of Immunology in late 2006 detailed the immunization of mice with self-IL-17 using either a protein carrier (ovalbumin), or an IL-17-conjugated virus-like particle [22, 23]. Both studies reported long-lasting IL-17 antibodies, with titers maintained for longer periods of time following OVA-IL-17 immunization, whereas higher titers were reached with VLP-IL-17 immunization. Both of these vaccines were shown to be highly effective at preventing EAE. In fact, OVA-IL-17 immunization resulted in zero incidence as evidenced by a lack of

neurological deficit or weight loss. Both immunization methods generated highly specific antibody responses against IL-17A, but not against any of the other IL-17 isoforms. As discussed in Rohn et al. [23], this may have important implications pertaining to the increased susceptibility of IL-17$^{-/-}$ mice to certain bacterial infections. It was noted that IL-17A and IL-17F partially overlap in function, especially with regard to neutrophil recruitment. Thus, specifically targeting IL-17A may not impair IL-17F-mediated neutrophil recruitment and may not increase susceptibility to bacterial infections. While both of these studies demonstrated efficacy of IL-17 immunization in prevention of EAE onset, neither study addressed the potential of using IL-17 immunization against ongoing CNS inflammation, despite employing a chronic progressive model of EAE. Such a demonstration would be desired before proceeding with clinical trials in MS.

Aside from cytokine secretion, autoreactive T cells must also migrate into the CNS for pathology to occur, and several therapeutic agents target this process. The prevention of T cell infiltration of the CNS is accomplished by treatment with the monoclonal antibody Natalizumab, which targets the $\alpha 4\beta 1$ integrin (VLA4) [1, 12, 13]. Natalizumab does not deplete lymphocytes like the mAb treatments described above, but prevents the extravasation of circulating lymphocytes across endothelium separating the vessels and the CNS. Natalizumab was shown to be effective in 6- and 12-month clinical trials for limiting CNS lesions and reducing the relapse rate, and gained FDA approval for use in MS treatment. However, use of Natalizumab was soon associated with the development of progressive multifocal leukoencephalopathy (PML) due to an infection with or reactivation of JC virus, and its use for MS therapy is currently restricted.

Agents targeting multiple processes

In addition to the three distinct approaches already discussed, there are three drugs or classes of drugs that affect multiple pathways of CNS inflammation. While their characteristic mechanisms of action are known (i.e., two of them – Fingolimod and the statins – have previously been shown to interfere with leukocyte migration), antagonistic effects on Th17 differentiation and function are also now being described.

Fingolimod, which is currently in a Phase III clinical trial, targets the $S1P_1$ expressed on T lymphocytes [24]. It is known to sequester lymphocytes in lymph nodes, thereby preventing their migration into the CNS, where they can cause pathology. In fact, therapeutic doses resulted in a paucity of circulating lymphocytes and a consequential increase in the risk of upper respiratory infections. However, Fingolimod treatment resulted in a decrease in CNS lesions and in the relapse rate of MS patients. Recently, a second mechanism by which Fingolimod suppresses autoimmunity was proposed. Liao and colleagues [25] showed that the addition of

S1P (an $S1P_1$ ligand) to T cell cultures supportive of Th17 differentiation (containing a TCR stimulus, TGF-β, and IL-6) resulted in a high degree of conversion to the Th17 lineage, accompanied by IL-17 secretion. While the numbers of IL-17$^+$ T cells were comparable in cultures with or without the addition of IL-23, the cultures containing this cytokine elicited greater IL-17 secretion. Conversely, the addition of Fingolimod, an $S1P_1$ negative agonist, to Th17 cultures decreased the amount of secreted IL-17 by up to 80%. This brief report, as well as the characterization of an $S1P_1$ transgenic animal, has made it clear that the S1P-$S1P_1$ axis makes a contribution to Th17 immunity, but that IL-23 may still be necessary for full Th17 function. A direct demonstration of reduced autoreactivity unrelated to trafficking is lacking, and would provide evidence that antagonism of Th17 differentiation or function is a major contributing mechanism of Fingolimod protection in MS/EAE.

Recently, statins, which are currently prescribed for lowering cholesterol, have been shown to exert Th17 antagonism and to have beneficial effects on MS/EAE [26]. A proposed mechanism by which statins regulate inflammation is through binding to LFA-1, and a general decrease in cell trafficking and lymphocyte activation is associated with this binding. Statins have also been shown to interfere with the activation of Rho-GTPases, which are integral components of several signaling pathways involving lymphocyte activation and differentiation [26]. Current data suggest that statin treatment results in significant reductions in disease and infiltration of leukocytes into the CNS both in animal studies and MS patients. Notably, EAE studies suggest that the Th landscape is shifted toward the Th2 effector arm, as evidenced by a slight decrease in IFN-γ and IL-17, but a significant increase in IL-4 [27]. These results are encouraging, and patients have generally responded well in small, preliminary clinical trials, but further investigation is required. While a decrease in CNS lesions was observed in patients receiving statins alone, the increased number of lesions and faster relapse rate in patients receiving statin/IFN-β combination therapy needs to be addressed. Definitive double-blind, placebo controlled studies are currently ongoing [26].

Finally, glatiramer acetate (GA) is an FDA approved treatment for MS that has several immunomodulatory effects [28]. GA is a mixture of synthetic amino acid polymers consisting of glutamic acid, lysine, alanine, and tyrosine, and is similar to the major immunogenic sequence in MBP. GA suppresses immune reactions against self-CNS peptides by acting as an inhibitor of T cell activation/proliferation, competing for TCR/MHC binding with myelin antigens, and by shifting pro-inflammatory conditions to an anti-inflammatory Th2 state. The latter of these three mechanisms of GA is evidenced by an increase in IL-10 and IL-4 and a coincident decrease in IL-12. Furthermore, Th2 lineage cells appear in the CNS secondary to GA treatment, and this is associated with a decrease in disease severity. Literature addressing the role of GA in regulating Th17 responses is scant, although it is expected that the skewing of the immune response toward Th2 and away from Th1 axis would affect the Th17 compartment as well.

Summary

This review has discussed the immunomodulatory agents that target the Th17 compartment according to their primary mechanism of action: T cell depletion, blockade of Th17 development, and blockade of Th17 function. Those agents that target multiple pathways of Th17 autoimmunity confound the model depicted in Figure 1 slightly, and a more thorough summary appears in Table 1. T cell ablation therapy is generally effective, especially against aggressive forms of MS, and although the increased risk of opportunistic infections can be managed by antimicrobial prophylaxis, the elevated incidence of leukemias, alternative forms of autoimmunity, and toxicity make this approach unfavorable. Despite the availability and long-standing use of such ablative agents, they seem inferior to alternative treatments that specifically antagonize Th17 cells and preserve immune competence. Interfering with Th17 development can be accomplished either by blocking the process completely by targeting cytokines or transcription factors required for the lineage's differentiation, or by immune deviation using exogenous cytokines antagonistic to Th17 cells but supportive of other lineages. Immune deviation strategies should be approached with caution however, for while Th2 skewing is associated with disease improvement, skewing toward Th1 responses is associated with disease exacerbation. A cogent description of the individual contributions to disease by Th17 cells and Th1 cells remains a goal of contemporary MS/EAE research. The final strategy discussed above, the prevention of Th17 function, is accomplished by interfering with leukocyte trafficking or by neutralizing IL-17. As is the case with global immune suppression, lymphocyte sequestration results in a loss of immune surveillance and the risk of opportunistic infections and leukemia is elevated. Neutralizing IL-17 is effective in limiting autoimmunity in animal studies, but some reports suggest that it is not as effective as neutralizing IL-23. Specific targeting of IL-17A and not other IL-17 isoforms may simultaneously limit or prevent autoimmune disease, but also mitigate the susceptibility to infection observed in organisms deficient in the IL-23/IL-17 pathway.

Aside from the direct risks associated with immunomodulation, it is important to consider the different forms of MS as well as the various contributors to CNS autoimmunity. Certain regimens, such as Alemtuzumab treatment, are effective against relapse-remitting MS (reducing the relapse rate by 94%), but have limited benefit for severe-progressive MS patients (whose condition deteriorates over the long term) [12]. Likewise, the use of strong immunosuppressive agents is advisable only against aggressive forms of MS [14]. Furthermore, while the Th17 lineage appears to be a logical therapeutic target, there are non-T cell mediators of CNS inflammation as well as neurodegenerative processes unrelated to autoimmunity to be considered [1]. Approaches that target several of these processes simultaneously as GA does, (i.e., GA inhibits proliferation, encourages anti-inflammatory Th2 activity, and perhaps facilitates remyelination) would be prime candidates for further study.

