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IL-17, IL-22 and Their Producing Cells: Role in Inflammation and Autoimmunity

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Editors

IL-17, IL-22 and Their Producing Cells: Role in Inflammation and Autoimmunity

Springer

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Preface

The IL-17 cytokines represent a novel family of cytokines, which extends the Th1-Th2 paradigm and defines a new effector T cell, the Th17 cell. Th17 cells may co-express at least IL-17A and IL-17F and IL-22. IL-17A and IL-17F can be produced by CD4+ T cells (Th17 cells), CD8+ T cells (Tc17), γδ T cells, iNKT, NK cells, lymphoid tissue inducers cells, innate lymphoid cells, mast cells, neutrophils and Paneth cells. IL-17F can be expressed also by epithelial cells. IL-22 can be produced by CD4+ T cells (Th22) as well as other cell types. The regulation of IL-17 family members and the effector cells and mechanisms are an area of intense current research, and include as an example the nuclear receptors RORgt and proinflammatory cytokines such as IL-1, IL-6, TGFβ, IL-21 and IL-23. Some experimental data suggest that IL-17A may have a dual function – proinflammatory and anti-inflammatory – suggesting that IL-17A may also terminate inflammation. Further, the reciprocal regulation of Th17 and regulatory T cells including the role retinoic acid is highlighted.

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

Experimental studies confirmed that IL-17A is induced and is critical for the development of allergic lung inflammation, arthritis, inflammatory bowel disease, experimental allergic encephalomyelitis and other inflammatory conditions and organ transplantation. Recent investigation revealed a dual role of IL-22 in inflammation, and IL-22 can be considered as an important protecting factor at the mucosal and cutaneous barriers. A newly defined innate lymphoid cell type emerged as an important source of IL-22 which activates STAT3-dependent protecting peptides at the barrier sites. IL-17A neutralization inhibited experimental arthritis opening a new therapeutic possibility to treat rheumatoid arthritis.

Neutralization of IL-17A, however, might alter host defense to microbial pathogens, such as mycobacteria and opportunistic extracellular pathogens including fungi, and tumor host responses. Therefore the introduction of neutralizing therapies

may require special cautions. However, even if safety data in clinical studies are limited, they have been favorable to date.

Tools for experimental investigations such as recombinant proteins, neutralizing antibodies and gene deficient mice have been developed and are discussed in more detail.

Clinical studies in psoriasis, rheumatoid arthritis, ankylosing spondylitis and psoriatic arthritis suggest that IL-17 antibody neutralization may be an interesting addition for the treatment of these diseases. However studies in Crohn's diseases have not kept the promise. Clinical studies in other autoimmune diseases and in asthma are ongoing and new insights in the pathogenesis of these diseases are expected.

The fully revised multi-author contribution with experts in the field will be very useful for scientists and medical doctors exploring novel mechanisms of inflammation and therapy. This comprehensive review on IL-17, IL-22 and Th17 cells is an updated summary by experts and a digest of the literature, which exploded in the last years. Finally, we wish to thank the authors who dedicated their precious time with expert contributions which make this an outstanding volume in Inflammation Research.

V. Quesniaux
B. Ryffel
F. Di Padova

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Part V Novel Tools and Therapeutic Avenues

Part I
Discovery and Basic Aspects of IL-17

IL-17A Family, Receptors, Proinflammatory Effects, and Production

Franco Di Padova, Bernhard Ryffel, and Valerie Quesniaux

Abstract IL-17A is a proinflammatory cytokine with critical effects on many cell types. It is part of a larger family of cytokines with important roles in protection against pathogens. The IL-17A receptors are IL-17RA and IL-17RC. They are expressed on most cell types. IL-17A, in synergy with other cytokines such as TNF-α, induces the production of cytokines, chemokines, and mediators of tissue destruction in several cell types. The identification of IL-17A and, later on, of Th17 cells has modified the established Th1 and Th2 paradigm, led to the definition of a new CD3+ CD4+ effector T 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 infections and autoimmune models. IL-17A is in fact produced by several other cell types involved in host defense, autoimmunity, and inflammation, and they might also be involved in IL-17A induced pathology.

Keywords IL-17A • IL17F • IL-17RA • IL17RC • Th17 cell

1 IL-17A and Other IL17 Family Members

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 protein. This sequence

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had homology to an open reading frame encoded within a T cell-tropic c-herpesvirus, Herpesvirus Saimiri, and they suggested that it might be a novel cytokine [1]. Later on, the protein described was found to be a rat protein [2]. The human homologue was reported in 1995 and was mainly produced by activated CD4+ T cells, induced the secretion of IL-6 and IL-8 in human foreskin fibroblasts, and enhanced 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].

Human IL-17A is a glycoprotein containing 155 amino acid residues and shares 63 % amino acid identity with murine IL-17A (147 amino acids). Both human and mouse IL-17A are secreted as disulfide-linked homodimers. Now, the IL-17 family includes 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). In the mouse system, IL-17F has 45 % amino acid homology to IL-17A, followed by IL-17C (24 %), IL-17B (21 %), IL-17D (16 %), and IL-17E (16 %) [5]. IL-17A and IL-17F are secreted by overlapping populations of T cells and form both homodimers and heterodimers [6, 7]. IL-17B, IL-17C, and IL-17E differ substantially from IL-17A and IL-17F in the N terminus, having longer extensions. Furthermore, IL-17B is secreted as a noncovalent dimer [8]. Common features of the IL-17 family are a highly conserved carboxyl terminus and five spatially conserved cysteine residues. Four of these cysteines form a cystine knot fold. Transforming growth factor (TGF)-β, bone morphogenic protein, and nerve growth factor superfamilies show structural similarities but have an additional disulfide bond [8].

The genes for IL-17A and IL-17F are located on human chromosome 6p12.2, while the genes of the other IL-17 family members are located on different chromosomes: IL-17B on Chr 5q32, IL-17C on Chr 16q24.3, IL-17D on Chr 13q12.111, and IL-25 on Chr 14q11.2. Polymorphisms in IL-17A (rs2275913) gene have been weakly associated with rheumatoid arthritis [9], while polymorphism in IL-17F (rs763780) gene has been associated with a protective role in asthma [10]. Polymorphism at the 197A (IL-17A) and 7488T (IL-17F) alleles may influence the susceptibility to and pathophysiological features of ulcerative colitis [11]. In the Chinese population, rs2275913 and rs763780 are associated with immune diseases and infectious diseases, including asthma, Vogt–Koyanagi–Harada syndrome, bacterial bronchiolitis, and neuromyelitis optica [12–14].

2 The Receptors

When the mouse and the human IL-17 receptors (IL-17RA) were cloned, IL-17RA was found to be ubiquitously expressed and unique. It consisted of a single transmembrane receptor with an unusually long cytoplasmic tail [15, 16]. Now, five different receptors have been described, IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE [17]. The IL-17R family contains conserved structural characteristics such as extracellular fibronectin III-like domains and cytoplasmic similar expression to fibroblast growth factor, IL-17R, and Toll-IL-1R family

(SEFIR) domains. IL-17A and IL-17F signal through a receptor complex composed of IL-17RA and IL-17RC [18]. In humans IL-17RA binds with higher affinity to IL-17A than to IL-17F, whereas IL-17RC associates with both IL-17A and IL-17F [18, 19]. Cristal structure of the IL-17F-IL-17RA interaction reveals the presence of a loop of IL-17RA which interacts with the deep groove at the interface of IL-17F homodimer [18]. IL-17RD has also been associated with the IL-17R complex and may contribute to IL-17A signaling [20]. IL-17RB is the receptor chain for IL-25 and IL-17B [21, 22]. The receptor for IL-17C appears to be a heterodimeric complex formed by IL-17RA (low-affinity interaction) and IL-17RE (high-affinity interaction) [23–25].

In conclusion, IL-17RA is shared among the members of the IL-17 family, while the specificity of the interaction mainly resides in the second subunit of the heterodimeric receptor complex. IL-17RC is common to IL-17A and IL-17F homo- and heterodimers; IL-17RB is specific for IL-25 and IL-17B, IL-17RE for IL-17C, and IL-17RD may be part of the IL-17A receptor complex [23–25]. Since IL-17RA is ubiquitously expressed, also the tissue specificity of the responses resides in the second receptor chain which is differently expressed in various tissues.

3 IL-17A as a Proinflammatory Cytokine

Several reports suggest that IL-17A has a critical role in the protection of the organism against bacteria and fungi due to the ability of IL-17A to recruit neutrophils to the infected organs/tissues [26, 27]. IL-17A and IL-17F can induce the production of proinflammatory cytokines (IL-1, IL-6, TNF-α, G-CSF, and GM-CSF), chemokines (CXCL1, CXCL5, IL-8, CCL2, and CCL7), antimicrobial peptides (defensins, lipocalin2, and S100 proteins), and matrix metalloproteinases (MMP1, MMP3, and MMP13) from different cell types. IL-17A also controls the expression of intercellular cell adhesion molecule 1 (ICAM-1) in keratinocytes as well as iNOS and cyclooxygenase-2 in chondrocytes. IL-17F is a weaker inducer of proinflammatory cytokine but seems to be produced by a wider range of cell types, including innate immune cells and epithelial cells. Not unexpectedly, IL-17A and IL-17F act in concert with other cytokines such as TNF-α, with which they strongly synergize in in vitro cellular assays and in vivo. It is expected that this characteristic is shared by the other IL-17 family members. Moreover, IL-17A contributes to germinal center formation and class switch recombination by its action on B cells [28, 29].

In summary, IL-17A, acting in synergy with other cytokines, contributes to modulate and control the inflammatory response. Some events, e.g., neutrophil recruitment, are indirectly mediated through the activity of IL-17A on cells which secrete chemotactic factors for neutrophils. Therefore, the mediators, secreted by activated cells, modulate the final outcome of the response to IL-17A.

4 IL-17A-Producing Cells

In 2000, Infante-Duarte et al. [30] recognized that IL-17A cannot be categorized as either a Th1 or a Th2 cytokine, but a major advance in IL-17 research came with the recognition of a novel T cell lineage producing IL-17A and/or IL-17F [31, 32]. 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.

In the mouse, IL-17A and/or IL-17F are however produced by several other cell types involved in host defense, autoimmunity, and inflammation, including subpopulations of CD8+ T cells (Tc17), γδ T cells [33], invariant natural killer (iNKT) cells [34], lymphoid tissue inducers [35], neutrophils [36], and macrophages [37]. In addition, IL-17A is produced by intestinal Paneth cells in an IL-23 independent fashion being one of the causes of a systemic inflammatory response syndrome [38], and IL-17F mRNA is expressed in colonic epithelial cells [39]. In particular, Jensen et al. [33] 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).

Yoshiga et al. [40] demonstrated that also iNKT cells synthesize and release IL-17A, accelerate IL-17 production by Th17 cells, and play a role in the development of collagen-induced arthritis (CIA). 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 [40]. 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 [41]. Addition of exogenous IL-10 abolishes IL-17A production both in Th17 cells and in macrophages [41], again suggesting that IL-17A production is highly regulated.

In humans, IL-17A has been detected in several T cell subsets, such as naive, central memory, and effector memory CD4+ IL-17+ T cells [42], NKT-like cells [43], macrophages [44], astrocytes [45], oligodendroglia [45], mast cells [46], neutrophils [47], and also a human myeloma cell line [48].

The large number of cell types able to secrete IL-17A and IL-17F, the migratory potential of the cells secreting these cytokines, the ubiquitous distribution of the receptor, and the synergism with other proinflammatory cytokines allow IL-17A and IL-17F to exert effects in multiple pathological conditions [26, 49]. In addition, Th17 cells act in close contact with their target cell, and IL-17A might have a short range of action and act in synergy with other cytokines secreted by Th-17 cells [50]. The role of other family members might be more restricted due to a more limited expression and tissue distribution of their receptors.

A pathological role for IL-17A in several autoimmune models of disease, such as experimental autoimmune encephalomyelitis (EAE) and arthritis, has been documented [5], and increased production of IL-17A has been reported in various

human autoimmune and allergic diseases, such as rheumatoid arthritis [51], multiple sclerosis [45], psoriasis [52], and asthma [53]. All these findings suggest a pathogenic role in several human diseases which has been confirmed by the clinical efficacy of IL-17A neutralization in different disease [54].

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IL-22 and IL-17: An Overview

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Abstract Many studies from recent years have shown that cytokines like IL-22, IL-17A, and IL-17F play a major role in both the defense against certain microbes and the development and maintenance of chronic inflammatory diseases. These mediators are often secreted by subpopulations of T-helper cells called Th17 cells and Th22 cells, respectively. This chapter provides an overview about 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, most basic aspects of IL-22 and IL-17A/IL-17F are different.

1 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, but they also regulate hematopoiesis, wound healing, angiogenesis, and physiological and pathological tissue reorganization. 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. Due to their similarities in regard to genome location, gene structure, secreted protein structure, and receptors used, various cytokines were grouped

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into families. It is important to note that members of the same family do not necessarily exhibit similar biological effects.

The application and repression of the cytokine action set the most successful strategy for therapy of infections and autoimmune diseases, respectively. Many studies from recent years have shown that mediators like IL-17A and IL-22 play important roles in chronic immune-mediated diseases. While IL-17A belongs to the IL-17 cytokine family, IL-22 is a member of the IL-10 cytokine family.

The IL-10 family comprises IL-10, IL-19, IL-20, IL-22, IL-24, IL-26, IL-28A, IL-28B, and IL-29 [1]. IL-28A, IL-28B, and IL-29 (also designated as interferon-λ s) are the "youngest" members of IL-10 family 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 comprising the genes for IL-10, IL-19, IL-20, and IL-24 on chromosome 1q; the second comprising the IL-26- and IL-22-encoding genes located on chromosome 12q; and the third comprising the genes for IL-28A, IL-28B, and IL-29 on chromosome 19q [1–3]. Apart from the high amino acid (aa) identity within the interferon-λ subgroup, the aa identity between the members of the IL-10 family is approximately 13–25 %. However, characteristic aa positions are conserved. Despite the relatively low sequence identity, all family members show a strikingly similar secondary structure; these α-helical proteins are built up of six to seven helices in an antiparallel conformation [4]. All IL-10 family members exert their biological effects via heterodimeric receptor complexes composed of an R1 subunit and an R2 subunit [1]. The subunits 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 [1, 2]. 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 subunit has the longer intracellular moiety able to bind signal transducers and activators of transcription (STAT) molecules. For IL-10 and IL-22, it is believed that ligand binding initially occurs to the R1 subunit (IL-10R1 and IL-22R1, respectively). This induces a conformational change in the respective cytokine that enables it to bind secondarily to the R2 subunit (IL-10R2 in both cases), leading to an aggregation of the two receptor subunits and initiation of signal transduction. Interestingly, in the case of IL-19, IL-20, and IL-24, the initial binding occurs to the R2 subunit. Importantly, the IL-10 family members share receptor subunits (the IL-10 family comprises nine cytokines; there are only four R1 subunits and two R2 subunits for these cytokines; Table 1). For instance, the IL-10R2 subunit 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 subunits are shared among

Table 1 Combination of receptor subunits 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	?

different IL-10 family members but even whole receptor complexes (Table 1). For instance, the complex composed of IL-20R1 and IL-20R2 is used by IL-19, IL-20, and IL-24. Despite the structural relation and the use of similar or partly identical receptors, there are great differences with respect to the biologic function between several of the IL-10 family members [3, 5–9].

The IL-17 family consists of six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (IL-25), and IL-17F [10, 11]. The genes encoding these mediators have an only moderately similar structure, which is completely different from that of the IL-10 family genes. The genes for IL-17A and IL-17F are located on chromosome 6q; the IL-17B-encoding gene is located on chromosome 5q; that of IL-17C is on chromosome 16q, the IL-17D-encoding gene lies on chromosome 13q, and the gene for IL-17E was mapped to chromosome 14q. The IL-17 family members are also structurally related. The characteristic motif is the C-terminal, so-called cysteine-knot. The highest sequence similarity exists between IL-17A and IL-17F (47.8 % identity between the 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. The receptor subunits differently assemble into receptor complexes for IL-17A and IL-17F (IL-17RA/IL-17RC), IL-17B and IL-17E (IL-17RA/IL-17RB), and IL-17C (IL-17RA/IL-17RE), while the functional receptor complex for IL-17D is not known so far [10]. Interestingly, IL-17A and IL-17F mediate in part similar biological effects [10, 12].

2 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 kbp and 99 kbp upstream from the *IL26* and *IFNG* locus, respectively, and has the same transcriptional orientation as these two adjoining genes (reverse strand) [13]. The *IL22* gene is 5,257 bp long and is comprised of five exons with the following lengths: 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, National Center of Biotechnology Information (NCBI), February 2012). The first 53 bp of exon 1 encode the 5′-untranslated region. The other portion of exon 1 (186 bp), the exons 2–4, and the first portion (75 bp) of exon 5 contain the

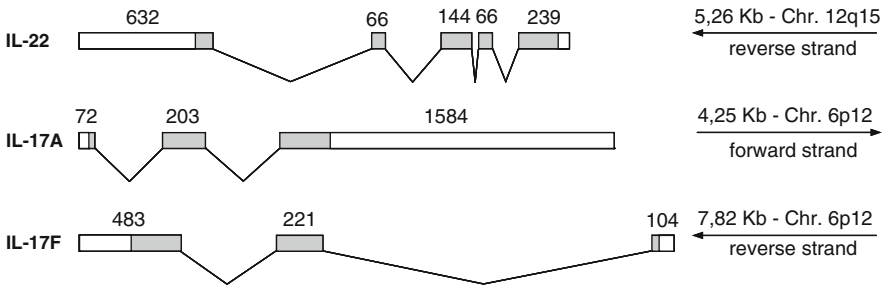

Fig. 1 *The IL-22 and IL-17A/IL-17F coding genes have different structures*. Shown are the exon–intron structures of the genes. Exons (1 cm corresponds to 250 bp) are presented as *boxes* comprising noncoding regions (in *white*) and coding regions (in *gray*). Introns (1 cm corresponds to 875 bp) are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

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 [11, 14]. The distance between these genes is 46 kbp. *IL17A* is located on the forward strand and has a length of 4,252 bp, and *IL17F* is located on the reverse strand and has a length of 7,815 bp. Both genes are comprised of three exons with the following lengths: exon 1, 72 (*IL17A*)/104 (*IL17F*) bp; exon 2, 203 (*IL17A*)/221 (*IL17F*) bp; and exon 3, 1,584 (*IL17A*)/483 (*IL17F*) bp. The IL-17A mRNA straddles 1,859 bp (NM_002190, NCBI, February 2012) and IL-17F mRNA 808 bp (NM_052872, NCBI, December 2011). The first 45 bp and 71 bp of *IL17A* and of *IL17F* exon 1, respectively, encode the 5′-untranslated region. The other portion of exon 1 and 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.

3 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 (33 aa), is secreted as a polypeptide of 146 aa [15]. Amino acid sequence alignment (mature proteins) revealed that human IL-22 has an 80.8 % identity to murine IL-22 [13]. Like all other IL-10 family members, IL-22 has an α-helical structure. The six helices (referred to as helices

A to F) are arranged in an antiparallel conformation resulting in a monomeric, bundle-like protein. This structure was proved upon its resolution by X-ray diffraction [16, 17]. Monomeric conformation of IL-22, at least at physiologically relevant concentrations, was also confirmed by gel filtration chromatography and dynamic light-scattering studies [16, 18]. It should be noted that in the IL-10 structure, 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 to D from one partner and helices E and F from the other partner [19, 20]. 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 [16].

The theoretical molecular weight 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 [21, 22]. 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) [22]. Interestingly, the glycosylation is not associated with a noteworthy change of the tertiary structure of IL-22 [16, 17]. 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 [22].

IL17A and *IL17F* encode proteins of 155 aa and 163 aa, respectively, in length. After splitting off the signal peptides (23 aa for IL-17A and 30 aa for IL-17), the secreted polypeptides are 132 aa (IL-17A) and 133 aa (IL-17F) long. The crystal structure of IL-17A and IL-17F was resolved and shows that the proteins form similar disulfide-linked homodimers [23, 24]. The core of the IL-17F monomer is composed of two pairs of antiparallel 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 [23]. The structure of the IL-17F homodimer includes a classical Cys-knot motif, which is also found in the transforming growth factor (TGF)-β and nerve growth factor superfamilies [25]. One difference in the cysteine-knot motif of IL-17F compared with the other known Cys-knot protein families is that it only utilizes four Cys instead of the classical six Cys to form the knot. In addition to IL-17A and IL-17F homodimers, an IL-17A–IL-17F heterodimer also exists. The theoretical molecular weight of secreted human IL-17A and IL-17F is 15.1 and 14.9 kDa, respectively.

4 The Cellular Sources of IL-22 and IL-17

During immune responses, IL-22 and IL-17A/F are often simultaneously present at high levels in inflamed tissues. Compatibly, these mediators can be secreted by numerous identical types of cells from the lymphoid lineage. In humans, these cell

types include activated $CD4^+$ T cells [26–28], $CD8^+$ T cells [26, 29, 30], and γδ T cells [31] as well as various innate lymphoid cells such as NK cells [26, 32–34], NKT cells [35, 36], lymphoid tissue inducer (LTi) [37, 38], and LTi-like cells [39, 40]. However, in humans IL-22 and IL-17A/F are rarely secreted by exactly the same cell population. For example, regarding the $CD4^+$ T cells, IL-22 is produced by the Th22 [41, 42] and the Th1 subpopulation [26, 43]. In contrast, IL-17A/F are secreted by the Th17 subpopulation [27, 28], which does not produce IL-22 [43–45]. Likewise, the simultaneous expression of IL-22 and IL-17A/F by the same γδ T cell [31] or NKT cell [35] is rather an exception. Interestingly, in humans the IL-17 production by $CD8^+$ and γδ T cells is often accompanied by IFN-γ secretion [29, 31].

It is important to note that no expression of IL-22 was found in monocytes, macrophages, immature or mature DCs that were generated in vitro from human blood monocytes, or mast cells that were isolated from the skin of healthy donors [26, 46]. Furthermore, non-hematopoietic tissue cells do not produce IL-22 either [46–49]. In contrast to IL-22, the cellular sources of IL-17A/F do not seem to be restricted to hematopoietic cells. In fact, IL-17A production has been described by Paneth cells, highly specialized epithelial cells of the small intestine [50].

The above-mentioned Th1 cells develop in the presence of IL-12 as IFN-γ-producing, IL-12Rβ2 and CXCR3, and mostly CCR6 chemokine receptor-expressing cells. They are programmed by the transcription factors T-bet and STAT4 [51].

Th22 cells particularly develop from naive $CD4^+$ T cells via interaction with pDCs in dependency of TNF-α and IL-6 [41]. Apart from IL-22, these cells do not produce any other known Th prototypical mediator such as IFN-γ, IL-4, or IL-17. Th22 cells carry a unique cell surface set of chemokine receptors (CCR6/CCR4/CCR10) and strongly express the aryl hydrocarbon receptor (AHR) as transcription factor [41, 42]. Via induction of endogenous AHR stimulators, Notch signaling then drives the production of IL-22 by these cells [52]. Interestingly, AHR ligation inhibits the production of IL-17 by human $CD4^+$ cells [42, 53].

Like Th1 and Th22 cells, Th17 cells are characterized by their cytokine production: IL-17A, IL-17F, and IL-26. Furthermore, they specifically express transcription factors such as RORγt and a cytokine/chemokine receptor pattern including IL-23R, CCR6, and CCR4, while lacking the expression of IL-12Rβ2, CXCR3, and CCR10. It was first in 2005 that two independent groups demonstrated that the activation of murine naïve T cells with mature dendritic cells (DCs) in the presence of IL-6 and TGF-β upregulated the receptor for IL-23 (p19/p40) on the T cells and, together with IL-23, induced their development into Th17 cells [27, 28]. In the human system, the combination of IL-23, TGF-β, IL-1β, and IL-6 appears to be necessary for development of Th17 cells. Many years later, Th17 differentiation-promoting activities were also assigned to PGE_2 and TLR2 stimulation [54–56]. The Th1 cell cytokines IL-12 and IFN-γ as well as the Th2 cell cytokine IL-4 actively suppress the development of Th17 cells [27, 28]. Interestingly, whereas in the human system Th22 and Th1 cells seem to be most important Th cell sources of IL-22 (as mentioned above), in the murine system Th17 cells were suggested to be the major IL-22 producers [43–45].

These observations suggest a profound species difference and imply caution in extrapolating mouse data onto humans.

Which type of cells actually contributes to IL-22 or IL-17A/IL-17F production in vivo depends on the kind (acute or chronic) and extent (local or systemic) of inflammation, the type of pathogen, and the site of pathogen entry. Corresponding to the preferential production of IL-22 and Th17A/F by Th1/Th22 and Th17 cells, respectively, elevated levels of these cytokines exist in chronic diseases, which are mediated by these T cells. Indeed, high IL-22 and IL-17A mRNA levels can be found in lesional skin from psoriasis patients, whereas IL-22 and IL-17A were completely absent in healthy skin of control donors [43, 47, 48, 57]. Importantly, besides its high cutaneous expression, IL-22 is also systemically present in these patients, and circulating IL-22 levels strongly correlate with the disease severity [48]. This is in contrast to other Th cell cytokines overexpressed in lesional psoriatic skin, making IL-22 a unique mediator. In addition to psoriasis, high cutaneous levels of IL-22 are present in the chronic T cell-mediated skin disease atopic dermatitis [47], whereas both IFN-γ and IL-17A are not or at low level present in lesions of these patients [43, 47, 58]. Interestingly, in another chronic skin disorder, acne inversa, the contrary situation exists: Cutaneous IFN-γ and IL-17A are strongly elevated, whereas IL-22 expression is only minimally upregulated [43]. Abundant presence of IL-22 and IL-17 has been demonstrated in active Crohn's disease and ulcerative colitis lesions [59, 60]. As in psoriasis, systemic IL-22 levels in Crohn's disease patients correlate with disease activity, although other disease-associated Th cell cytokines are scarcely detectable [60, 61]. A massive IL-22 and IL-17 presence was also found in inflamed tissues of patients suffering from rheumatoid arthritis [62–64]. However, in contrast to IL-22, IL-17 does not appear to be present in the blood of rheumatoid arthritis patients [65, 66]. Finally, IL-22 expression was found during various infectious diseases (e.g. *Mycobacterium tuberculosis* infection, *Campylobacter jejuni* infection, abdominal sepsis, and HIV infection) [67–69].

It is important to note that in experimental mice models, innate lymphoid cells are often the source of IL-22. For example, the high IL-22 levels in mice, found in certain organs during acute systemic inflammation after LPS application or during intestinal infection with *Citrobacter rodentium*, seem to derive from IL-23- and RORγt-dependent LTi(-like) cells as demonstrated by the use of T cell-deficient (Rag2-/-), p19-/-, or RORγt-/- mice (Sabat and Wolk, unpublished data, [70, 71]). In contrast, NKT cells have been demonstrated as being principal IL-22 sources in experimental *influenza A* virus lung infection [72].

5 Receptors

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

The IL-22 receptor complex is composed of IL-22R1 and IL-10R2 (Table 1 and [15, 21, 73]). In accordance with the CRF2 characteristics, both transmembrane subunits have an extracellular moiety containing two tandem FNIII domains with several aa positions conserved within this receptor family [2]. The human IL-22R1-encoding gene (*IL22RA1*) is located on chromosome 1p36.11 (reversed strand), near the *IL28RA* locus, whereas the IL-10R2-encoding gene (*IL10RB*) is located on 21q22.11 (forward strand), near the *IFNAR1*, *IFNAR2*, and *IFNGR2* loci. *IL22RA1* and *IL10RB* have similar structures being comprised of seven exons with exons 2–7 containing the sequences for the 574 (sequence under NP_067081) and 325 (sequence under NP_000619) aa mature proteins, respectively. 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 motifs indicating putative STAT recruitment sites [74]. 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 which receptor subunit is the primary IL-22-binding subunit. The initial studies published by Xie et al. and Kotenko et al. described direct IL-22 binding to IL-10R2 [15] and to both IL-22R1 and IL-10R2 [74], respectively. In contrast, Logsdon et al. determined the kinetic binding data by surface plasmon resonance techniques showing that IL-22 has a high affinity toward IL-22R1 ($K_D <$ 20 nM) but no actual affinity for IL-10R2 [18, 22]. However, IL-10R2 showed a measurable affinity for the IL-22/IL-22R1 complex. Moreover, Fouser's group described 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 [75]. This work additionally provided the initial evidence for a conformational change of the IL-22 molecule during the interaction with its receptor subunits. Our own experiments aimed to identify the possible binding sites for the interaction between IL-22 and IL-10R2 using scans of overlapping peptides derived from the protein aa sequence. By this approach we showed 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) [76]. 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).

The crystal structure analysis of the IL-22/IL-22R1 complex revealed key residues for interaction at the IL-22/IL-22R1 interface [77]. The identified residues of the IL-22 molecule contacting IL-22R1 include Phe47, Gln49 (pre-helix A), Thr53, Ser64 (helix A), Asp67, Thr70, Asp71, Arg73 (the AB loop) and Lys162, Gly165, Glu166, Asp168, and Arg175 (helix F). On the IL-22R1 side, the binding interface comprises residues of the L2–L6 loops (Lys58, Tyr60, Gly61, Glu62, Leu88, Thr89, Glu90, Tyr93, Arg112, Ser114, Pro206, Thr207), whereby IL-22 AB

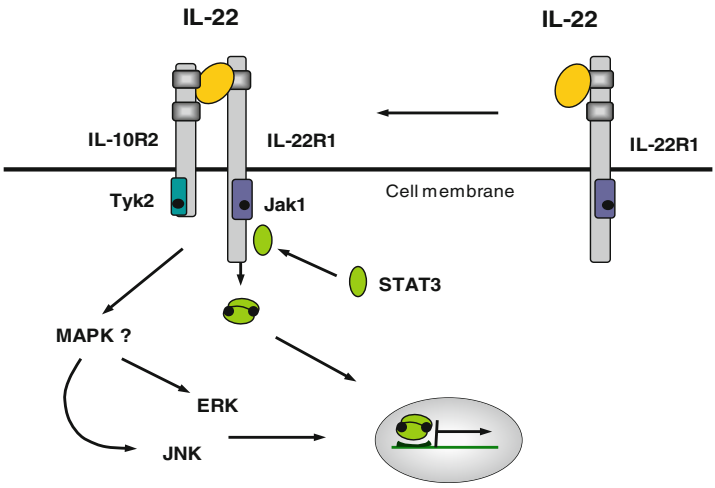

Fig. 2 *The interaction between IL-22 and the IL-22 receptor complex*. IL-22 affects their target cells via the IL-22 receptor complex consisting of IL-22R1 and IL-10R2. There is evidence that IL-22 binds first to the high-affinity chain IL-22R1. This induces a conformational change in the cytokine allowing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

loop and helix F contact IL-22R1 loops L2–L4 (D1 domain) and the IL-22N-terminal helix A interacts with IL-22R1 loops L5–L6 (D2 domain). Essentially, site-directed mutagenesis confirmed the IL-22R1 residues Tyr60 and Lys58 as functionally critical and important aa for IL-22 binding, respectively [77].

The current knowledge regarding the IL-22 residues interacting with IL-10R2 binding is based on homology models and on experimental data. 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 IL-22's aa sequence [76]. Our data revealed distinct binding of IL-10R2 to the N-terminal end of helix 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 [16], these two separate segments in the primary sequence form a discontinuous epitope in the IL-22 molecule, which is located next to a region which has been proposed as a binding site for IL-22R1. Our data basically are 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 of IL-22 to soluble IL-10R2 in the presence of soluble IL-22R1 [22]. As noted above, Asn54 represents the residue whose glycosylation is important for the interaction between IL-22 and IL-10R2 [22]. 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. However, there are no published data clarifying whether there is any interaction between both receptor subunits within the ternary IL-22/IL-22R1/IL-10R2 complex.

The receptor complex for IL-17A and IL-17F is composed of IL-17RA and IL-17RC [78–80]. IL-17RA and IL-17RC together with IL-17RB, IL-17RD, and IL-17RE are transmembrane proteins that constitute a receptor family. Together with TLR and IL-1 receptors, they are assigned to the so-called SEFIR (similar expression to fibroblast growth factor genes, IL-17 receptors, and Toll–IL-1R) protein family, which is defined by a conserved cytoplasmic SEFIR domain. The human IL-17RA-encoding gene (*IL17RA*) is located on chromosome 22q11.1 (forward strand), whereas the IL-17RC-encoding gene (*IL17RC*) 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 [81]. *IL17RA* and *IL17RC* have limited similarity and contain the sequences for the 866 (sequence under NP_055154) and 791 (sequence under NP_703191) aa mature proteins, respectively. IL-17RA is composed of a 293 aa-long extracellular domain, 21 aa-long transmembrane domain, and 525 aa-long intracellular moiety. IL-17RC shares 23 % aa identity with IL-17A. IL-17RC is the binding subunit 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 ca. a 1,000-fold lower affinity [78]. By means of computational modeling, Kramer et al. identified two FNIII domains in the extracellular part of IL-17RA connected by a nonstructured linker [82]. The resolution of the crystal structure of IL-17F bound to IL-17RA revealed a unique mechanism of complex formation. In this complex, the two fibronectintype domains of IL-17RA engage the cytokine in the groove of the homodimer [83].