Table 1. Agents and mechanisms of Th17 antagonism

Agent	Target/mechanism	Clinical trial phase	Anti-Th17 activity*
Mitoxantrone	DNA replication	IV/approved	1
Cladribine	Adenosine deaminase	III	1
Cyclophosphamide	DNA replication	II	1
Cyclosporine A	Calcineurin	None	1, 2
Alemtuzumab	CD52	Suspended	1
Anti-IL-23	IL-17 synthesis/secretion	None	2
IL-23 siRNA	IL-17 synthesis/secretion	None	2
IL-25 (IL-17E)	Th17 → Th2 shift	None	2
IL-27	Th17 → Th2 shift	None	2
IFN-β	Th17 → Th2 shift	IV/approved	2
Anti-IL-17	IL-17	None	3
IL-17 vaccination	IL-17	None	3
Natalizumab	VLA4	Approved/restricted usage	3
Fingolimod	S1P-S1P$_1$	III	2, 3
Statins	HMG-CoA	II	2, 3
Glatiramer acetate	TCR-MHC binding	Approved	2, 3

**Anti-Th17 activities: (1) T cell depletion; (2) impedes Th17 differentiation from naïve precursors; and (3) prevents proper Th17 migration/function. Adapted from [13].*

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IL-17 cytokines in asthma

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Abstract

The current chapter scrutinizes the published evidence from humans that IL-17, as well as more recently discovered members of the IL-17 cytokine family, may be involved in the pathogenesis of asthma by contributing to the mobilization of granulocytes. Whereas there is indicative published evidence from patients with asthma arguing for IL-17 and, to lesser degree, for IL-17F, involvement, the current case for the other IL-17 cytokines remains weak in terms of clinical evidence. For the future, there is a need for more studies on human patients with well-characterized phenotypes and consistent medication, to more firmly establish the pathogenic role of IL-17 cytokines in asthma and the pharmacotherapeutic potential of targeting these intriguing molecules.

Introduction

The idea that the T cell cytokine interleukin (IL)-17 (also known as IL-17A) is involved in obstructive airway disease including asthma was originally forwarded in a review article published by Lindén and co-workers in 2000 [1]. That particular review article summarized the findings of three, at that time, novel experimental studies documenting the neutrophil-mobilizing effects of recombinant IL-17 protein in rat airways *in vivo* and in human bronchial epithelial cells *in vitro* [2–4]. In essence, the early experimental studies indicated that IL-17 induces the production and release of neutrophil-mobilizing cytokines, including IL-8 and IL-6 from bronchial epithelial cells, and thereby indirectly recruits and possibly also activates neutrophils locally in the airways. Then, in 2001, Molet and co-workers [5] published the first supportive clinical evidence obtained from patients with asthma. These early publications have subsequently inspired additional experimental and clinical studies on IL-17 in the context of asthma. The current chapter scrutinizes the available evidence from humans that IL-17, as well as more recently discovered members of the IL-17 cytokine family, may be involved in the pathogenesis of asthma.

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IL-17 in the airways of patients with asthma

Data on bronchoalveolar lavage samples

Molet and co-workers [5] reported in their early study that the concentration of soluble IL-17 protein in bronchoalveolar lavage (BAL) fluid is almost 70% higher in patients with mild asthma. Approximately half of these patients displayed a positive skin prick test, indicating allergen sensibilization. Some of the patients were ex-smokers but none had a history of severe smoking. No patient was treated with glucocorticoids and no patient had a respiratory infection during the 6 weeks preceding the study inclusion. Molet and co-workers also demonstrated that the intracellular, "immunocytochemical" signal for IL-17 protein is approximately three times more frequent among BAL cells from patients with mild asthma than among BAL cells from control subjects. Notably, even though it was not statistically significant in either case, Molet and co-workers reported a trend towards an increase in the relative number of BAL neutrophils in the patients with asthma, paralleling the increase in IL-17. As expected, Molet and co-workers reported that the relative number of eosinophils is moderately increased in patients with asthma.

Data on induced sputum

In line with their corresponding findings in BAL fluid and cells that were published in 2001, Molet and co-workers [5] also demonstrated that the intracellular, "immunocytochemical" signal for IL-17 protein is almost three times more frequent among sputum cells from patients with mild asthma than among sputum cells from control subjects. Their data confirmed that the relative number of eosinophils is substantially increased in sputum samples from patients with asthma, whereas there was only a trend towards a corresponding increase for neutrophils.

In 2003, Barczyk and co-workers [6] published a study indicating an association between an increased concentration of IL-17 in induced sputum and bronchial hyperreactivity to metacholine in a mixed patient group, of patients with asthma or chronic bronchitis. The study by Barczyk and co-workers showed that the increase in sputum IL-17 among the patients with bronchial hyperreactivity is modest, around 40%, compared with control subject without bronchial hyperreactivity. Interestingly, according to this study on sputum, the concentration of IL-17 correlates with the actual sensitivity index (PC20) for metacholine. The patients with asthma had mild to moderate disease and some of these patients were treated with inhaled glucocorticoids. The reviewed study was small and it was probably because of this and the inconsistency in terms of treatment with glucocorticoids, that the difference in sputum IL-17 between patients with asthma and control subjects did

not reach statistical significance. The same is likely to be true for the difference in IL-17 between patients with chronic bronchitis and control subjects. Notably, this study did not demonstrate a strict association between increased concentration of IL-17 and neutrophils in sputum.

Compatible with the study by Barczyk and co-workers, Sun and co-workers [7] reported a fourfold concentration of IL-17 protein in induced sputum from patients with severe asthma, compared with control subjects. This increase in IL-17 is accompanied by an approximately twofold higher relative number of neutrophils, compared with control subjects. Sun and co-workers also included patients with mild and moderate asthma in their study and, interestingly, when analyzing IL-17 in all three stages of disease, they showed that the concentration of sputum IL-17 corresponds to the severity of disease. The same is true for the concentrations of myeloperoxidase and IL-8, in spite of the fact that infections during the 6 weeks preceding the study were an exclusion factor. None of the patients with asthma was treated with a glucocorticoid and these patients were non-smokers. When a mixed group comprising all three degrees of disease severity was treated with an inhaled glucocorticoid for 1 week, this treatment reduced the IL-17 concentration only to a minor degree, even if this impact was statistically significant. The relative number of neutrophils in sputum was not altered by this treatment.

Bullens and co-workers [8] quite recently published evidence on IL-17 in patients with asthma. They demonstrated that there was an increase in the mRNA for IL-17 in sputum cells from these patients and that the increase in mRNA was of a similar magnitude in mild and moderate to severe asthma. They also found no pronounced difference in this respect for patients with allergic and non-allergic asthma. Bullens and co-workers were able to demonstrate a positive and statistically significant correlation between the percentage of neutrophils among inflammatory cells in sputum and the mRNA for IL-17, thereby providing evidence for IL-17 being associated with neutrophil accumulation in patients with asthma, even though a causative relationship remains to be proven in this setting.

Data on exhaled breath condensate

In 2006, Matsunaga and co-workers [9] published an innovative study in which they demonstrated that the relative concentration of IL-17 protein in exhaled breath condensate is almost 50% higher in patients with mild asthma than in control subjects. Interestingly, according to this study, the increase in IL-17 is associated with a similar increase in the chemokine IL-8 as well as in RANTES, TNF-α and TGF-β. In this study, all patients were non-smokers and none was treated with glucocorticoids.

Data on bronchial tissue

So far, the most striking clinical evidence for an association of IL-17 with asthma in humans was published by Chakir and co-workers in 2003 [10]. By including patients with moderate to severe asthma, they generated data demonstrating that there is an immunohistochemical signal for IL-17 protein in almost six times as many cells in the subepithelial bronchial layer of patients with severe asthma, compared with healthy control subjects. The patients with moderate to severe asthma had never smoked and were not treated with glucocorticoids. Infections during the last 6 weeks preceding the study inclusion formed part of the study exclusion factor. An even more dramatic, relative increase in IL-17 signal was obtained when analyzing the intraepithelial bronchial layer. Among the patients with severe asthma, there was a substantial increase in type I collagen and TGF-β. The major part of the immunohistochemical signal for IL-17 protein proved to be sensitive to 2 weeks of treatment with a systemic glucocorticoid.

IL-17 in the blood of patients with asthma

The first published study addressing IL-17 in patients with asthma was in fact conducted by Wong and co-workers in 2001 [11]; this was actually a couple of months before the seminal study by Molet and co-workers. The study by Wong and co-workers was conducted on blood cells and plasma. Interestingly, the results revealed a trend towards a twofold higher plasma concentration of soluble IL-17 protein in patients with allergic asthma, compared with healthy control subjects without allergy, but the limited material did not yield statistical significance for this trend [11]. Subsequently, the trend demonstrated by Wong and co-workers was supported by data published by Molet and co-workers, demonstrating an increase in the number of blood eosinophils containing immunocytochemical signal for IL-17 in patients with mild asthma [5].

Cellular sources of IL-17 in patients with asthma

The early documentation of IL-17 in BAL, sputum and blood eosinophils that was published by Molet and co-workers consisted of data obtained using three different techniques: immunocytochemistry, Western blot and *in situ* hybridization [5]. Importantly, in view of the collective evidence from experimental studies, the findings published by Molet and co-workers remain controversial and have yet to be confirmed in terms of functional relevance [1, 12–17]. Until now, there is no published study demonstrating that live eosinophils isolated from human subjects

can actually release IL-17 protein in response to any stimulus, despite the increasing research activity in this area.

In contrast to that reported early by Molet and co-workers, most current experimental evidence on the cellular source of IL-17 in humans originates from studies of peripheral blood mononuclear cells *in vitro* and links IL-17 production to subsets of T lymphocytes (CD3 cells), but there is insufficient documentation that this is actually the case in patients with asthma [1, 12–17]. Ivanov and co-workers [18] reported an immunocytochemical signal for IL-17 in BAL cells displaying a cellular morphology similar to that of lymphocytes, as well as a substantial increase in mRNA for IL-17 among BAL cells, obtained from healthy, human volunteers exposed to organic dust. Even though not conclusive in terms of asthma and specific cell characteristics, the work of Ivanov and co-workers is fully compatible with the notion that CD3 cells are important sources in human airways. The more recent evidence from Bullens and co-workers [8] provides more specific support for this idea, by showing that there is a positive and strong correlation between the amount of mRNA for IL-17 and the amount mRNA for CD3γ in sputum from patients with moderate to severe asthma. Currently, there are no published studies establishing whether IL-17-producing cells in human lungs display the typical "Th17" characteristics that have been described for human blood cells producing IL-17 [16, 17, 19–25]. Moreover, it remains unknown whether the IL-17 response to stimulation with anti-CD3 and anti-CD28 antibodies and extract from house dust mite in blood mononuclear cells from patients with allergic asthma mirrors activation of such "Th17" cells [26].

Additional IL-17 cytokines in patients with asthma?

Apart from IL-17 (IL-17A) *per se*, there are two additional members of the IL-17 cytokine family that currently can be linked to asthma and allergy, IL-17F and IL-25 [14–17].

Like IL-17A, IL-17F is potentially interesting in the context of acute and severe asthma in humans [14–17]. This is mainly because of findings published in 2001 by Kawaguchi and co-workers [27]. This study demonstrates that recombinant IL-17F does induce the production and release of neutrophil-mobilizing cytokines, including IL-8 and IL-6 in human bronchial epithelial cells, and that allergen-challenge of patients with allergic asthma increases the immunohistochemical signal for IL-17F mRNA in BAL cells [27]. Moreover, it is noteworthy that, as opposed to IL-17, IL-17F is believed to be produced and released by several different cell types, including basophils, epithelial cells, mast cells, monocytes and, just like IL-17, from certain T lymphocytes [14–17, 27]. Of potential clinical interest is the finding by Kawaguchi and co-workers [27] that a genetic mutation makes the IL-17F molecule biologi-

cally inactive *in vitro* and this mutation is absent among patients with asthma or COPD but can be present in healthy control subjects, even though the frequency of this mutation is fairly low among the control subjects. However, contradicting data exist, so this issue is in need of further evaluation [28].

Fort and co-workers [29] demonstrated in 2001 that interleukin-25 (originally named IL-17E) is the "odd sibling" in the IL-17 family of cytokines [14–17]. This is due to its eosinophil-mobilizing profile, probably exerted *via* its ability to induce IL-4, IL-5 and IL-13 in the airways [14–17, 29]. Currently, there is evidence that this "Th2" cytokine can be release from T lymphocytes, mast cells and alveolar macrophages [14–17]. Even more than is the case for IL-17F, the current evidence for IL-25 being involved in asthma has its origin in experimental studies on animal and cell models.

Should we target IL-17 in asthma?

That asthma is considered to be a T cell-driven disease as well as the fact that the T cell cytokines IL-17 and IL-17F can accumulate neutrophils and neutrophil-derived bioactive compounds makes it tempting to take the elevated IL-17 level in the airways of patients with asthma into account as an argument for blocking these two IL-17 cytokines pharmacologically. This is particularly true for patients with acute and severe asthma, a condition where exaggerated neutrophil activity seems to constitute a pathogenic problem [16, 17]. However, before studies of this pharmacotherapeutic approach are undertaken, it is important to consider the convincing evidence from animal studies that IL-17 constitutes an important orchestrating molecule in host defense against bacteria and fungi [14–17]. Although it is not yet confirmed that IL-17 plays a similar role in host defense among humans, the mere idea that IL-17 inhibition can result in detrimental immunosuppression constitutes an argument for caution in targeting IL-17. For this reason, local inhibition may be safer than systemic inhibition.

Conclusions

Even though the published evidence from patients with asthma is compatible with IL-17 and, possibly, IL-17F being involved in mediating the mobilization of neutrophils in this disease entity, there is a need for more solid clinical information to strengthen the case for these intriguing T cell cytokines as key pathogenic factors. Experimental data on IL-17 and IL-17F in allergic and non-allergic airway inflammation in animal models are providing strong support for conducting more elaborate clinical studies [30–33]. Because of inconsistencies in previous clinical studies, it is still unclear to what extent the pathways mediated by IL-17 and IL-17F are

sensitive to drugs that are now in clinical practice. It is therefore important that new studies are designed so that well-characterized patient groups have consistent medication, in particular with reference to glucocorticoids and/or other anti-inflammatory drugs. Moreover, in future studies, it may prove useful to also address the pathogenic importance of down-stream signaling for IL-17 and IL-17F, by examining the IL-17A/C receptor complex in patients with various stages of asthma [16, 17, 34]. Finally, to evaluate the case for IL-25 in the pathogenesis of asthma, the first important step is to evaluate its expression in various stages of disease in well-characterized patients.

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IL-17/23, potential targets for Crohn's disease

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Abstract

Biological agents have profoundly changed the therapeutical management of Crohn's disease and ulcerative colitis, the major clinical subentities of inflammatory bowel disease (IBD). In the gut mucosa, the interleukin (IL)-23 drives the development of the effector Th17 lineage, which plays an essential role in maintaining tissue homeostasis and in repelling enteropathogenic infections. Conversely, aberrant IL-17- and IL-23-dependent signaling have been recently linked to the predisposition of IBD, prioritizing IL-17 and/or IL-23 signaling as potential therapeutical targets in such common immunopathologies. Clinical trials are currently evaluating the safety and efficacy of fully human recombinant immunoglobulins neutralizing IL-12p40 or IL-17. Consistent with a physiopathological role of IL-17 and IL-23 in Crohn's disease, preliminary data showed encouraging results in regards to tolerability and beneficial effects. Long-term follow-up monitoring is now eagerly awaited to provide evidence of a durable protective role of IL-12/IFN-γ in host defense against pathogens, as well as additional clinical trials to assess the efficacy of anti-IL-23p19 treatment.

Introduction

Crohn's disease (CD) and ulcerative colitis (UC) are common relapsing-remitting intestinal illnesses, defined as the major clinical subentities of inflammatory bowel disorder (IBD). While UC lesions are limited to the colonic and rectal mucosa, CD is associated with transmural inflammatory lesions, which can potentially concern any part of the gastrointestinal tract. IBD mostly affects young adults, with a combined prevalence of about 250–300 cases per 100 000 individuals in Western European and North American countries. The IBD patient's quality of life is profoundly affected by severe diarrhea, abdominal pain, fever, weight loss, malnutrition and bleeding. Certain subsets of IBD sufferers can also experience additional complications, such as fistulae, abscesses, bowel strictures and/or extra-intestinal manifestations (mostly involving the eyes, skin, joints and liver) [1]. The natural history of IBD is unpredict-

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able and an overall 25–30% therapy refractoriness is seen, regardless of the drug, resulting in accumulation of adverse events and indicating that the treatment of IBD is, at present, far from optimal [2].

There is now good evidence to suggest that IBD might result from a defective innate immune response at the gut mucosa in genetically predisposed individuals, leading to an overly aggressive immune response to microbial antigens [2]. T helper (Th) cells and inflammatory cytokines play essential roles in the integrity of the gut mucosal immune system, prioritizing human cytokine-based therapy in IBD [3]. Notably, mutations in the interleukin-23 receptor (IL-23R) have been recently linked to the predisposition to IBD [4]. Composed of the p40 subunit of IL-12 and of a specific p19 subunit, IL-23 is an essential regulator of the development of effector memory T cells and the Th17 cell lineage [5, 6]. Both the pro-inflammatory cytokines IL-17 and IL-23 are key effectors in the pathogenesis of IBD (see below). Here we review the concept of cytokine-based therapy in CD and next discuss the recent clinical advances using IL-17/23-targeting molecules.

What have we learned from cytokine-based therapy in CD?