6 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 toward 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 is ubiquitously expressed [47], which can be explained by its function as part of several cytokine receptors [1]. Therefore, the expression of the IL-22R1 subunit determines whether a cell is an IL-22 target or not. Our numerous studies demonstrated lacking IL-22R1 expression in bone marrow, blood mononuclear cells, thymus, and spleen, as well as in resting and activated primary immune cells including monocytes, B cells, T cells, NK cells, macrophages, and DC populations [46, 47, 49, 76]. In accordance with these results, no signal transduction was induced by IL-22 in any of these cells [47]. 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 [47, 76].

In contrast to immune cells, our data showed that a few organs expressed IL-22R1 and therefore contain putative target cells of IL-22 [47]. 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 [47]. These quantitative results are in line with the semiquantitative data published earlier by Aggarwal et al. [84] and Parrish-Novak et al. [85]. Interestingly, most IL-22R1-expressing tissues form outer body barriers and contain epithelial cells. Regarding isolated cell populations, IL-22R1 is expressed on keratinocytes, bronchial and intestinal epithelial cells, intestinal subepithelial myofibroblasts, hepatocytes, pancreas acinar cells, and a range of respective tissue cell-derived cell lines [13, 86]. Interestingly, the expression of IL-22R1 and IL-10R2 in keratinocytes and fibroblasts can be further upregulated by IFN-γ and TNF-α suggesting an amplification of IL-22 effects in the presence of these cytokines upon inflammation [47, 87].

As mentioned above, IL-17A/F signals through the IL-17RA/IL-17RC receptor complex [10]. Whereas IL-17RA is broadly expressed in hematopoietic and non-hematopoietic cells [80], IL-17RC is thought to be mainly expressed by non-hematopoietic cells [81]. There seems to be one exception, namely, for macrophages, expressing both IL-17RA and IL-17RC [88]. Therefore, it appears that numerous cell types are sensitive toward IL-17 action.

7 Signal Transduction

IL-22 and IL-17A/IL-17F use different signal transduction pathways to activate target cells.

Like other IL-10 family members, IL-22 activates in its target cells a signaling cascade via the JAK/STAT pathway resulting in tyrosine phosphorylation of STAT3. Upon detailed studies using primary human cells, this was first demonstrated by our own research groups for keratinocytes and subsequently by the Fujiyama, Kolls, and Mizoguchi research groups for intestinal subepithelial myofibroblasts as well as bronchial and intestinal epithelial cells [47, 48, 59, 89, 90]. However, most studies investigating IL-22-induced signal transduction in cells with endogenous receptor expression refer to tumor cell lines. In the cell lines, activation of STAT1 and/or STAT5 additionally to STAT3 tyrosine phosphorylation was often observed [9, 47]. Using the H4IIE rat hepatoma cell line, Lejeune et al. clearly demonstrated that intracellular events involve tyrosine phosphorylation of Jak1 and Tyk2, but not Jak2 [91]. Since Tyk2 has been known to be associated with IL-10R2, these studies suggest Jak1 as the kinase associated with the IL-22R1. Despite four potential STAT binding sites on the IL-22R1, this receptor was recently shown to be pre-associated with STAT3 independently of these sites and to forward the IL-22 binding signal by this alternative mechanism [92]. In addition to tyrosine phosphorylation of STAT molecules, serine phosphorylation of STAT3 and an activation of the three major MAP kinase pathways (p38 kinase, JNK, and ERK1/2)

by IL-22 was reported [91]. An activation of MAP kinase pathways by IL-22 was also shown for primary human keratinocytes (ERK1/2 and JNK), intestinal subepithelial myofibroblasts (ERK1/2, JNK, p38 kinase), and synovial fibroblasts from rheumatoid arthritis patients (ERK1/2, p38) [48, 59, 62].

Following interaction with its receptor complex, IL-17A/F preferentially activate the NFκB and MAP kinase (p38 kinase, JNK, and ERK1/2) pathways [93, 94]. 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. Unlike IL-1R signaling, the SEFIR domain of the IL-17 receptor mediates signal transduction independently of classical TIR adaptors like MyD88 or TRIF [94]. In its coiled-coil region at the C-terminus, Act1 also contains a SEFIR domain. Afterward, TAK1 kinase (TGF-β-activated kinase 1) and the E3 ubiquitin ligase TRAF6 (TNFR-associated factor 6) are recruited to the IL-17 receptor complex followed by NFκB and C/EBPβ/δ activation [95–97]. Already in 2002, it was demonstrated that TRAF6 is necessary for IL-17 activation of NFκB and JNK [98]. Additionally, Act1 may directly lead to p38 activation. The phosphorylation of C/EBPβ inhibited proinflammatory gene expression induced by IL-17 [96]. Moreover, IL-17 treatment of target cells increased the Act1-independent phosphorylation of JAK1/2-activated PI3K signaling [99].

8 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 and IL-17F are typical proinflammatory mediators (Fig. 3).

As described above, cells of the digestive tract, skin, lung, and kidney appear to be the most important target cells of IL-22, whereas immune cells are not responsive to this cytokine [47].

The effects of IL-22 that are most precisely characterized to date concern those on the biology of epithelial cells, in particular of keratinocytes. Following our initial study that worked out human keratinocytes as important target cells and demonstrated the first effects of IL-22 on these cells, the induction of antimicrobial molecules [47], few independent studies were conducted that identified a broad range of IL-22 effects on these cells [48, 87, 100–102]. These studies demonstrated that IL-22 basically influences five different keratinocyte functions. It enhances the innate immunity of these cells, inhibits their terminal differentiation (indicative of a regenerative state induction), increases their cellular mobility, induces the production of a few chemokines, and shows mechanisms that prolongs its own effects. These effects are described now in more detail:

IL-22 enhances the innate immunity of keratinocytes by strong upregulation of the expression of numerous antimicrobial molecules like the β-defensin (BD) 2 and

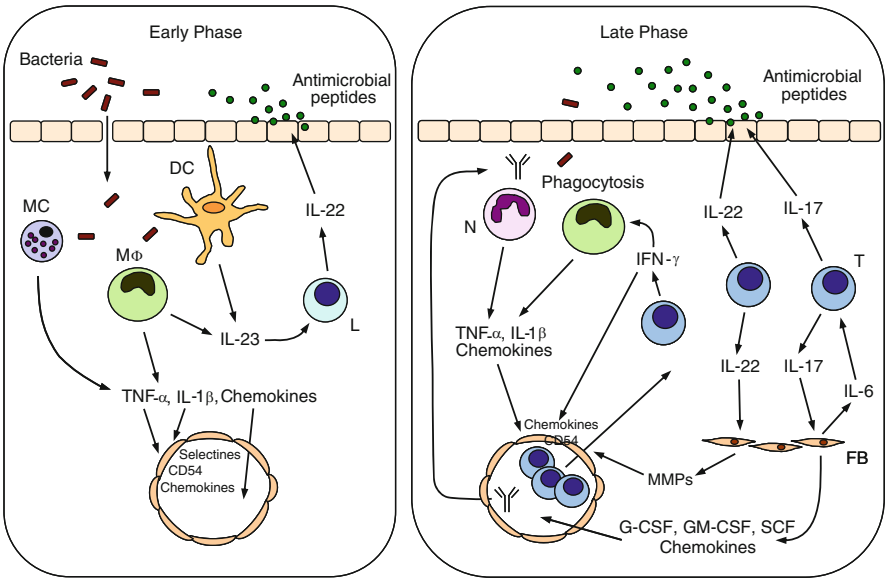

Fig. 3 *The role of IL-22 and IL-17A/IL-17F during mucosal infections (see text). MC* mast cell, *MΦ* macrophage, *DC* dendritic cell, *L* Lymphocyte, *N* neutrophile granulocyte, *T* T cell, *FB* fibroblast

BD3, as well as S100A7, S100A8, and S100A9 [43, 47, 48, 100]. Next, IL-22 inhibits the expression of profilaggrin, keratin (K) 1 and K10, calmodulin-like 5, keratinocyte differentiation-associated protein, kallikrein 7, desmocollin-1, and late cornified envelope protein 1B [48, 87]. 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 impressive consequences of this IL-22 influence were demonstrated using a three-dimensional epidermis model: acanthosis (thickening of the epidermis), parakeratosis (retention of nuclei in the stratum corneum), and hypogranularity (loss of keratohyalin granules) [87, 102]. Interestingly, the influence of IL-22 on the keratinocyte differentiation is not shared by the Th1 and Th17 cell lead cytokines, whereas the latter also have a clear impact on antimicrobial protein (IFN-γ, IL-17; see below) and MMP (IFN-γ) expression [87]. The third group of IL-22-sensitive genes encode the matrix metalloproteinases (MMPs) 1 and 3, which are increased by IL-22, and annexin A9, which is decreased by IL-22 [48, 100]. Their regulation might be responsible for the IL-22-induced increase of the keratinocyte cellular mobility. Furthermore, IL-22 slightly enhances the expression of neutrophilic granulocyte-attracting chemokines: CXCL1, CXCL2, CXCL5, and CXCL8 [87, 100]. Finally, once exerting its effects on keratinocytes, IL-22 promotes an amplification circle by driving positive feedback regulation mechanisms [87, 101]. In fact, IL-22 induces the mRNA and protein expression of STAT3, the major and essential IL-22-signaling element [87]. Moreover, IL-22 induces the production of another member of the IL-10-family with

similar effects as its own and the capacity to strengthen and prolong the IL-22 effects: IL-20 [101]. Interestingly, inflammatory mediators like TNF-α, IL-1β, and IL-17 enhance some IL-22 effects [43, 48, 87, 101].

In other epithelial cells, IL-22 induces effects similar to those in keratinocytes. In fact, the Kolls group found that human bronchial epithelial cells respond to IL-22 stimulation by upregulation of BD2, S100A7, and S100A12; the neutrophilic granulocyte-attracting chemokines CXCL1 and CXCL5; thyroid oxidase 2; and G-CSF [89]. Likewise, in mouse tracheal epithelial cells, IL-22 enhanced the expression of the antimicrobial protein lipocalin (LCN)-2, of CXCL5, polymeric immunoglobulin receptor, and the mucus-associated protein MUC1 [89]. In colonic epithelial cells isolated from mice, locally overexpressed IL-22 enhanced the expression of MUC1, MUC3, MUC10, and MUC13, which are important for mucus layer formation and mucosal damage protection, as found by the Mizoguchi group [90]. Furthermore, IL-22 upregulates the expression of Reg family members (RegIIIβ, RegIIIγ, Reg1α) in these cells [103].

The few non-epithelial tissue cells the IL-22′ influence on was reported involve hepatocytes and pancreas acinar cells. In hepatocytes IL-22 strengthens the production of acute-phase proteins such as serum amyloid A (SAA), α1-antichymotrypsin, haptoglobin, and LPS-binding protein (LBP) [61, 73, 76]. These hepatic proteins display systemic inflammation-limiting and protective roles. In fact, α1-antichymotrypsin favors the degradation of proteases known to be produced in large amounts during inflammation, SAA enhances the opsonization and phagocytosis of gram-negative bacteria, haptoglobin protects against hemoglobin-induced oxidative tissue damage, and high LBP levels neutralize bacterial components and therefore limit their immunostimulatory capacity. Regarding the exocrine pancreas, Gurney's group showed that IL-22 induced the upregulation of mRNA expression of two tissue-protective proteins, pancreatitis-associated protein 1 (PAP1/RegIIIα), and osteopontin [84]. Only the last years provided more and more evidence that PAP1 plays an important role in the protection against tissue injury. Besides its anti-apoptotic effects and the promotion of cellular immune responses by influencing Th1 cell development, osteopontin plays an important role in mucosal tissue protection and wound healing.

The above-described IL-22 effects suggest that this mediator might have an important role for the avoidance/clearance of epithelial and mucoepithelial infections and the regeneration and protection against damage in some chronic inflammatory cutaneous, pulmonary, and intestinal diseases. The effect of IL-22 on keratinocyte functions is indeed mirrored by the keratinocytes from psoriasis patients [47, 48, 87, 101]. These data, together with the high IL-22 expression levels in the plaques and the blood of psoriasis patients, led us to consider IL-22 as a key mediator in the dramatic keratinocyte functional alterations in psoriasis [104, 105]. A role of IL-22 was also demonstrated in psoriasis-like skin alterations in mice, which were induced either by the transfer of T cells to pathogen-free scid/scid mice [106] or by overexpression of IL-22 [87]. Regarding chronic inflammatory pulmonary diseases, it seems that IL-22 is required for the onset of allergic asthma, while functioning as a negative regulator in established allergic inflammation [107].

Few studies suggest a protective role of IL-22 in inflammatory bowel disease. Using a mouse model for ulcerative colitis (TCRαKO mice), Mizoguchi's group demonstrated that mice locally supplemented with IL-22 showed attenuated severity of colitis. This attenuation was attributed to the IL-22-induced STAT3-dependent expression of mucus-associated molecules and restitution of mucus-producing goblet cells [90]. Additionally, a protective role for IL-22 was demonstrated in two further murine colitis models. In fact, a more severe phenotype and higher mortality was associated with IL-22 deficiency in DSS-induced, innate immunity-mediated colitis and in Th1-mediated colitis induced by the adoptive transfer of IL-22-deficient compared to wild-type $CD4^{+}CD45RB^{++}CD25^{-}$ T cells into $Rag1^{-/-}$/$IL\text{-}22^{-/-}$ mice [108].

Furthermore, there might be some protective action of systemic IL-22, whose levels are elevated in CD compared to healthy individuals and correlate with the disease activity [61]. These levels might contribute to the prevention of systemic inflammation provoked by LPS present in the blood of CD patients through its induction of hepatic LBP. The role of IL-22 in chronic inflammatory diseases that do not primarily involve epithelial tissues is less characterized. In rheumatoid arthritis IL-22 is assumed to play a pathogenetic role. This assumption was mainly based on the observed minimally reduced susceptibility of $IL\text{-}22^{-/-}$ mice to collagen-induced arthritis [109]. Furthermore, IL-22 does not seem to play a major role in the murine model of multiple sclerosis [110].

Since IL-22 prominently stimulates the production of antimicrobially acting proteins in various epithelia (see above), it is not surprising that IL-22 seems to play an important role in the innate immune defense, especially against extracellular bacteria. Two studies in mice confirmed the significance of this property of IL-22. Zheng et al. demonstrated that IL-22-deficient mice died as a result of intestinal infection with *Citrobacter rodentium* [103]. Furthermore, the Kolls group found that neutralization of IL-22 during pulmonal *Klebsiella pneumoniae* infection led to animal death [89]. Furthermore, we very recently found a relative IL-22-deficiency of the affected skin of patients suffering from acne inversa [43]. This may contribute to the persistence and propagation of bacteria in the lesions and consequently inflammation and superfluous exudate of purulence that heavily overloads the patients. In contrast to epithelial infections, little to no role for IL-22 is assumed for the control of facultative intracellular bacteria [89, 111–113].

In line with the above-described protective and regenerative features of IL-22, this cytokine plays a protective role in experimental (ConA, CCl_4, or FAS ligand-induced) liver injury [114, 115]. IL-22 also protected $p35^{-/-}$ mice during *Salmonella enteritidis* infection from hepatic necrosis [111]. A recent study by the Gao group nicely showed that IL-22 protected mice from cerulein-induced pancreatitis. This protection likely involved the inhibition of autophagosome formation by upregulating BcL-2 and BcL-XL protein in the acinar cells [111].

In contrast to IL-22, IL-17A and IL-17F have clear proinflammatory properties and act on a broad range of cell types.

IL-17A and IL-17F induce the production of many neutrophilic granulocyte-attracting chemokines, such as CXCL1, CXCL2, CXCL5, and CXCL8, and of T

cell- and myeloid cell-attracting chemokines CCL20, CCL2, and CCL7 in fibroblasts, epithelial cells, endothelial cells, and keratinocytes [28, 87, 116–118]. In dendritic cells IL-17A reduces the production of CCL17, a chemokine that is responsible for the recruitment of Th2 cells to the site of inflammation [119]. Furthermore, IL-17A and IL-17F enhance the expression of G-CSF, GM-CSF, and stem cell factor in tissue cells, macrophages, and T cells and thereby led to strengthened granulopoiesis [88, 120, 121]. In line with this, adenovirus-mediated gene transfer of the murine IL-17A cDNA resulted in a transiently transgenic phenotype, with dramatic effects on the in vivo granulopoiesis. There was a significant increase (tenfold) in the absolute neutrophil count in the peripheral blood that was associated with a doubling in the spleen size [122]. By means of the enhancement of chemokine production and granulopoiesis, IL-17 might lead to a significant increase in the immigration of neutrophilic granulocytes to the site of inflammation. Furthermore, the treatment of tissue cells with IL-17A and IL-17F induces the expression of IL-1β, TNF-α, IL-6, and a variety of metalloproteinases (MMP1, MMP2, MMP3, MMP9, and MMP13) [93, 116, 123–126]. IL-17A also upregulates the IL-1β and TNF-α production in human macrophages [88, 127]. Additionally, IL-17A/IL-17F increases the production of a range of antimicrobial proteins including BD2, S100A7, S100A8, S1200A9, lipocalin-2, and LL-37 in their target cells [43, 128–130]. In primary human tracheobronchial epithelia, IL-17A enhances the expression of the mucin genes MUC5B and MUC5AC [131]. Interestingly, further investigation of MUC5B expression demonstrated that the IL-17 effect is at least partly mediated through IL-6 by an autocrine/paracrine loop. Furthermore, the increase in the production of cyclooxygenase 2 (COX-2)/prostaglandin-2 (PGE_2) and inducible NO synthetase (iNOS) seems to also belong to the repertoire of IL-17 action on tissue cells [93, 116, 132]. 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 [133].

All these observations suggest that IL-17 has an important role in the host protection against specific pathogens. Indeed, as demonstrated by means of IL-17RA-deficient animals, IL-17 was critical for the protection against *Klebsiella pneumoniae* [134], *Citrobacter rodentium* (in later stages) [88], *Toxoplasma gondii* [135], and systemic and oropharyngeal candidiasis [136, 137]. It should be noted that IL-17A/IL-17F do not play an essential role in every infection since IL-17RA-deficient mice do not show increased susceptibility to most infections with intracellular pathogens such as *Mycobacterium tuberculosis* or *Listeria monocytogenes*. The role of IL-17 in antiviral protection remains controversial so far.

IL-17A and IL-17F are also important mediators of certain autoimmune diseases. Already in 2002, Nakae et al. 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 [138]. Lubberts et al. showed that blocking of endogenous IL-17A/IL-17F by means of an IL-17RA-Fc fusion protein in the autoimmune collagen-induced arthritis model results in suppression of arthritis, whereas various studies show that overexpression

of IL-17 enhanced collagen-induced arthritis [139, 140]. This clear proinflammatory effect of IL-17 seems to be TNF-α and IL-1β independent under arthritic conditions [141]. Furthermore, the treatment of rats suffering from adjuvant-induced arthritis with IL-17RA-Fc fusion protein also led to significantly reduced paw volume and radiographic scores [142]. Regarding the role of IL-17 in EAE, the development of this murine multiple sclerosis model was significantly suppressed in IL-17A-deficient mice. These animals exhibited delayed onset, reduced maximum severity scores, ameliorated histological changes, and early recovery [88, 143]. Interestingly, IL-17A, but not IL-17F, seems to play an important role in the development of experimental contact delayed-type hypersensitivity, airway hypersensitivity, arthritis, and EAE [88, 144]. The role of IL-17 in experimental colitis in mice is less clear. It was described that IL-17F deficiency resulted in reduced DSS colitis, whereas IL-17A deficiency or neutralization caused more severe colitis [144, 145]. Furthermore, Zhang et al. found that IL-17RA-deficient mice were significantly protected against TNBS-induced weight loss and colonic inflammation [146].

Interestingly, some effects of IL-22 and IL-17 are additive. This is the case for the induction of antibacterial proteins in epithelial cells [43, 130]. However, it becomes clear from all these data that we do not have a complete understanding of the biological functions of IL-17A/IL-17F and IL-22. 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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IL-17 Receptor Family: Structure, Signal Transduction, and Function

Fang Shen

Abstract Since the discovery of a unique population of T helper cells which secrete interleukin 17 (IL-17), IL-17 is no longer viewed as "yet-another interleukin" but stands out as a unique cytokine in host defense and autoimmunity. To date, the IL-17 cytokine family has been expanded to six members: IL-17A, IL-17B, IL-17C, IL-17D, IL-17E, and IL-17F. They elicit overlapping and yet divergent functions through different receptors. IL-17 receptor family currently consists of five members: IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE. They are type I transmembrane proteins that tend to form heteromeric receptor complexes on cell surface. This chapter will cover recent update on structure, signaling pathway, and function of IL-17 receptor family.

Keywords IL-17 receptor • Signal transduction

1 Introduction

The discovery of the Th17 population has expanded our view on T helper cell function beyond the classical Th1/Th2 diagram [1, 2]. It is now recognized that Th17 cells represent one of the major pathogenic T helper cell populations in autoimmune diseases like multiple sclerosis, rheumatoid arthritis, psoriasis, and inflammatory bowel diseases [3–5]. On the other hand, Th17 cells also play a nonredundant role during host defense again extracellular pathogens [6–8].

Although Th17 cells secrete several other cytokines including IL-21, IL-22, and IL-26, they are named by two major cytokines they produce, IL-17A and IL-17F. IL-17A was first cloned in 1993 [9]. IL-17F shares the greatest homology (55 %)

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with IL-17A among other IL-17-like cytokines. The crystal structure of IL-17F/F [10] and IL-17A/A [11] homodimers revealed a "cysteine knot"-like structure similar to nerve growth factor (NGF). IL-17A/F heterodimer was later identified from activated human and mouse CD4+ T cells [12, 13]. Although initially found in the adaptive immune system, IL-17A and IL-17F can also be produced by other innate immune cells in vivo [14]. Both of IL-17A and IL-17F are able to mount a strong inflammatory response and have quickly become major therapeutic targets under consideration for autoimmune diseases [15].

IL-17E (also known as IL-25) is expressed in Th2-polarized CD4+ T cells [16], mast cells [17], and epithelial cells. Systemic delivery of IL-17E or overexpressed IL-17E results in enhanced Th2 response, increased serum IgE levels, and blood eosinophilia in vivo [16, 18, 19]. Recently, it was reported that IL-17E induced a special lineage-negative innate-like cell in vivo which plays an important role in Th2 response and pulmonary inflammation [20, 21]. Other IL-17 family members (IL-17B, IL-17C, and IL-17D) have been discovered based on homology to IL-17A [22]. However, we only have limited understanding of their functions.

Contrary to mountains of data on Th17 biology, the studies on the IL-17 receptors are somewhat lagging. Five IL-17 receptors have been cloned: IL-17RA, IL-17RB, IL-17RC, IL-17RE, and IL-17RD.

2 Cloning and Tissue Distribution

Murine IL-17RA was the first cloned from the cDNA library of a murine thymoma cell line by its ability to bind both mouse IL-17 and HSV13 gene products from *Herpesvirus saimiri* [23]. Human IL-17RA was later cloned from human peripheral blood leukocyte cDNA library using mouse IL-17RA cDNA as the probe [24]. Mature human IL-17RA contains an N-terminal 293-amino acid extracellular domain, 21-amino acid transmembrane domain, and C-terminal 512-amino acid cytoplasmic domain. IL-17RA is ubiquitously expressed on various tissues including spleen, kidney, lung, liver, brain, heart, skeletal muscle, and testes. IL-17RA expression spans both immune cells (T cells, B cells, monocytes, and NK cells) and nonimmune cells (fibroblast, epithelial cells, osteoblastic cells, and stromal cells).

IL-17RB was cloned based on homology to IL-17RA [25] and independently as an oncogene (Evi27) in BXH2 murine myeloid leukemia cells [26]. IL-17RB protein shares 26 % amino acid identity to IL-17RA [27]. Compared to other family members, IL-17RB has the shortest intracellular tail of less than 200 amino acids. IL-17RB gene transcripts show multiple splicing variants [26]. Some of the mRNAs encode soluble proteins lacking transmembrane and intracellular domains. Murine IL-17RB is highly expressed in various tissues including liver, testes, brain, and lung, while human IL-17RB is expressed highly in kidney, moderately in brain, liver, and testes. Of note, IL-17RB is also considered a biomarker for tamoxifen resistance in breast cancer patients [28].

IL-17RC was identified based on similarity to IL-17RA cytoplasmic domain [29]. IL-17RC protein is 22 % identical to IL-17RA. Analysis of the human EST database revealed extensive alternative splicing of IL-17RC gene. In human prostate cancer cells, there are over 90 different splicing isoforms expressed [30]. Most IL-17RC isoforms vary in the N-terminal ligand-binding domain. However, some soluble receptor isoforms lack transmembrane/intracellular domain and might act as "decoy receptors." IL-17RC is highly expressed in non-hematopoietic tissues such as kidney, liver, lung, heart, and muscle.

IL-17RD was first cloned from zebra fish and named Sef (similar expression to fgf genes) because of its co-expression with FGF during embryonic development [31, 32]. At least three different splicing variants were reported in human: full-length hSef, hSef-s which lacks the N-terminal 144 residues [33], and hSef-b in which the 42-amino acid lead sequence in full-length sef is replaced by an unrelated 10-amino acid sequence [34]. IL-17RD is highly expressed on epithelial cells but not on hematopoietic cells. It is also expressed in various tissues including ovary, breast, brain, spinal cord, heart, skeleton muscle, and kidney.

IL-17RE was discovered based on similarity to IL-17RC [35]. IL-17RE mRNAs also undergo alternative splicing control. At least six splicing variants in mouse and three splicing variants in human have been identified. IL-17RE is expressed in various tissues including kidney, stomach, testes, and lung.

In summary, IL-17 receptors share similar primary structure: glycosylated extracellular domain, single transmembrane domain, and cytoplasmic tail. Except for IL-17RA, other IL-17 receptors have different isoforms due to alternative splicing. IL-17RA, IL-17RB, and IL-17RE seem to express more broadly on both immune cells and nonimmune cells, while IL-17RC and IL-17RD expressions seem to be limited in the non-hematopoietic compartment.

IL-17RA and IL-17RD genes may be the most evolutionally ancient genes in the family. Both of them could be found in the genome of basal chordate amphioxus, while IL-17RB, IL-17RC, and IL-17RE might have originated from later gene duplication events [36]. In mouse, the genes of IL-17RA, IL-17RC, and IL-17RE are located in chromosome 6 while IL-17RB and IL-17RD are on chromosome 14. In human, IL-17RA is on chromosome 22 while all other members are clustered on chromosome 3 (see Table 1).

3 Receptor Complex and Ligand Binding

3.1 IL-17A/A, IL-17F/F, and IL-17A/F Binds to IL-17RA/IL-17RC Complex

IL-17RA and IL-17RC were originally thought to be the receptor for IL-17A/A homodimer [24] and IL-17F/F homodimer [37], respectively. Affinity data from surface plasmon resonance (SPR) analysis seem to support this hypothesis: Human

Table 1 IL-17 receptor family gene structure and expression pattern

Receptor	Chr	Splicing variants	Protein	Alias	Tissue distribution	References
hIL-17RA	22q11.1	None	866 aa	IL-17R	Spleen, kidney, lung liver, brain, heart, skeletal muscle, testes	[23, 24]
mIL-17RA	6F1	None	864 aa			
hIL-17RB	3p21.1	2	502 aa (FL)	IL-17RH1	Kidney, brain, liver, testes	[25–27]
mIL-17RB	14 B	Multiple	499 aa			
hIL-17RC	3p25.3	Multiple	720 aa (FL)	hIL-17RL	Kidney, liver, lung, heart, and muscle	[29]
mIL-17RC	6 E3	Multiple	674 aa (FL)			
hIL-17RD	3p14.3	3	739 aa (FL)	SEF	Ovary, breast, brain, spinal cord, heart, skeleton muscle, and kidney	[31, 32, 45]
mIL-17RD	14 A3	?	738 aa			
hIL-17RE	3p35.3	3	667 aa		Kidney, stomach, testes, and lung	[35]
mIL-17RE	6 E3	6	637 aa (FL)			

From NCBI web site and other literatures. *FL* full length

IL-17A/A binds better to IL-17RA-Fc than IL-17RC-Fc; IL-17F/F binds better to IL-17RC-Fc than IL-17RA-Fc [13]. Interestingly, mouse IL-17F only binds to full-length mouse IL-17RC, while human IL-17F can bind to both full-length human IL-17RC and an IL-17RC splicing variant lacking exon 7 [37]. Although both IL-17F and IL-17RA are glycosylated, the IL-17F binding seems to be not affected by endoglycosidase H treatment of IL-17RA [38]. The crystal structure of IL-17F complexed with the IL-17RA extracellular domain has been resolved [38]. As predicted by computational approach [39], IL-17RA extracellular domain folds into two fibronectin III-like domains, namely, FN1 and FN2. The IL-17RA/IL-17F-binding interface spans FN1, linker, and FN2 domain with a unique "side-on" orientation which is significantly different from other cytokine receptor complex.

Dean et al. unexpectedly found that human IL-17RA cDNA could not compensate for the loss of function in IL-17RA knockout (KO) mouse embryonic fibroblasts (MEFs). The deficiency of ligand binding and downstream response in IL-17RA KO MEFs is only rescued by co-expression of human IL-17RA and IL-17RC [40]. As predicted, MEFs lacking IL-17RC fails to respond to either IL-17A/A or IL-17F/F [41, 42]. Later Wright et al. demonstrated that IL-17A/F signaling was impaired if either IL-17RA or IL-17RC is blocked by neutralizing antireceptor antibody or knockdown of receptor expression using siRNA [13].

One of the models of IL-17RA and IL-17RC complex assembly is described as a "stepwise pairing": IL-17A/A could bind to either IL-17RA or IL-17RC first; but once the initial receptor-ligand complex forms, the affinity for the second identical receptor decreases dramatically, while the 1:1:1 complex of IL-17RA: ligand: IL-17RC is favored [43]. However, later study revealed that the IL-17RA associates with IL-17RC even before exogenous ligands were added [42]. Unlike Toll-like receptors (TLRs), this interaction happens between the extracellular domain of IL-17RA and IL-17RC. Nonetheless, upon IL-17A/A or IL-17F/F treatment, IL-17RC showed inducible association with a glycosylated, slow-migrating IL-17RA isoforms [42].

3.2 IL-17E Binds to IL-17RA/IL-17RB Complex

When IL-17E was cloned, IL-17RB is thought to be its receptor [27]. Inspired by the IL-17RA/IL-17RC complex story, Rickel et al. demonstrated that splenocytes deficient in either IL-17RA or IL-17RB could not induce Th2 cytokine production after IL-17E stimulation. IL-17RA KO or IL-17RB KO mice did not mount robust lung inflammation after intranasal IL-17E challenge. Neutralization antibodies against IL-17RA or IL-17RB could abrogate inflammatory effects of IL-17E in mouse and human cells in vitro [44]. Although IL-17RA does not bind to IL-17E directly by itself [27, 44], the stepwise binding of IL-17RA to IL-17RB/IL-17E complex has been demonstrated similar to IL-17RA/IL-17RC/IL-17A [43]. The conformational change in IL-17E after binding to IL-17RB might increase its affinity to IL-17RA.

3.3 IL-17RD Is an Orphan Receptor and Functions Without Known Ligands

The direct association of IL-17RD and FGFR has been known since its discovery. IL-17RD protein inhibits FGFR1/2 signaling in both zebra fish [31, 32] and human umbilical vein endothelial cells [45]. Surprisingly, the extracellular domain of IL-17RD is dispensable for this interaction. Mutated IL-17RD lacking extracellular domain is still capable of inhibiting FGF-induced ERK activation, while deletion of IL-17RD cytoplasmic tail will abrogate its function [33, 45]. One report suggests that IL-17RD associates with IL-17RA and positively impacts IL-17RA signaling [46]. However, the functional significance of this interaction is yet to be tested in IL-17RA- or IL-17RD-deficient mice in vivo. Given no reports on other known cytokines as ligand, IL-17RD remains to be the orphan receptor in the family.

3.4 Other IL-17 Receptors

IL-17C is secreted by epithelial cells in response to bacteria and proinflammatory cytokines. IL-17C functions in an autocrine manner binding to the IL-17RA-IL-17RE heterodimeric receptor complex, to promote innate host defense responses from tissue epithelial cells (Ramirez-Carrozzi et al. Nat Immunol. 2011;12(12):1159). IL-17B was reported to bind to IL-17RB with much lower affinity compared to IL-17E [25]. The receptor for IL-17D has not been identified yet. IL-17B, IL-17C, and IL-17D have showed overlapping biological effect compared to IL-17A. They can induce expression of many proinflammatory genes in various cells [47–49] (see Fig. 1).