In IBD, several lines of evidence suggest the physiopathological role of a large set of inflammatory cytokines, for which antagonistic molecules have been evaluated in clinical trials for the development of new therapeutics (Tab. 1). To restore the integrity of the mucosal immune system, the first approach aimed at using recombinant cytokines to treat CD patients, such as the subcutaneous administration of recombinant human IL-10 (e.g., tenovil). IL-10 is an anti-inflammatory cytokine that inhibits both antigen presentation and subsequent release of pro-inflammatory cytokines, thereby attenuating mucosal inflammation. Despite encouraging preliminary results from a 24-week multicenter, prospective, randomized, double-blind, placebo-controlled, and sequential-escalating-dose study [7], further clinical data failed to show any difference in the induction of clinical remission in the Tenovil-treated group when compared to placebo [8]. Similarly, subcutaneous treatment of Tenovil failed to prevent post-operative recurrence in CD [9]. Recently, a clinical Phase I trial showed the safety and efficacy of a daily oral administration of a *Lactobacillus lactis* strain engineered to secrete IL-10 [10], suggesting that local (i.e., at the intestinal mucosa) administration of such anti-inflammatory cytokine might be one clue for efficient treatment in CD. Similar to IL-10, recombinant IL-11 showed limited efficacy in a large cohort [11] and no beneficial effect was reported for the use of recombinant type I interferons. Lastly in CD, the safety and efficacy of subcutaneous administration of recombinant human granulocyte-colony-stimulating factor (rhuG-CSF, filgrastim) and recombinant human granulocyte/monocyte-colony-stimulating factor (rhuGM-CSF, sargramostim) have been evaluated in Phase I and III clinical trials, respectively [12, 13]. After 12 weeks of follow-up, the admin-

istration of rhuG-CSF was apparently safe and led to encouraging results, as shown by the induction of clinical remission in about 25% of patients and by a significant decrease of at least 70 points of the clinical disease activity index (CDAI) score in 55% of patients. Mild transient bone pain was the most common reported side effect [12]. Similarly, in a 56-day Phase II placebo-controlled trial on active CD patients, the daily administration of rhuGM-CSF was efficient in inducing significant remission rate and in reducing the CDAI score of at least 100 points at days 15, 29 and 57. However, a high remission rate in the placebo group may explain the failure in achieving the primary endpoint at days 15 and 57. Besides injection side reactions, which were the most common drug-induced adverse event, migraine, demyelinating syndrome and ischemic heart disease were reported in 3 of 81 patients [14]. In addition to filgrastim, the safety and efficacy of sargramostim was evaluated recently in a Phase III study (referred as N.O.V.E.L. for New Opportunities to Verify Evolving Logic in CD). Despite encouraging results in Phase II [14], the remission and response rates at 56 days were not statistically different between the placebo ($n = 93$) and sargramostim-treated (*via* subcutaneous injection 6 µg/kg/day, $n = 193$) group (22.6% *versus* 22.3% and 33.3% *versus* 41.1%, respectively) [13]. However, there was an extreme heterogeneity between the placebo groups of each country involved in this clinical trial (Argentina, Australia, Brazil, Canada, New Zealand, Russia, Switzerland, Ukraine and United Kingdom). Further clinical trials are now awaited to improve the safety and pharmacokinetics of recombinant cytokines, e.g., by defining the optimal routes of administration. Together, the above clinical data support the notion that harnessing the immune system using recombinant cytokines might be a valuable therapeutic approach for CD treatment.

As a complementary approach, pharmacological blockade of cytokine and their receptors might be achieved using neutralizing monoclonal antibodies. Until now, tumor necrosis factor-alpha (TNF-α) is the only targeted cytokine used to treat IBD, reinforcing its physiopathological role in such chronic inflammatory disorders (Fig. 1, p. 217). TNF-α is a 17-kDa pleiotropic cytokine produced by macrophages, monocytes and T lymphocytes. TNF-α signaling influences multiple cellular processes, including the expression of inflammatory cytokines, adhesion molecules and the activation of neutrophils and lymphocytes [2]. Approved in 1998, infliximab is a chimeric monoclonal IgG1 with a high affinity to TNF-α. The half-life of infliximab after intravenous infusion of 5 or 10 mg/kg is 8–9.5 days. It proved to be efficient in inducing both biological and clinical response [15] and in maintaining clinical remission not only in CD [16], but also in UC [17]. Scheduled maintenance infusions of infliximab (at a dose of 5 mg/kg every 8 weeks) are now recommended rather than episodic treatment, with regards to efficacy and immunogenicity. More recently, another anti-TNF-α antibody has been approved for the treatment of CD (referred to as adalimumab). Adalimumab is a fully human recombinant IgG1 that shows a longer half-life than infliximab and binds with high affinity to both soluble and membrane-bound TNF-α. Subcutaneous administration of adalimumab was

Table 1 - Cytokine-based therapeutics evaluated in inflammatory bowel disease

Targeted cytokine	Generic/ brand Name	Alias	Manufacturer	Compound class	Mode of injection	Half-life (days)	Phase study (ClinicalTrials.gov identifier)	
							CD	UC
G-CSF	Filgrastim	Neupogen®	NIAID	Recombinant cytokine	s.c.	0.1	I (NCT00025805)	N/A
GM-CSF	Sargramostim	Leukine®	Bayer HealthCare Pharmaceuticals, Inc.	Recombinant cytokine	s.c.	0.1	III (NCT00329537)	N/A
IL-2	Daclizumab	Zenapax®	PDL Biopharma, Inc.	Humanised mAb	i.v.	20	N/A	II (NCT00073047)
IL-2	Basiliximab	Simulect®	Cerimon Pharmaceuticals	Chimeric mAb	i.v.	7	N/A	II (NCT00430898)
IL-6	C326	–	Avidia	Avimer	i.v.	7	I (NCT00353756)	N/A
IL-6	Tocilizumab	MRA, R-1569, atlizumab, Actemra®	Chugai Pharmaceuticals	Humanized mAb	i.v.	4	II	N/A
IL-10	Tenovil®	–	Schering-Plough	Recombinant cytokine	s.c.	0.1	III	N/A
IL-11	Oprelvekin	Neumega®, rhIL-11	Wyeth	Recombinant cytokine	Oral	0.1	II (NCT00040521)	I (NCT00038922)
IL-12/ IL-23p40	ABT-874	J695	Abbott	Humanized mAb	i.v.	9	II (NCT00562887)	N/A
IL-12/ IL-23p40	Ustekinumab	CNT0 1275	Centocor, Inc.	Fully human mAb	i.v., s.c.	20	II	N/A
IL-12/IL-23	Apilimod mesylate	STA-5326	Synta pharmaceuticals, corp.	Small molecule	Oral	N/A	II (NCT00234741)	N/A

IL-15	AMG-714	HuMax IL-15™	Genmab, Amgen, Immunex	Fully human mAb	s.c.	N/A	N/A	N/A
Interferon-β	r-IFN-β 1a	Rebif®	EMD Serono	Recombinant cytokine	s.c.	3	N/A	II (NCT00303381)
Interferon-β	r-IFN-β 1b	Betaseron®	Bayer HealthCare Pharmaceuticals, Inc.	Recombinant cytokine	s.c.	0.1	N/A	II (NCT00303381)
Interferon-γ	Fontolizumab	HuZAF™	PDL Biopharma, Inc.	Humanized mAb	s.c.	80	II	N/A
TNF-α	Etanercept	Enbrel®	Wyeth	Recombinant cytokine receptor	s.c.	0.6	II	N/A
TNF-α	Onercept	r-TBP-1	EMD Serono	Recombinant cytokine-binding molecule	i.v.	14	N/A	N/A
TNF-α	CDP-571	Humicade®	UCB Pharma	Chimeric mAb	i.v.	18	II (NCT00072943)	N/A
TNF-α	Infliximab	Remicade®	Schering-Plough	Chimeric mAb	i.v.	7	Approved	Approved
TNF-α	Adalimumab	D2E7, Humira®	Abbott	Fully human mAb	s.c.	15	Approved	III (NCT00573794, NCT00385736, NCT00408629)
TNF-α	Certolizumab pegol	CDP-870	UCB Pharma	Humanized Fab′ fragment	s.c.	14	III (NCT00552058)	N/A
TNF-α	Golimumab	CNTO 148	Centocor, Inc.	Fully human mAb	i.v., s.c.	7	N/A	III (NCT00488631, NCT00488774)