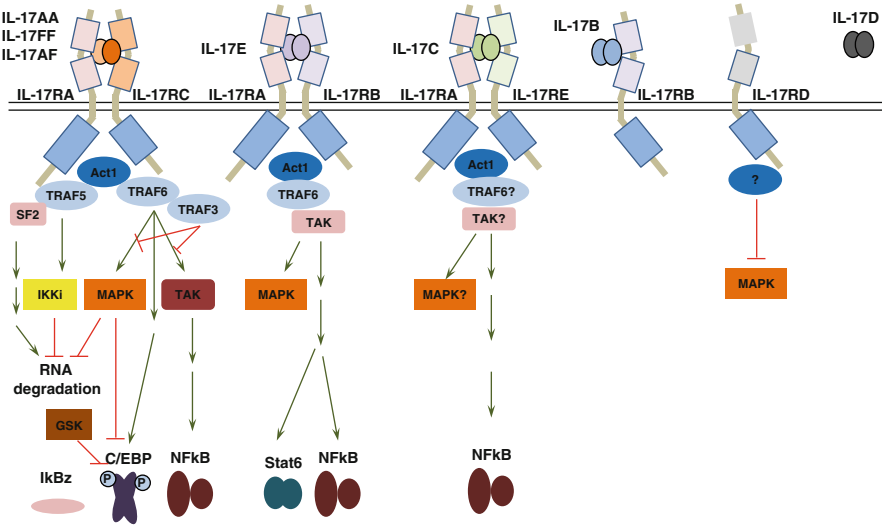

Fig. 1 IL-17 receptor family and signal transduction

4 Signal Transduction

4.1 SEFIR Domain Is the Key Signaling Motif Homologous to TIR Domain

Despite of the size of their intracellular tails, IL-17 receptors did not display any known signaling motifs at first glance. Later bioinformatics analysis revealed a conserved signaling pattern shared among the family members [50]. This domain is called "SEFIR" domain because it is distantly homologous to TIR domain which was first identified in TLRs and IL-1Rs. TIR domain consists of three conserved boxes that span 135 ~ 160 amino acid and folded as central five-stranded parallel β-sheet surrounded by five helices [51].

The functional significance of SEFIR domain was first demonstrated by mutagenesis study in IL-17RA KO fibroblasts [52]. Only mutants bearing intact SEFIR domain are able to rescue IL-17A-induced target genes expression in KO cells. The boundary of SEFIR in IL-17RA seemed to extend beyond the C-terminal side of previously defined SEFIR [53]. Similar to TLR4, IL-17RA has a BB-loop-like structure (conserved box 3 in TIR domain) outside of the SEFIR domain. Point mutation of critical Val553His in BB loop of IL-17RA impairs downstream signaling which resembles the loss of function mutation of Pro712His in TLR4 [52]. Surprisingly, the BB-loop-like structure is unique to IL-17RA. Other IL-17 receptors only have conserved box 1 and box 2 of TIR but do not have a distinct BB loop. Recently, the SEFIR domain in IL-17RC was also profiled [42]. Similarly, the SEFIR domain alone in IL-17RC is not sufficient to drive downstream

signaling. Instead a C-terminal extended region ending in amino acid 658 – 668 is required to recapitulate full-length IL-17RC function.

4.2 Act1 Is the Unique Proximal Adaptor Protein

TRAF6, an adaptor protein in IL-1R/TLR pathway, was connected to IL-17RA signaling soon after the discovery of IL-17A [54]. However, there are no canonical TRAF6-binding sites in IL-17RA cytoplasmic tail. The other intermediate adaptor proteins in TLR pathway, including MyD88, TRIF, and IRAK, are dispensable for IL-17RA signaling [55]. XiaoXia Li's group first demonstrated that IL-17RA signaling requires Act1 [56] which is previously linked to CD40 and BAFF-R signaling [57]. Act1 have three functional domains: The C-terminal SEFIR domain directly associates with IL-17RA SEFIR domain, the N-terminal putative TRAF6-binding site [58, 59], and the U-box type 3 ubiquitin ligase domain near center [60].

Besides TRAF6, Act1 also signals through other TRAF adaptor proteins. One of the early puzzling data showed that stabilization of CXCL1 mRNA by IL-17A is dependent on Act1 but independent of TRAF6 [61]. So there must be some other components downstream of Act1 but upstream of TRAF6. Later, it was showed that IL-17A treatment induces interaction of Act1/TRAF2/TRAF5/arginine- and serine-rich splicing factor SRSF1 (SF2 (ASF)). SF2 could bind to 3′-UTR of target mRNA and promote mRNA degradation, while TRAF2 and TRAF5 enhance mRNA stability [62]. Another report showed that TRAF3 negatively regulates IL-17RA signaling by interfering withTRAF6 binding to Act1. TRAF3 transgenic mice showed delayed disease progression in EAE model [63]. It is well known that IL-17RA/IL-17RC signaling synergistically amplifies proinflammatory responses from TNF receptor. The co-engagement of TRAF6 and TRAF5 pathways might partially explain why IL-17 target genes are highly enriched for neutrophil-recruiting chemokines, acute phase protein, and other inflammatory mediators [64].

Besides IL-17RA/IL-17RC, Act1 also directly interacts with IL-17RB SEFIR domain [65]. IL-17E signaling through IL-17RB is dependent on TRAF6 but not TRAF2 or TRAF5 [66]. Cells lacking TRAF6 are deficient to IL-17E-induced NF-kB activation, and overexpression of dominant-negative TRAF6 will impair IL-17E-induced target gene expression in MEFs. One of the other unique features of IL-17E signaling is that dependent on Stat6 in vivo [67].

4.3 NF-kB Pathway

One of the hallmark features of IL-1R and TLR signaling is NF-kB activation. The terminal components of canonical NF-kB pathway are indeed shared by IL-17RA/IL-17RC signaling: Signaling through IL-17RA/IL-17RC leads to TRAF6-dependent TGFβ-activated kinase 1 (TAK1) activation, which in turn activates IKK complex; the latter phosphorylates inhibitor of NF-kB alpha (IkBa); IkBa

then is degraded in ubiquitination-dependent mechanism; the NF-kB p50/p65 complex enters the nuclears and initiates transcription. There is one report showing that dominant-negative NIK could inhibit IL-17-induced chemokine expression [68]. More data is needed to support the alternative NF-kB pathway downstream of IL-17RA.

One recent study showed that inducible inhibitor of NF-κB (IκB) kinase (IKKi, also known as IKKε) could directly phosphorylate Act1 and regulate Act1 interaction with TRAF6/TRAF2/TRAF5. IKKi-deficient cells failed to upregulate some IL-17 target genes (CXCL1, CXCL2, TNFa, IL6, and CSF3) after stimulation. IKKi knockout mice also showed reduced lung inflammation after intranasal IL-17 challenge [69].

Nuclear factor of kappa light polypeptide gene enhancer in B cell inhibitor, zeta (NFKBIZ, also known as MAIL), is upregulated after IL-17 stimulation as early as 2 h [70]. Unlike IkBa, NFKBIZ is a positive regulator of NF-kB in IL-1R/TLR signaling [71]. It is reported that NFKBIZ is required for IL-17A-induced human beta-defensin 2 in epithelial cells [72]. NFKBIZ overexpression could induce other IL-17A target genes expression (Lcn2, Csf3, and CEBPd) without exogenous stimulation [73]. It is possible that NFKBIZ is a cofactor to recruit other transcription factors (p65 or C/EBPb) binding to DNA in a promoter-specific fashion [73].

4.4 CCAAT/Enhancer-Binding Protein Pathway

CCAAT/enhancer-binding proteins (C/EBP) are basic-leucine zipper (bZIP) containing transcription factors. There are six family members (C/EBPα, C/EBPβ, C/EBPγ, C/EBPδ, C/EBPε, and C/EBPζ) which form homo- or heterodimers and play important role on tissue development, cancer, and inflammation [74]. IL-17A stimulation leads to new protein synthesis of C/EBPβ and C/EBPδ [70]. C/EBP-binding sites are enriched in proximal promoter of many IL-17 target genes including IL-6 and Lcn2 (also known as 24p3) [64]. C/EBPβ- and C/EBPδ-deficient cells failed to upregulate target genes in response to IL-17A stimulation [75]. C/EBPβ and C/EBPδ are functionally redundant since reconstitution of either protein rescues the loss of function in C/EBPβ/δ double knockout cells [75]. IL-17A regulates C/EBPβ function at both the transcriptional and posttranslational level. Upon IL-17 stimulation, C/EBPβ undergoes rapid phosphorylation at Thr188 by ERK and later at Thr179 by glycogen synthase kinase 3β (GSK3β) [76]. Surprisingly, increased expression reporter genes were observed in cell's overexpression of mutated C/EBPβ which is resistant to phosphorylation of Thr188 and Thr179. This suggests that transcriptional activity C/EBPβ is downregulated by phosphorylation of Thr188 and Thr179. Since C/EBPδ has been implicated in persistent gene transcription downstream of TLR [77, 78], it is possible that NF-kB and C/EBPβ drive early gene transcription while C/EBPδ takes over at later time point.

4.5 MAPK Pathway

MAPK pathway is a three-level protein kinase module which is highly conserved from yeast to human. IL-17A can activate many components in MAPK pathway. Among them, ERK activation is most consistently reported in many different studies [79–83]. ERK1/2 activation could stabilize mRNA of IL-17 target genes like IL-6 and CXCL1 [84]. Some reports showed activation of c-jun NH [2]-terminal kinase (JNK) after IL-17A stimulation [85]. JNK is connected to activation of transcription factor AP1 (activator protein-1) [81]. However, mutation of AP1-binding site in IL-6 promoter did not impact IL-17-induced IL-6 transcription [86]. There are also several reports on IL-17-induced activation of p38 MAPK [81–83, 85]. IL-17E signaling through the IL-17RA/IL-17RB complex could also activate MAPK pathway in eosinophils [87].

Unlike IL-17RA/IL-17RC, IL-17RD has been reported to inhibit MAPK activation. The classical Ras-dependent MAPK activation is modulated by IL-17RD signaling [31, 33]. It is still not clear why the SEFIR domain activates MAPK in IL-17RA/IL-17RC but inhibits MAPK in IL-17RD.

4.6 Negative Signals Through IL-17RA/IL-17RC

Emerging data suggest that signaling through IL-17RA/IL-17RC might negatively regulate T cell differentiation. For example, increased frequency of Th17 cells or increased IL-17A/IL-17F/IL-22 production was observed in mice lacking IL-17 signaling components like IL-17RA [88], Act1 [89], or IL-17RC [41]. One of the hypotheses is that IL-17F binding to IL-17RA on T cells forms a negative feedback loop which inhibits IL-17A production from Th17 cells [90].

IFNg has been reported to inhibit Th2 cell differentiation. Could Th17 differentiation condition block Th1 differentiation? In the CD45RBhi transfer colitis model, both IL-17A KO and IL-17RA KO mice develop more aggressive wasting diseases due to uncontrolled Th1 response [91]. However, elevated Th1 priming is not seen in T cells lacking IL-17RC [41] which suggests other T cell extrinsic factors contribute to these inhibitory effects. IL-17A has been shown to inhibit DC functions in established allergic asthma model [92]. More studies are needed to dissect the molecule mechanism of the feedback loop in different cell populations.

5 Functions and Therapeutic Implications

The function of TH17 cells and Th17 cytokines will be discussed in other chapters of this book. The IL-17 receptor-related data in both mouse models and human diseases are summarized here.

5.1 Host Defense

In 2001, Ye et al. showed that IL-17RA knockout mice have increased mortality in intrapulmonary *Klebsiella pneumoniae* infection [93]. After the first report linking IL-17RA to host defense, the impaired host defense in IL-17RA knockout mice has been demonstrated in many other infectious models including systemic *Toxoplasma gondii* infection [94], systemic [95] and oral [96] *Candida albicans* infection, *Helicobacter pylori* infection [97], systemic *Listeria monocytogenes* infection [98], cutaneous *Staphylococcus aureus* infection [99], vaginal *Neisseria gonorrhoeae* infection [100], and *Herpes simplex virus* infection of the cornea [101, 102]. Similarly, IL-17RC-deficient mouse also has difficulty clearing infections of *Candida albicans* [42]. In most cases, the clearance of extracellular pathogens is dependent on neutrophil-recruiting chemokines [93, 94, 100] or bacteria-limiting acute phase protein [103] expression. Impaired humoral responses can also contribute to the phenotype [97]. In some rare occasions, IL-17R signaling is actually causing increased mobility due to excessive inflammatory response [104].

The contribution of IL-17RA to host defense is well translated into human. A nonsense mutation in the extracellular domain of IL-17RA gene leads to premature stop during translation and loss of IL-17RA protein expression. Patients carrying this homozygous mutant allele developed chronic mucocutaneous candidiasis disease and, to a lesser extent, *Staphylococcus aureus* infection. Patients carrying mutant IL-17F gene and producing mutant IL-17F with weak affinity to IL-17RA are also susceptible to *Candida albicans* infection [105].

5.2 Inflammatory Diseases

It is well established that Th17 cells are one of the major drivers for inflammation during experimental autoimmune encephalomyelitis (EAE) development, a mouse model of multiple sclerosis. Conventional knockout mice deficient in either IL-17RC [41] or Act1 [56] showed delayed disease onset and reduced severity in MOG-EAE. Further analysis revealed that IL-17A or IL-17F targets astrocytes but not endothelial cells or macrophages in the central nervous system [89].

Th17 cells are also critical to the development of inflammatory bowel diseases. Similarly, mice lacking either IL-17RA or treated with IL-17R IgG1 fusion protein showed significantly attenuated colonic inflammation in TNBS-induced colitis [106]. Selectively knockout of Act1 in colonic epithelial cells also reduced symptoms in dextran sulfate sodium (DSS)-induced colitis [56].

In arthritis model, Act1 is required for initiation of collagen-induced arthritis and anti-type II collagen antibody production [107]. Interestingly, Act1 is dispensable in myeloid cell driven collagen antibody-induced arthritis.

Although the significance of IL-17E in human lung inflammation has not been firmly established, IL-17E does play an important role in Th2 responses in several

mouse models. IL-17RA KO or IL-17RB KO mice fails to develop pulmonary inflammation after intranasal IL-17E challenge [44]. Act1 knockout mice are deficient in IL-17E-induced Th2 cytokine production, eosinophil recruitment, mucus hypersecretion, and airway hyperreactivity in vivo [65, 108, 109]. Unlike IL-17RA/IL-17RC which targets mostly non-hematopoietic cells, IL-17RA/IL-17RB signaling in T cells is clearly required for IL-17E-induced lung inflammation [109].

IL-17RA also plays an important role in other models of inflammatory diseases, including western-type diet-induced atherosclerosis in LDLr-deficient mice [110], hapten-induced contact hypersensitivity responses [111], polymicrobial sepsis [112], and TNF-induced shock [113].

6 Perspective

IL-17 receptors are unique family of cytokine receptors. In vitro and in vivo animal studies with genetically modified mice have provided valuable insights regarding IL-17 receptors configuration, downstream signaling, and disease association. Blockade of IL-17 receptor signaling is considered as a viable therapeutic strategy. Secukinumab and Ixekizumab (anti-IL-17 antibody) showed variable responses in psoriasis, rheumatoid arthritis, and chronic noninfectious uveitis [114]. The clinical trials of Brodalumab (anti-IL-17RA antibody) on psoriasis are ongoing (see www.clinicaltrials.gov).

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Contributions of IL-22 to TH17 Responses: Repairing and Protecting Peripheral Tissues

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Abstract Despite its structural relationship with IL-10, IL-22 does not share any activity with this cytokine and appears, 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 and/or infections in both the 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.

Keywords IL-22 • Keratinocytes • Psoriasis • Crohn's disease • Inflammatory bowel disease • Epithelial cells • Hepatitis • Multiple sclerosis • Acute phase response

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Interleukin-22 (IL-22) was originally identified as a gene upregulated 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-10Rß chain is part of both receptor complexes when it associates with the IL-10Rα or the IL-22R chain, respectively [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 has completely distinct patterns of expression with very little or no overlap [4, 5]. Whereas the IL-10Rα chain 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, as well as lung and intestinal epithelial cells [4, 6, 7].

Investigations about the pathophysiological significance of IL-22 have been biased by the finding that this cytokine is produced by TH17 lymphocytes, which are considered to be responsible for major autoimmune diseases. However, recent data suggest that innate lymphoid cells (ILCs) represent a major source of this cytokine, playing a key role in antimicrobial defenses. In this chapter, we will try to 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.

1 IL-22 Production

Many in vitro models have highlighted the role of T helper cells as a major source of IL-22. 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, TGFß and IL-6 are the main factors promoting TH17 differentiation of naïve mouse T cells, 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 in vitro differentiation of TH17 cells from naïve T cell precursors. The current

dogma is that TGFß and IL-6 represent the minimal requirement for 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 TGFß, IL-6, IL-1ß, TNF-α, and IL-23, they were shown to express 100 times more IL-22 than TH1 and 1,000 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 TGFß actually inhibited this effect [11]. This suppressor effect of TGFß depends on the c-Maf transcription factor, which is induced by TGFß and directly binds to the IL-22 promoter to repress its activity, whereas IL-17 expression is increased by this transcription factor [13].

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 TGFß and IL-6 does not seem to induce Th17 differentiation of naïve CD4$^+$ cells, but IL-23 is a major inducer of IL-17A and IL-22 [14, 15]. Alternatively, IL-1 and IL-6 can also promote the differentiation of human T cells producing both IL-17A and IL-22 [16]. By contrast, TGFß rather inhibits the production of these cytokines by human TH17 cells [14].

In contrast with these reports that focused on in vitro TH17 differentiation from naive 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 [17]. Further studies highlighted the fact that IL-22 can be specifically expressed by human CD4 T cells that were designated TH22 cells [18–20]. These cells are localized to the skin and express CCR6 and skin-homing receptors such as CCR4 and CCR10. Differentiation of TH22 cells can be induced after TCR stimulation in the presence of IL-6 and TNF [18] or of IL-1 and IL-23 [21]. Epidermal Langerhans cells and dermal dendritic cells promote the differentiation and the activation of memory TH22 cells [19]. However, whether these TH22 cells must be defined as a separate lineage, like the TH1, TH2, and TH17 subsets, and the existence of this population in mice require further investigations.

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 [22]. Another transcription factor, the aryl hydrocarbon receptor (AhR), was found to play a critical role in IL-22 production by TH17 cells [23]. 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 toward 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 open interesting perspectives regarding the influence of environmental factors on autoimmune processes [23]. However, a number of more physiological ligands have been described for this receptor, and its activity as a dioxin receptor might have overshadowed its genuine function. For instance, recent observations demonstrated that natural dietary compounds such as indole-3-carbinol found in broccoli and Brussels sprouts or flavonoids found in fruits and vegetables can activate AhR and promote IL-22 expression in vivo in the intestinal mucosae [24–26].

It must also be stressed that TH17 T lymphocytes are not the only source of IL-22. In fact, the main source of IL-22 differs from one pathological situation to another, and γδ T cells represent a major source of both IL-17A and IL-22, particularly in skin inflammatory models [27–30]. However, IL-17 production often remains restricted to T cells, whereas several experimental models showed that IL-22 production is often not affected by T cell depletion because innate lymphoid cells (ILCs) produce large amounts of this cytokine, particularly in the intestinal mucosae. ILCs consist of a heterogeneous family of cells belonging to the innate immune system but can be classified based on their cytokine production like T helper subsets: Type 1 ILCs consist of NK cells which produce IFNγ; type 2 ILCs include nuocytes and natural helper cells that produce IL-5 and IL-13; and type 22 ILCs include $NKp46^{+}$ ILCs, LTi-like cells, and $Sca1^{+}Thy1^{high}$ ILCs, which produce IL-22 and, to a lesser extent, IL-17 [31]. LTi-like cells can be also be found in the spleen, representing up to 1 % of the spleen of Rag2 -/- mice, and produce large amounts of IL-22 in response to IL-2 or IL-23 produced during inflammatory processes triggered by various TLR ligands [32, 33].

2 Association Between IL-22 and Diseases

2.1 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 [34]. Several reports have shown that IL-22 production is also associated with this disease. Using quantitative RT-PCR, Wolk and colleagues have shown that high IL-22 levels were present in skin from patients suffering from psoriasis and correlated with the expression of ß-defensins, which are well-described disease

markers, whereas IL-22 is not detectable in healthy skin [35]. High IL-22 levels in psoriatic skin were also associated with strongly upregulated cutaneous S100A7, S100A8, S100A9, and MMP1 expression. Moreover, psoriatic patients showed elevated IL-22 plasma levels, which correlated with disease severity [36, 37]. 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 [37], and treatment with cyclosporine A downregulated both IL-17 and IL-22 gene expression in the skin of psoriasis patients [38].

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 [34]. Interestingly, keratinocytes constitutively express the IL-22 receptor, and IL-22 activates STAT3 in these cells [35, 37, 39]. 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 [40]. In vitro, IL-22 upregulates the expression of a series of proinflammatory molecules including S100A7, S100A8, S100A9, and ß-defensins by keratinocytes [35, 39], alone or in cooperation with IL-17 [9]. In addition, IL-22 promoted keratinocyte migration in an in vitro injury model, downregulated the expression of genes associated with keratinocyte differentiation, and induced hyperplasia of reconstituted human epidermis [39].

In vivo, the role of IL-22 in psoriasis is supported by data from three murine models related to the human disease. In a first model, injection of IL-23 in 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 antimicrobial 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 ß-defensin gene expression [41].

Such mouse models of skin inflammation triggered by injections of IL-23 or naïve T cells could be criticized because they do not perfectly recapitulate the pathophysiological processes underlying human psoriasis disease. Recently, topical treatment with imiquimod, a ligand for TLR7 and TLR8, was presented as a novel mouse model for psoriasis-like skin inflammation, inducing acanthosis, parakeratosis, and a mixed inflammatory infiltrate [42]. The relevance of this model is supported by the fact that imiquimod is used in humans, for topical treatment of superficial basal cell carcinoma, and that this treatment can also induce psoriasis or exacerbate the disease in patients with a well-controlled psoriasis [43]. Interestingly, scaly skin lesions and neutrophil infiltration induced by imiquimod in

wild-type mice were almost totally absent in IL-22-deficient mice or in mice treated with an anti-IL22 blocking antibody [30]. As neutrophils do not directly respond to IL-22, this observation likely reflects the specific role of IL-22 in the induction of chemotactic factors such as CXCL3 and CCL3 in this imiquimod model. In addition to these factors, blocking IL-22 decreased biomarkers reflecting the antimicrobial and hyperproliferative response of keratinocytes, including S100A8, S100A7, and keratin 14 [30].

2.2 *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 the acute phase response, and IL-22 was indeed found to upregulate 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 [44]. 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 [45]. 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 antiapoptotic molecules [46, 47]. IL-22 also protects hepatocytes against necrosis in a *Salmonella* infection model [48]. Moreover, administration of anti-IL-22 antibodies worsens liver injury in the ConA-induced hepatitis model [47], and similar results have been obtained in IL-22 -/- mice [49]. However, the clinical relevance of these mouse models of hepatitis for human disease remains elusive at this stage.

The protective effect of IL-22 is also observed in a model of alcohol-induced hepatotoxicity and for the regeneration of liver tissue after partial hepatectomy [50, 51]. In fact, IL-22 treatment in alcoholic liver injury decreases the expression of fatty acid transport protein and increases several antioxidant, antiapoptotic, and antimicrobial genes [51, 52]. In contrast to its protective role against liver injury, IL-22 seems to promote tumors in the liver as well as in the adipose tissue [53, 54]. In vivo, diethylnitrosamine-induced mouse hepatocellular carcinoma is decreased in IL-22-deficient mice [55], whereas liver-specific IL-22 transgenic mice were more susceptible to this type of cancer [53].

2.3 *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 upregulate the expression of

typical acute phase genes such as serum amyloid A and α1-antichymotrypsin, suggesting that this cytokine could modulate intestinal inflammation [56]. 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 upregulated 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 [57].

Interestingly, IL-22 gene expression is increased in inflamed colonic lesions of patients with Crohn's disease and, to a lesser extent, in patients with ulcerative colitis [58, 59], whereas high IL-22 serum concentrations have also been described in Crohn's disease patients [57]. 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 [57, 58]. 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 [60]. In the TCRα KO model, further IL-22 gene delivery significantly reverted goblet cell depletion typically associated with colitis. IL-22 stimulated the expression of mucin genes MUC1, -3, -10, and -13 by colon epithelial cells in a STAT3-dependent manner, and coadministration of the mucolytic agent N-acetylcysteine abolished the protective effect of IL-22 [60]. In the DSS mouse colitis model, anti-IL-22 antibodies significantly delayed the recovery from acute colitis as judged by body weight loss or disease scores [60], and the inflammation was similarly exacerbated in IL-22-deficient mice [61], illustrating the anti-inflammatory role of endogenous IL-22 in this model.

A similar anti-inflammatory and protective effect of IL-22 has been reported during intestinal infection by *Citrobacter rodentium* [62]. A few days after infection, IL-22 is induced in the colon of wild-type mice, together with IL-17 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-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 [62].

During *C. rodentium* infection, the expression of a few genes was found to be strictly dependent on IL-22, including RegIIIß and RegIIIγ, which belong to a family of secreted C-type lectin proteins. Most importantly, administration of exogenous RegIIIγ could protect IL-22 -/- mice from weight loss upon *C. rodentium* infection, and approximately 50 % of RegIIIγ-treated IL-22 -/- mice survived infection, whereas all control-treated IL-22 -/- mice became moribund or died [62]. 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 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. In addition, other antimicrobial proteins are likely to contribute to the protective effect of IL-22 during bacterial infections as shown in *Salmonella typhimurium* infection for lipocalin-2, which is upregulated by IL-22 and binds bacterial siderophores, rendering them inaccessible for intestinal pathogens [63]. Besides these specific antimicrobial mechanisms, the protective effect of IL-22 also relies on the STAT3-dependent regulation of the cellular stress response, apoptosis, and pathways associated with wound healing in epithelial cells [64].

Interestingly, IL-22 production induced by *C. rodentium* infection or by DSS treatment was 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 [61, 62]. *C. rodentium* inoculation induces IL-22 production by NKp46^{+} ILCs from the intestinal mucosae [65, 66]. Activation of the AhR transcription factor by phytochemicals present in grain-based diets is required for the postnatal expansion of this ILC cell population and for IL-22 induction upon infection, and AhR-deficient mice are therefore highly susceptible to *C. rodentium* infection [24–26].

2.4 IL-22 and Rheumatoid Arthritis

Rheumatoid arthritis (RA) is another autoimmune disease in which TH17 lymphocytes are supposed to play a significant role. The IL-22 mRNA has been detected by RT-PCR in RA synovial tissues and mononuclear cells isolated from RA synovial fluid samples. Correlation was established between IL-22 serum levels and the incidence of erosion [67]. Expression of the IL-22R was also detected in synovial tissues, and IL-22 was shown to upregulate the expression of the MCP-1 chemokine by synovial fibroblasts from RA patients, suggesting that IL-22 plays a proinflammatory role in this disease [68]. In a mouse model of collagen-induced arthritis, IL-22-deficient mice were partially protected [69], and a similar protective effect of anti-IL-22 treatment was reported in spontaneous arthritis developed by IL-1Ra-/- mice [70]. The mechanisms underlying this pathogenic effect require further investigation but might reflect a regulation of osteoclastogenesis [69, 71].

2.5 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, experimental autoimmune encephalomyelitis (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 [72]. 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 [72]. 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 correlates nicely with the disease, IL-22 -/- mice develop EAE exactly as control mice, suggesting that this cytokine does not exert any nonredundant activity in this mouse autoimmune model [12].

2.6 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 ß-defensin2, S100A7, as well as inflammatory cytokines [73]. A model of pulmonary infection by *Klebsiella pneumoniae* has been used by Aujla and colleagues to assess the involvement of IL-22 in mucosal host defense in vivo. Whereas IL-17 -/- mice showed 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 [73]. In addition, IL-22 is also required for *Aspergillus fumigatus* clearance upon lung infection [74].

Lung inflammation frequently results in fibrosis, as highlighted by silica- and bleomycin-induced lung fibrosis experimental models in which expression of both IL-17A and IL-22 is upregulated. Upon silica particle administration, IL-17 seems to play the major role during the early inflammatory stage as lung neutrophil infiltration was reduced in IL-17R-deficient mice but not in IL-22 -/- mice. However, neither IL-17 nor IL-22 seems to play any significant role in subsequent development of fibrosis [75]. In the bleomycin model, IL-17 plays a similar proinflammatory role as IL-17-deficient mice showed much less pulmonary inflammation than wild-type mice. More surprisingly, neutralization of IL-22 had completely opposite results depending on the presence or absence of IL-17. In wild-type mice, blocking IL-22 protects mice from airway inflammation including neutrophil infiltration, disruption of lung architecture, and body weight loss. By contrast, the same treatment exacerbated the inflammatory response in

IL-17-deficient mice [76]. Finally, in a third model of lung inflammation and fibrosis triggered by repeated exposure to the *Bacillus subtilis*, IL-22 neutralization increased collagen deposition in the lung whereas injection of recombinant IL-22 inhibited lung fibrosis. In addition, defective signaling by the AhR transcription factor led to decreased IL-22 production and increased lung fibrosis, confirming that this transcription factor represents a potential target for drug development aiming at regulating IL-22 production in vivo [77].

Allergic asthma is mainly a TH2-driven pathology, and IL-22 is usually undetectable in a classical mouse asthma model such as BALB/c mice sensitized by ovalbumin and alum adjuvant. However, TH17 cells have been shown to be involved as well in other murine asthma-like models, and IL-22 appears to be upregulated in the serum of asthmatic patients [78] and in several mouse asthma models [78–80]. In these experimental settings, administration of IL-22 before or with antigen challenge had an inhibitory effect against inflammation [78–81]. This protective effect of IL-22 has been attributed to an inhibition of IL-13 activity on lung epithelial cells either for CCL17/TARC induction [79] or for IL-25 production [80]. Inhibiting IL-22 in vivo, through antibody treatment or by gene targeting, increased expression of these inflammatory mediators, infiltration by eosinophils, and bronchial hyperresponsiveness [78–80].

3 Concluding Remarks

Although our understanding of the biology of IL-22 has significantly progressed over the last 2 years regarding the definition of its main cellular source and physiological targets, it remains uneasy 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 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 antimicrobial 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 in order to help IL-22 to keep focusing on its beneficial wound healing and protective activities. Depending on the context, both IL-22 agonists and antagonists could have clinical applications, as well as molecules regulating the transcription factors such as AhR, which tightly controls in vivo IL-22 production and is expected to become a promising target for drug development.

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Central Role of Th17 Cells in Adaptive Immune Responses

Franco Di Padova, Bernhard Ryffel, and Valerie Quesniaux

Abstract Th17 cells have emerged as a crucial T helper effector subset in various pathological conditions including autoimmune diseases. The type and the amount of antigens as well as the degree of activation of various pattern recognition receptors (PRRs) determine the quality of the response of antigen-presenting cells (APCs), which influence the generation of induced T regulatory (iTreg) cells or of effector T cells. Properly activated dendritic cells (DCs) can secrete cytokines such as IL-1β, IL-6, and IL-23 which direct the generation of Th17 cells over iTreg cells. Moreover, DCs are fully equipped only for the generation of Th17 effector cells. For the generation of Th1 and Th2 effector cells, the involvement of other cell types is often required. NKT cells contribute IFN-γ for the generation of Th1 effector cells, while basophils and other cell types facilitate the generation of Th2 cells by providing IL-4. In addition, available data suggest that Th17 cells are often activated also in responses to intracellular pathogens and parasites, which are considered typical Th1 and Th2 responses. There is flexibility in the immune response and inflammatory cytokines, such as IL-1β, can convert iTreg cells into Th17 cells, and IFN-γ and IL-23 or IL-4 might act on Th17 cells to convert them into populations of cells able to secrete both IL-17A and IFN-γ or IL-17A and IL-4.

Keywords CD4+ T cell • iTreg cells • Th17 cell • Th1 cell • Th2 cell • IL-17A

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1 Critical Roles of Antigens, PAMP, and Cytokines in Directing the Adaptive Immune Response

An adaptive immune response involves the activation of naïve CD4+ T cells which develop into different types of mature T helper cells. These cells orchestrate the overall immune response leading in most cases to elimination or control of the pathogens. Under certain circumstances, the immune response is directed toward self and causes autoimmunity.

In vivo, an adaptive immune response is strictly associated with a concomitant innate immune response, which often initiates with the recognition of invariant motifs, such as pathogen-associated molecular patterns (PAMPs) present in commensal or pathogenic microbes or danger-associated molecular patterns (DAMPs) released by damaged host tissues [1], by specialized germ line-encoded pattern recognition receptors (PRRs) [2]. Many PRRs, such as Toll-like receptors (TLRs) and different families of lectins, are involved. These receptors are expressed on the surface or in the cytoplasm of various types of dendritic cells (DCs), macrophages, and neutrophils. During the immune response, monocytes, macrophages, and neutrophils normally provide phagocytosis and killing, while DCs, once stimulated through the PRR and other receptors such as CD40, mature into professional antigen-presenting cells (APCs) with upregulated expression of MHC class II, CD40, CD80, CD83, and CD86 [3]. In this way, they become more efficient at delivering antigenic peptides and costimulatory signals. In addition, they start to produce different cytokines which have a role in directing the type of the adaptive immune response. Cytokines, secreted by APC, are, among others, TGF-β, IL-6, IL-1, IL-23, and IL-12 [4–7].

Activation of extrathymic naïve αβ CD4+ T cells is a crucial step in the initiation of an adaptive immune response. In the mouse, naïve T cells are long lived and can remain in interphase for weeks or months [8]. They are in a resting state and show high expression of lymph node homing receptors CD62L and CCR7 and low expression of CD44, an activation marker. These cells are kept alive in the peripheral lymphoid tissues by contact of their T cell receptors (TCRs) with self-peptide–MHC complexes on APCs and exposure to IL-7 [9, 10]. Signals to the different clonotypic TCRs of naïve CD4+ T cells are provided by APCs which present different exogenous but also endogenous antigen-specific oligopeptides in the context of the MHC class II molecules [11–13]. Critical events for the expression of the antigen by APCs are the routes of endocytosis through clathrin-coated pits, caveolae, or macropinocytosis, its cleavage by endosomal and lysosomal proteases, and competition among oligopeptides for the MHC class II molecules [13]. It is expected that during an immune response to a pathogen, the frequency of certain antigenic specificities might increase above a critical threshold allowing the naïve T cells to reach a novel critical level of activation at the TCRs. This event can be modulated not only by its magnitude, i.e., the actual number of a specific epitope presented by the APC but also by the different affinities of the oligopeptides for the MHC class II molecules and of the oligopeptide/MHC complexes for the clonotypic TCRs [14].