efficient in increasing the clinical response rate and in maintaining clinical remission in CD [18–20]. Besides infliximab and adalimumab, Certolizumab has been shown to be efficacious in inducing clinical response and in maintaining remission in CD [21, 22]. Certolizumab (also referred to as CDP-870) is a poly(ethyleneglycol) (PEG)ylated humanized Fab' fragment that does not induce apoptosis of T cells or monocytes, and shows a higher affinity and increased half-life when compared to infliximab. Additional molecules targeting soluble, but not membrane-bound, TNF-α have also been tested, but show no efficacy for induction and maintenance of response or remission in CD (CDP-571, etanercept and onercept). Together, the use of certain anti-TNF-α in management of CD is effective for maintenance of remission, steroid-sparing, and mucosal healing. However, important concerns still remain in that serious therapeutic complications have also been reported, such as increased risk of developing neurological disorders, neoplasia (such as hepatosplenic T cell and plasmablastic lymphomas), sepsis and/or opportunistic infections (e.g., tuberculosis, listeriosis, and endemic fungal infections) [23, 24]. Furthermore, about a third of patients fail to achieve clinical response (equally distributed between no response at all or partial response) and about half of responders showed evidence of loss-of-response, acute and delayed infusion reactions and immunogenicity, which influence the pharmacokinetics of anti-TNF-α. Lastly, a Japanese Phase I/II clinical trial has recently reported a potential therapeutical benefit of blocking the activation of the IL-6 signaling, by administrating intravenously a recombinant monoclonal anti-IL-6R antibody (referred to as tocilizumab). Tocilizumab binds with high affinity to both the soluble and the membrane-bound forms of the IL-6R. At the 12-week follow-up, the infusion of tocilizumab was seen to be tolerated and induced a clinical response (in 8 of 10 patients in the drug-treated group *versus* 4 of 13 in the placebo group, $p = 0.019$). Biological response was also improved, as shown by the reduced serological expression of erythrocyte sedimentation rate, C-reactive protein (CRP), serum amyloid A protein and fibrinogen. However, the study did not meet the secondary-end points, including clinical remission, which might be due to the small size of the groups [25]. Lastly, monoclonal anti-IL-2R antibodies (Daclizumab and Basiliximab) were shown to be tolerated, but failed to show any marked clinical efficacy in UC [26, 27]. Other molecules in the pipeline will be evaluated in the near future, e.g., anti-IL-15 (AMG-714) and anti-IL12/23 antibodies (Tab. 1).

Targeting the IFN-γ/IL-12 axis in CD

IFN-γ might also play a pivotal role in the pathogenesis of IBD. Fontolizumab is a humanized monoclonal antibody that binds and blocks IFN-γ. Two European clinical trials [28, 29] independently evaluated the safety and efficacy of fontolizumab in adult patients with moderate to severe CD. In both studies, treatment was generally well tolerated and a significant induction of the remission rate was

observed in about 60% of the treated patients when compared to the placebo group. Consistently, subcutaneous administration of fontolizumab triggered biological response, as shown by the decrease of serum CRP level [28, 29] and/or the median CD endoscopic index of severity [29]. Together, these clinical data are encouraging and await the results from a third, North American, Phase II, double-blind, placebo-controlled study.

More recently, small molecule inhibiting IL-12-related cytokines, such as apilimod mesylate (Synta pharmaceuticals, corp.), are currently being evaluated. Following selection from an 80 000-compound library and optimization, apilimod mesylate, also known as STA-5326, is a unique morpholinopyrimidine derivative, which is efficient in selectively down-regulating the IFN-γ/*Staphylococcus aureus*-induced expression of both IL-12 p35 and IL-12/IL-23 p40 in human peripheral blood monocytes without affecting their viability [30] (Fig. 1). Oral administration of STA-5326 at 10 mg/kg was also effective in improving the severity of T cell-mediated colitis in mice by inhibiting the secretion of IFN-γ by lamina propria mononuclear cells [30]. Despite the observation of dizziness, nausea, headache, and

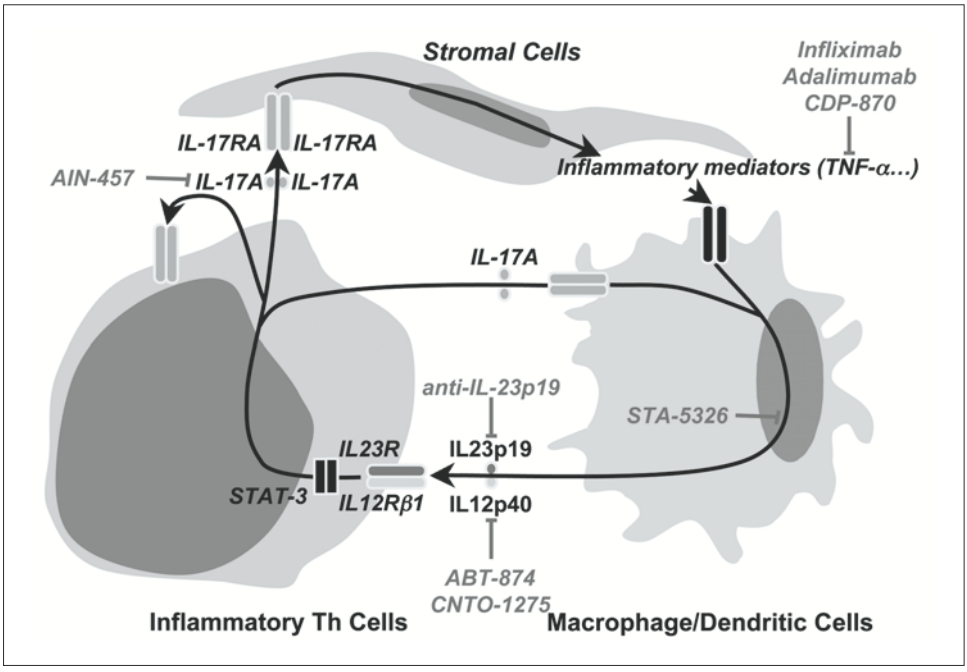

Figure 1
Overview of biological agents targeting IL-17 and IL-23 in Crohn's disease.

fatigue as the most common STA-5326-induced side effects, an open-label trial has reported encouraging preliminary results with regards to the safety and effectiveness of daily oral administration of STA-5326 [31]. A randomized, double-blinded and placebo-controlled Phase IIb clinical trial is now ongoing to evaluate the effectiveness of 4-week oral administration in adult patients with moderate to severe, active CD (NCT00234741). A better understanding of its biological mode of action, in general, and its role on Th1 cell apoptosis, in particular, might also provide clues to limit serious adverse events, such as increased risk of bacterial infections.

A human recombinant full-length IgG1λ genetically modified to recognize the IL-12 p40 protein (ABT-874/J695; Wyeth Research and Abbott Laboratories in collaboration with Cambridge Antibody Technology) has been evaluated in a multicenter, randomized, placebo-controlled, double-blind, Phase II clinical trial [32] (Fig. 1). Despite decreased expression of IL-12, IFN-γ, TNF-α and IL-17 in mononuclear cells of the colonic lamina propria [32, 33], subcutaneous administration of ABT-874 at 3 mg/kg was safe and 75% of ABT-874-treated patients experienced clinical response at the end of the 7-week treatment. However, at 18-week follow-up, it failed to be effective in inducing clinical response and remission [32], suggesting a pharmacodynamic effect. The efficacy (defined as a CDAI score of <150 points after 6 weeks), safety (at 12- and 24-week post-administration of placebo or ABT-874) and pharmacokinetics of intravenous infusions are now currently being evaluated in a Phase II clinical trial for the treatment of moderate to severe CD in adults (NCT00562887). Additional biologics remain on the horizon and might be promising. Notably, a multisite, randomized, placebo-controlled Phase IIa clinical study is now evaluating, over a 54-week period, the safety and efficacy of intravenous and subcutaneous infusion of CNTO 1275 in patients with moderate to severe CD (Centocor Inc. in collaboration with Medarex). CNTO 1275, also referred as Ustekinumab, is a fully human IgG1κ monoclonal antibody, which binds to the IL-12/23 p40 subunit with high affinity, thereby neutralizing subsequent activation of the IL-12/23-dependent signaling by blocking its binding to IL-12Rβ1 in activated human peripheral blood mononuclear cells [34] (Fig. 1). At the 8-week follow-up, CNTO 1275 induced clinical response, as defined by a reduction from baseline in the CDAI of ≥ 100 points (49.0% of CNTO 1275-treated patients *versus* 30.2% of placebo-receiving patients, $p = 0.05$). CNTO 1275 showed greater efficiency in patients previously treated with infliximab ($p = 0.02$) [35]. Consistently, short-term intravenous treatment with CNTO 1275 was sufficient to trigger biological response in patients with moderate to severe CD despite conventional therapy, as shown by reduced serological expression of inflammatory mediators [36]. Long-term follow-up of the safety and efficacy of CNTO 1275 is now eagerly awaited. Taken together, novel biological therapy targeting the IL-17/23 axis might be a more efficacious cytokine-based therapy, when compared to antibodies neutralizing both IL-12 and IL-23 (i.e., monoclonal anti-IL-23p19 and anti-IL-17 immunoglobulins, which are currently in development by Schering-Plough and Novartis Pharma GmbH, respectively).