The signals through the TCRs are further amplified by signals originated through costimulatory molecules. CD28, which interacts with CD80 (B7.1) and CD86 (B7.2) on APCs, is the best characterized costimulatory molecule expressed by T cells. This second signal contributes to enhance the level of activation of the naïve CD4+ T cells leading to its survival or to proliferation and differentiation with upregulation of surface markers such as CD25, CD44, and CD69. In the absence of this second signal, the T cells are clonally deleted through programmed cell death or apoptosis or become anergic [15]. However, costimulatory receptor pairs, such as CTLA-4/CD80-CD86, can also deliver inhibitory signals and contribute to T cell unresponsiveness [15].

Moreover, the proliferation and differentiation of activated CD4+ T cells are mainly dependent on the presence of cytokines in the extracellular milieu, which through the activation of their respective receptors and transcription factors induce the expression of central "master regulators" which control lineage commitment and differentiation. Naïve CD4+ T cells can differentiate into induced T regulatory (iTreg) cells, characterized by the expression of Foxp3 [16], Th17 cells expressing RORγt, Th1 cells which are T-Bet+, and Th2 cells expressing GATA3 [17–20].

The genetic background, the site of the reaction, the type and the entity of the signals originated through the activation of different PRR, and the type of APCs involved contribute to determine the type and the entity of specific signature cytokines released by APCs. These cytokines work together with cytokines released by other immune cells to promote the maturation and differentiation of naïve CD4+ T cells [21, 22]. Several cytokines and transcription factors are involved in this process, and some of them show self-reinforcing activities, promoting and stabilizing the maturation of that lineage but also inhibiting the development of other lineages. The outcome of an immune response appears to depend on a few critical decision steps which are clearly influenced by the amount and the type of the antigens and the concomitant presence of different amounts of PAMP and DAMP.

2 APCs as Crucial Determinant for the Generation of iTreg Cells and Th17 Cells

A crucial step for the naïve αβ CD4+ T cell, once engaged with an APC activated through specific antigens, is to become an iTreg cell or an effector cell (Fig. 1). Available data suggest that this decision is controlled by APCs, which have the choice to direct the immune response toward suppression by generating iTreg cells or response by favoring Th17 cells. For this task, DCs are properly equipped as they not only present the antigen in the presence of costimulatory stimuli but also can secrete the critical cytokines TGF-β, IL-6, IL-1β, and IL-23 [4–7]. The generation of Th1 and Th2 effector cells could be less favored in the direct contact between DC and naïve CD4+ T cell, as it requires the involvement of additional cell types which provide IFN-γ or IL-4 (Fig. 2) [23–25].

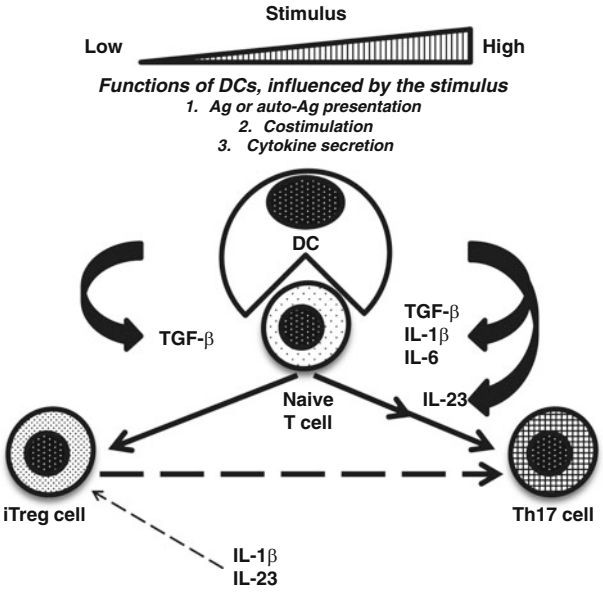

Fig. 1 The amount and the type of the stimulus determine the degree of activation of DCs, the type and amount of cytokines secreted and finally the type of T cell response. TGF-β is critical for the generation of iTreg cells from naïve T cells, but in combination with IL-6, IL-1β, and IL-23 contributes to the generation of effector Th17 cells. Under certain conditions, inflammatory cytokines such as IL-1β or IL-23, can convert iTreg cells into effector Th17 cells

Some experiments seem to confirm an early overlap between iTregs and Th17 cells. Naïve T cells, stimulated with TGF-β, upregulate both Foxp3 and RORγt and progressively lose RORγt expression as they differentiated into iTreg cells [26]. Similarly, early Th17 differentiation induced by TGF-β and IL-6 is accompanied by coexpression of RORγt and FoxP3. FoxP3 disappears as the Th17 development progresses. However, in this context, it is unclear whether FoxP3 might actually be an activation marker [26, 27]. It is even possible to envisage that in vivo, both iTreg cells and Th17 cells could be generated during the same immune response depending on the local maturation of APCs linked to availability of antigens and PAMP. Only later on, one of the two would prevail. However, other scenarios are feasible. The final result might depend on the amount and type of antigens, the entity and range of PRR activation, and finally on the production of activating or suppressing cytokines. In this context, also the site, in which the innate immune response is initiated, has an important role [28]. For example, CD103+ DCs from the small intestine and mesenteric lymph nodes produce both TGF-β and retinoic acid (RA) and seem to favor the generation of iTreg cells over effector T cells [29, 30]. Moreover, depletion of inflammatory macrophages reduces the numbers of Th17 cells and increases the numbers of iTreg cells, suggesting that reduction of inflammatory cytokines such as IL-6 and IL-23 may regulate the generation of these T cell subtypes [31].

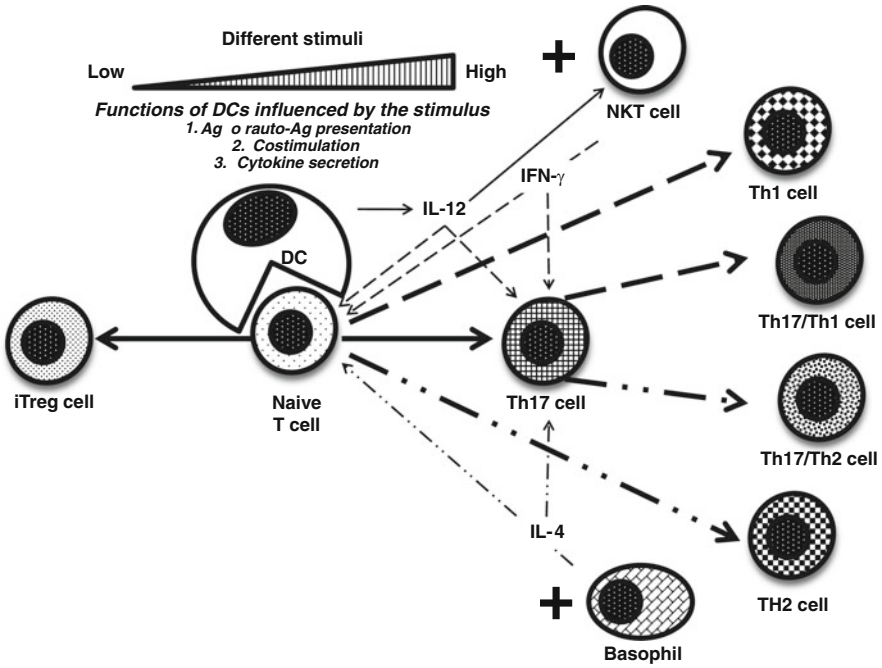

Fig. 2 Schematic representation of an adaptive immune response. DCs alone can control the generation of iTreg cells or Th17 cells, but need the help of cytokines secreted by other cells to generate effector Th1 or Th2 cells. IL-12 secreted by DC, is relevant for activation of NKT cells to release IFN-γ. The combination of IFN-γ and IL-12 directs naïve T cells to differentiate into Th1 cells and redirects Th17 cells toward the Th1 phenotype (Th17/Th1 cells). IL-4 released by basophils or other cells directs naïve T cells toward Th2 cells and Th17 cells toward Th17/Th2 cells

3 Induced Regulatory T(iTreg) Cells

There are two major types of CD4+ FoxP3+ regulatory T cells: those generated in the thymus, i.e., the natural T regulatory (nTreg) cells, and those generated in the periphery from naïve T cells (iTreg cells) [32, 33]. nTreg and iTreg cells are phenotypically and functionally similar as they show high expression of CD25 (high-affinity IL-2 receptor), GITR, and CTLA-4 and low expression of CD127, synthesize anti-inflammatory principles such as IL-10, TGF-β, IL-35 (mouse only), adenosine, IDO, and granzyme/perforin, and function in a contact-dependent manner [34]. However, these two populations differ in TCR signal strength and the costimulatory and cytokine requirements for generation and expansion [35] and perhaps in the expression of Ikaros family member transcription factor, Helios [36]. nTreg cells appear to be a more stable cell subset, whereas iTreg cells have been described as less stable [37].

The generation of iTreg cells requires an active process [38]. When subimmunogenic conditions (i.e., low antigen dose, immature DC, plasmacitoid DC, B cells, high production of IL-10, and TGF-β) are encountered, naïve T cells

tend to acquire FoxP3 expression and become iTreg cells [39, 40]. In particular, TGF-β, at high concentrations, favors the generation of iTreg cells, blocks the expression of IL-23R on Th17 cells, and suppresses Th1 and Th2 differentiation [27, 41]. The generation of iTreg cells is facilitated by the capacity of the same cells to produce TGF-β [42] and by the presence of IL-2 [43], RA [44], and vitamin D, which favors the generation of iTreg cells both indirectly by downregulating costimulatory molecules on DCs and directly upregulating CTLA-4 [45], a functional marker of iTreg cells. Also, activation of the aryl hydrocarbon receptor (AhR), a transcription factor of the bHLH/PAS family, which regulates the biochemical and toxic effects of environmental chemicals, has been shown to promote epigenetic regulation and facilitate the differentiation of iTreg cells [46] even if some data suggest a more complex regulation [47]. The relationship between Th17 cells, iTreg cells, and IL-2 has also received special attention. iTreg cells are able to scavenge IL-2 from Th1 responder cells and to dampen Th1-mediated pathology [48]. However, this might actually favor the differentiation of naïve T cells toward the Th17 program, by removing an inhibitory cytokine. In conclusion, iTreg cells might actually promote Th17 development and effector function by scavenging a negative regulator such as IL-2 [49–51]. On the other hand, cytokines like IL-6, IL-21 secreted by Th17 cells, IFN-γ (Th1), and IL-4 (Th2) tend to block iTreg cells development suggesting that generation of iTreg cells might be controlled by other T cell subsets [52].

4 Th17 Cells

TGF-β, at low concentrations and in the presence of inflammatory cytokines such as IL-6 or IL-1β, promotes the expression of the IL-23 receptor (IL-23R) and the acquisition of Th17 cell effector functions [53, 54]. As DCs can secrete both IL-1β and IL-6, they can influence directly the development of Th17 cells [4]. In vitro, full acquisition of pathogenic functions by effector Th17 cells is mediated by IL-23, another cytokine secreted by APC [55], and later on by IL-7 [56]. Th17 cell differentiation is also promoted by IL-21, a cytokine produced by Th17 cells [57–59]. On the other hand, many more cytokines such as IL-4, IFN-γ, IL-2, IL-10, IL-12, IL-25, and IL-27 act as negative regulators of the differentiation of Th17 cells, suggesting that the generation of Th17 cells is tightly controlled [18, 19, 44, 60–63]. IL-27 appears to have a relevant role in the control of IL-17A production by Th17 cells and represents an important checkpoint under the control of DCs and innate immunity. In particular, IL-27, not only has direct immunosuppressive effects on various T cell subsets, but also is able to induce the release of IL-10 by CD4+ and CD8+ T cells. IFN-γ and IL-4 are other important mediators involved in the control of IL-17A production and Th17 cell differentiation [19]. It appears therefore that Th17 cell generation is strictly controlled by other T cell subsets.

The mouse Th17 cell subset is a heterogeneous population that produces IL-17A, IL-17F, IL-17A/F heterodimer, IL-21, IL-22, TNF-α, and under specific

circumstances, GM-CSF, IL-6 [61, 64], and IL-10 (55). Production of IL-17A and IL-17F [60, 65] 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 [66, 67].

The mechanisms that regulate IL-17A and IL-17F may also be different; IL-17F seems to be expressed earlier than IL-17A [68], deficiency of RORα selectively reduces IL-17A production [69], and IL-17A expression is more sensitive to TCR signaling [70]. Moreover, in mice, CD4+ IL-17A single-positive T cells seem to be prevalent in the lamina propria and intestinal intraepithelium [67]. Expression of IL-22 by Th17 cells appears to be more dependent on IL-23 than IL-17A and IL-17F [71]. 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 [72], 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 [72]. Another interesting aspect is the identification of 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. [65] 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. It is reasonable to propose that the function of Th17 cells may be influenced by the levels of surface expression of molecules in the CD28 or the TNF superfamily [65]. Both iTreg cells and Th17 cells express CCR6 that facilitate homing to tissues and not follicles, suggesting that both cell types migrate to the same inflammatory sites [73]. Among other properties, Th17 cells appear uniquely equipped to promote osteoclastogenesis as a result of RANKL expression [74] and to induce generation of ectopic lymphoid follicle-like structures. Th17 cell produce IL-17A and express podoplanin on their surface [75]. Podoplanin deficient mice have a defect in the formation of secondary lymphoid structures [75]. RANKL+ Th17 cells have also been shown in the lesions of arthritic patients [76].

Human Th17 cells are also characterized by the variable production of IL-17A, IL-17F, IL-22, IL-21, IL26, and TNF-α and by the expression of a unique pattern of surface receptors, including CCR4, CCR6, and CD161 [77, 78]. Their differentiation pathway is slightly different from that of the mouse, as stimulation of naïve CD4+ T cell precursors with TGF-β and IL-6 is not so efficient [78]. However, also in humans, IL-1β, IL-21, and IL-23 are critical for Th17 cell differentiation, growth, survival, and effector functions [78].

Several transcription factors or pathways have been involved in the signaling from the cytokine receptors. It is interesting to observe that IL-17A producing CD4+ T cells express the IL-10 receptor α and that IL-10 leads to a decrease in Th17 and Th17/Th1 CD4+ T cells [79]. It is intriguing that IL-6, IL-21, and IL-23 which favor the generation of Th17 cells but also IL-10 and IL-27, which inhibit

their generation, signal mainly through STAT3 [79, 80]. The number of specific cytokine receptors, which is variable with time, might actually control the entity of the response. As for other T cell lineages, the generation of iTreg cells and Th17 cells is associated with epigenetic modification such as histone remodeling and DNA demethylation [81–83].

5 Plasticity of iTreg cells

Presently, it is not fully clear whether mature iTreg cells retain substantial developmental plasticity and are able to convert into Th17 cells (Fig. 1) or whether the appearance of Th17 cells in a Treg cell-dominated environment is due to the expansion of novel T cell clonotypes. However, it has been reported that, in the presence of IL-1β, TGF-β, and IL-23, human Th17 cells can originate from a population of Treg cell precursors [84]. In addition, a hybrid subpopulation coexpressing Foxp3 and RORγt with regulatory functions and production of IL-17A has been described [85, 86]. Fully differentiated Treg cells can be converted into Th17 cells in mice [27, 87, 88] and humans [89], and stimulation of memory Treg cells in the presence of IL-1β allows them to become Th17 cells [89, 90]. Osorio et al. [91] showed that mouse CD25+ FoxP3+ Treg cells can be converted into a hybrid T cell population characterized by the expression of FoxP3 and RORγt and the production of IL-17A. Conversion was observed upon coculture with DCs selectively activated via dectin-1, a C-type lectin receptor involved in fungal recognition, and was linked to IL-23 production by DCs. These data suggest that there are several potential cross talks in the differentiation pathways of Treg cells and Th17 cells and that an antigen-specific immune response can be generated, when the proper stimuli are applied, supporting the view of plasticity of the immune response. However, while it has been documented that, under inflammatory conditions, a Treg cell-dominated environment can convert into a Th17 cell-dominated immune response, the opposite has not been shown. The conversion of Treg cells into Th17 cells may be an irreversible process, like the conversion of Th17 precursors into Th1 cells. Similarly, it has been shown that Th1 cells do not develop into Treg cells [92]. It is also interesting that, in animal models, Treg cells tend to appear during the recovery phase [93], again suggesting that when the antigen load and the inflammatory environment are reduced, the generation of new iTreg cells is favored.

6 Th17 Cells and Autoimmunity

In summary, cytokines secreted by APCs and various metabolites crucially contribute to the control of Treg cells and Th17 cell development, differentiation, and function. Th17 cells have a major role in mediating the immune responses against some extracellular bacterial and fungal pathogens [54, 94–96] but have also been

implicated in the response to some intracellular bacteria [97], intracellular [98, 99] and extracellular parasites [100, 101], and viruses [102, 103], in the pathogenesis of several autoimmune disorders, including multiple sclerosis, rheumatoid arthritis, psoriasis [61, 104, 105] and in allergic reactions [106]. During an enhanced or not properly suppressed response to a self-antigen or to a foreign antigen mimicking self or in concomitance with tissue damage or infection or in the presence of a defect in costimulatory inhibitory signals, a T cell response to an autoantigen could be initiated. In conclusion, a minor derangement in the finely tuned regulation of the immune response to self-antigens might tip the balance toward an immune response. The prevalent role of Th17 cells in many animal models of autoimmunity can be related to the fact that these cells can be directly generated through the encounter of naïve αβ CD4+ T cells with properly activated APCs.

7 Th1 or Th2 Immune Responses Require the Involvement of Additional Cells and Can Coexist with Th17 Cell Activation

Th1 responses to intracellular bacteria or Th2 responses to parasites can be associated with a concomitant and/or transient Th17 cell response [97, 107–109]. The generation of a Th1 or Th2 cell response in vivo often depends not only on the activation of APCs but also on the involvement of additional cell types. A different function for APCs in the generation of Th17 or Th1 cells is supported by the fact that a Th17 response is favored by activated APC and that disruption of the APC–T cell interactions with anti-MHC antibodies can block IL-17A but not IFN-γ production [110]. Infections with intracellular bacteria or protozoa such as leishmania promote immune responses dominated by Th1 cells, but a Th17 component has been documented in several cases [97–99].

For the generation of Th1 cells, invariant NKT (iNKT) cells appear to have a relevant role [23, 111]. The recognition of PAMP, associated with bacteria and viruses, induces the production of IL-12 by APCs [112]. The IL-12R is constitutively detected on iNKT cells which are characterized by the expression of a single invariant TCRα chain, associated with a highly restricted set of Vβ chains [23]. iNKT cells appear to be constantly activated by endogenous glycolipids presented by CD1d, a monomorphic MHC-like class Ib molecule [23]. IL-12 acts on iNKT cells to enhance their production of IFN-γ [113] and, later on, in association with IFN-γ, causes the activation and clonal expansion of naïve αβ CD4+ T cells into Th1 cells. Sequential activation of STAT1 by IFNγ and STAT4 by IL-12 drives optimal expression of T-bet, the central transcription factor for Th1 programming. Stability of this developmental program is maintained through the production of IFNγ by mature Th1 effector cells. IFN-γ acts as an autocrine cytokine feedback loop for Th1 cell generation and as an inhibitor of iTreg cells and Th17 cell generation [52]. However, some Th1 responses can be generated through other pathways. For example, the Th1 response to certain viruses is independent from

IL-12 [114–116]. There is also evidence for temporal differences in the appearance of Th17 and Th1 cells, as the Th17 response often peaks earlier, followed by the appearance of Th1 cells [93, 117–119].

Parasites or allergens induce Th2-biased responses that can be associated with a Th17 response [100, 101, 120]. Murine *Trypanosoma congolense* [121] and *Trichinella spiralis* infections [107] but also intranasal immunization of mice with house dust mite induce both Th2 and Th17 responses [122–124]. The generation of Th2 cells appears to require contributions of innate sources of IL-4, IL-13, and thymic stromal lymphopoietin (TSLP) [24, 25]. Nuocytes, basophils, FcεRI-expressing inflammatory dendritic cells [125], and follicular T helper cells [126, 127] but also other cell subsets have a critical role in Th2 induction, and several cytokines, including IL-4, IL-13, IL-25 (IL-17E), IL-33, and TSLP, are involved [127]. Under certain conditions, basophils can rapidly release IL-4, IL-9, IL-25, and TSLP upon activation and direct Th2 cell polarization [128]. The protease allergen papain, injected subcutaneously, induces the migration of basophils into lymph nodes where they promote Th2 differentiation by acting as APCs and by secreting IL-4 and TSLP [129–132]. However, also cooperation, via ROS-mediated signaling, between migratory dermal DCs and basophils positive for IL-4 has been described [133], suggesting that multiple pathways of activation might be in place or that different stimuli might have different capabilities to activate DCs through PRR. Activation of STAT6 by IL-4 upregulates GATA3 which is central to Th2 programming. However, some Th2 responses in vivo can be independent of IL-4, including those to some parasites [134, 135]. Stability of the Th2 developmental program is reinforced in part through production by mature effector T cells of IL-4, which acts as a potent inhibitor of early Th17 development.

8 Th17 Cell Plasticity and Mixed Th17/Th1 and Th17/Th2 Cell Subpopulations

It has also been shown that Th cells can coexpress IL-17A with a variety of other cytokines, including IFNγ, IL-4, or IL-10. IL-17A-secreting effector cells, generated in vitro by TGF-β, IL-6, and IL-23, fail to reexpress IL-17A in the absence of the original inducing signals, even after repeated instructive restimulations [136]. They convert to IFN-γ expression when stimulated with IL-12 or to IL-4 expression when stimulated with IL-4, suggesting substantial developmental plasticity [68, 136–139]. Once differentiated, Th17 cells coexpress the IL-12Rβ1 with the IL-23R and with low levels of IL-12Rβ2. The expression of both receptors enables them to respond to either IL-23 or IL-12 [68]. In contrast, Th1 cells do not upregulate the IL-23R and are unresponsive to IL-23 [68]. As already mentioned for iTreg cells, which can convert into Th17 cells, Th17 cells can acquire the characteristics of Th1 cells, challenging the model of a stable Th lineage commitment. This conversion is associated with abrogation of IL-17A production,

gain of IFN-γ production, and concomitant changes in histone methylation at the Il17a and Ifng locus [27, 68, 138]. Thus, Th17 cells can give rise to progenies that retain high levels of IL-17A expression, silence IL-17A, and express high levels of IFNγ or acquire a mixed phenotype expressing both IL-17A and IFNγ. A switch of mouse Th17 cells into Th1 cells has also been documented during the development of type 1 diabetes and experimental autoimmune encephalomyelitis (EAE) [140, 141]. Interestingly, the final destination of Th17 cells is strongly influenced by the cytokine milieu [142] as they can acquire IFN-γ expression in the context of EAE but not in the context of a *Candida albicans* infection.

Human Th17 cells have also the capacity to acquire expression of IFN-γ, but often IL-17A production is maintained and the resulting cells have an intermediate Th17/Th1 phenotype [143, 144]. This suggests that differentiated human Th17 cells are relatively resistant to conversion toward the Th1 lineage [145]. The reciprocal conversion of Th1 cells or Th2 cells to Th17 is less likely to occur [146] as the Rorc promoter in these cells is repressed [18, 68].

More recently, an additional subset of Th2 memory/effector cells that coexpress the transcription factors GATA3 and RORγt and coproduce Th17 and Th2 cytokines has been described [147]. In a mouse model of allergic lung diseases, IL-17A-producing CD4+ Th2 cells were induced in the inflamed lung and persisted as the dominant IL-17A-producing T cell population during the chronic stage of asthma. In man, IL-17+ Th2 cells are significantly increased in blood of patients with atopic asthma. In addition, CCR6+ CD161+ CD4+ T cell clones able to produce both IL-17A and IL-4 have been described [148]. These clones produce also IL-5, IL-8, IL-9, IL-13, IL-21, and IL-22 and displayed the ability to induce the in vitro secretion of IgE. A very few IL-17A+ Th2 cells were found among circulating CD4+ T cells from normal subjects, but their proportions were significantly increased in the circulation of patients with chronic asthma [148].

These findings highlight the plasticity of Th2 memory cells and suggest that IL-17A-producing Th2 cells may represent the key pathogenic Th2 cells promoting the exacerbation of allergic asthma [147].

9 Conclusion

In conclusion, an adaptive immune response is triggered by the activation of different clonotypic naïve CD4+ T cells which have reached their specific threshold of activation at the level of TCRs, costimulatory signals, and accessory signals. Diversity and flexibility of the immune response is provided by the intensity of the activation of different T cell clones, and, in this context, the accessory signals and cytokines contributed by APCs and by other cell types have a prominent role in defining the sequential polarization and imprinting of naïve T cells into iTreg, Th17, Th1, and Th2 cells.

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Regulatory T cells Versus Th17: Differentiation of Th17 Versus Treg, Are They Mutually Exclusive?

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Abstract Naive $CD4^+$ cells differentiate into T helper (Th1, Th2, Th9, Th17) and regulatory T (Treg) cells to execute their immunologic function. Whereas TGF-β suppresses Th1 and Th2 cell differentiation, this cytokine promotes Th9, Th17, and $Foxp3^+$ regulatory T cells depending upon the existence of other cytokines. IL-6 promotes Th17 but suppresses regulatory T cell differentiation. Moreover, natural but not TGF-β-induced regulatory T cells convert into Th17 cells in the inflammatory milieu. This chapter discusses the updates of these cell differentiation and conversion, as well as underlying mechanisms.

Keywords T helper cells • Th1 • Th2 • Th9 • Th17 • Tfh • $Foxp3^+$ regulatory T cells • Cytokine • Differentiation • Autoimmunity

Naive $CD4^+$ cells have to differentiate into T helper (Th1, Th2, Th9, Th17) and regulatory T (Treg) cells to execute their functional activities. Th1, Th2, and Th17 cells play an important role in the protective immune response against intracellular pathogens and extracellular parasites; nonetheless, excessive immune responses exerted by these T helper cells also cause autoimmune and inflammatory diseases. $Foxp3^+$ Treg cells are essential for the immune tolerance and play a crucial role in the limitation of the excessive immune and inflammatory response executed by these T helper cells. Although Treg and Th17 cells have a completely different function in the immune responses, the differentiation of both cell subsets does need TGF-β. In this chapter, I will discuss the differentiation and relation of Tregs and Th17 cells,

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in particular Treg cell conversion to Th17 cells. I have also discussed the underlying mechanisms and functional significance of these differentiations and conversions.

1 Functional Characteristics and Categories of Regulatory T Cells

It is now well accepted that $CD4^+CD25^+Foxp3^+$ regulatory or suppressor cells are critically involved in immune tolerance and homeostasis. In the early 1970s, Gershon and colleagues initially reported that thymocytes from his experimental animal model contained such cell population they called "suppressor T cells" and assumed they belong to $CD8^+$ cell subset [1]. This suggestion was not appreciated until Sakaguchi et al. found that a population of $CD4^+CD25^+$ cells rather than $CD8^+$ T cells in the thymus did indeed possess immunosuppressive activity that is now referred to as "regulatory T cells or natural regulatory T cells, nTregs" [2].

$CD4^+CD25^+$ cell population also exists in humans, although only the $CD4^+CD25^{bright}$ cell population appears to display an immune suppressive activity. A better approach for the identification of human Treg cells is to target the $CD4^+CD25^+CD127^{-/low}$ population [3]. More recently, $CD4^+CD25^+$ $CD127^-$ $CD45RO^+Foxp3^+$ cells are identified as real human suppressor cells [4].

CD25 is also an activation marker for lymphocytes. Thus, the utility of CD25 expression as a Treg marker is limited since it does not completely discriminate between activated T effector cells and Tregs. Fortunately, the nuclear transcription factor Foxp3 has been identified as a much more specific marker for Treg cells. Foxp3 is critically involved in the development and function of Treg cells [5]. In mice, the lack of functional Foxp3 expression results in a fatal lymphoproliferative disorder known as scurfy, and mutations of the human FOXP3 gene result in a human syndrome known as IPEX (immune dysregulation, polyendocrinopathy, enteropathy, X-linked), which is characterized by autoimmune disease expression in multiple endocrine organs [6].

Despite the fact that Foxp3-GFP "knock-in" studies clearly demonstrate that there is a very broad spectrum of CD25 expression on Treg cells and that the intranuclear location of Foxp3 makes it difficult to use this protein for immunoaffinity-based purification methods although recent study has identified a new technique to improve the isolation of the live Treg cells [7], $CD4^+CD25^+$ cells are still widely used in the field of the biology of Treg cells without using genetically modified tissues, particular in human studies. Although Foxp3 is considered as a specific marker for Tregs in mouse, this may not be the case for human Tregs. Recent data demonstrate that FOXP3 (FOXP3 for human cells and Foxp3 for mouse cells) may be upregulated in rapidly proliferating human T cells and might be viewed as an activation marker for human T cells [8]. More studies are needed to determine how FOXP3 might also be expressed on rapidly proliferating human T

effector cells, and more specific molecular markers to identify human Tregs are also desirable.

Many studies have revealed that the numbers of $CD4^+CD25^+$ cells and $CD4^+FOXP3^+$ cells in patients with various autoimmune diseases are diminished and that this Treg deficit is associated with disease severity and activity [9]. The peripheral Treg deficit in patients with autoimmune diseases is not resultant from their redistribution to different organs [10]. Diminishment of Tregs in the autoimmune diseases is not a universal finding. Other groups have actually observed the converse that the numbers of human $CD4^+CD25^+$ cells can be increased under these circumstances [11]. Since CD25 and FOXP3 can also be classified as activated makers, this aspect may reflect the disparity between these findings. Miyara et al. have further classified human $FOXP3^+$ cells into three cell subsets: $CD45RA^+FOXP3^{low}$, $CD45RA^-FOXP3^{hi}$, and $CD45RA^-FOXP3^{low}$. Functional assay demonstrated that the $CD45RA^-FOXP3^{low}$ subset contains non-suppressor cells, that the $CD45RA^+FOXP3^{low}$ subset contains resting Tregs, and that active Tregs are found in the $CD45RA^-FOXP3^{hi}$ subset. Using these criteria, they found that Treg cell numbers were indeed diminished in patients with active autoimmune disease [4].

In addition to Treg frequency, others have also reported that the functional activity of Tregs has been altered in some autoimmune diseases. For example, the suppressive activity of $CD4^+CD25^+$ cells isolated from patients with active rheumatoid arthritis was significantly reduced [12]. It is possible that some intrinsic defect in $CD4^+CD25^+$ cells in these patients accounts for their reduced functional activity. Similarly, the frequency of $CD4^+CD25^+$ cells in patients with multiple sclerosis (MS) is unaltered; however, the functional activity of these cells to suppress T cell immune responses including antigen-specific or nonspecific stimulation is also diminished [13–15]. These results suggest that the manipulation of nTregs to restore their numbers and function may be therapeutic.

Although most people claim that $CD4^+CD25^+$ cells in peripheral blood mononuclear cell (PBMC) belong to natural Treg cells, we and others would suggest that $CD4^+CD25^+$ cells in PBMCs consist of a mixture of both thymic-derived nTregs and those induced in the periphery (induced Tregs, iTregs) [16–18]. Until today, there are no specific markers that can distinguish nTregs from iTregs. Although Shevach's group recently reported that Helios, an Ikaros family transcription factor, may be helpful for distinguishing these cell populations [19], others reported that Helios is also highly expressed on Th2 and T follicular helper cells and may be associated with the differentiation of these cells [20]. We recently observed activated $Foxp3^-$ T cells also express Helios (unpublished data); therefore, it is necessary to develop more reliable molecular marker(s) to distinguish various subsets of Treg cells.

It has been well known that the adoptive transfer of nTregs can prevent the appearance and development of autoimmune diseases in many animal models. Nonetheless, there are also considerable numbers of studies demonstrating that the therapeutic effect of nTregs on established autoimmune diseases is fairly unsatisfactory. For example, the efficacy of adoptive transfer of nTregs to

established collagen-induced arthritis (CIA) is poor for controlling the disease progression [21]. Injection of nTregs to established lupus had mild protective effects, and it was unable to suppress lupus glomerulonephritis and sialoadenitis [22, 23]. Moreover, adoptive transfer of nTregs failed to control Th17-mediated autoimmune gastritis [24].

There are several possibilities that could explain the inability of nTregs to treat CIA and other autoimmune diseases. First, proinflammatory cytokines may hamper their suppressive activity. Pasare et al. have reported that Treg suppressive activity can be abolished by IL-6 [25]. Valencia et al. also revealed that elevated TNF-α may interfere with the suppressive capacity of nTregs in patients with rheumatoid arthritis (RA) [12]. There is no question that these proinflammatory cytokines are elevated in patients with RA and other autoimmune diseases [26]. Secondly, Th17 cells may be resistant to the suppressive effects exerted by nTregs. This could explain how nTregs are able to prevent development of disease before Th17 cells become established, while demonstrating ineffective suppression after disease expression is evident where Th17 cells have been developed. Third, nTregs are inherently unstable and can be converted to Th1, Th2, Th17, and Tfh effector cells when they encounter an inflammatory milieu [21, 27–31].