IL-17/23, potential magic bullets in CD

Several lines of evidence suggest that Th17 cells play a predominant role in the pathogenesis of uncontrolled intestinal inflammation. In CD, Th17 cells, some of which produce both IL-17 and IFN-γ (Th17/Th1), are present in the inflamed mucosa of patients [33, 37, 38]. Expressing IL-23R, Th17 exhibit certain functional features similar to Th1 cells, such as their low cytotoxicity, ability to help B cells and poor potential to control regulatory T cells [37]. Besides its pro-inflammatory role, IL-23-dependent signaling events have been shown to protect against enteropathogens, such as *Citrobacter rodentium* [39] and *Helicobacter hepaticus* [40]. The transforming growth factor-β promotes responsiveness to IL-23 and subsequent commitment to Th17 development by regulating the expression of IL-23R on T cells [39]. Interestingly, commitment to Th17 development is potentiated by bacterial muramyl dipeptide, which is a cognate agonist of the CD-associated molecule NOD2 [41]. Similarly, Th17 differentiation and IL-17 secretion is dampened in germ-free mice [42], as well as in animals deficient for MyD88 – an essential adaptor of Toll-like receptor signaling [43]. In mice, IL-17 and IL-23 have been shown to be elevated in spontaneous models of IBD, the IL-10- and RAG1-deficient mice, respectively [42, 44, 45]. In addition, Th17 cells have a strong ability to induce colitis in a dose-dependent manner in the SCID transfer model [46]. In a SCID transfer model, inhibition of IL-23p19 by neutralizing monoclonal antibody was effective in both prevention and treatment of active colitis [46]. This pro-inflammatory role of Th17 cells has been confirmed in several mice models of colitis, including IL-10-deficient mice. Indeed, mice lacking IL-23 ($p19^{-/-}$) do not develop colitis, while knockout mice for the Th1 cytokine IL-12 ($p35^{-/-}$) strongly develop IL-10-dependent [6] and TCR-α-dependent colitis [47]. Resistance to colitis in IL-23-knockout mice correlated well with the absence of IL-17-producing $CD4^+$ T lymphocytes despite normal presence of antigen-specific-IFN-γ-producing Th1 cells [6]. Similarly, deficiency in protein kinase C θ is sufficient to decrease IL-17 production by colonic $CD4^+$ T cells and to give protection from spontaneous development of T cell-mediated colitis in both TCR-α- and IL-2-deficient mice [48]. Taken together, the IL-17/23 axis of inflammation represents a relevant therapeutical target in IBD.

Similar to monoclonal antibody targeting of the p40 subunit of IL-12/23, both pharmacological blocking using an IL-17R IgG fusion protein and genetic inactivation of IL-17R improved hapten-induced colitis in mice [49]. Recent clinical trials in asthma and autoimmune diseases prompted a clinical evaluation in IBD. Since January 2008, a multicenter, randomized, double-blind, placebo-controlled, parallel-group study (NCT00584740) in North America is now evaluating the efficacy, safety and tolerability of intravenous infusions of an anti-IL-17A monoclonal IgG1 (referred as AIN457; Novartis Pharma GmbH) in patients with moderate to severe active CD (Fig. 1). Primarily secreted by $CD4^+$ and $CD8^+$ lymphocytes, the isoform IL-17A is a homodimeric glycoprotein involved in several immunological

processes by triggering the maturation of hematopoietic progenitor cells and the synthesis of cytokines, chemokines, prostaglandins and metalloproteinases. Upon recognition by a type I transmembrane receptor termed IL-17R, IL-17A has a synergistic effect on the cellular response to additional cytokines. Isolated from a hybridoma, the recombinant AIN457 exhibits an affinity of 0.227 nM for interacting with human IL-17 and a dissociation equilibrium constant for binding to IL-17 of about 0.188 ± 0.036 nM, as defined by BIAcore measurements (WO/2007/117749). Additional monoclonal antibodies are also currently in development, such as an Fab fragment antibody attached to PEG moieties (WO/2008/001063, UCB Pharma SA). The affinity and half-life of such PEGylated molecules are increased (ranging from 133 to 365 pM), which might allow subcutaneous administration of these molecules. Taken as a whole, the therapeutical use of monoclonal anti-IL-17/23 neutralizing antibodies is encouraging and awaits additional clinical data.

Concluding remarks

Despite tremendous progress in mice, targeting the IL-17/IL-23 axis is still challenging in humans, as an impaired IL-12/IFN-γ function results in primary immunodeficiency leading to recurrent microbial infections [50]. The potential use of molecules antagonistic to IL-17/23 function now warrants long-term vigilance for the occurrence of serious side effects, including microbial infections and malignancies. Advances in our understanding of the function of both IL-17 and IL-23 might provide crucial clues to the development of more rational therapeutic methods directed towards the restoration of the integrity of the mucosal immune system in IBD [37].

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Novel tools and therapeutic avenues

IL-17A and Th17 cells as therapeutic targets for autoimmune diseases

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Abstract

The definition of the $CD3^+$ $CD4^+$ Th17 cell subset and the identification of the IL-23–Th17 axis have introduced new paradigms to explain the origin of autoimmune events in animal models, subverting the established Th1–Th2 paradigm. IL-17A has been pivotal for the discovery of the Th17 lineage, which probably evolved as an arm of the adaptive immune system for host protection against extracellular bacteria and fungi. IL-17A, is the founding member of the IL-17 family composed of six members. Th17 cells and IL-17A have been implicated in a variety of inflammatory and autoimmune diseases in rodents. In these models, Th17 cells are pivotal in the pathogenesis of the disease and IL-17A appears to be the main mediator, but the situation might be different in humans. In some human pathological conditions, in addition to Th17 cells, other IL-17A-producing cells have been described, including $CD8^+$ T cells, astrocytes, macrophages and Langerhans cells. The therapeutic effect of some new biologics can now, at least in part, be explained by their interference with mediators involved in the generation of Th17 cells, but more specific treatments would be valuable to dissect these intricate networks. An antibody neutralizing IL-17A is being evaluated under different autoimmune conditions. This approach might not only benefit patients, but, by neutralizing IL-17A selectively, might also help to define the role of this cytokine in autoimmune disorders and contribute to a new wave of selective and targeted therapies.

Introduction

The definition of the $CD3^+$ $CD4^+$ Th17 cell subset and the identification of the IL-23–Th17 axis have introduced new paradigms to explain the origin of autoimmune events in animal models, subverting the established Th1–Th2 paradigm [1]. IL-17A has been pivotal for the discovery of the Th17 lineage that probably evolved as an arm of the adaptive immune system for host protection against extracellular bacteria and fungi. Th17 cells and IL-17A are involved in the pathogenesis of several experimental autoimmune models of arthritis, colitis, psoriasis, uveitis, and encephalomyelitis. In these pathological conditions, Th17 cells are present and contribute

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to the inflammatory condition, neutralization of IL-17A ameliorates disease, and IL-17A-deficient animals are protected [2].

In mice, TGF-β and IL-6 appear to be involved in the commitment of naive T cells to the $CD4^+$ Th17 lineage. When IL-6 is lacking, as in knockout mice, IL-21 can step in and initiate an alternative pathway to induce pro-inflammatory Th17 cells [3]. Indeed, IL-21 is produced by Th17 cells and may be the main driver of an autocrine amplification loop. Once, IL-23R is expressed on the cell, IL-23 then drives Th17 cell survival. Still, several details of the process are missing. The Th17 cell population is probably regulated differently in man, where IL-1β in addition to IL-6 and IL-23 is required [4]. CCL20 is up-regulated in human Th17 cells and is a defining feature of the Th17 'signature', along with IL-17A, IL-17F and IL-22 [4].

Also in humans, several studies support a role for Th17 cells and IL-17A in autoimmune disorders. However, IL-17A can be produced by other cells including $CD8^+$ T cells, γδ T cells, a fraction of natural killer (NK) cells, neutrophils [1], macrophages [5], astrocytes and oligodendrocytes [6], and possibly Langerhans cells [7]. Therefore, it will be important to establish whether other $IL\text{-}17^+$ cells contribute to the pathogenesis of autoimmune disorders.

Differentiating between the role of Th17 cells and that of IL-17A is also relevant. While, in some instances, IL-17F and IL-22, cytokines released by Th-17 cells, might contribute to pathology, in other cases, the additional cell types able to produce IL-17A might participate. The introduction of more specific and targeted therapies, which might benefit patients reducing the risk of untoward side effects, remains an important goal.

IL-17A and IL-17F

IL-17A, previously called IL-17, is the founding member of the IL-17 family comprising at least six members [8, 9]. In the mouse, the IL-17 cytokine family consists of IL-17A (IL-17/CTLA-8), IL-17B (CX1/NERF), IL-17C (CX2), IL-17D , IL-17E (IL-25), and IL-17F (ML-1) [1]. IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F are normally secreted as homodimers [10–12]. IL-17A and IL-17F can be secreted by the same cell, and share 55% identity [10, 13].

In most studies, IL-17A does not act as a growth factor, but as an inflammatory cytokine, like IL-1 and TNF. IL-17A has a major role in inflammation, PMN recruitment, inflammatory angiogenesis, formation of germinal centers and granulomas, and tissue remodeling and destruction. IL-17A exhibits pleiotrophic activities on various parenchymal cells, including the induction of IL-6, IL-8, G-CSF, leukemia inhibitory factor, PGE2, ICAM-1, and matrix metalloproteinase in fibroblasts and other stromal cells, induction of TNF-α and IL-1β in macrophages, production of NO, inducible NO synthase, and cyclooxygenase-2 by chondrocytes, and induction

of RANKL in osteoblasts, as well as stimulation of granulopoiesis, maturation of dendritic cells, and costimulation of T cell proliferation [1].