There are still other reasons that could hamper the utilization of nTregs as therapeutics. First, the intranuclear location of Foxp3 makes it difficult to purify human nTregs for functional study. Second, nTregs constitute only 1–2 % of human $CD4^+$ T cells, and this is also difficult to gain the sufficient numbers for therapeutic requirement. Although several groups have claimed that expansion of human nTregs in vitro can overcome this problem [32], other laboratories have reported that repeated expansion alters Treg phenotype and function [33]. Third, the expansion of nTregs from patients with RA and MS for therapeutic purposes may be problematic due to potential other intrinsic defects in RA and MS nTregs. nTreg instability, Teff cell resistance, and the influence of an inflammatory milieu may individually or collectively account for the inability of nTregs to control established autoimmune diseases.

Of great interest, the plasticity or instability of nTregs under inflammatory conditions could be overcome with cytokines or other compounds. Our group recently reported that while nTregs become Th17 cells in the presence of IL-6, these cells also lost their suppressive role in the progression of the lupus-like syndromes and CIA. We also determined that pretreatment of nTregs with IL-2 combined with TGF-β, or *all-trans* retinoic acid (atRA), a vitamin A metabolite, can render these nTregs resistant to Teff cell conversion and allow them to begin to suppress lupus and CIA progression [7, 31]. Recently, we found that atRA also maintains the stability of nTregs in human (unpublished data). This indicates that the manipulation of nTregs still holds a potential promise in the treatment of autoimmune diseases.

Like nTregs, iTregs generated ex vivo with IL-2 and TGF-β also share similar phenotypes related to Treg cells and suppress immune responses and immune cell-mediated diseases. Importantly, adoptive transfer of iTregs not only prevents autoimmune diseases in many animal models but also attenuates the disease syndromes when iTregs were infused when or after diseases are established

[34–41]. It is very likely that iTregs are stable and sustain their immune suppressive activity in the inflammatory condition [31]. Because sufficient numbers of iTregs can be easily gained and antigen-specific iTregs can be easily developed in the certain environment, it implicates that the manipulation of iTregs has a great potential to treat autoimmune and inflammatory diseases.

2 iTregs and Their Differentiation and Development

$CD4^+$ Treg subsets can be further classified into three main populations, thymus-derived, naturally occurring $CD4^+CD25^+Foxp3^+$ cells (nTregs) described as above; endogenous-induced Tregs in vivo; and those that can be induced ex vivo from $CD25^-$ precursors in peripheral lymphoid organs (iTregs) [42]. Although IL-10-induced Tr1 cells represent another cell population of iTregs, they do not express Foxp3 and produce considerable levels of IL-10 [43]. As IL-10 may promote autoimmune response through stimulating B cell activation and its level is highly increased in patients with active systemic lupus erythematosus (SLE) [44], Tr1 may not be suitable for the treatment of SLE and other autoimmune diseases. TGF-β-induced Tregs will be defined as iTregs in this chapter.

While Yamagiwa et al. reported that TGF-β promotes endogenous $CD4^+CD25^+$ nTreg cell expansion [45], our group first reported that TGF-β does have an ability to induce $CD4^+CD25^-$ cells to become $CD4^+CD25^+$ Treg cells in vitro [42]. When Foxp3 was identified as Treg marker, several groups immediately found that TGF-β can induce Foxp3 expression in iTregs [34, 40, 46]. Additionally, other studies have also clearly demonstrated that the development of $Foxp3^+$ Tregs in vivo is also through TGF-β-dependent mechanism [47].

Phenotypically, both nTregs and iTregs express similar molecules such as CD25, CD122, CTLA-4, GITR, CCR4, CD62L, PD1, and Foxp3 and express $CD45RB^{low}$ in mice and CD45RO in humans. $CD4^+CD25^+Foxp3^+$ cells in the periphery have been considered as a mixed population comprised of nTregs and iTregs. Although Helios might possibly help to distinguish nTregs from iTregs [19], more specific molecular markers are needed to distinguish both Treg cell populations.

Although both nTreg and iTreg subsets share similar phenotypes and display comparable suppressive activity, several factors distinctly affect their development, stability, and function (Table 1). First, nTregs develop in the thymus through recognition of self-antigens. A high- and medium-affinity cognate interaction between self-peptide–MHC complex and T cell receptor is required for this process. They also require CD28 costimulation because they do not develop in CD28-deficient mice [48]. Although IL-2 and TGF-β play an important role in the maintenance of the pool size of nTregs, both cytokines are redundant for their development since both IL-2 and TGF-β knockout mice contain $CD4^+CD25^+Foxp3^+$ regulatory T cells in the thymus [49]. Although one group recently reported that TGF-β is essential for the nTreg cell development [50], most

Table 1 Differences between nTreg and iTreg cells

		nTreg	iTreg
Origin		Thymus	Periphery
TCR stimulation	Antigen	Self	Self and environmental
	Affinity	High to medium	Suboptimal
Co-stimulations	Essential	CD28	CTLA-4
	Ox40/Ox40L	Function deficient	Generation deficient
Cytokines	Generations requirement	(−) or TGF-β?	TGF-β
		(−) or IL-2?	IL-2
	Maintenance requirement	IL-2	IL-2
	Function requirement	TNF-α (+)	TNF-α (−)
Stabilities	IL-6 or others	Convert to Th17, Th1, Th2 and Tfh	No conversion
Phenotype		Helios (+)	Helios (−)
Human		No cytokine production	Cytokine production

researchers believe that TGF-β is redundant in nTreg development although this cytokine is important for maintaining pool size of the nTreg cells [51].

By contrast, the generation of iTregs is dependent upon the presence of both TGF-β and TGF-β receptor signals since the absence of TGF-β or TGF-β receptors or blocking the TGF-β receptor signal prevents the induction of Foxp3 expression and the subsequent functional suppressive capacity [27, 52]. Similarly, IL-2 plays an essential role in the differentiation of $Foxp3^+$ iTregs. TGF-β fails to induce $Foxp3^+$ iTregs from naïve $CD4^+CD25^-$ precursor cells in IL-2-deficient mice [53]. The conversion of $CD4^+CD25^-$ cells in the periphery to $CD25^+$ iTregs requires a suboptimal TCR stimulation, and thus environmental antigens may sufficiently trigger iTreg development. The absence of CD28 costimulatory molecules does not affect the differentiation of iTregs (Lan and Zheng unpublished data), but inhibitory CTLA-4 costimulation and CTLA-4/B7.1 signaling are crucially required for the generation of iTregs [54]. This conclusion is further documented by an observation that the blocking of CTLA-4/B7.1 signal abolished the capacity of TGF-β to induce iTregs in wild-type mice [55]. OX40/OX40L, an alternate CD28/B7-independent costimulatory pathway, also negatively regulates the development and function of both nTregs and iTregs. While stimulation of mature nTregs by OX40 results in the loss of suppression of T cell proliferation and cytokine production, the generation of iTregs is completely abolished by OX40 although OX40 does not affect the generation of nTregs [56].

Recently, Housley et al. reported that while the TNF-R2 expression is essential for nTreg-mediated suppression of colitis, its expression is not required for iTreg-mediated suppression (Housley et al. 2011). Differing IL-2 and costimulatory molecule requirements for Treg development and TNFRII expression requirements

for the suppressive function of both nTregs and iTregs suggests that nTregs and iTregs are possibly heterogeneous populations and that integration of both Treg subsets is required for the maintenance of normal immune homeostasis. It is also likely that both nTreg and iTreg subsets can act either in concert or separately on different targets. In addition, as anti-TNF-α therapy has been widely used in treating patient with rheumatoid arthritis, further studies are required to understand whether this therapy differentially regulates nTregs and/or iTregs development in individual diseases.

3 Function and Differentiation of Th17 Cells

Recent studies have provided a line of new evidence demonstrating the existence of a third subset of effector $CD4^+$ cells in addition to the classic Th1 and Th2 cells, the differentiation and growth of which is directed by a combination of TGF-β1 and IL-6 or IL-21 [57–60]. These T cells have been designated Th17 cells based on their production of IL-17A and F. Despite the evidence for the role of Th1 cells in autoimmune disease, recent incontrovertible findings have revealed that pathologies previously attributed to Th1 cells may in fact be mainly mediated by Th17 cells in some autoimmune diseases. The best evidence for this comes from studies using anti-IFN-γ-treated mice, IFN-γ- or IFNR-deficient mice, and mice deficient in IL-12p35, IL-12 receptor β2 (IL-12Rβ2), or STAT1, which are critical molecules in IL-12/IFN-γ–Th1-mediated responses and are capable of developing even severe collagen-induced arthritis (CIA) or experimental autoimmune encephalomyelitis (EAE) despite interference of the proper functioning of the prototypic Th1 cytokine IFN-γ [61–66]. Moreover, these findings concur with reports demonstrating that CIA is suppressed in IL-17-deficient mice and that administration of neutralizing anti-IL-17 antibodies at preclinical and advanced stages significantly reduces disease severity [67]. Similarly, IL-17R-deficient mice or IL-17R IgG1 fusion protein significantly attenuates colonic inflammation in acute trinitrobenzenesulfonic acid (TNBS)-induced colitis although this protection occurred in the presence of equivalent induction of local IL-23 and higher levels of IL-12p70 and interferon-gamma in IL-17R knockout mice compared with wild-type mice [68].

Interleukin-17 (IL-17A) is one of the prototypic IL-17 family members that are predominately produced by $CD4^+$ memory cells [69]. IL-17 receptor is ubiquitously expressed and ligand binding causes the secretion of a range of other factors known to drive inflammatory responses such as rheumatoid arthritis (RA), multiple sclerosis (MS), and colitis [61, 63, 68]. IL-17 stimulates epithelial, endothelial, and fibroblastic cells to secrete proinflammatory factors such as IL-6, IL-8, GM-CSF, CXCL1, CCL20, as well as prostaglandin E2 [70]. Secreted CXCL1 and CCL20 result in the recruitment of neutrophils and/or macrophages to the area of inflammation and enable cell movement and tissue damage. Thus, research directed at controlling autoimmune inflammatory diseases will require a better characterization of the developmental and functional properties of Th17 in these diseases.

Naive $CD4^+$ T cells can be induced to differentiate toward Th1, Th2, Th9, Th17, and Treg phenotypes according to the local cytokine milieu. While IL-12 favors the differentiation toward Th1 cells through transcription factor T-bet [71], IL-4 toward Th2 via GATA-3 [72], and IL-2 and TGF-β toward iTregs via Foxp3 [34, 40, 42], the combination of TGF-β and IL-6 induces Th17 cells through transcription factor orphan nuclear receptor RORγt [73]. IL-21 could substitute for IL-6 to promote Th17 differentiation in this condition [60]. IL-23 was initially considered as a key cytokine to induce Th17 cell production [74]; however, recent studies revealed it is dispensable for the differentiation of Th17 cells but critical for Th17 expansion and survival [75]. IL-1β also promotes the allergic asthma by enhancing Th17 cell differentiation [76]. TGF-β signal is crucial for Th17 cell differentiation although one group recently reported that Th17 cells can be differentiated in the lack of TGF-β signal (John O'shea).

It has been demonstrated that the skewing of naïve T cells toward Th17 and iTreg is mutually exclusive. While we and others have recently revealed IL-2- critically drives TGF-β-treated $CD4^+$ cells to differentiate into $Foxp3^+$ iTreg cells [53, 77], IL-6 or IL-21, however, exclusively drives TGF-β-treated T cells to become Th17 cells [57–60]. It seems IL-6 has a dominant effect on promoting Th17 cells compared to IL-2 promoting Treg cell differentiation. Our data demonstrated that naive $CD4^+$ cells preferentially differentiate into Th17 rather than Tregs in the presence of IL-6, IL-2, and TGF-β no matter whether low or high doses of IL-2 are included in the cultures although others have reported that IL-2 constrains Th17 cell differentiation [78].

4 $Foxp3^+$ Tregs Conversion to Th17 and Other T Effector Cells

While $Foxp3^+$ nTregs suppress Th1 and Th2 cell differentiation and function, it is less defined whether these cells similarly suppress Th17 differentiation and function. Yang et al. have reported that Foxp3 inhibited Th17 differentiation by antagonizing the function of the transcription factors RORγt and ROR. However, IL-6 overcame this suppressive effect of Foxp3 on Th17 differentiation [79]. It has been known that adoptive transfer of nTregs to Th17-mediated diseases was less therapeutic [21, 24], suggesting that nTregs may be less effective on suppressing Th17 cell differentiation, function, and Th17-mediated diseases.

In fact, nTregs can convert into Th17 cells and other T effector cells in the certain environments. As TGF-β either promotes $Foxp3^+$ iTreg, Th9, or Th17 cells depending upon other cytokines involved (Fig. 1), and as nTreg cells express a membrane-bound form of TGF-β and this TGF-β has functional activities, it is reasonable to assume that IL-6 can convert nTregs to become Th17 and other T helper cells [30]. To demonstrate this, Xu et al. used the purified nTregs from Foxp3 GFP knock-in mice to exclude the possibility that $CD4^+CD25^+Foxp3^-$ non-Tregs made this conversion. We used both wild-type and Foxp3 GFP knock-in mice to confirm this observation [31]. Endogenous TGF-β produced by nTregs is critically

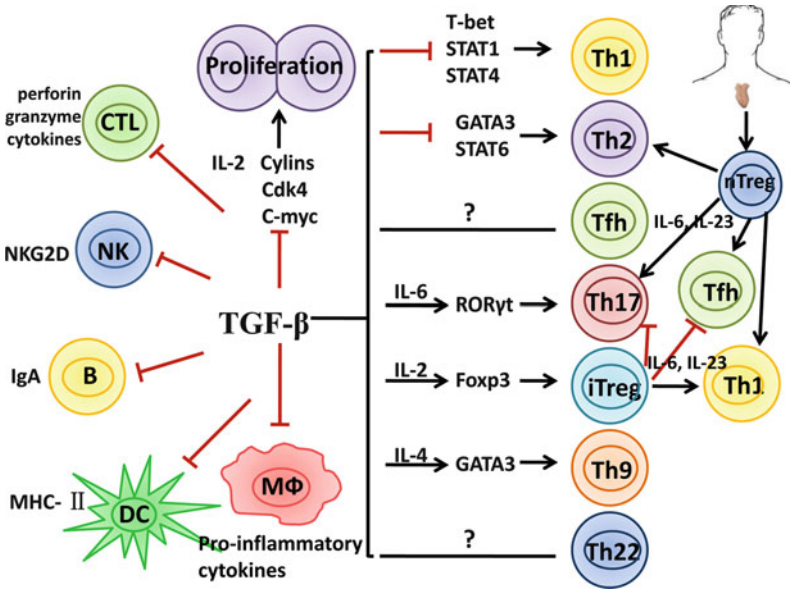

Fig. 1 Multieffects of TGF-β on regulatory and effector T cells and interrelation between Treg and T effector cells. TGF-β inhibits the differentiation, proliferation, and function of various immune cells including Th1, Th2, and Tfh cells. TGF-β also promotes iTreg, Th9, and Th17 cell differentiation depending upon the cytokine environment. Additionally, TGF-β inhibits maturation and function of other immune cells such as $CD8^+$ CTL, NK cell, DC, and macrophages. Both nTregs and iTregs suppress Th1 and Th2; only iTregs suppress Th17 cells. nTregs convert into Th1, Th2, Th17, and Tfh cells and lost suppressive activity; iTregs are resistant to T effector cell conversion except Th1 but maintain suppressive activity

required for this conversion since blocking TGF-β receptor I signal or using nTregs from TGF-β receptor II-dominant mice resulted in the failure of Th17 conversion [27, 31]. Moreover, activation of nTregs with IL-6 resulted in decreased Foxp3 expression and suppressive activity both in vitro and in vivo. Furthermore, adoptive transfer experiments revealed that nTregs treated with IL-6 ex vivo lost their ability to protect mice from a lupus-like disease [31]. Moreover, it has recently been demonstrated that nTregs can be converted into Th17 cells in an in vivo model (Kong and Zheng manuscript submitted). Others have also reported that Th17 cells derived from Tregs share common features with Th17 cells generated from naive precursors, including expression of the chemokine receptor CCR6 [80]. This conversion is not the result of outgrowth of a contaminating Th17 precommitted population because it is indicated by the demonstration of double-positive cells for the Treg transcription factor Foxp3 and IL-17 [31, 81]. Human nTregs also can convert into Th17 cells when stimulated with IL-1 and IL-6 although the role of TGF-β in this conversion is less clear. IL-17 production from Treg cells also occurs in vivo [82]. Thus, in an IL-1- and/or IL-6-rich inflammatory milieu, nTregs may be unstable and lose the functional activity.

In addition to Th17 cell conversion, nTregs can be converted into other subsets of T effector cells. Wan and Flavell found that Foxp3$^+$ nTregs can convert to Th2 cells when endogenous Foxp3 gene expression is attenuated in these cells [29]. Interestingly, T cells expressing decreased Foxp3 still preferentially became Th2 effectors even in a Th1-polarizing environment. It is therefore likely that these cells instructed Th2 differentiation of conventional T cells, which contributed to the immune diseases observed in these mice [29]. Moon et al. also reported that acetylsalicylic acid also changes Th17-type into Th2-type inflammation cells in mice model with asthma [83]. When these cells were strongly stimulated with antigen or anti-CD3/CD28 antibodies, nTregs also lost Foxp3 and became Th1 cells [27]. IL-2 also promotes Treg cells to become Th1-like cells although Foxp3$^+$INF-γ^+ cells are still suppressive [84]. In human, nTregs can also be converted into Th1 cells and most lost suppressive activity (Lu and Zheng Blood, 2012 manuscript submitted). Moreover, these cells can become T follicular helper (Tfh) cells [28]. Tsuji et al. have demonstrated that in adoptive transfer of nTregs to immune-deficient mice, Foxp3$^+$ CD4$^+$ cells can differentiate into Tfh cells in mouse Peyer's patches. The conversion of Foxp3$^+$ T cells into Tfh cells requires the loss of Foxp3 expression and subsequent interaction with B cells [28].

Previous studies have clearly demonstrated that naive rather than memory CD4$^+$ cells preferentially differentiate into Foxp3$^+$ Tregs cells in the presence of exogenous TGF-β [53]. It is not surprising that Th1, Th2, and Th17 cells are unable to differentiate into Foxp3$^+$ Treg cells even if they have been primed with exogenous TGF-β (Zheng SG lab unpublished data). However, Th17 cells can be converted into Th1 and Th2 cells, suggesting that Th17 cells are not stable phenotypes [85, 86]. The functional significance of Th17 to Th1 and Th2 cell conversion is unclear so far. In addition, the epigenetic modifications were remarkably stable during these cells' conversion [87].

In sharp contrast, TGF-β-induced iTregs were found to be completely resistant to the Th17 conversion by IL-6. This difference cannot be explained by insufficient production of TGF-β by iTregs since both nTregs and iTregs expressed similar levels of membrane-bound TGF-β (20–25 %) and secreted similar levels of active TGF-β (about 40 ng/ml). Furthermore, the resistance of iTregs to Th17 conversion also cannot be explained by alterations in TCR stimulation since anti-CD3/CD28-activated nTregs can still differentiate into Th17 cells upon IL-6 stimulation. To account for this difference between nTregs and iTregs, we found that the combination of IL-2 and TGF-β downregulated IL-6 receptor expression and function in activated T cells. We have observed that both cytokines markedly decreased IL-6 receptor alpha-chain (CD126) and beta-chain (CD132) expression on CD4$^+$ cells and these cells expressed significantly lower level of phosphorylated STAT3 when stimulated by IL-6 [31]. Unexpectedly, Yang et al. had a conversed report showing iTregs can convert into Th17 cells in the presence of proinflammatory cytokines [79]. To solve this contradiction, O'Connor et al. reinvestigated the fates of both nTregs and iTregs in the proinflammatory condition. They did find that iTregs are completely resistant to Th17 conversion although nTregs do in the presence of IL-6, IL-23, and TGF-β [88]. Interestingly, they also demonstrated that iTregs rather than nTregs became Th1-like cells. Although these Th1-like cells began to express T-bet

and produce INF-γ, they are less pathogenic compared to conventional Th1 cells; conversely, they still suppressed naive T cell clonal expansion and protected against the development of EAE [88].

We further observed the differences of stability of both Treg cell subsets in vivo. About 50 % of nTregs converted to Th17 cells in draining LNs 10 days after cells transfer to established collagen-induced arthritis. Conversely, iTregs were completely resistant to Th1, Th2, and Th17 cell conversion. When these cells were sorted for in vitro analysis, nTregs mostly lost suppressive activity, whereas the functional activity of iTregs was mostly intact (Kong and Zheng unpublished data). These results indicate iTregs are stable and functional in the inflammatory condition and may have a greater advantage to treat autoimmune and inflammatory diseases compared with nTregs.

Nonetheless, others have also reported that TGF-β-induced iTregs were unstable in vitro [89] and in vivo following antigen stimulation [90] and lack protective activity to prevent lethal graft-versus-host disease (GVHD) [89, 91]. It has been claimed that the Foxp3 promoter on TGF-β-induced iTregs but not nTregs is methylated and accounts for their instability [89]. However, we have recently observed that the methylation status in Foxp3 gene loci does not affect Foxp3 stability. Moreover, addition of atRA to TGF-β promoted iTreg stability and maintenance in vitro and in vivo, and this effect is unrelated to CpG methylation in Foxp3 promoter but related to acetylation of Foxp3 histone [92]. Others have also observed protective human TGF-β-induced Tregs that exhibit methylated Foxp3 [32]. To explain these controversial results, we consider the technical reasons are possibly responsible for the generation of unstable, ineffective TGF-β-induced iTregs in these groups. They have used high concentrations of plate-bound anti-CD3 with TGF-β, whereas our group has used suboptimal concentrations of anti-CD3- and anti-CD28-coated beads with IL-2 and TGF-β. It has been known that strong, sustained TCR stimulation activates the mTOR/Akt signaling pathway which facilitates Teff cell differentiation and inhibits Foxp3 expression and Treg differentiation [93]. Treg generation is best established with suboptimal TCR stimulation that facilitates Foxp3 expression [16].

These studies also raise the possibility that nTregs and iTregs may have distinct roles in the adaptive immune response. In response to microbial infections, nTregs could possibly serve as a first line of host defense by differentiation to IL-17-producing cells, which contribute to neutrophil mobilization and have other proinflammatory effects. After eradication of invading pathogens, the late appearance of TGF-β-induced iTregs would not only terminate the antigen-specific response but also prevent the emergence of nonspecifically stimulated or cross-reactive self-reactive T cells. Accordingly, failure of this mechanism could result in an immune-mediated disease.

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Other Sources of IL-17: iNKT 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. Similar 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 iNKT 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.

Keywords iNKT cells • IL-17 • NK1.1 • CD1d • Inflammation • Autoimmunity • Asthma

Abbreviations

iNKT	Invariant natural killer T
IL	Interleukin
Th	T helper

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α-GalCer	α-galactosylceramide
TCR	T cell receptor
APCs	Antigen-presenting cells
LPSs	Lipopolysaccharides

1 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]. Some years ago, a new effector population called Th17 has been identified by its capacity to produce IL-17A (or IL-17), IL-17 F, and IL-22 [2]. IL-17 is the founder member of this newly identified cytokine family composed of six proteins: IL-17A, IL-17B, IL-17 C, IL-17D, IL-17E (IL-25), and IL-17 F [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 will ultimately mediate tissue infiltration and destruction. Th17 cells recognize peptide antigens (Ag) 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 Ag presentation pathways which 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 Ag 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] that we will focus on in this chapter.

2 Natural Killer T Cells: Generalities

The term “natural killer T (NKT) cells” has initially been used to designate T lymphocytes expressing NK markers, such as NK1.1 (NKR-P1A or CD161). Later on, this population turned out to be quite heterogeneous, and it is now well established that it 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 “invariant natural killer T (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, which is composed of five members, namely, CD1a, b, c, d, and e, CD1d is the only one which 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, kidney, and blood [4–6, 9].

iNKT cells recognize a limited number of synthetic or naturally occurring α- and β-linked glycolipids bound to CD1d expressed on Ag-presenting cells (APCs), such as dendritic cells (DCs) [4–6, 10, 11]. These CD1d-restricted Ags 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 the α-galactosylceramide (α-GalCer), originally isolated from a marine sponge [12]. 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 [13].

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 [14] followed by other endogenous glycolipids such as charged β-linked glucosylceramide(s) [15]. Most recently, it was reported that β-D-glucopyranosylceramide (β-GlcCer) was a potent iNKT cell self-antigen in mouse and human [10]. Exogenous lipids like galacturonic acid-containing glycosphingolipid from nonpathogenic *Sphingomonas* bacteria, but also from *Borrelia burgdorferi* spirochetes or *Streptococcus pneumoniae*, Gram-negative or Gram-positive bacteria responsible for Lyme disease or community-acquired pneumonia, respectively, are also potent iNKT cell activators [16–18]. These studies 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* [17, 18]. 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. It turned out that iNKT cells are effectively implicated in tumor immunity, infections, autoimmune diseases, as well as 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 antitumor responses, while IL-4 is necessary for their detrimental effect in experimental allergic asthma [19–21].

These observations raise two fundamental questions, namely, (1) which factors induce the particular cytokine profile of iNKT cells that will lastly 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 toward predominant IL-4 or IFN-γ production [22–24]. 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, 25].

In this line of evidence, we have recently identified a particular subset of iNKT cells that generated high amounts of IL-17 together with 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.

3 RORγt^{pos} iNKT IL-17-Producing Cells

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

It has been reported that 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 neither transform NK1.1^{pos} iNKT cells into IL-17-producing cells by culturing them in the presence of both TGF-β and IL-6 that are required for driving the differentiation of naïve mouse CD4 T cells into Th17 cells, nor did the addition of IL-23 that enhances IL-17 production by conventional T cells increase their IL-17 production, suggesting that NK1.1^{neg} and NK1.1^{pos} cells are indeed functionally distinct. 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 naive T cells.

We used the lack of NK1.1 expression as a distinctive feature of peripheral IL-17-producing iNKT cells 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 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 [26]. Indeed, unlike conventional autoreactive T cells,

autoreactive iNKT cells are not deleted in the thymus but 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 is 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 ration decreases [26]. In the thymus, $NK1.1^-$ iNKT cell precursors produce high levels of IL-4 and 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 [26]. Thus, it was important to determine whether iNKT17 cells exist in the thymus, whether the iNKT $NK1.1^-$ precursors that 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. We recently answered this question by reporting that a particular thymic iNKT cell subset produces IL-17 [27]. In fact, to address this issue, we assessed the functional capacities of iNKT cells sorted from the thymus at different phenotypically defined stages of differentiation. We show that IL-17 is massively produced by the $CD44^{high}NK1.1^{neg}CD4^{neg}$ subset, which indicates that this faculty is encoded in the developmental program of thymic iNKT cell differentiation. Using an EGFP reporter mouse, we found that the ability to produce large amounts of IL-17 is conferred by the expression of ROR-γt (retinoic acid receptor-related orphan receptor γt) in thymic $CD44^{high}NK1.1^{neg}CD4^{neg}$ iNKT cells. Hence, we demonstrated that the IL-17-producing iNKT cell subset (*iNKT17* cells) derives from a new thymic pathway of differentiation (see Fig. 1) dependent on the transcription factor RORγt that, in contrast to the mainstream iNKT cell subset, is maintained in mature peripheral iNKT17 cells. Considering that NK1.1 expression may be modulated following iNKT cell activation, we proposed to use RORγt expression associated to CD1d-tetramer to better identify IL-17-producing iNKT cell subset.

4 IL-17-Producing iNKT Cells and Infections

IL-17-producing iNKT cells represent 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 lipopolysaccharides (LPSs) instillation is significantly reduced in iNKT cell-deficient $J\alpha18^{-/-}$ mice, which produced 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 were implicated in several infection disorders suggesting that iNKT cells could represent at least one of the sources of this cytokine.

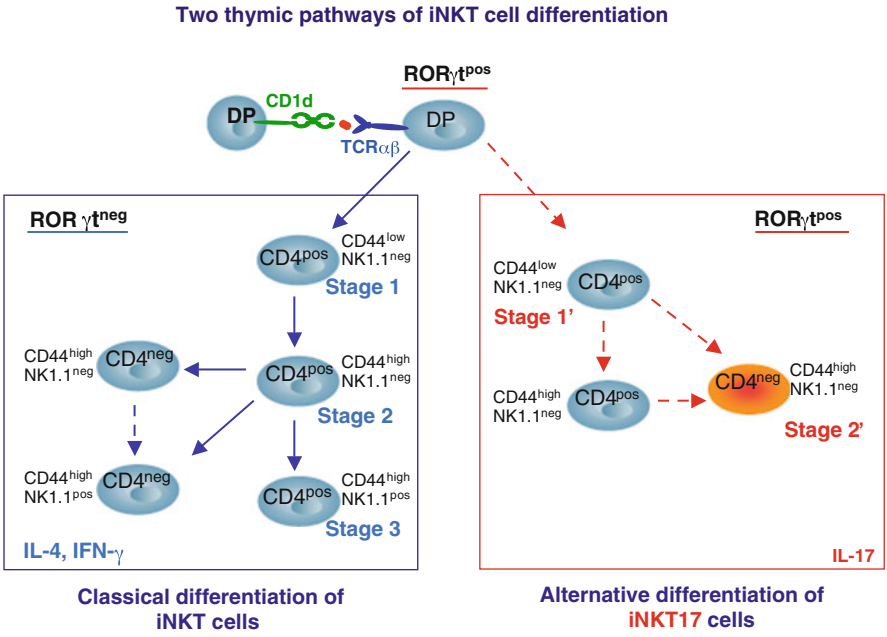

Fig. 1 Two thymic pathways of "invariant natural killer T" (iNKT) cell differentiation: (1) iNKT RORγt^{neg} cells: IL-4 and IFN-γ producers; (2) iNKT RORγt^{pos} cells: IL-17 producers

For instance, it was already demonstrated that IL-17 is 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 the lung and blood [28, 29]. In this model, a correlation exists between the higher numbers of pulmonary neutrophils, γδ-T cells but also NKT cells, and the protection observed. However, it remains to be determined whether iNKT17 cells could be implicated in the protection afforded by CpG treatment. Knowing that iNKT cells can be indirectly stimulated by CpG to produce IFN-γ [15], it will be important to determine whether this or other 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 activate and induce IL-17 production by iNKT cells [7, 30].

5 IL-17-Producing 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, a coup of evidences supported the involvement of Th1 cells in the induction of autoimmune inflammation and tissue injury. However, some predictions of the Th1/Th2 hypothesis were erroneous, and the results were just the opposite [31], as, for instance, 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 incorrect conclusions were published probably because IL-23 was only identified in 2000 [32]. 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 CIA (collagen-induced arthritis). 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.

The implication of iNKT cells was also reported 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 NOD (nonobese diabetic) mice probably by shifting the immune response toward an anti-inflammatory Th2 type [4–6, 33]. In autoimmune diabetic NOD mice, it was reported a numerical and functional deficit in IL-4-producing iNKT cells [34] in parallel to an enhanced frequency of IL-17-producing iNKT cells [35]. The adoptive transfer of IL-17-producing iNKT cells enhanced the autoimmune severity in these mice [35], suggesting a balance between the protective and deleterious role of respectively IL-4- and IL-17-producing iNKT cell subsets in experimental autoimmune diabetes.

6 IL-17-Producing iNKT Cells and Asthma

Asthma is a heterogeneous and complex pathology associated or not with atopy. Consequently, diverse mechanisms have been proposed to explain the pathogenesis of various asthma phenotypes, and iNKT cells and IL-17 are potentially implicated in the severity of some phenotypes. iNKT cells were associated with asthma severity in experimental models, and these cells are present in the bronchoalveolar lavage of asthmatic patients [36]. It was also recently reported that IL-17 contributes to experimental allergic asthma [37]. 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 in order to obtain the major asthma symptoms. IL-17-producing iNKT cells can induce airway neutrophilia, and it was reported that the presence of iNKT cells and the production of IL-17 were required for airway hyperreactivity (AHR) induced by repeated exposure of mice to ozone [38]. 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 in order to potentially lead to new approaches to inhibit asthma progression.

7 IL-17-Producing Human iNKT Cells

In mice, iNKT17 cells comprise a distinct lineage that retains ROR-γt expression during their development and does not express the NK1.1 marker and, contrary to conventional T cells, emigrate from the thymus fully capable to secrete IL-17 without polarization [7, 27]. We found that human IL-17-producing iNKT cells behave differently. Indeed, they originate from a lineage-committed precursor bearing the CD161 marker as human CD161+ iNKT cells. These CD161+ iNKT cells are intrinsically endowed with the capacity to secrete IL-17. However, in contrast to mouse iNKT cells, the capacity of human iNKT cells to secrete IL-17 requires the presence of TGF-β, IL-1β, and IL-23 during the stimulation. Thus, it is possible that IL-17-producing iNKT cells could contribute to some inflammatory disorders, including inflammatory bowel diseases (IBDs). Enhanced numbers of IL-17-producing T cells have been evidenced in the gut together with increased expression of the pro-inflammatory cytokine IL-23 in the intestinal mucosa of Crohn's disease (CD) patients. Among these T cells, we found that a fraction of iNKT cells from CD patients were capable of producing high levels of IL-17 without the exogenous addition of TGF-β, IL-1β, and IL-23 [25]. These studies were performed in peripheral blood and are not necessarily representative of the in situ inflammation. Consequently, further studies using T lymphocytes from the inflammatory sites are required to determine the possible influence of IL-17-producing iNKT cells in CD and other inflammatory disorders.