IL-17A has been implicated as an agent contributing to progression and pathology of a variety of inflammatory and autoimmune diseases in humans and in mouse models of human diseases [14, 15]. On the other hand, the role of IL-17F in human diseases is still uncertain. Recently, the analysis of animals deficient in either of the two genes has revealed distinct functions of IL-17A and IL-17F in inflammatory responses [16]. Additional studies with neutralizing antibodies selective for these two cytokines are required to clarify further the contribution of IL-17A and IL-17F under inflammatory conditions.

Moreover, it is not yet clear how the production of IL-17A and IL-17F is regulated at the single-cell level in Th17 cells. In mice, the genes encoding IL-17A and IL-17F are located on chromosome 1, and Th17 cell development seems to be accompanied by epigenetic changes at both the IL-17A and the IL-17F gene promoters [17]. However, at the single-cell level, IL-17A and IL-17F are not always coexpressed, as IL-17A and IL-17F single-positive cells are detected at relatively high frequencies [16, 18]. Moreover, in mice, $CD4^+$ IL-17A single-positive T cells seem to be prevalent in the lamina propria and intestinal intraepithelium [16]. It is not clear whether this differential expression results from the existence of different cell types, of various stages of maturation or of environmental factors.

More recently, an IL-17A/F heterodimer has been described both in man and in mice [18–20]. In a Th17 cell adoptive-transfer model characterized by increased neutrophilia in the airways, Liang et al. [18] were able to show that IL-17A homodimers are the most biologically active, with IL-17A/F heterodimers displaying a moderate activity and IL-17F homodimers showing the lowest activity. The role of the heterodimer in inflammatory diseases in mice and humans needs further investigations.

IL-17A and IL-17F receptors

The receptor for IL-17A, once cloned, was named IL-17RA [9]. Bioinformatic analysis led to identification of four additional IL-17 receptor-related molecules and these are now designated IL-17RB, IL-17RC, IL-17RD, and IL-17RE [10]. Despite the relatedness of the IL-17 and IL-17R family, the receptors for only three members of this cytokine family have been identified. In humans, hIL-17A binds to hIL-17RA and hIL-17RC, hIL-17F binds to hIL-17RC [21] and hIL-17E binds to hIL-17RB [22].

Kuestner et al. [21] have recently raised questions about the recognition of IL-17A and IL-17F by their receptors. They showed that, in humans, hIL-17A binds to hIL-17RA and to hIL-17RC with high affinity and that hIL-17F binds to hIL-17RC. Binding of IL-17F to hIL-17RA occurs with a 1000-fold lower affin-

ity, suggesting that, under physiological conditions, hIL-17F does not bind to this receptor. In addition, they reported that, while hIL-17RA is expressed ubiquitously, with higher levels observed in hemopoietic cells, hIL-17RC is expressed only in non-hemopoietic tissues. In summary, in humans, non-hemopoietic tissues should respond to both hIL-17A and -F, whereas hemopoietic cells should respond only to hIL-17A. In contrast, in mice, mIL-17RA binds both mIL-17A and -F, whereas mIL-17RC binds only mIL-17F. This implies that mouse cells expressing mIL-17RA respond to both mIL-17A and -F, whereas those expressing mIL-17RC respond only to mIL-17F. Therefore, the relative contributions of IL-17A and -F in inflammation could be different in humans and mice. If these data are confirmed, the role of hIL-17A could be more prevalent in human pathological conditions, as hIL-17A should be able to activate cells through both hIL-17RA and hIL-17RC. However, a formal proof of the function of the different receptors *in vivo* is still lacking. The presence in man and mice of spliced variants of IL-17RC, which are not functional, further complicates the picture, opening the question of the role of the non-functional receptors.

Different results were obtained by Zrioual et al. [23], who described the expression of IL-17RA and IL-17RC in peripheral whole blood from normal subjects and an overexpression in rheumatoid arthritis (RA) patients. In hemopoietic cells, protein and mRNA levels of IL-17RA were higher than those of IL-17RC. Both receptors were expressed in $CD14^+$ monocytes and $CD19^+$ B cells. In $CD3^+$ T cells, lower levels of IL-17RA were measured, while IL-17RC was almost completely absent [23]. In addition, both receptors were broadly expressed in whole synovium tissues from RA and osteoarthritic patients with stromal and infiltrating immune cells positively stained [23].

These data support the observation that hIL-17RA and hIL-17RC may function as a heterodimeric receptor complex for hIL-17A and perhaps also for hIL-17F [24]. The picture might be further complicated by the presence of IL-17A/F heterodimers [19]. At present, no information is available on the relative binding of IL-17A/F heterodimers to the IL-17RA and IL-17RC in humans and mice.

Cells that produce IL-17A in human pathological conditions

Increased levels of IL-17A are detected in several human pathological conditions but few studies have addressed the cells that produce IL-17A. The identification of the IL-17A-producing cells is essential to define their contribution to disease pathogenesis and to design targeted approaches. In the peripheral blood of healthy subjects, T cells can produce IL-17A. The $IL\text{-}17A^+$ T cells reside in more than one subpopulation and they are distinguished by the differential expression of specific chemokine receptors [25]. However, in pathological conditions, the situation might be more complex and different cell types may contribute to the production of IL-17A.

In RA, Kotake et al. [26] not only reported elevated IL-17A protein levels in synovial fluid but also identified a subset of $CD4^+$, $CD45RO^+$ memory T cells producing IL-17A in synovial tissue. T cells making IL-17A were also detected in synovial fluid from patients with Lyme arthritis [27]. Flow cytometry was used to analyze the phenotype, cytokine production and chemokine receptor expression of IL-17A-producing T cells in peripheral blood and synovial fluid of children with juvenile idiopathic arthritis (JIA) [28]. IL-17A-expressing T cells were highly enriched in the inflamed joints and their number directly correlated with the clinical phenotype. In the joint, IL-17A was produced by $CD4^+$ T cells. These cells were present at significantly higher numbers in the joint than in either the paired blood samples from JIA patients or the blood of healthy controls. The IL-$17A^+$ T cells had the characteristics of memory $CD4^+$, $CD45RO^+$ cells and included cells that also produced IL-22 and IFN-γ, but not IL-4. Moreover, while, in peripheral blood, IL-$17A^+$ T cells were clearly distinct from either Th1 cells (defined by IFN-γ production) or Th2 cells (defined by IL-4 production), in the joints, more than half of the cells producing IL-17A were also positive for IFN-γ production. In PBMCs, the expression of CCR4 on IL-$17A^+$ T cells mirrored that of CCR6, while the synovial IL-$17A^+$ T cells expressed CCR6, but showed variable expression of CCR4. Therefore, circulating IL-$17A^+$ T cells seem to have different characteristic from synovial IL-$17A^+$ T cells. Flow cytometric data from synovial fluid T cell analyses estimated that IL-17A is produced by about 1% of synovial tissue T cells. The authors also suggested that Th17 cell migration to the joint may set up a positive feedback loop for further recruitment through the autologous production of CCL20.

Page et al. [29] identified IL-17A-producing cells in activated lymph nodes, in RA synovium, and in the endomysium of patients with dermatomyositis. In this study, IL-17A-producing T cells had a plasma cell-like morphology, expressed the CD4 but not the CD3 and/or B/plasma cell markers, and some cells expressed both IL-17A and IFN-γ. Even though the authors showed that IL-17A and/or IFN-γ producing cells can down-regulate the expression of CD3 markers *in vitro* after activation, the absence *in vivo* of the CD3 marker is somehow intriguing as, in other studies, the CD3 marker was used to identify IL-17^+ T cells [6, 27]. In conclusion, even though there is compelling evidence from several studies that T cells are involved in the production of IL-17A in RA, additional analysis should be performed to confirm the origin of the IL-17^+ $CD4^+$ $CD3^-$ cells. It might be interesting here to bear in mind that plasmacytoid dendritic cells are $CD4^+$ and $CD3^-$, that they were initially described as plasmacytoid T cells/monocytes, and that they are localized in the T cell areas of human reactive lymph nodes [30]. Moreover, it has been shown that, under specific culture conditions, some dendritic cells can also produce IFN-γ [31] and more recently, that Langerhans cells, a specialized subset of dendritic cells, have been shown to produce IL-17A [7].

In psoriatic patients, IL-17 messenger RNA (mRNA) was identified within lesions [32, 33], but an increase in serum protein levels was not observed [34].

$CD45RO^+$ memory effector T cells, either $CD4^+$ or $CD8^+$, seem to play a role in the pathogenesis of psoriasis [35]. $CD4^+$ T cells are abundantly present in the upper lesional dermis, whereas in the lesional epidermis $CD8^+$ T cells are in the majority [36, 37]. More recently, IL-17-producing T cells were recovered at increased levels in the lesional epidermal and dermal samples of psoriatic patients [38]. However, no further characterization of the IL-17-secreting cells was reported.

In patients with active ulcerative colitis (UC) and Crohn's disease (CD), Fujino et al. [5] reported significantly elevated serum IL-17A levels, while Nielsen et al. [39] described an increased gene expression. In addition, Fujino et al. [5] described a marked increase in $IL\text{-}17^+$ cells in the inflamed regions of active UC and CD patients. In active UC patients, $IL\text{-}17^+$ cells were localized mainly within the lamina propria but, in active CD patients, they were scattered throughout the submucosa and muscularis propria. In this study, both T cells and monocytes/macrophages were identified as a source of IL-17. In this study an anti-CD68 antibody was used for the identification of monocytes/macrophages. Even though CD68 is considered a marker specific for monocytes/macrophages, other cell types can express it [40], and additional studies might be useful to confirm a consistent production of IL-17A by monocytes/macrophages in these diseases.

Another pathological condition, in which IL-17A seems to have a prominent pathogenic role, is multiple sclerosis (MS). Recently, Tzartos et al. [6] performed a systematic analysis of $IL\text{-}17A^+$ cells in the brains of MS patients. Using double immunofluorescence and *in situ* hybridization, they showed that IL-17A immunoreactivity was detected in T cells, astrocytes and oligodendrocytes. $CD8^+$ as well as $CD4^+$ T cells were equally immunostained for IL-17A. $IL\text{-}17A^+$ T cells were particularly prominent in perivascular spaces in acute and chronic active MS lesions, where they accounted for the majority of $CD3^+$ T cells. They proposed that the large numbers of $IL\text{-}17^+$ T cells, found in both active and chronic active lesions, implicate that IL-17A is involved not only in initiating the lesions but also in their persistence. Moreover, $CD8^+$ T cells were predominantly found in the central nervous system parenchyma, whereas $CD4^+$ T cells remained primarily in the perivascular regions, suggesting a prevalent role of $CD8^+$ T cells in actual tissue destruction. The linkage between CD8 T cells and IL-17A may also influence future strategies for the treatment of MS. A potential role of $CD8^+$ T cells in the pathogenesis of MS has recently emerged due to genetic association of MS with MHC class I alleles, the failure of therapies directed to $CD4^+$ T cells and the presence of clonally expanded $CD8^+$ T cells in inflammatory central nervous system plaques [41]. In addition, Tzartos et al. [6] reported IL-17A immunoreactivity in astrocytes and oligodendrocytes. IL-17A immunostaining was abundant in the highly activated astrocytes in acute lesions and in the borders of chronic active lesions, but was barely detectable in astrocytes in control brain. These findings suggest that in an inflammatory environment astrocytes and oligodendrocytes may also contribute to IL-17 production and confirm previous data showing that IL-17A is expressed by human astrocytes cultured in the

presence of TNF-α and IL-1β [42]. Therefore, it seems likely that astrocytes (and perhaps oligodendrocytes) can be induced to produce IL-17 under the influence of activating stimuli. These findings corroborate previous microarray analyses of MS lesions obtained at autopsy that demonstrated highly increased transcripts of IL-17A [43], and elevated IL-17A levels in the cerebrospinal fluid of patients with opticospinal MS [44].

The fact that other cells in addition to Th17 cells might produce IL-17A in pathological conditions is stressed by the findings in Langerhans cell histiocytosis (LCH), where large amounts of IL-17A are detected in serum, and Langerhans cells are the main producers of IL-17A [7]. In this study, intralesional IL-17A expression was analyzed by triple staining (Langerin, CD3 and IL-17A), and IL-17A$^+$ dendritic cells were detected in skin and bone lesions. Very few IL-17A$^+$ T cells were found. Moreover, multinucleated giant cells always expressed large quantities of IL-17A [7]. Interestingly, in this study monocyte-derived dendritic cells were positive for IL-17A and were able to spontaneously secrete this cytokine, implying that they were activated *in vivo*. The authors concluded that the main cellular source of IL-17A in LCH is a dendritic cell subpopulation. Expression of IL-17A mRNA in activated peripheral blood monocytes/macrophages was also detected by Fujino et al. [5]. As it is not yet clear whether LCH is a neoplastic or a reactive disease [45], it is not possible to infer whether the ability to secrete IL-17A is acquired and due to an oncogene that has transformed the cells or whether it is intrinsic and due to the response of a subset of dendritic cells to an altered environment.

Some studies have relied on the isolation or extraction of cells from physiological fluids or tissues and on the analysis of cultured and stimulated cell populations by FACS. These studies have almost uniformly identified CD4$^+$ T cells as the main source of IL-17A. On the other hand, studies that have used histochemistry have often come to the conclusion that different cell types contribute to the production of IL-17A. Both approaches are valuable, but they may provide different information. The first approach offers a more precise identification of the cells but selectively enriches the sample for cell populations that are more easily extracted or apt to culture conditions. The main limitation of the histochemical approach is linked to the difficulty to precisely identify cell surface markers and often to the lack of specificity of the reagents. In conclusion, additional studies are required to more precisely identify the cells contributing to the production of IL-17A. and to identify the cells that secrete IL-17F, IL-17AF heterodimer and IL-22. IL-17F and IL-22 are expressed at increased levels in several diseases [46, 47].

Targeting Th17 cells and IL-17A and IL-17F in humans

Biologics not only provide clinicians with a new armamentarium to treat autoimmune diseases but also represent tools to dissect pathogenic events and to define the

role of cells and cytokines involved in autoimmune disorders. Several new biologics are or will become soon available to treat patients with autoimmune disorders. Many of these principles have several mechanisms of action, target molecules involved in many biological events, and it is often impossible to define a critical step that explains their therapeutic effect. Some of them also show an activity on Th17 cells.

Etanercept is a recombinant TNF blocker made from the combination of two soluble human 75-kDa TNF receptors linked to the Fc portion of an IgG1. Etanercept has shown beneficial effect in many clinical conditions including RA, psoriasis and ankylosing spondilitis. Recently, Zaba et al. [48] have shown that, in psoriasis, one of the mechanisms of action of etanercept is to inhibit Th17 cells by reducing the production of IL-23 by dendritic cells. It will be interesting to verify whether this mechanism of action is shared by other anti-TNF approaches and whether it is effective in other clinical conditions that benefit from an anti-TNF treatment.

Abatacept (CTLA-4-Ig) is another promising approach for treatment of RA patients. It is a soluble fusion protein that consists of the extracellular domain of human cytotoxic T lymphocyte-associated antigen 4 (CTLA-4) linked to the modified Fc (hinge, CH2, and CH3 domains) portion of human IgG1. The mechanism of action is to block costimulatory signals between antigen-presenting cells and T cells. The beneficial effect of an anti-CTLA4 approach in RA patients supports a role for T cell costimulation and might suggest a possible involvement of Th17 cells in the pathogenesis of this disease [49]. In fact, CD28 is not only a major costimulator for CD4 and CD8 naive T cells, it is also involved in the activation of memory Th17 cells [50].

Targeting IL-6 with a humanized anti-IL-6 receptor antibody is effective in several clinical studies in RA [51]. Several studies have shown that IL-6 is involved in the commitment of naive T cells to the Th17 cell lineage [1], and this mechanism of action may explain at least in part the therapeutic effect of this approach.

Ustekinumab, an antibody that neutralizes the effects of IL-12 and IL-23 by binding their shared p40 subunit, has shown dramatic effects in patients with psoriasis [52, 53]. IL-23 is directly involved in the survival of Th17 cells and this suggests that Th17 cells and their products might be involved in the pathogenesis of the disease. However, this antibody targets not only IL-23 but also IL-12, and it is not possible to differentiate the effects on Th17 cells *versus* those on Th1 cells. Therefore, to dissect further these cytokine networks, it would be important to develop even more specific tools and selectively block the effects of only one cytokine. Such targeted biologics might not only be of therapeutic value for the patients but also reduce further the risk of untoward side effects. With this goal in mind, we have generated a high-affinity fully human monoclonal anti-human IL-17A antibody of the IgG1/kappa isotype, which is now being developed for the potential treatment of several autoimmune disorders. This antibody is specific for IL-17A and does not recognize IL-17F. Therefore, it targets only one of the cytokines secreted by Th17

cells, and it will be important to assess its therapeutic effects in comparison with less selective approaches such as anti-p40 antibodies. On the other hand, in addition to Th17 cells, other cells may contribute to production of IL-17A in various pathological conditions. In conclusion, each therapy might have its own specific signature and it will be important to plan clinical trials able to highlight these differences, and to optimize treatment for the patients.

An additional approach is to use a soluble IL-17RC-Fc fusion protein [21]. This soluble fusion protein will probably consist of the extracellular domain of a human IL-17RA/RC-Fc fusion protein and will neutralize the biological effects of both hIL-17A and hIL-17F. By targeting IL-17F in addition to IL-17A, a different therapeutic profile might be expected.

In conclusion, through the development of new approaches targeting new cytokines and cell networks, not only will the patients have access to effective and more sophisticated therapies that might have a lower incidence of side effects, but the scientific community will acquire new knowledge about the role of cytokines and cells under different pathological conditions.

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