8 IL-17 Production by γδ T Cells

Although the study of IL-17 production by T cell has been focused on $CD4^+$ αβ Th17 cells, several reports have shown that γδ T cells are also a major innate source of IL-17 [39], whose rapid response is implicated in host protection and the development of immunopathologies. Like iNKT cells, these are unconventional T lymphocytes with innate and adaptive properties, playing a crucial role in tissue immunosurveillance. They predominantly express recurrent families of TCR with reactivity to a small set of conserved self-structures. The specificity of most γδ T cell subsets remains nevertheless unclear, although they have a tendency to recognize stressed, activated, or transformed cells and are implicated in various infectious conditions. These cells acquire their functional properties, including IL-17 production, early in their thymic development, which allows rapid induction of their effector functions following the detection of tissue stress [40].

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* bacilli Calmette-Guérin-infected mice [41, 42], 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 [43]. It is noteworthy that the 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 is 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 (antigen-presenting cells) 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 APC [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 [44]. 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 Vγ4 + Vδ4+ but not Vγ1+ T cells from draining lymph nodes produce IL-17, thereby increasing the severity of the disease [45].

9 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. By now, it is well established that iNKT cells respond to glycolipids rather than peptides, a mode of recognition, which 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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Retinoic Acid in Mucosal Immune Regulation

Daniel Mucida and Hilde Cheroutre

Abstract The vitamin A metabolite, retinoic acid (RA), and transforming growth factor-beta (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 T_H1 and T_H2 cytokine polarization concomitant with the generation of regulatory T cells (Tregs), and paradoxically, along with inflammatory cytokines such as IL-6, it also induces the differentiation of pro-inflammatory IL-17 producing CD4 helper T cells (T_H17). RA, in contrast, is able under certain conditions to stimulate T_H2 differentiation, and it is a profound inhibitor of IFN-γ synthesis. Additionally, RA has been shown to efficiently promote gut tropism. We described RA as a key modulator of TGF-β-driven immune deviation capable of suppressing T_H17 differentiation while promoting $Foxp3^+$ Treg generation. More recently, RA signaling was also shown to be essential for efficient intestinal immune responses. Here we will discuss how RA can affect mucosal immune regulation.

Keywords TGF-β • Retinoic acid • Foxp3 • T_H17 • IL-6 • Mucosal immune regulation • Oral tolerance • Nuclear receptors

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 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

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Scheme 1 *Production of retinoic acid*. 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)

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 CD8$\alpha\alpha$/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.

Regarding their regulatory capacity, it was shown that antigen presentation by mucosal DCs in the MLN 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 CD8$\alpha\alpha^+$ 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 was also demonstrated that mucosal DCs from mesenteric lymph nodes (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 reversible catalytic process mediated by alcohol dehydrogenases (ADH). The subfamily of enzymes ADH 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].

Retinoic acid (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 T_H1 and T_H2 cytokine polarization concomitant with the generation of Tregs [9]. Although the so-called thymus-derived naturally occurring Treg cells (nTreg) are important for the control of a variety of autoimmune processes, it has been shown, using monoclonal nonself antigen-specific TCR transgenic mice (such as OVA-specific TCR transgenic mice) which lack nTreg, 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 Treg cells [10]. RA, in contrast, is able under certain conditions to stimulate T_H2 differentiation, whereas it strongly inhibits IFN-γ synthesis [11]. More recent reports, however, have shown that RA can efficiently suppress both T_H1 and T_H2 differentiation in vitro [12, 13].

The RA produced by MLN, LP, and PPs DCs mediates upregulation of gut-specific molecules on the T cells they prime, such as integrin α4β7 and chemokine CCR9, which interact 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 lamina propria (LP) immune cells in the intestine [7]. Additionally, 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 envelop 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 retinoic acid signaling pathways [20]. Moreover, mice with a deficiency in RALDH-2 die before birth with several developmental defects and reduced TGF-β1 [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 expand 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 Treg cells in the LP in normal mice, oral tolerance induction, and therefore mucosal immune regulation [25, 27]. We 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 T_H17 cells in vivo, whereas injection of 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 $Foxp3^+$ Treg in the gut, as well as for the mucosal in situ control of T_H17 cells, is not yet known.

Two groups described that $CD103^+$ DCs are the main population involved in the RA production. Coombes et al. 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 [28]. Sun et al. described 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 Treg cells in the presence of exogenous TGF-β, when compared to spleen DCs [27]. More recently, Denning et al. 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 T_H17 cells as compared to the $CD11b^+$ DCs, they could not

find any increased ability to induce Tregs [31]. Therefore, the diversity of mucosal APCs is more extended and more complex than previously appreciated.

Our study shows that RA signaling through RAR receptors in the T cell blocks the inhibitory effects of inflammatory cytokines, such as IL-6, on the TGF-β-mediated Foxp3 induction. Although a report suggests that RA works indirectly, by antagonizing inhibitory effects of inflammatory cytokines [32], a series of studies demonstrated a direct role of RA signaling in promoting Foxp3 induction [33, 34]. 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, 35]. 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 [36], an autoimmune disease shown to correlate with increased production of IL-17 [37]. Finally, we showed that RA directly inhibits retinoic acid orphan receptor gamma T (RORγt) which is involved in T_H17 differentiation [38]. It is not known, however, whether RA antagonistic effects on IL-6 signaling extend to the recently described IL-21 pathway of T_H17 differentiation [39–41].

Despite these early evidences, the assumption that RA has only broad, anti-inflammatory effects on immune responses has been recently challenged. Uematsu and coworkers have suggested that production of RA by $CD11c^{hi}CD11b^{hi}$ LP DCs, when stimulated by the TLR5-ligand flagellin, promoted, rather than suppressed, a modest differentiation of antigen-specific Th17 and Th1 cells, suggesting that innate stimuli may induce contrasting effects of RA on either DCs or T cells [42]. This idea was elegantly explored by DePaolo and coworkers, demonstrating that RA, in the presence of IL-15, has synergistic effects on differentiation and function of inflammatory DCs, leading to enhanced pathological responses and *coeliac* disease-like inflammation [43]. Additionally, using a model of *Toxoplasma gondii* infection, Hall et al. have recently suggested that RARα signaling is actually required for development of efficient Th1/Th17 intestinal immune responses, inferring a general role of the RA pathway in adaptive immune responses [44]. Although the dose of RA has been suggested to play a crucial role in determining its effects, the literature is still elusive regarding this issue. Physiological doses such as 1–2.5 nM of RA [25, 35] or even spontaneous RA release from (mesenteric lymph node) MLN DCs [25] have been shown to suppress Th17 cell development in vitro. Nevertheless, the contrasting results obtained using exogenous RA and mice deficient in RA receptors require further investigation [35, 44]. These results demonstrate how one metabolite may have strikingly different effects depending on microenvironment milieu and cell target of this metabolite. They also point out how deleterious are the consequences when the robust mechanisms of tolerance induction in the mucosal surfaces are broken.

The transcription factors STAT5 and STAT3 have been shown to be important for the transcription of Foxp3 and IL-17, respectively [29, 38, 45]. 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 [46]. STAT5 and RARs have even been shown to physically interact in vivo to promote RAR-mediated transcription [47]. In addition, the STAT5 consensus binding site directly overlaps with a RAR-response element which may lead to coordinated transcription activity rather than competition for the same site [47]. The cooperation between STAT5 and RARs results in STAT5-enhanced responsiveness of the RARs to RA-induced transcription of target genes [47]. It was further demonstrated that RAR and STAT5 can bind the same repressor of transcription, SMRT, which can be released by RA [48]. The RA-mediated effects made 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 finding reported that, similarly to IL-6, the T_H2 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 therefore 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 [49].

The immune regulatory mechanism 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 in physiological conditions have an immune quiescent state [50]. Therefore, an improper balance between inflammatory and suppressive immunity can jeopardize mucosal homeostasis. The abundant production of RA by the intestinal epithelium and dendritic cells, 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. Under inflammatory conditions, however, both RA and TGF-β may help busting protective immune responses.

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Part II
Experimental Models of Disease

Critical Role of IL-17 in Experimental Arthritis

Marije I. Koenders and Wim B. van den Berg

Abstract Since the discovery of IL-17 expression in synovial fluid and biopsies in RA patients, the role of this proinflammatory 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 proinflammatory cytokines and chemokines. IL-17 also contributes to joint destruction directly by upregulation of MMPs 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 will be discussed.

Keywords IL-17 • Animal models • Experimental arthritis

1 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

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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 will discuss the potential therapeutic value of these mediators.

2 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 nonimmune 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, and subsequently the IL-17R-deficient mice developed inflammation and cartilage proteoglycan depletion comparable to wild-type controls [1]. In contrast to acute, nonimmune 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 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 by 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 proof 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 did no longer affect T cell immunity, since no differences in T cell responses to murine collagen peptides were found. Also during rat antigen-induced arthritis, treatment with interleukin-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, although 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.

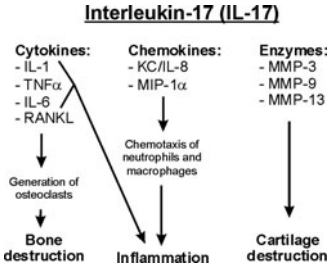

Fig. 1 Pathological effects of IL-17 during experimental arthritis

3 IL-17: Inducer and Accelerator of Inflammation and Destruction

3.1 Overexpression of IL-17 Induces Joint Pathology

IL-17 is a proinflammatory 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 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 naïve 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 PG depletion comparable to wild-type controls.

Besides 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]. The therapeutic effect of such a combination blocking was recently shown in collagen-induced arthritis, where neutralizing IL-17 in addition to TNF further improved protection against joint damage and was even effective during late-stage disease [10]. Also when IL-17 is overexpressed during an established arthritis, IL-17 contributes 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, 11].

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 [12]. The enhanced osteoclast activity caused by IL-17 could be inhibited by OPG treatment, thereby restoring the RANKL/OPG balance.

3.2 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 collagen-induced arthritis reduced joint inflammation, cartilage destruction, and bone erosion [13]. 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 the chronic SCW arthritis. In this arthritis model, mice receive repetitive intra-articular injections with fragments of Streptococcal cell wall (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 [1]. In comparison to CIA and chronic SCW 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 IL-17-positive T 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 [14]. During antigen-induced arthritis, anti-IL-17 treatment administered shortly before the flare completely prevented cell influx and suppressed the local expression of IL-1, TNF, and RANKL [15]. In this strongly T cell-driven AIA-flare model, the anti-IL-17 treatment seemed far more powerful than during collagen-induced arthritis 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.

4 Manipulating the Role of IL-17-Producing Th17 Cells in Experimental Arthritis

4.1 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

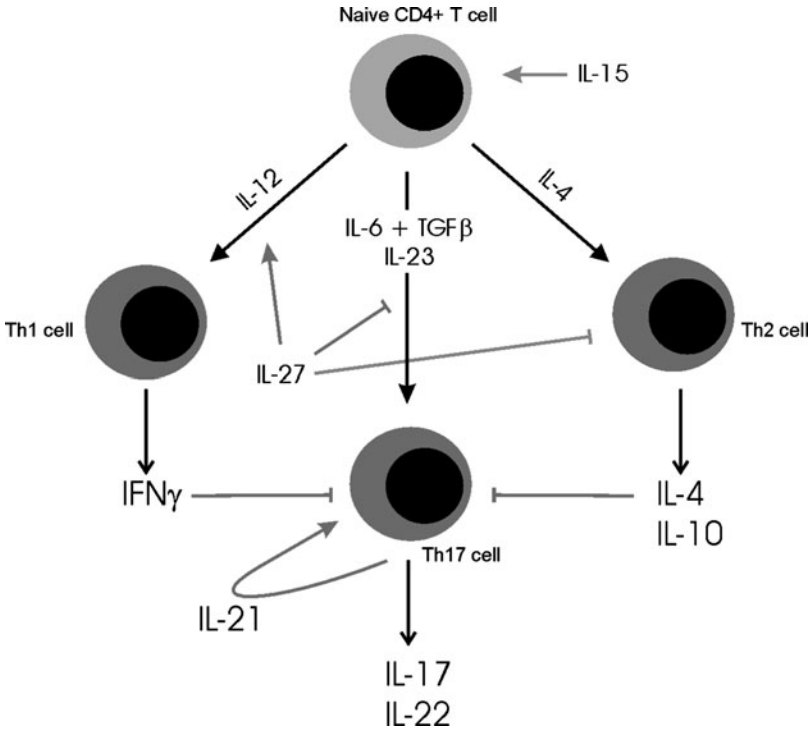

Fig. 2 Cytokines influencing T cell differentiation and maturation

IL-12 decreased IL-17 levels [16]. Without the knowledge of Th17 that time, Murphy et al. 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, whereas loss of IL-12 exacerbated collagen-induced arthritis [17]. 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 NFκB pathway in CD4+ T cells, but also indirectly, by inducing the expression of the costimulatory molecule OX40 on $CD4^+$ T cells [18]. The position of IL-23 upstream of IL-17 suggests that targeting IL-23 could be an interesting approach in the treatment of RA.

4.2 IFNγ Restrains IL-17 Production

Not only studies on the Th1-promoting cytokine IL-12 but also on its downstream mediator interferon-γ (IFNγ) 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 IFNγ paradoxically enhanced collagen-induced arthritis [19]. The discovery of Th17 cells led to the rejection of this Th1 concept, and with IFNγ-/- mice and anti-IFNγ antibodies, it was demonstrated that this Th1 cytokine suppresses IL-17 production during experimental arthritis [20] but also inhibits the expansion of Th cells [21]. Interestingly, DBA-1 mice which are prone to collagen-induced arthritis 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 IFNγ on IL-17 production does not mean that IFNγ and Th1 are not contributing to arthritis. On the contrary, in proteoglycan-induced arthritis (PGIA), severe arthritis is dependent on the production of IFNγ and is independent of IL-17 [22]. In addition, also IL-17 + IFNγ + T cells are found in the arthritic joint during experimental arthritis (Marijnissen and Koenders, manuscript in preparation). Both Th1 and Th17 as well as double-positive Th1/17 cells therefore might contribute to the arthritic process, and the cytokine environment seems to have a great influence on the balance of Th cell subset that will be found at the site of joint pathology.

4.3 IL-4: Directly or Indirectly Suppressing IL-17 Secretion

Not only the Th1-related cytokines IL-12 and IFNγ but also the Th2 cytokine IL-4 influences IL-17 expression during arthritis. During collagen-induced arthritis, severe bone erosions could be prevented by IL-4 gene therapy [23]. Local IL-4 overexpression suppressed IL-17 and RANKL expression in the synovium and inhibited the formation of osteoclast-like cells but could also prevent collagen type I breakdown in human bone explants directly. Targeting IL-17 via genetically modified murine dendritic cells that secrete IL-4 led to similar findings [24]. 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 IFNγ. Since blocking of IL-12, IL-23, or IFNγ 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.

4.4 IL-6, a Pleiotropic Cytokine Influencing Th17 Development

IL-6 is a pleiotropic proinflammatory 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 TGFβ together induced the differentiation of Th17 cells from naive T cells (Fig. 2), thereby inhibiting the generation of TGFβ-induced Treg cells. Although the exact role of IL-6/TGFβ is still subject of debate, also in experimental arthritis

models, a role for IL-6 in IL-17 production was demonstrated. The 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 IFNγ deficiency exacerbated arthritis [25]. 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 antigen-induced arthritis, IL-6 deficiency resulted in less IL-17 production, fewer osteoclasts, and reduced arthritis severity [26]. In clinical trials, the anti-IL-6 receptor monoclonal antibody has demonstrated to improve 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.

4.5 Enhanced T Cell Activation by IL-15

Similar as with IL-6, also for IL-15, the first clinical trials with a neutralizing antibody have shown promising results in the treatment of RA. IL-15 regulates T and NK cell activation and proliferation and also directly stimulates IL-17 production by T cells. Blocking of IL-15 during collagen-induced arthritis suppresses incidence and severity of arthritis [27, 28]. IL-15 deficiency slightly decreased incidence and severity of arthritis, while IL-15 transgenic mice showed increased arthritis [29]. Both IFNγ and IL-17 levels were reduced by inhibition of IL-15 signaling, whereas the levels of antigen-specific IFNγ and IL-17 production were significantly higher in IL-15 transgenic mice compared to WT 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 IFNγ-producing Th1.

4.6 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 collagen-induced arthritis and rat adjuvant arthritis reduced the clinical and histological signs of arthritis [30]. Interestingly, treatment with this murine IL-21 receptor Fc fusion protein resulted in enhanced expression of IFNγ in the paws and lymph node cultures, whereas antigen-specific IL-17 production was suppressed. In this chapter, 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). IL-21 was shown to induce Th17 differentiation of both human and murine naïve CD4+ T cells in the presence of

TGFβ [31–33]. 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. Anti-IL-22 treatment in arthritic IL-1Ra-deficient mice resulted in reduced joint inflammation and destruction [34], and in psoriasis-like skin inflammation, it was demonstrated that blocking of IL-22 suppressed development of disease and reduced Th17 cytokine expression [35]. However, the proinflammatory role of IL-22 in other autoimmune diseases is still subject of debate, since IL-22 deficiency during experimental autoimmune encephalomyelitis showed that IL-22 was not involved in the pathogenesis of EAE [36].

IL-27, the latest member of the IL-12 family, is produced by antigen-presenting cells in response to IFNγ. This IL-12 family member seems to have a conflicting role in immunity, having both proinflammatory and anti-inflammatory properties. IL-27 contributes to Th1 cell development by increasing the IL-12-responsiveness of naïve T cells, upregulation of the Th1 transcription factor T-bet, and downregulation 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 IFNγ production [37]. In the IFNγ-dependent proteoglycan-induced arthritis, IL-27R deficiency resulted in delayed development of arthritis and suppressed severity [38]. The knee joints of these IL-27R-/- mice not only have reduced expression of IFNγ, but also IL-17 expression was suppressed. 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 the experimental autoimmune encephalomyelitis (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 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. An elegant study in IL-1Ra-/-xTLR-4-deficient mice showed that despite similar disease incidence, TLR-4 deficiency resulted in suppressed arthritis severity in the chronic phase of arthritis [39]. TLR4 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.

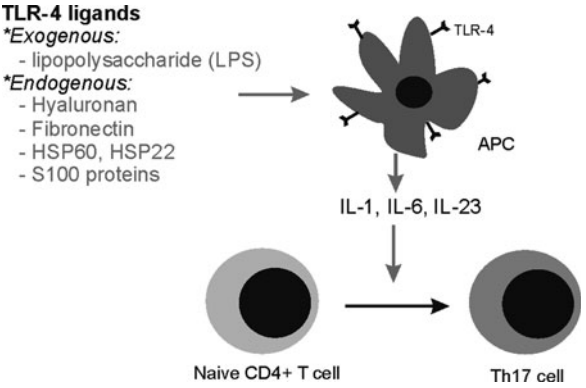

Fig. 3 TLR-4 activation contributing to arthritis progression via Th17 activation

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Dual Role of Th17 Cytokines, IL-17A,F, and IL-22 in Allergic Asthma

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Abstract The proinflammatory role of T helper (Th) 17 cells and therefore of its cytokines, IL-17 (IL-17A), IL-17F, and IL-22, in autoimmune disorders has been favored, although there is evidence that not only IL-17A but also IL-17F and IL-22 have a dual role as negative regulators. Here we review the concept of the dual function of IL-17A, IL-17F, and IL-22 in the light of recent strategies to use neutralization of these cytokines as potential alternative to neutralizing TNF and IL-1 treatments in chronic inflammatory disorders. Expectedly, in allergic lung inflammation, neutralization of IL-17A inhibited neutrophil recruitment. However, this IL-17A antibody treatment concomitantly increased eosinophil recruitment by neutralizing IL-17A's dual role as negative regulator. IL-17A negatively regulated dendritic cell function and activation of T helper cell (Th)2 cytokine production. Furthermore, IL-17A inhibited Th2-characteristic chemokine and adhesion molecule expression. On a mechanistic level, IL-17A acted on IκB-β by preventing degradation and in turn leading to reduced NF-κB activation or IL-17A inhibited transcription factor IRF-1. Therefore, anti-IL-17A therapy, although presenting a promising lead in chronic inflammatory disorders, bears a potential risk of exacerbating allergic asthma.

1 IL-17A, IL-17F, and IL-22 Production by Antigen-Specific T Helper Th17 Cells Inhibits Th2 Response

Interleukin-17A (IL-17A, aka IL-17) 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.

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IL-17A and IL-17F homodimers and IL-17F/IL-17A heterodimer transduce their signals through the receptor composed of IL-17RA and IL-17RC [2, 3].

IL-17A and IL-17F are produced by the memory T cells termed Th17, a T helper cell lineage distinct from Th1 and Th2 cells, which is negatively regulated by interferon-γ and IL-4 [4, 5]. 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 heretofore prototypical "Th1" disease models [6, 7].

The IL-23-Th17 cell axis has been implicated to contribute to the allergic Th2 response [8]; for review, see [9]. IL-23 as well as the co-expressed Th17 cells cytokines IL-17A and IL-22 [10] is found in the lung of allergic patients [11–13] and in lung homogenates of ovalbumin (OVA)-sensitized and challenged mice [8, 14]. In vitro production of IL-17A and IL-22 was triggered with IL-23 and even further enhanced in the presence of OVA, in cultures of mediastinal lymph node (MLN) cells isolated from antigen OVA-sensitized and challenged mice [8, 14]. Therefore, the role of IL-17A and IL-22 was addressed in the allergic response.

IL-17A was indeed required during antigen sensitization to develop a Th2 response in allergic asthma, as shown in IL-17R-deficient mice [8]. Neutralization of IL-17A, however in this model, augmented the allergic response, while recombinant IL-17A administration reduced pulmonary eosinophil recruitment and bronchial hyperreactivity. Recombinant IL-17A reduced eosinophil-chemokine eotaxin (CCL11) and thymus- and activation-regulated chemokine (TARC/CCL17) in lungs in vivo, and antigen uptake by dendritic cells and IL-5 and IL-13 production in regional lymph nodes were also reduced by recombinant IL-17A [8]. These findings demonstrated a novel negative regulatory role of IL-17A. A beneficial role of IL-17A has been confirmed in the model of chronic fungal-induced asthma. In this model, the protective role of TLR6 was dependent on IL-23 and the production of IL-17A. TLR6 deficient mice showed reduced IL-23 and IL-17A expression and an exacerbated Th2 response, which was normalized by addition of recombinant IL-23 which recovered the IL-17A production [15]. Furthermore, Murdoch and Lloyd provide evidence in the model of acute allergen-induced response that the γδT cell-dependent normalization of lung function and resolution of inflammation was dependent on the production of IL-17 [16]. Therefore, endogenous IL-17A has a dual role. While it is essential during antigen sensitization to establish allergic Th2 response, in sensitized mice IL-17A attenuates the Th2 response.

A new role as negative regulator of antigen-driven Th2 response was ascribed for IL-17F. IL-17F dampened antigen activation of DCs resulting in a reduced Th2 response and reduced inflammation [14]. These findings may provide a possible explanation why Yang and colleagues found an increased allergic response in IL-17F-deficient mice [17].

IL-22, similar to IL-17A [7, 18–20], has been found in diseased tissues from patients with different chronic inflammatory diseases, involving infiltrating activated T cells, such as rheumatoid arthritis, psoriasis, inflammatory bowel disease, and COPD [21–25]. IL-22 is expressed by IL-9-activated mast cell and by Th17 cells initiated by TGF-β in the context of IL-6 and other proinflammatory

cytokines [10]. IL-22 is increased in lung homogenates of OVA-sensitized and challenged mice [14]. Neutralization of endogenous IL-22 in OVA-sensitized mice increased the eosinophilic response [14], whereas administration of recombinant IL-22 attenuated the acute allergic response [26, 27].

These data provide evidence that IL-17A,F and IL-22 besides their inflammatory role have a negative regulatory function in allergic lung inflammation.

2 Increasing Evidence Supports This Novel Role of IL-17A and IL-22 as Down Modulators of a Committed Immune Response

It has in the past abundantly been described that full acquisition of pathogenic function in experimental autoimmune encephalomyelitis (EAE) by effector Th17 cells is mediated by IL-23. However, as shown most recently, stimulation of the myelin-reactive T cells with TGFβ plus IL-6, instead of IL-23, completely abrogated their pathogenic function despite upregulation of IL-17A production [28]. These regulatory Th17 cells failed to upregulate 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 [28] did not show whether IL-17A directly conveyed negative regulation of inflammation, which was rather due to co-expressed IL-10 in EAE. Several experimental approaches listed below have indeed demonstrated direct inhibitory functions of recombinant and endogenously produced IL-17A in vitro and in vivo. Furthermore, an IL-17A-induced expression of the anti-inflammatory IL-10 has been demonstrated in macrophages, yet IL-17A has moderate effects on monocytes and macrophages [29].

First, expression of recombinant murine IL-17A in *Vaccinia* virus increased viral virulence significantly in mice [30], suggesting that IL-17A negatively regulated the antiviral host defense. Second, administration of recombinant IL-17A ameliorated and negatively regulated the late phase of experimental autoimmune neuritis (EAN), a model of peripheral nerve demyelination [31]. Third, in vitro studies provided possible mechanisms of how IL-17A acts as a suppressor. IL-17A inhibited the chemokines RANTES (CCL5), Fractalkine (CX3CL1), and CTACK (CCL27) [32–34] and the mononuclear leukocyte adhesion molecule VCAM-1 in TNF-activated mesenchymal cells [35]. 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-17A needs to be revisited in multiple inflammatory and immune disorders.

Revisiting the role of IL-17A in multiple immune responses would help address the question as to whether IL-17A acts like a regulatory T cell (Treg) cytokine such as TGFβ or IL-10, which reduces allergic pulmonary challenges as well as vast T

cell responses [36–38]. Indeed, in initial experiments IL-17A was described as a Treg cytokine in cell cultures, inhibiting vast T cell responses [36, 39]. Furthermore, Treg cells and TGFβ promote under proinflammatory conditions the development of Th17 cells and production of IL-17A [40]. Therefore, IL-17A as a downmodulator of the dendritic cells and Th2 response provides evidence for a novel feedback mechanism by which Treg cells may control a Th2 response in the effector phase of allergic asthma.

Protective role of IL-22 has also been ascribed in other models than allergic lung inflammation. IL-22 provided protection to hepatocytes during acute liver inflammation [41] and protected against ConA- or tetrachloride-induced liver injury [42]. Second, delivery of IL-22-Ig fusion gene ameliorated experimental autoimmune myocarditis (EAM) in rats [43]. Third, local IL-22 gene delivery led to rapid amelioration of intestinal inflammation in a mouse model of ulcerative colitis, and conversely, inhibition of IL-22 activity by local overexpression of its antagonist, IL-22 binding protein, prevented recovery and goblet cell restitution in acute colitis (DSS) [44]. Therefore, even though IL-22 is an upregulator of proinflammatory gene expression and has proinflammatory function, there is growing evidence that IL-22 has, similar to IL-17A, a protective role in inflammatory diseases such as colitis, EAE, and allergic lung inflammation.

3 Excess Endogenous IL-17A and IL-22 Production in the Absence of IL-4 Signals In Vitro and In Vivo

IL-17A production is induced by TGFβ in a proinflammatory milieu, including presence of IL-6, IL-1, or TNF, and its production is sustained by IL-23. In contrast, IL-17A production is inhibited by IL-4, IFNγ, IL-25 (IL-17E), or IL-27. In lungs, IL-17A has been shown to originate from antigen-specific Th17 cells as well as from an iNKT subpopulation, which is NK1.1 negative [45]. In an allergic lung response, IL-17A production was induced by IL-23 and controlled by IL-4 receptor signaling [8]. In mice lacking IL-4 responsiveness, IL-17A was overproduced correlating with reduced effector functions of allergic asthma. The inhibition of the Th2 response was indeed ascribed to endogenous IL-17A, as assessed using IL-17A neutralizing antibody treatment in vivo.

In the absence of IL-4 responsiveness, not only IL-17A but also IL-22 production was significantly increased upon allergen challenge, and similar to IL-17A, inhibition of endogenous IL-22 by neutralizing antibodies increased the allergic response, indicating that both IL-17A and IL-22 contribute to the inhibition of the Th2 response.

This increase in IL-17A and IL-22 in the absence of IL-4 responsiveness was not due to differences in IL-23 concentrations since pulmonary OVA-induced IL-23 concentrations are equal in IL-4Rα KO and WT mice [8]. Therefore, the IL-4 signals affect directly IL-22 and IL-17 production and do not act on IL-23

production [8]. However, the molecular mechanism of IL-22 expression, being either IL-23 dependent or independent, in allergic lung inflammation needs to be investigated in future experiments.

Neutrophil involvement has been ascribed to both infectious disease and allergic inflammation [46–48]. In allergy, neutrophil recruitment is in part due to endogenous IL-17 [8, 49], but not due to IL-22, since neutralization of IL-22 did not diminish neutrophil but increase recruitment in the allergic response. This is in line with a previous report, which demonstrated that IL-22 alone and in synergy with IL-10 decreased IL-8 production by human alveolar epithelial cell lines [50].

Therefore, a novel mechanism of how IL-4 promotes a Th2 response was proposed, by suppression of the novel suppressor molecules IL-17A and IL-22. 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 [8, 51–53].

4 Molecular Mechanism of Negative Regulatory IL-17A and IL-22 Effects

On a mechanistic level, IL-17A elicits dual effects and reportedly promotes expression of proinflammatory (hemopoietic, CXC-chemokines, acute phase) factors [54, 55], whereas it inhibits the production of mononuclear cell recruiting molecules like TNF-induced VCAM-1 and CC-chemokine RANTES [35]. This dual effect of IL-17A 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 CCR4-positive Th2 cells. IL-17A 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, was also reduced by IL-17A and accompanied by reduced eosinophil infiltration in the airways. Indeed, reductions or absence of these CC chemokines reportedly ablates allergic asthma [56–58]. Therefore, diminished cell attraction seems to be the pivotal mechanism of how IL-17A attenuates the allergic inflammation.

Further IL-17A effects like acute phase IL-6 and prostaglandin (PG)E2 elevations may also have corroborated to reduce locally the allergic inflammation in the lungs. IL-6 elevations inhibited aeroallergen-induced Th2 inflammation [59, 60]. PGE2 elevations reduced pulmonary allergy specifically via the E3 receptor [61]. Therefore, while IL-17A may upregulate 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-17A 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-17A represented a reduction rather than a complete blockade. Intact anti-allergen IgE concentrations in the circulation may explain why IL-17A did not completely block but rather reduced pulmonary allergy and asthma. Elevated IgE concentrations reportedly correlate with and contribute to allergic reactions [62], although it is not sufficient for the development of allergy. Therefore, IL-17A acts as negative regulator of established Th2 response locally in lungs.

In the experimental models of allergic lung inflammation, IL-22 dampened the hallmarks of an allergic Th2 response in vivo, by inhibition of DCs and their expression of co-stimulatory molecules upon antigen treatment [14]. IL-22 attenuates further the allergic response by inhibiting the induction of TARC (CCL17), IL-13 and IL-25 as shown in vivo [26, 27]. In vitro, IL-22 prevented TNF-α/IL-13-induced TARC and IL-13 production in murine Clara cells [26] and IL-25 production in the lung epithelial cell line MLE-15 induced by IL-1β or LPS [27]. Therefore, these data corroborate that IL-22 not only has a negative regulatory function in experimental models of autoimmunity, and inflamed liver and colon but also in established Th2 response in the lung.

5 High Efficiency of Inhibitory Function of IL-17A In Vivo and In Vitro

Intranasal administration of recombinant IL-17A reduced eosinophil recruitment and a Th2 response, while neutrophil recruitment was not induced when applied locally at low doses of 2.5 μg/kg IL-17A to allergen-treated mice [8]. These findings were supported by the following cell culture data [35]. IL-17A inhibited TNF-induced chemokine RANTES expression in human synovial fibroblasts and mouse lung fibroblasts. This inhibitory activity of IL-17A was sixfold more potent than its stimulatory activity on TNF-α-induced IL-6 or IL-8 secretion (IC50 = 0.2 ng/ml vs. ED50 = 1.2 ng/ml), measured in the same cells. Furthermore, neutralization of the human IL-17A receptor (IL-17R) by antibodies competitively reversed the IL-17A-induced IL-6 upregulation. However, anti-IL-17R antibody only partially neutralized the inhibitions of RANTES production by IL-17A. Yet, IL-17R was essential for the RANTES inhibition, as assessed in IL-17R-deficient cells. Therefore, inhibitory and stimulatory functions of IL-17A involve receptor 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-17A functions and may explain why a net negative regulatory effect of IL-17A manifests in chronic inflammation in vivo where IL-17A production is low.

6 Molecular Signaling of Inhibitory Effects of IL-17A and IL-22

IL-17A interferes at TNF-activated NF-κB signaling in human synoviocytes [35]. This inhibition is immediate, within 20 min, and proposes a direct effect of IL-17A 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-17A 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 [63, 64]. The fact that IκB-β but not IκB-α is affected by IL-17A further supports the possibility that IL-17A is implicated in the regulation of the chronic phase of inflammation and immunity.

However, interferences of IL-17A on TNF-induced NF-κB activity virtually depend on the cell type and promoter targeted by TNF. For example, unlike the synoviocytes described above [35], TNF-induced NF-κB binding was only moderately and not statistically significantly reduced by IL-17A in colonic myofibroblasts [32]. In those cells, it was proposed that IL-17A 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-17A inhibited TNF expression transiently, the effect of IL-17A being biphasic with an early decrease of TNF release (at less than 30 min) and a marked stimulation later on (by 6 h) [29]. After 60 min and later, IL-17A also inhibited cAMP production and the transcription factor activities of CREB, AP-1, as well as NF-κB in the macrophages [29].

IL-22, on the molecular and cellular level, acts by activating STAT3. In vitro stimulation of epithelial cell with IL-22 resulted in the phosphorylation of STAT3, and diminished TNF/IL-13 induced TARC and IL-13 production [26]. Beneficial effect of IL-22 through STAT3 activation has previously been published. So IL-22 enhanced hepatocyte survival, by enhancing expression of transcription factor STAT3, Bcl-2, and Bcl-xL in inflamed liver and colon [41, 42]. STAT3 activation in intestinal epithelial cells and IL-22 was linked to mucosal wound healing [65]. IL-22 activated STAT3 and induced IL-10 by colon epithelial cells [66]. Prevention of DC activation and antigen (OVA) sensitization induced by transfer of ex vivo OVA-loaded DC has previously been ascribed to the prototype immune regulator IL-10 [25] and newly for IL-22 [14]. Whether the negative regulatory effects of IL-22 in allergic lung inflammation are due to IL-22 or indirectly induced needs further investigation.

7 IL-17 and IL-22 Have Similar Effects in Allergic Lung Inflammation but Are Still Distinct

The Th17-derived IL-22 and IL-17A negatively regulated allergy and DC functions. Its expressions were further induced by allergic stimulation together with IL-23 and were controlled by IL-4Rα signals. While neutralizing IL-22 or

IL-17 antibodies augmented the allergic response, IL-22, IL-17A, and IL-17F reduced the response by inhibiting DC functions. Therefore, the data demonstrate that IL-22 and IL-17 are novel endogenous negative regulators of allergy, and IL-22, IL-17, or Th17 cells may represent an interesting therapeutic target in lung allergy.

IL-17A and IL-22 are both co-expressed in Th17 cells [10], yet they are induced through independent pathways. Recent studies showed that IL-22 but not IL-17A and IL-17F production strongly depends on aryl hydrocarbon receptor signals [67].

Furthermore, IL-22 production is induced in absence of IL-6, while IL-17A production depends on the presence of IL-6 [22]. Additional IL-22 production is ascribed to IL-9 activated mast cells [68]. We found that IL-17A, but not IL-22 production, was markedly dependent on the intracellular signaling of the IL-1 receptor and its adaptor MyD88 pathway in lymph node cell cultures originating from OVA-treated mice [14]. A review by Eyerich and colleagues provides an overview on overlaps and differences between IL-17 and IL-22 [69]. Together this suggests collaborative and nonredundant pathways leading to Th17 cytokines and allergy.

8 Anti-IL-17 and Anti-IL-22 Therapies: A Risk or Advantage?

The pathological role of IL-17A, IL-17F, and IL-22 in autoimmune disorders has convincingly been documented and hence favored so far, although there is emerging evidence that IL-17A, IL-17F, and IL-22 also have a beneficial role as negative modulator in antigen-specific immune processes and allergic asthma [8, 14, 26–28, 31].

Increased IL-17A concentrations in allergic asthma, chronic bronchitis, chronic obstructive pulmonary disease (COPD), cystic fibrosis, and acute respiratory distress syndrome (ARDS) (for review, see [11]), but also rheumatoid arthritis (RA), were linked to the pathology of the diseases [20]. IL-17A neutralization inhibits experimental murine arthritis [20] and is a potential alternative therapy to TNF neutralization in rheumatoid arthritis. The novel negative regulatory function of IL-17A indicates, however, that such a therapy bears the potential risk of exacerbating allergic asthma. Therefore, an anti-IL-17A 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-17A antibodies may exclude respective groups at risk from such a therapeutic anti-IL-17A treatment.

Because of the proinflammatory function of IL-17A and IL-22, neutralizing therapies targeting either IL-17 or IL-22 are considered in allergic asthma [70, 71]. However, inhibiting IL-17A or IL-22 may bear the potential risk of opportunistic infections. IL-22 together with TNF-α was found to be important to keep the epidermal integrity during infection with *Candida albicans* [72]. Mutations in STAT3 in animal models and humans confer a defect in IL-17 function, resulting

in increased susceptibility to respiratory infections with bacteria and fungi [70, 73, 74]. Neutralization of IL-22 resulted in exacerbation of bacterial infections, suggesting a protective role in mucosal/epithelial host defense [75]. And IL-22 was protective during the development of lung fibrosis induced by chronic exposure to *Bacillus subtilis* [76].

Still, because of the novel function of IL-22 and IL-17 as endogenous negative regulators of allergy, IL-22, IL-17, or Th17 cells may represent an interesting therapeutic target in lung allergy.

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Inflammasome: IL-1/IL-17 Response in Lung Inflammation

Isabelle Couillin, Dieudonnée Togbe, Mamdouh Sedhom, Marc Le Bert, Hana Čipčić Paljetak, Francois Erard, René Moser, and Bernhard Ryffel

Abstract Airway exposure to endotoxin and other microbial TLR agonists induces a rapid production of mediators including IL-1 and IL-17, neutrophil recruitment, and bronchoconstriction which are abrogated in mice deficient for distinct TLRs or the common adaptor molecule myeloid differentiation factor 88 (MyD88). Furthermore, administration of IL-1β mobilizes neutrophils and induces IL-17 production in the lung. Therefore, IL-17 participates in IL-1β-induced lung inflammation. Importantly, lung injury by particles, chemicals, and allergens activates the NLPR3 inflammasome complex leading to the cleavage of pro-IL-1β into active IL-1β- and IL-17-dependent inflammation and repair. In conclusion, TLR agonists and lung injury induces the activation of the NLPR inflammasome with maturation of IL-1β leading to IL-17-dependent lung inflammation.

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Abbreviations

LPS	Lipopolysaccharide
TLR	Toll-like receptor
TIR	Toll-interleukin 1 receptor
TIRAP	TIR-domain-containing adaptor protein
TRIF	TIR-domain-containing adaptor-inducing interferon-β
Penh	Enhanced respiratory pause

1 TLR-Dependent Neutrophil Recruitment

The innate immune system senses microbial products by Toll-like receptors (TLRs), which are broadly distributed in the lung and the body [1, 2]. The TLR engagement by microbial products causes cell activation which may result in an inflammatory 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 or lipopolysaccharide (LPS) plays an important role in the development and progression of chronic respiratory diseases including asthma [3–5]. In the 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 is TLR4 expression dependent. Endotoxin-induced bronchoconstriction and neutrophil recruitment in the lung are abrogated in mice deficient for the LPS receptor complex, TLR4/CD14/MD2. Further, TNF, IL-12p40, and KC production and protein leakage are also dramatically reduced in the absence of the LPS receptor [8]. Other bacterial products such as MALP-2 or 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 respectively. Similarly, viral ssRNA have TLR3 agonistic properties. Poly I:C is a synthetic TLR3 agonist which causes acute neutrophil recruitment into the BAL and lung inflammation, which is absent in TLR3-deficient mice. This is not an exhaustive list of agents activating TLRs and other pattern recognition receptors leading 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 showed recently that the TLR adaptor 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-33R, or the focal adhesion kinase, FAK [14]. In certain infection models, the extreme sensitivity of MyD88 deficient mice may however be ascribed, at least in part, to deficient IL-1R/IL-18R signaling, as shown recently for cutaneous *S. aureus* infection [15] and for mycobacterial infection on IL-1R [16].

2 Inflammasome Activation and IL-1β Maturation

In addition to innate immune recognition receptor of the TLR family, NOD-like receptors (NLRs) represent another essential cytosolic receptor family, which senses endogenous danger signals upon cell injury. Danger signals or "alarmins" include uric acid, ATP, heat shock proteins (HSPs), HMGB1, IL-1α, IL-33, and many other endogenous molecules generated by damaged or dying cells [44]. These endogenous mediators activate the innate immune system by engaging the sensing receptor NLPRs as recently reviewed [17]. The NLPR proteins form a large, signal-induced multiprotein complex, the inflammasome, resulting in the activation of proinflammatory caspases [18]. Uric acid was identified in dying cells [19] and was implicated in IL-1R-dependent inflammation [20, 21]. Uric acid crystals induce activation of the NLPR3 inflammasome complex leading to IL-1β and neutrophil recruitment in the peritoneal cavity [22]. Macrophages from mice deficient in various components of the inflammasome such as caspase-1, ASC, and NALP3 are defective in uric acid crystal-induced IL-1β activation. Furthermore, chemical lung injury with bleomycin causes uric acid release and NLPR3-dependent maturation of IL-1β, leading to lung inflammation and fibrosis [23]. Bleomycin-induced inflammation and fibrosis depend on ASC, which is a critical adaptor protein of the NLPR3 inflammasome complex [24]. Mediators or endogenous danger signals activating the inflammasome complex are a topic of broad interest. Lowering intracellular K+ level may be a mechanism of NLPR3 activation [25, 26].

We revisited the role of the NLPR3 inflammasome complex and IL-1β in the allergic response [27] and found NLPR3 is required for the response. Further, IL-1 receptor signaling and of both IL-1β and IL-1α is essential in the inflammatory response to ovalbumin challenge in immunized mice in the absence of adjuvant alum [41].

Therefore, the activation of the inflammasome complex cleaving pro-IL-1β leading to mature IL-1β plays a major role in a broad range of insults resulting in IL-1-dependent inflammation and has been reviewed recently by us [40].

3 Critical Role of IL-1 in Lung Inflammation

Recent investigations confirmed a critical role of IL-1β in models of lung inflammation.

First, in acute cigarette smoke-induced inflammation in mice, we found that neutrophil recruitment in the alveolar space depended on TLR4 and IL-1R signaling as the response was ablated in gene-deficient mice [43]. Cigarette smoke induced IL-1 and heat shock protein 70, which is a known agonist of TLR4 explaining the absence of inflammation in TLR4-deficient mice, and the response was IL-1R signaling dependent [43].

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 [24]. Indeed, IL-1R-deficient mice or neutralization of IL-1β abrogated bleomycin-induced inflammation and fibrosis [23, 24].

Third, the inflammatory response to uric acid crystals as observed in clinical gout has been demonstrated to be IL-1β dependent [22, 28] and now is confirmed in lung [24] and can be blocked by neutralizing antibodies.

Further, the inflammatory response to intranasal mycobacteria administration was drastically increased in the absence of IL-1R signaling. We found that a protective host response required functional IL-1β, as IL-1R1-deficient mice succumbed to uncontrolled infection [16]. Lastly, rmIL-1β administered by the intranasal route induces lung inflammation [24].

Therefore, the different models suggest a direct and critical role of IL-1 in the inflammatory response, and NLPR3 inflammasome activation leads the maturation and release of IL-1β.

4 IL-17 Contributes to the Inflammatory Response in the Lung

The initial investigations suggested that the T cell-derived cytokine IL-17 participates in the acute pulmonary inflammatory response ([29, 30, 44]). 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 MIP-2 production in the BAL, which were both inhibited by neutralizing IL-17 antibodies [31]. In order to link the proinflammatory effects of endotoxin with the proinflammatory effects 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 [32]. Further, neutralization of IL-6 and MIP-2 inhibited the neutrophil accumulation. Ferretti and colleagues reported a first peak at 24 h followed by a second peak of neutrophil recruitment in the airway upon LPS exposure, the latter coincided with IL-17 production in the BAL fluid and lung [33]. 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 [33]. The finding that non-T cells produce IL-17 is interesting and needs additional confirmation.

Our team investigated the role of IL-17A in the chemical lung injury model with bleomycin. We found that IL-1β-dependent pulmonary IL-17 expression and lung inflammation and fibrosis were attenuated in the absence of IL-17RA signaling or by neutralizing IL-17A antibodies [34].

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 interferon-γ and IL-4 [35]. NK1.1(neg) iNKT cells produce IL-17 upon synthetic (alpha-galactosylceramide [alpha-GalCer] or PBS-57) as well as natural (lipopolysaccharides or glycolipids derived from *Sphingomonas wittichii* and *Borrelia burgdorferi*) stimulation. NK1.1(neg) iNKT

cells are found more frequent in the lung, which is consistent with a role in natural immunity to inhaled antigens. Airway neutrophilia induced by alpha-GalCer or lipopolysaccharide instillation was significantly reduced in iNKT-cell-deficient Jalpha18 mice and was abolished by neutralizing IL-17 monoclonal antibody. Therefore, NK1.1(neg) 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* was used as another way to test the role of IL-17 in neutrophil recruitment and host resistance [36]. IL-17 receptor-deficient mice succumbed rapidly to intranasal *P. pneumonia* challenge with uncontrolled infection, delayed and diminished neutrophil recruitment in the alveolar space, and reduced MIP-2 and G-CSF production in the lung [36]. Therefore, for an acute inflammatory response and to clear the bacilli, IL-17RA signaling is critical.

IL-17 has been found in sera and BAL fluids from asthmatic patients suggesting a role in allergic reactions [37]. 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 [38]. We showed IL-17 receptor signaling-dependent IL-23 production and elevated levels of IL-17 in the lung of allergic mice [39]. 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 downregulating the hypersensitive response [39]. See also accompanying contribution by Linden. Further, we found a role of the related Th17 family member IL-22, which has different roles in the development and resolution of allergic lung inflammation [42].

However, the link between IL-1β and IL-17 and the need of IL-17 as a critical inflammatory effector cytokine in lung inflammation needs to be explored further. We have recently reviewed the IL-1–IL-1 axis in the initiation and resolution of inflammation [40].

In conclusion, present knowledge about injury-induced inflammasome activation and maturation of IL-1β and IL-17 is emerging. The knowledge on the role of IL-1α and IL-33, two alarmins released upon cell death, is an exciting area needing further investigations. There is now substantial evidence that lung injury leads to inflammasome complex formation with caspase-1 activation resulting in mature IL-1β, which may elicit IL-17 production and inflammation.

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 diseases. Genetically susceptible mice injected with cardiac self-antigen-loaded dendritic cells or immunized with cardiac self-antigen together with strong adjuvants develop overt cardiac inflammation. Later on, affected mice develop heart failure and a cardiac phenotype, which closely resembles the morphological picture of dilated cardiomyopathy in humans. IL-17-producing T cells contribute to EAM development, but the absence of IL-17A signaling on CD4+ T cells can be compensated by other heart-reactive T helper cell subsets. IL-17A, however, has proven to be critical for progression of acute myocarditis to an end-stage heart failure phenotype. Accordingly, cytokines promoting Th17 CD4+ expansion and/or IL-17 release, such as IL-23, IL-6, TGF-β, and IL-1, are key players in EAM and most likely in inflammatory dilated cardiomyopathy. Understanding the specific role of distinct cytokines during induction and progression of EAM will expand our knowledge on the pathogenesis of dilated cardiomyopathy and will help us to develop novel treatment strategies in the future.

Keywords Autoimmunity • Mouse model • Myocarditis • T helper cells • Interleukin-1 • Interleukin-6 • Interleukin-17 • Interleukin-23 • Interferon gamma • Dendritic cells • Monocytes/macrophages • Toll-like receptors • Nitric oxide • Innate immunity • Inflammatory cardiomyopathy

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1 Autoimmunity in Inflammatory Heart Disease

Inflammatory dilated cardiomyopathy represents a 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, Parvo-, hepatitis C, human immunodeficiency, HHV-6, HHV-7, Cytomegalo-, Epstein-Barr viruses have been detected in the hearts of patients with inflammatory dilated cardiomyopathy [2, 3]. Virus infections not only contribute to cardiac tissue destruction [6] but also trigger heart-specific autoimmunity, which plays a relevant pathogenetic role in many patients with inflammatory dilated cardiomyopathy [7]. In fact, many affected patients develop heart-specific autoantibody responses [5, 6]. In addition, immunosuppressive 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 type B3 (CVB3) virus 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 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 histologic 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.

2 The Experimental Autoimmune Myocarditis (EAM) Model

Immunization models offer the advantage to study the 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-2d) and A/J (H-2a) mice [16, 17]. The most pathogenic epitope for BALB/c mice has been

identified on the alpha myosin heavy chain protein [17]. Activation of antigen-presenting cells (APCs), taking up and processing 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. In fact, 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. The decisive role of CD4+ T cells for EAM induction can be shown in adoptive transfer experiments: injection of in vitro restimulated CD4+ T cells isolated from spleens and draining lymph nodes of mice with EAM results in myocarditis, albeit at a low severity grade, in severe combined immunodeficient (SCID) mice or LPS pretreated wild-type animals [12]. The efficacy of adoptive transfer can be greatly improved if heart-specific CD4+ T cells are specifically enriched over several cycles of in vitro restimulation/expansion.

3 Role of IL-17 and IL-17-Producing CD4+ T Cells in EAM

EAM represents a CD4+ T cell-mediated disease. Three major CD4+ T cell subsets have been defined according to their cytokine production pattern: IFN-γ-producing Th1 cells, IL-4-releasing Th2 cells, and IL-17-producing Th17 cells. Signaling through the receptor of the key Th2 cytokine IL-4 is dispensable for EAM development [19, 20]. The fact that the majority of heart-infiltrating cells express a monocyte/macrophage phenotype would suggest a Th1 predominant phenotype. A substantial proportion of these monocyte-like cells, however, express a pattern of surface markers, which labels them as immature myeloid-derived precursors with the capacity to differentiate within the inflamed microenvironment either in mature monocytes/macrophages or GM-CSF dependently into granulocytes, the latter being a hallmark of Th17-mediated inflammation [21, 22]. Interestingly, 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, 23, 24]. In addition, mice lacking T-bet, a T-box transcription factor essential for Th1 lineage differentiation, show enhanced myocarditis severity [23]. These apparently contradictory findings can be explained by analysis of gene-targeted mice with mutations in the Th1-driving cytokine IL-12 and its receptor. IL-12p70, which binds 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 an IL-12Rβ1 and an IL-12Rβ2 subunit and induces the recruitment of

JAK2 and STAT4 activation. Experiments with knockout mice revealed that both the IL-12β1- and the IL-12p40 subunit were essential for myocarditis induction [23, 24]. In contrast, IL-12p35-deficient mice developed more severe autoimmune myocarditis compared to wild-type littermate controls [23]. These apparent paradoxical findings were 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, however, promotes expansion and survival of Th17 cells [25–28]. Th17 cells are involved in chronic and autoimmune inflammation in experimental autoimmune encephalomyelitis [28], graft-versus-host disease [29], or allergic asthma [30]. In the context of experimental autoimmune myocarditis, 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 yields heart-specific Th17 T cell lines, which are highly pathogenic after transfer in wild-type mice [31]. In addition, treatment of myosin-peptide immunized mice by active vaccination [32] or with an IL-17-depleting antibody [31] ameliorates EAM severity. In line with these findings, Cruz-Adalia et al. linked enhanced myocarditis susceptibility and heart failure progression in CD69-deficient mice to an exacerbated Th17 response [33]. In contrast, IL-17A signaling seems dispensable for EAM induction because IL-17A-deficient mice developed similar myocarditis severity scores as wild-type controls 3 weeks after immunization [34]. Of note, EAM was also induced in double knockout mice lacking both IL-17A and Th1 cytokine IFN-γ [34]. These latter findings suggest that neither IL-17 nor IFN-γ compensates for each other in EAM induction at least not in the knockout model.

The utility and relevance of the EAM model mirroring human disease relies on the fact that mice with EAM develop dilated cardiomyopathy. Interestingly, the absence of IL-17A did not prevent acute myocarditis, but almost completely protected from progression to cardiomyopathy by inhibiting pathological remodeling and cardiac fibrosis [34]. Similar protective effects are observed in anti-TGF-β-treated, myosin/CFA-immunized mice [22] or in animals lacking the IL-1 receptor-linked, downstream adaptor molecule MyD88 [21]. Both TGF and IL-1 are closely linked to IL-17. In fact, increasing evidence suggests a critical role for IL-17 in chronic progressive inflammatory responses and fibrogenesis that goes beyond its role as key cytokine of a distinct helper cell subset. Despite the not yet clarified role for IL-17 in EAM induction, IL-17 therefore appears as promising target for the prevention of heart failure progression.

4 Activation of Self-Antigen-Loaded Dendritic Is Critical for EAM Induction

The efficient priming of heart-specific, autoreactive T cells requires cooperation between the innate and adaptive activation signals beyond an individual and genetically defined threshold level. Dendritic cells are sentinels of the immune system scavenging foreign pathogens as well as cell debris and necrotic tissues.

Nonspecific activation of dendritic cells through Toll-like receptors 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 [35].

Several lines of evidence point toward 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 [36]. 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 [37]. The number of these cells and upregulation of their MHC class II expression parallels the development of cardiac inflammation [38].

The precise role of these heart resident dendritic cells is not clear. As mentioned above, dendritic cell activation is largely mediated by Toll-like receptors. Toll-like receptors (TLRs) represent a family of evolutionarily conserved transmembrane receptors [39]. Importantly, different TLRs 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, such as cardiac myosin, for example [40]. 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 [41]. 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 [35]. Furthermore, mice lacking the common adaptor molecule MyD88 for different Toll-like receptors are protected from autoimmune myocarditis [42]. 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 a well-known clinical observation: It might explain why some patients develop heart-specific autoimmunity after noninfectious tissue damage, that is, after cardiac surgery or myocardial infarction [43, 44]. 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 [45, 46].

Activation of antigen-presenting dendritic cells through Toll-like receptors results in upregulation 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 antigen-presenting cells as well as T cells. Major pro-inflammatory cytokines released by TLR activated dendritic cells include IL-12p40, IL-1β, IL-6, and TNF-α, which are all critical for EAM induction and progression [18, 20, 42, 47]. 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 [35]. As aforementioned, IL-12p40 represents a subunit of IL-23, which has been recognized as a critical mediator for the expansion and survival for the pathogenic heart-specific Th17 T cell subset [25–27]. IL-1 and IL-6, together with TGF-β, are required for the priming of autoreactive Th17 T cells [28, 48]. From this point of view, it is not surprising that mice lacking IL-6 as well as IL-1 receptor type I-deficient mice are protected from experimental autoimmune myocarditis [18, 47]. 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 restimulation 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). Nevertheless, disease resistance of IL-1 receptor 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-1 receptor type I signaling in the T cell compartment. Further studies are needed to identify cytokines and mediators compensating for the absence of IL-1 signaling in Th17 priming.

5 Opposing Roles for IFN-γ and Th17 Cells in Experimental Autoimmune Myocarditis

Immunization of BALB/c mice with MyHC-α-peptide/CFA results in a mixed T cell response. In fact, in vitro restimulation 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 Th1 and Th17 subsets. In fact, EAM can be induced with Th1 and Th17 heart-specific T cell lines (Blyszczuk and Eriksson, unpublished data). Furthermore, double knockout mice lacking both IL-17A and Th1 cytokine IFN-γ are susceptible to EAM. Thus, the T helper cell phenotype rather determinates the specific disease phenotype, and – even more important – the development of end-stage heart failure, than myocarditis susceptibility by itself. Nevertheless, in diseased hearts of wild-type mice, Th17 cells predominate, and IFN- γ released from other heart-infiltrating cells, such as nonspecific CD8+ T cells, for example, negatively regulates autoimmune myocarditis [23]. Accordingly, mice lacking IFN-γ or IFN-γ receptor develop more severe, progressive disease [19, 20].

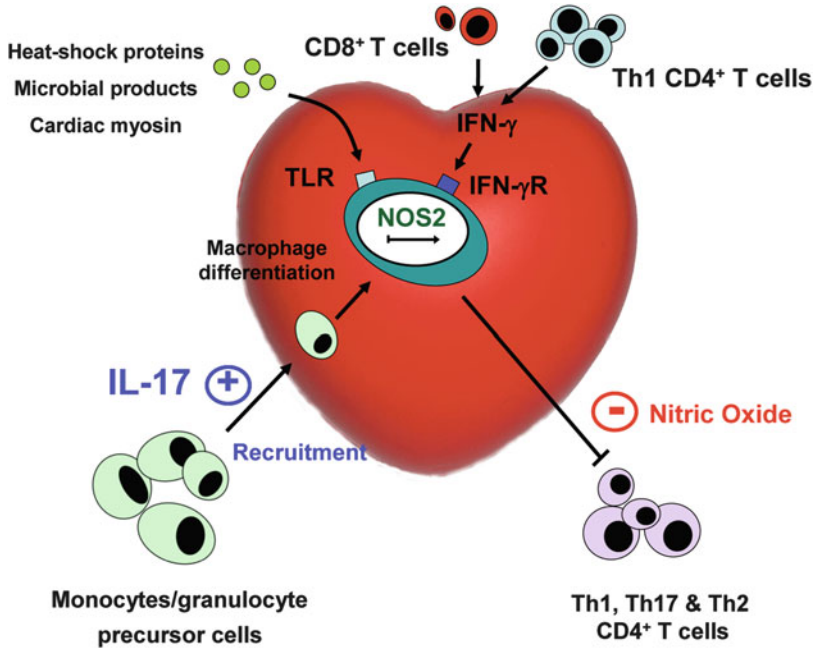

Fig. 1 IL-17 promotes the recruitment of myelo-derived precursor cells to the inflamed heart. Precursors differentiate into granulocytes or macrophages. In macrophages, interferon-gamma and/or Toll-like receptor activation triggers inflammatory cytokine release and upregulation of nitric oxide synthase 2. Nitric oxide, on the other hand, induces growth arrest of autoreactive $CD4^+$ T cells and confines heart-specific inflammation

These data are in line with observations from the experimental autoimmune encephalomyelitis model, where 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 [25–29]. In experimental autoimmune myocarditis, IFN-γ impairs the apoptosis of CD25 + CD4+ regulatory T cells [49] and promotes nitric oxide-mediated T cell growth arrest [19, 31]. Mac-1 (CD11b) expressing monocytes represent the major fraction of heart-infiltrating cells in experimental autoimmune myocarditis [31]. In vivo experiments with IL-17-depleting antibodies demonstrated that IL-17 directly mediates the recruitment of CD11b expressing myeloid-derived monocytes to the heart. So far, monocytes have been considered an active, disease-promoting substrate of inflammation. Accordingly, factors influencing monocyte/macrophage differentiation can regulate EAM severity [50]. Interestingly, specific monocyte subsets even exert protective effects. Heart infiltrating, CD11b + cells, for example, might also represent a key element in the IFN-γ-dependent negative feedback mechanism confining autoreactive T cell expansion and cardiac inflammation. Mechanistically, IFN-γ induces NOS2 (nitric oxide synthase 2) upregulation in monocytes. NOS2 upregulation promotes local release of nitric oxide, 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 promote the 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 (Fig. 1).

6 Conclusions

Experimental autoimmune myocarditis is a helpful in vivo model to study the pathogenesis of human inflammatory heart diseases. It illustrates the cooperation between innate and adaptive immunity as the critical event triggering heart-specific autoimmunity and experiments with gene-targeted mice lacking specific cytokines and cytokine receptors greatly advanced our knowledge of the decisive pathogenetic pathways. 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. Meanwhile, insights from the autoimmune myocarditis model nevertheless offer an attractive tool to develop innovative and novel treatment strategies.

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Th17 Cells in Transplantation: Actors or Innocent Bystanders?

Louis-Marie Charbonnier, Benoit Vokaer, Philippe Lemaître, and Alain Le Moine

Abstract The recent interest for Th17 cells immediately raised questions about their possible involvement in allogeneic processes. Indeed, IL-17-producing innate immune cells and Th17 cells have now been related to allograft rejection and ischemia-reperfusion injury in clinical settings and in experimental models. Some of them clearly established a link between the presence of these cells and the development of long-term graft dysfunction. A pivotal role of a Th17-mediated pathway in transplant damage has been demonstrated independently of MHC disparities with T cells recognizing either autoantigen or minor transplantation antigen. The ambiguous relationship between regulatory T cells (Treg) and Th17 cells also complicates Treg-based therapy. Herein, we briefly discuss recent studies reporting Th17 and IL-17 involvements in allograft rejection processes.

1 Introduction

Th17 cells are now recognized as important players of immune responses, especially in responses against microorganisms or in autoimmunity as developed in the other chapters. A priori, there is no reason for excluding Th17 from the allogeneic response and to not consider them as potential effectors of allograft damage. Indeed, the presence of IL-17 has been described in allogeneic tissues or graft product during rejection. IL-17 has been observed in renal biopsies and urine during acute rejection in kidney-transplanted patients [1, 2]. This feature was correlated

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with a late renal allograft dysfunction [3]. In parallel, elevated levels of IL-23 (a Th17 booster) and IL-17 were measured in serum of patients presenting an acute rejection of hepatic or pulmonary transplant [4]. An abnormal production of IL-23 and IL-17 associated with the presence of neutrophils has been described in the bronchoalveolar lavage from patient suffering of bronchiolitis obliterans syndrome (BOS), which is considered the hallmark of chronic lung allograft rejection [5].

2 IL-17 and Ischemia-Reperfusion: The "Snow Ball Effect"

Previous studies established a direct role played by the IL-17 in the ischemia-reperfusion injury (IRI) at the early stage of transplantation. In a murine model of renal IRI, IL-17, or IL-23 neutralization improved renal function and prevented the inflammatory infiltrate [6]. In this context, polymorphonuclear neutrophils were the relevant source of IL-17. Neutrophil-derived IL-17 promotes the production of several chemokines by epithelial and endothelial cells (CXCL1, CXCL2) that in turn recruit other leucocytes. In a model of ischemic brain injury, the pathogenic role of IL-17 has also been established. In this system, an early burst of IL-17 released by the $\gamma\delta^+$ T cell subset orchestrates the inflammatory process and damages [7].

Whereas IL-17 acts directly in ischemia-reperfusion injury, recent studies demonstrated that IRI by itself promotes the Th17 differentiation, constituting therefore a positive feedback loop. Indeed, two independent works reported how IL-6, a typical pro-inflammatory cytokine abundantly released during IRI, promotes the Th17 differentiation of naive T cells [8, 9]. In these observations, IL-6 strongly induces the expression of the transcription factor HIF-1 (hypoxia-inducible factor-1), which in turn enhances the Th17 differentiation by activating RORγt, a transcription factor considered as the master regulator of Th17. The expression of HIF-1 was dependent of mTOR pathway and disappeared after rapamycin exposure [8]. This concept was supported by the experiments of Nakagiri et al. [10]. In this model of tracheal transplantation, the early IL-6 neutralization abolished the chronic obliterative airway disease by preventing late IL-17 production. At the same time, it seems that IL-17 by itself can end this vicious circle. Indeed, in a model of member ischemia, the IL-17-producing $CD4^+$ T cells exhibit angiogenic properties that support neovascularization [11].

Antigen-presenting cells (APCs) are classically described as a link between innate and adaptive immunity. Becoming mature under inflammatory conditions, they present allogeneic peptides in self-major histocompatibility complexes (MHC) to alloreactive T lymphocytes (the indirect pathway of alloantigen presentation), or as donor-derived cells they can directly present intact allo-MHC molecules to alloreactive lymphocytes (direct pathway). In a heart transplantation model, the early IL-17 production promotes the maturation of dendritic cells by increasing the expression of MHC and costimulatory molecules and by this way optimizes the sensitization of alloreactive lymphocytes [12]. Those IL-17-mediated effects are summarized in Figs. 1 and 2.

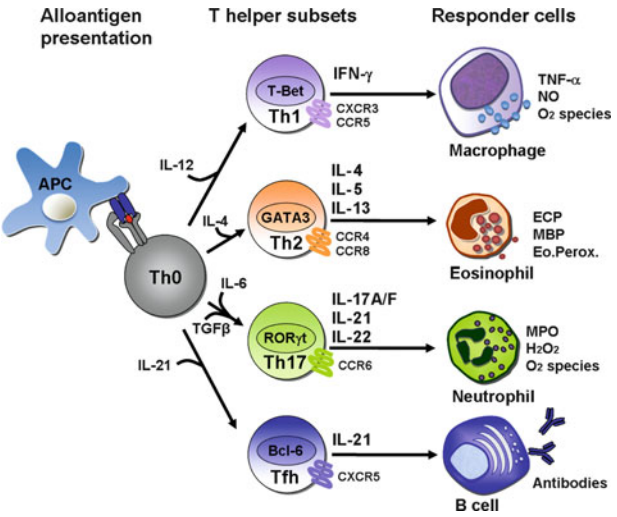

Fig. 1 T-helper subset differentiation and their implication in allograft rejection. When alloantigens are presented either as processed peptides (*indirect pathway*) or as donor intact MHC (*direct pathway*) on antigen-presenting cells (APCs), naive CD4+ T cells (Th0) are activated and differentiated into Th1, Th2, Th17, or follicular helper T cells (Tfh). T helper differentiation is triggered by polarizing cytokines leading to the expression of specific transcription factors: T-bet via IL-12 for Th1, GATA3 via IL-4 for Th2, RORγt via IL-6 plus TGF-β for Th17 and Bcl-6 via IL-21 for Tfh cells. Th1 cells express CXCR3 and CCR5, secrete IFN-γ which is critical for MHC II upregulation on cell surface and promote macrophage recruitment and release of TNF-α, nitric oxide (NO), and oxygen species. Th2 cells express CCR4 and CCR8 and produce IL-4, IL-5, and IL-13 leading to IL-5/eosinophil-mediated rejection by the release of eosinophil cationic protein (ECP), major basic protein (MBP), and peroxidase (Eo.Perox.) activity. Th17 cells express CCR6 and secrete IL-17A, IL-17F, IL-21, and IL-22 and promote neutrophil granulopoiesis, recruitment, and activation (the latter is characterized by myeloperoxydase (MPO) activity and release of H_2O_2 and oxygen species). Tfh cells are recruited to the B cell zone of lymphoid organs through the CXCR5 chemokine receptor and secrete IL-21 leading to B cell differentiation in plasma cells that secrete alloantibodies

3 The Multiple Roles of the IL-17 in the Anti-donor Immune Response

The involvement of human Th17 cells in the rejection process has been widely described in pulmonary transplantation [13] (summarized in Figs. 1, 2, and 3). During IRI following lung transplantation, cryptogenic fragments of type V collagen (col(V)) derived from the pulmonary basal membrane are released and processed by antigen-presenting cells. These new epitopes sensitize anti-col(V) $CD4^+$ lymphocytes, which differentiate toward an IL-17-producing phenotype. Several clinical and experimental studies in rats illustrate the implication of these lymphocytes in the bronchiolitis obliterans syndrome (BOS) [14], chronic form of the pulmonary graft rejection. However, in this syndrome, it seems more appropriate to speak about autoimmunity than true alloreactivity (reactivity against col(V)

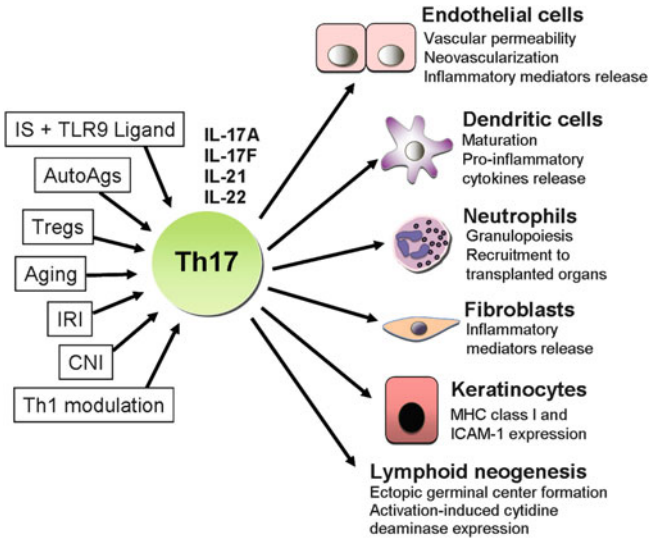

Fig. 2 Conditions favoring Th17-mediated rejection and responder cells. The bacterial product CpG, a TLR9 ligand, promotes IL-6-mediated Th17 pathway of rejection under immunosuppression with rapamycine and CD40 costimulation blockade. In clinical lung transplantation, release of type V collagen from the transplant promotes autoreactive Th17 cells that favor the bronchiolitis obliterans syndrome. In the context of minor antigen disparity, regulatory T cells (Treg) are critical for Th17-mediated neutrophil pathway of rejection. Memory Th17 cells increase with age and contribute to allograft rejection. Th17 cells play a critical role in inflammation and neovascularization after ischemia. Calcineurin inhibitor (CNI)-induced Th17/Treg imbalance and susceptibility to allograft dysfunction may rely on the dampening of Th1 reactivity and IL-2 inhibition (this remains to be clearly established in animal models). Th17 cells mediate cardiac allograft rejection and vasculopathy in T-bet-deficient mice. Effector pathways by which Th17 cells could contribute to allograft rejection are described on the right

rather than against MHC molecules). In a murine model of MHC-mismatched skin transplantation, the absence of IL-17 does not modify the survival of the graft [15]. Nevertheless, the neutralization of this cytokine in the context of minor antigen-disparate skin graft delays significantly the graft rejection. These observations suggest that, without any immunosuppressors, IL-17 in an untreated recipient does not seem to play a dominant role in MHC-mismatched graft rejection.

However, it is not excluded that a "Th17" pathway of rejection could emerge when the immune system is regulated by some treatment. For example, in a model of allograft tolerance induced by the costimulatory blockade (blockade of the CD40-CD154 pathway), an IL-6/IL-17-dependent pathway of rejection appears after APCs stimulation with a Toll-like receptor 9 agonist [16]. In a similar model of heart tolerance, Burell E. et al. showed that *T-bet* deficiency (a key factor of the Th1 differentiation) in the recipient causes an acute form of rejection depending on IL-17-producing $CD8^+$ lymphocytes [17]. In addition, it seems that graft rejection could spontaneously tip toward a Th17 phenotype in aging recipients [18]. Indeed, both in human and mouse, an increased pool of anti-donor IL-17-

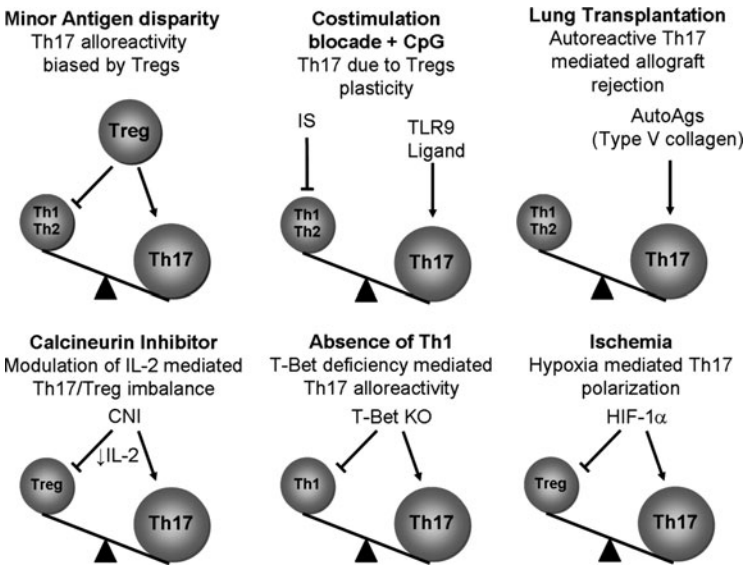

Fig. 3 Conditions favoring Th17 imbalance in transplantation. Tregs favor a Th17 bias of alloreactive T cells in the context of minor antigens disparity. By induction of IL-6 secretion, TLR9 ligand promotes Th17 pathway of rejection in the context of costimulation blockade. Type V collagen from the transplant promotes autoreactive Th17 cells leading to allograft rejection. Absence of Th1 differentiation due to T-bet deficiency leads to a Th17-dependant pathway of rejection. During ischemia, hypoxia induces HIF-1α that modulates the balance between Tregs and Th17

producing $CD4^+$ lymphocytes was observed in aged recipients. These cells exhibit a memory phenotype and could be responsible of an exacerbated form of rejection. Very recently, it has been shown that some immunosuppressive drugs could contribute to the emergence of a Th17 immune response among transplanted patients [19, 20]. Interestingly, this phenomenon could be linked to a long-term graft dysfunction.

By studying 11 human renal grafts detransplanted for terminal failure, the work of Deteix et al. established a correlation between the presence of Th17 lymphocytes and the short duration of life of the graft [21]. In this study, human Th17 cells were described to produce IL-21, which promotes lymphoid neogenesis in the renal interstitium. Another role played by the IL-17 in the chronic allograft rejection could be its pro-fibrotic effect. This was illustrated in a murine model of heart transplantation after $CD4^+$ cell depletion in which a progressive fibrosis is prevented by IL-17 neutralization [22]. Finally, renal tubular epithelial cells can also produce IL-17 during acute rejection. This phenomenon was illustrated by immunostaining of renal biopsies in transplanted patients presenting an acute antibody-mediated form of rejection. This production could be dependent on the activation of complement [23]. However, the direct involvement of IL-17 in these lesions remains unclear.

4 Crisscross Inhibition of Th17 and Th1 Alloimmune Response

The in vitro differentiation of a naive $CD4^+$ T lymphocyte into a Th17 phenotype is strongly inhibited by either pro-Th1 or pro-Th2 cytokine environment (Figs. 2 and 3). Many experimental arguments let us to expand this fundamental concept to transplantation. Indeed, by using T-bet-deficient recipients in a model of single class II MHC-mismatched cardiac allograft, the inhibition of the Th17 differentiation by Th1-related gene could be demonstrated in transplantation [24]. In this study, whereas cardiac grafts survived 40 days in wild-type recipients, an IL-17-dependent graft destruction occurred among T-$bet^{-/-}$ recipient at day 11 post-transplantation. Conversely, the inhibition of the Th1 pathway by IL-17 has also been described during graft-versus-host disease (GVHD) [25]. In this case, IL-17-deficient allogeneic T cells transferred into irradiated recipients led to an exacerbated Th1 response and a worsened disease. We also observed an increased Th17 alloreactivity in IL-4 KO recipients suggesting the inhibition of the Th17 alloimmune response by IL-4, a typical Th2 cytokine (unpublished data).

5 Relation Between Tregs and IL-17 in Transplantation

Although tolerance induction is still anecdotic in clinical transplantation, it has been already achieved in many experimental studies by using the tolerogenic properties of regulatory T cells (Fig. 3). Tregs constitutively express the α-chain of the high-affinity IL-2 receptor (CD25) and the transcription factor foxp3. Although their capacities to control both Th1 and Th2 alloimmune responses are well described, their interactions with Th17 cells remain under debate. Indeed, we have recently shown that a Th17 alloimmune response could escape to Treg-mediated suppression in vitro [26]. Furthermore, some evidences demonstrated that Tregs could promote a Th17 immune response. Indeed, $CD4^+$ T cells were induced to produce IL-17 when stimulated by allogeneic mature dendritic cells in presence of Tregs in a dose-dependent manner. In contrast, the Tregs in this setting led to a decreased production of the cytokines Th1 and Th2 [26]. Other teams obtained similar results after polyclonal stimulation by anti-CD3 [27]. We recently confirmed this concept in vivo by using a model of minor antigen-disparate graft rejection [15]. In this particular model, Treg-depletion prevented Th17 alloreactivity. In addition, the adoptive cotransfer of Tregs with naïve monospecific anti-donor T cells in a lymphopenic hosts biased the immune response toward Th17.

The mechanisms by which Tregs favor a Th17 immune response were recently clarified. Two independent teams described that $foxp3^+$ regulatory T cells promoted Th17 cell development in vivo and in vitro through regulation of interleukin-2 [28, 29]. Indeed, by limiting the availability of IL-2, Tregs consume a great inhibitor of the Th17 differentiation. Interestingly, the Treg-dependent TGF-β

production (a pro-Th17 factor) was not required for Th17 cell promotion. This point was demonstrated by the fact that specific deletion of the *tgf-β* gene in Tregs did not affect their ability to favor Th17 differentiation. Another nonmutually exclusive possibility relies in the Treg-mediated suppression of Th1 cells, which have been described as inhibiting Th17 differentiation [30, 31]. However, Treg-mediated inhibition of Th1 alone may not be sufficient to explain the pro-Th17 effect of Tregs. Indeed, the Treg-dependent Th17 differentiation still occurred even when Th1 cytokines are completely blocked [15, 29]. Altogether, these results suggest that the use of Tregs for tolerance induction should be carefully considered because of their ability to promote the emergence of a Th17-mediated rejection.

6 Conclusion

Several clinical studies have reported the presence of Th17 cells and related cytokines in association with particular forms of transplant rejection. Moreover, some of them clearly established a link between the presences of these cells and the development of long-term graft dysfunction. Interestingly, a pivotal role of the Th17 pathway in transplant damage was demonstrated independently of MHC molecule disparities. This is true for alloreactive Th17 cells in the context of minor antigen disparities and autoreactive anti-collagen (V) Th17 cells after lung transplantation. This suggests that the participation of Th17 pathway in transplantation operates in an autoimmune-like manner. As redundant immune pathways lead to allograft rejection, it seems unlikely that IL-17 neutralization alone could have an impact on MHC-mismatched allograft survival. However, it is not excluded that a "Th17" pathway of rejection could emerge when the immune system or the alloreactive repertoire is modified by some therapeutics.

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Part III
Role of IL-23-1 L-17 in Infection and Tumor Control

Is IL-17 Required to Control Tuberculosis?

Andrea M. Cooper

Abstract Herein we review the state of knowledge regarding the role of IL-17 in tuberculosis (TB). IL-17 is induced following exposure to mycobacteria in mice and humans, yet its role in protection and the immunopathologic consequences of infection is not defined. It appears that the IL-17 response to mycobacterial infection is dependent on IL-23 and that the induction of this cytokine is dependent upon pathogen-associated molecules on the surface of the mycobacterium. While there is evidence for a protective role, there is also evidence for a pathologic role; it is therefore critical to decipher the impact of IL-17 so that preventive and therapeutic measures can be appropriately tailored.

Keywords Tuberculosis • Inflammation • T cell activation • Th17 • Th1 • T cells • Human • Mouse • Immunity • IL-17 • IL-23 • IFN-γ • IL-12

1 Introduction

There are two main reasons for studying IL-17 and tuberculosis. The first is to determine whether IL-17 is important in disease. The second is that the dynamic interaction between host and this unique pathogen may allow us to identify as yet unknown functions for this cytokine. Therefore, when considering the role of IL-17 in tuberculosis, the ability of this cytokine to mediate and regulate antibacterial activity as well as its ability to impact the inflammatory response should be determined. In addition, the bacterial and host factors that mediate induction and regulation of this cytokine should be addressed.

Tuberculosis is a disease resulting from the interaction of a persistent yet inflammatory intracellular bacterium and a responsive but regulated immune

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response [1–3]. While cellular immunity is essential to control bacterial growth, the concurrent development of inflammation leads to tissue damage that then promotes dissemination. 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 [3]. From both animal 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 critical to controlling disease [1–3]. CD8 T cells have the ability to protect the host from disease and have unique antibacterial activity, but the absence of these cells can be overcome by the presence of CD4 T cells [1–3]. There is active regulation of the acquired immune response, and it is likely that this serves to limit not only antibacterial activity but also tissue damage during chronic disease [4, 5]. Taking into account the known functions of IL-17, this cytokine could impact the innate, acquired, and inflammatory response during TB.

2 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) [6]. 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 [7]. In mice, IL-17 production occurs rapidly upon a high-dose intratracheal challenge with *Mycobacterium bovis* BCG (BCG), and this response is dependent upon the γδ T cell population [8]. This population is also a major producer of IL-17 in response to Mtb infection in the lung [9]. 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 [8] (see 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 inflammatory response is altered [6]. It is tempting to speculate that the disrupted granulomatous response previously reported for Mtb-infected γδ T cell-deficient mice [10] 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 demonstration that unrestrained IL-17-producing γδ T cells can lead to tissue damage and death in a fungal model of infection [11]. Indeed, regulation of these IL-17-producing γδ T cells is superoxide dependent [11], and in the absence of superoxide, there is an increase in neutrophil accumulation in the TB granuloma in mice [12] suggesting that the ability of IL-17-producing γδ T cells to recruit neutrophils may also be regulated by superoxide. There are other innate sources of IL-17 in the lung such as invariant NK T cells [13], but the role of this response in TB has not been addressed.

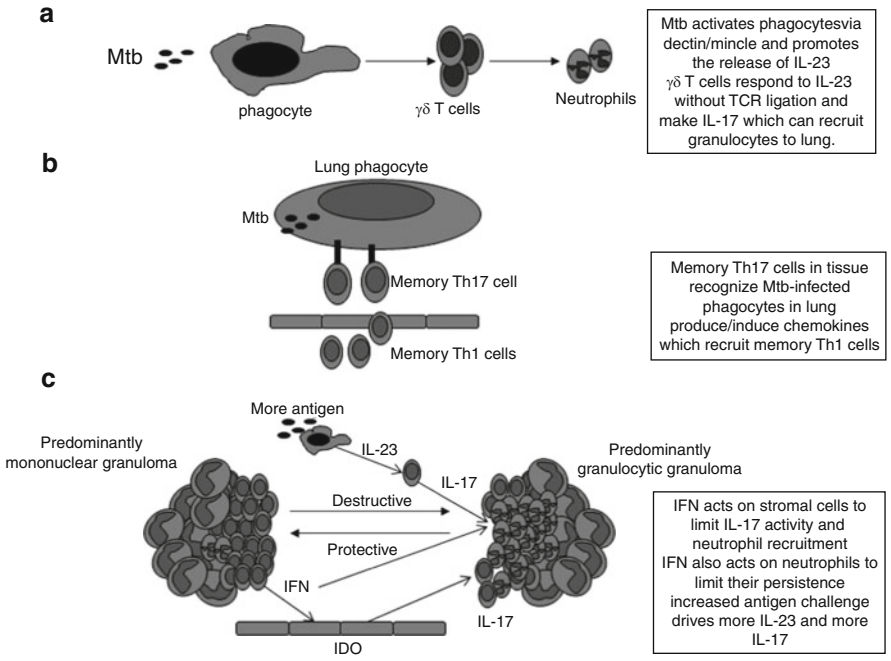

Fig. 1 *Functions of IL-17 during Mtb infection.* (**a**) Upon mycobacterial infection in the lung, phagocytes respond to the bacteria, generate IL-23, and promote γδ T cells to make IL-17 and thereby mediate recruitment of neutrophils. Absence of this response limits early neutrophil recruitment and 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 altered in the absence of IL-23 and IL-17; IFN and IL-17 appear to counterregulate the development of a mononuclear or granulocytic inflammation in response to Mtb

3 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 [14, 15]. In TB it appears that IL-17 is present both during the innate and acquired response. This has been shown in mice [6, 9, 16] and more recently in humans [7]. 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 [6, 16]. Interestingly, despite the reports of IFN-γ and IL-17 responses counterregulating each other and the fact that the IL-17 response is regulated by IFN-γ following BCG infection [17], the antigen-specific IL-17 response is maintained in the lung during Mtb infection despite the presence of a

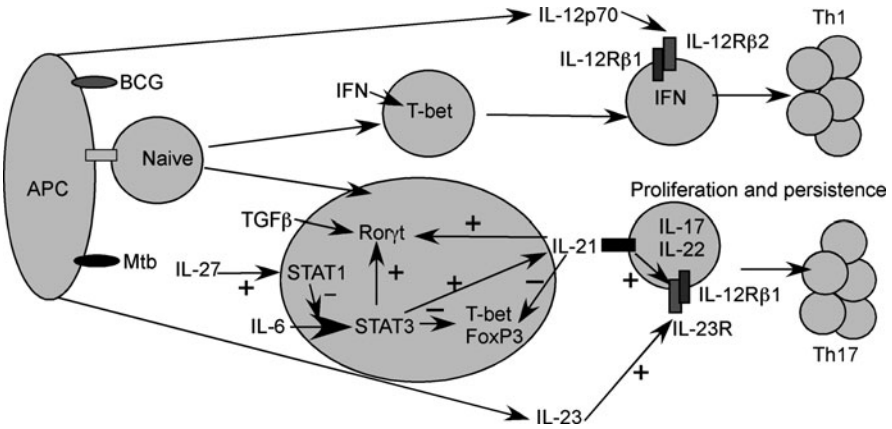

Fig. 2 *Polarization of Th17 cells during mycobacterial infection*. TGFβ drives induction of the transcription factor RORγt, if no inflammation is present, cells will tend toward a regulatory phenotype. If mycobacteria are present, however, IL-6 will induce IL-21 which in turn promotes more RORγt and 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. In some cases memory T cells and γδT can become IL-17 producers without TCR ligation in the presence of IL-1β and IL-23

strong IFN-γ response [6]. The accepted model for induction of IL-17-producing T cells (Th17) has a requirement for TGFβ to initiate the polarization process in both mice and humans [15, 18] and that inflammatory cytokines, such as IL-6, act to direct the cells to a Th17 rather than regulatory, FoxP3-expressing phenotype (Treg) [19]. IL-23 is thought then to promote the release of IL-17 by polarized cells [20] (see Fig. 2). IFN-γ limits the amount of IL-23 made by dendritic cells infected with mycobacteria [17], and this may be one way IFN-γ-producing T cells (Th1) regulate the Th17 response. In mycobacterial models, however, IL-1β in conjunction with IL-23 can drive IL-17 from γδ T cells (and $CD4^+$ cells) without the need for TCR engagement [21, 22]. Interestingly, IFN inhibits IL-1β production by mouse cells but enhances it for human cells [22], and thus, the impact of IFN on IL-17 regulation in mice and humans may differ.

It is likely that the nature of phagocyte activation and the local cytokine environment will determine the polarization of T cells both during priming and expression of effector function. These conditions will be defined to some degree by the impact of mycobacterial molecules on phagocyte pattern recognition receptors.

4 When and Where Is IL-17 Expressed During Human Disease?

Following the original demonstration of antigen-specific IL-17-producing $CD4^+$ T cells in PPD-positive individuals [7], many further studies have examined IL-17 production in both active and latent human tuberculosis. In the original publication, IL-17-, IFN-, and IL-22-producing cells were detected in the peripheral blood of patients with active TB; however, only IFN- and IL-22-producing cells were present in the bronchoalveolar lavage; it was suggested that IFN-γ limits the IL-17 response in the lungs [7]. In several recent studies, patients with active TB have been shown to have a stronger IFN response to mycobacterial antigens than do those with latent disease; however, the IL-17 response is less clearly associated with disease state [23, 24]. There is however a tendency for the IL-17 response to antigen to be higher in latent than active TB patients [23, 25] possibly due to the higher IFN response in the active cases inhibiting IL-17. While one study was able to correlate decreased IL-17 levels in serum during treatment with increased risk of death [26], a second study could not correlate IL-17 in plasma during treatment to disease state or response to treatment [27]. In the mouse model, a drop in IL-17 was found to occur during effective treatment [28].

The potential for patients to generate an IL-17 response following exposure or during disease seems to be associated with geographical location and/or the nature of the disease. In one study of a Swiss population, IL-17 was not detected strongly in response to purified protein derivative (PPD) of Mtb; cells that did respond were $CD45RA^-$, $CD25^-$, $CCR6^+$, and $CXCR3^+$ and produced mainly IFN-γ [29]. In contrast, in a South African population, the BCG-reactive cells were $CD45RA^-$ cells and those making IL-17 or IL-22 were predominantly $CCR7^+$ and $CD27^{+/-}$, whereas those making IFN-γ were predominantly $CCR7^-$ and $CD27^-$ [7]. In Chinese studies of cells from pleural effusions, IL-17 was produced by $CCR7^+$, $CD27^+$, CD4 T cells but only after restimulation [30, 31]. Again in contrast, a South African study of both pleural and pericardial effusions found very low to negligible IL-17 in these effusions although high levels of IL-22 and IFN were detected; a low level of $CD4^+$ cells capable of making IL-17 was also detected, but these did not appear to be specific for mycobacterial antigen [32].

5 Can IL-17 Mediate Protection?

IL-17 acts to regulate granulocyte homeostasis [33], chemokine expression and cell recruitment [34, 35] and to orchestrate germinal center formation [36]. IL-17 is required for protection against *Klebsiella pneumoniae* [34], *Citrobacter rodentium* [37], *Candida albicans* [38], and *Mycoplasma pulmonis* [39]. IL-17 has also been extensively implicated as a mediator of autoimmune-mediated disease (reviewed in [20]). These myriad functions make it difficult to immediately identify where within the complex interaction between host and bacteria IL-17 is likely to act.

In revising this chapter for 2012, I had thought to be able to more definitively answer the question raised in the title; however, the primary role of IL-17 in protection against Mtb remains unclear. Following low-dose aerosol delivery of Mtb to IL-23-deficient mice, there is no difference in the level of bacterial burden within the lung for the first 90 days of infection, this despite the fact that there is very little IL-17 mRNA or Th17 response detected [6]. Similarly in a BCG model, absence of IL-23 or IL-17 does not alter the bacterial burden [40]. 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 [6, 40]. 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 [41] suggesting that the natural IL-23/IL-17 response may be improved upon. In later studies we have shown that, although early control is not affected by the absence of IL-23, later control of bacterial growth following a low-dose challenge is lost. Importantly, this loss of protection does not appear to be related to the loss of IL-17, as bacterial growth is identical between wild-type and IL-17RA-deficient mice in this model over a prolonged period [42, 43]. In stark contrast to the data from low-dose aerosol delivery of Mtb, others have shown increased susceptibility to Mtb infection in mice lacking IL-17A when a moderately higher dose is delivered intratracheally [44]. The difference in bacterial growth between the wild-type and IL-17A-deficient mice following intratracheal infection occurs within the first 4 weeks and is associated with a defective inflammatory response. This differential requirement for IL-17 between the aerosol and intratracheal model is intriguing. It may be that the intratracheal model is more inflammatory and that therefore the cells recruited to the lung are different and more dependent upon IL-17 for effective function. In contrast, the aerosol model is known to induce very little inflammation early, and there may therefore be less of a need for early IL-17. The relevance of either model to natural human disease is a matter for discussion, but it is clear that defining why the two models differ so dramatically will be informative in the dissection of the function of IL-17 in lung disease.

One aspect of the role of IL-17 in protection may be related to its impact on dissemination of bacteria from the site of infection. Recent work showed an increase in IL-17 in the IL-10-deficient mouse, and while depletion of IL-17 had no effect on bacterial growth in the lung, it appeared that dissemination of bacteria to the spleen was increased in the absence of IL-17 [45]. Further, in a study of the dissemination of BCG from the skin to the draining lymph node, it was shown that absence of migratory IL-17-producing γδ T cells restricted the accumulation of BCG in the lymph node [46]. It is possible therefore that IL-17 plays a role in defining the cells which mediate dissemination of the bacteria. As the induction of the acquired response depends upon dissemination of bacteria from the lung to the draining lymph node [3], the kinetics of the acquired response may be delayed in the absence of IL-17. While this may not be critical in the low-dose aerosol model, it may be more so in the higher dose intratracheal model.

In a couple of recent reductionist studies, the ability of T cells making IL-17 to mediate protection in mice infected with Mtb was assessed. One group transferred

IFN-deficient, antigen-specific, IL-17-polarized T cells into immune-deficient mice and found that these cells could protect more efficiently than unpolarized, IFN-deficient, antigen-specific cells [47]. In a second study, the ability of cells to protect was dependent upon the cells retaining plasticity and thereby maintaining a mixed rather than fully polarized IL-17 phenotype [48]. These data suggest that while IFN is not essential for protection in these transfer models, IL-17 alone is not sufficient to mediate protection. Further studies are clearly warranted.

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 [49, 50], and that IL-23 drives efficient IL-17 responses in human T cells [51]. In view of the mouse 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 [52, 53] provides impetus and potentially useful subjects for analysis. As discussed above, there have been several studies on the expression of IL-17 in healthy-exposed, infected, and diseased subjects, and while there is some evidence that circulating IL-17 is seen in the healthier exposed individuals, there is no strong current evidence linking IL-17 to either protection or disease development.

6 How Does IL-17 Impact Vaccine-Induced Protection?

Despite the absence of a defined role in controlling bacterial burden, the absence of IL-23, and thereby IL-17, does result in reduced protection in a model of immunological memory in mice (see Fig. 1b). In this model, the accelerated memory Th1 response induced by a subunit vaccine is lost in the absence of IL-23, and this is associated with the absence of an IL-17-producing $CD4^+$ memory population in the lungs of vaccinated IL-23-deficient mice [16]. The expression of IL-17 mRNA in vaccinated lungs correlates 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 fails to occur, and thus, vaccine-induced protection is lost [16]. 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 noninflamed tissue and provide a surveillance function [16]. Further, in the absence of CCR4 using a BCG model, it was found that both IFN and IL-17-producing cells failed to be maintained and that recall granulomatous responses were defective [54].

When IL-23 is added to a DNA vaccine regimen, it is capable of increasing both IFN-γ and IL-17 responses and protection to a degree equivalent to IL-12 [55, 56]. In view of the downregulatory activity of IL-27 with regard to IL-17 cellular responses [57, 58], it is not surprising that this cytokine fails to improve

vaccine-induced protection [55]. In contrast to these studies, BCG vaccination did not require IL-23 to be protective against a subsequent BCG challenge [40]; 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 [17, 56]. A recent study of the mechanism of IL-17 induction during BCG vaccination has shown that BCG induces prostaglandin E2 and that this drives IL-10 which in turn limits the IFN response to BCG [59]. At the same time, however, BCG also induces IL-23 which promotes IL-17 production which in turn limits the IL-10 thereby promoting an overall IFN response; in the absence of IL-10, there is no need for IL-17 to promote the IFN response [59]. Thus, expression of IL-17 during BCG vaccination may promote the IFN response. Studies with genetically manipulated BCG have shown increased protection, and this is associated with an increased IL-17 response to the genetically manipulated BCG compared to the wild-type BCG [60].

Human and animal vaccine studies have increasingly measured IL-17 responses after vaccination. In the bovine model, a viral boost of BCG vaccination showed increased efficacy, and this was associated with an increased antigen-specific IL-17 response prior to challenge [61]. Boosting BCG-vaccinated people with virus-delivered antigen results in multifunctional antigen-specific cells with a preference for IL-17 in adolescents compared to children [62]. A higher dose of the virally delivered antigen promotes more IL-17-producing cells; however, previous exposure may limit the IL-17 response [63]. Detailed analysis of the cells responding to the virally delivered antigen suggests that the IL-17 response may be limited by regulatory T cells that act to limit levels of the inflammatory extracellular ATP [64].

Extensive studies using the whole-blood assay have measured the level of IL-17 to mycobacterial antigens in vaccinated individuals. Vaccination does result in an increased IL-17 production in infants [65], and while age at vaccination could impact size of the IL-17 response, this response evened out as infants aged [66]. The efficacy of BCG differs geographically, and so the whole-blood assay was used to compare responses in UK and Malawian infants receiving BCG. It was found that while the Malawian children had a greater general inflammatory response including IL-17, the UK infants had stronger type 1 responses; it was suggested that these different biosignatures may explain why BCG is more effective in the UK [67]. However, in an extensive study on the potential for a biosignature to predict protective efficacy of BCG vaccination, no difference in the cytokine profile (IL-2, IFN, TNF, or IL-17) or cell types was associated with the development of tuberculosis in vaccinated individuals [68]. It would seem therefore that the role of IL-17 in vaccine studies in humans is still undefined.

7 How Could IL-17 Impact Inflammation?

Dissecting the role of such a pluripotent entity as IL-17 in a chronic disease like TB is difficult. A major confounding factor is that the granulomatous structure, even within the tractable mouse model, is a constantly developing entity [69]. 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 [70]; however, in the absence of IL-23 and IL-17, there is a modest decrease in the inflammatory response early in infection [6] (see 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 wherein IL-23 and IL-17 downregulate the protective Th1 response and increase the pyogranulomatous nature of the inflammation [71]. Further, IL-23 and IL-17 improve survival and reduce the killing activity of neutrophils in this fungal model [71]. The potential for neutrophils to impact granuloma formation during Mtb infection [72] and the causal connection between early and enhanced neutrophil recruitment with susceptibility to TB [73] prompted us to examine the role of IL-17 and IL-23 in the Koch phenomenon. We found that repeated delivery of mycobacterial antigen drove an IL-23-dependent IL-17-response in the lungs of Mtb-infected mice that did not impact bacterial control but did increase the pyogranulomatous nature of the lesions [74] (Fig. 1c). In two other recent studies, the impact of IFN in regulating the inflammatory action of IL-17 has been highlighted. In the first study, the absence of the IFNγR on nonhematopoietic cells was found to limit the anti-inflammatory activity of these cells, and this resulted in enhanced IL-17 responses which compromised immunity [75]. In the second study, the absence of IFN in memory cells did not impact their ability to control bacteria but did limit their ability to limit neutrophil recruitment and thereby tissue damage [76] (Fig. 1c). These data demonstrate that IL-17 is associated with the potential for a more damaging inflammatory response to Mtb infection. In this regard, an intriguing paper from Argentina shows that patients with multidrug-resistant tuberculosis and high bacterial burdens had higher levels of IL-17 [77]. This is in contrast to the lack of apparent IL-17 in pleural and pericardial tubercular effusions (see discussion above).

An important aspect of the Mtb granuloma that is often dismissed is the accumulation of B cells in secondary lymphoid structures in both mice and humans [78–80]. Indeed, an altered granulomatous response [81] and reduced protection can be seen following Mtb infection of B cell-deficient mice [82]. The ability of IL-17 to mediate germinal centers [36] may impact the B cell response, as recent studies have identified IL-17 mRNA associated with the B cell follicles in human tuberculosis lesions in the lung [83]. In our recent studies, we have found that the absence of IL-17RA does not greatly inhibit the development of B cell follicles but that IL-23 and CXCL13 seem to be critical for these structures and for T cell recruitment to the granulomatous site [42, 84].

8 Induction and Regulation of the IL-17 Response

IL-23 is a key mediator of IL-17 secretion in memory T cells [85] and γδ T cells [9], and in its absence there is very little IL-17 produced in response to Mtb infection [6]. Mycobacteria can induce IL-23 in dendritic cells [9, 17], and this induction can be regulated by IFN-γ, at least for BCG [17]. 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 [86]. During mycobacterial infection, dectin-1 has been implicated as a mediator of macrophage [87] and dendritic cell [88] activation with a specific role for dectin-1 in induction of IL-12p40, a subunit of IL-23. Recently, dectin-1 ligation by β-glucan or Mtb on monocyte-derived dendritic cells was reported to result in IFN and IL-17 production in a mixed lymphocyte reaction, whereas ligation of DC-SIGN or the mannose receptor resulted in an IFN response [89, 90]. The role of dectin-1 in stimulating the phagocytic response to fungal bodies is dependent on developmentally regulated display of β-glucan moieties [91, 92]. It is possible therefore that the expression of β-glucan moieties on different mycobacterial species will regulate the amount of IL-23 and therefore the amount of IL-17 they induce. One could hypothesize therefore 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 to overcome Th1 regulation [6, 17]. The importance of the early cytokine milieu 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 [93].

Although it is technically difficult to assess the very earliest events during low-dose aerosol infection directly in vivo, the response of CD4 T cells and dendritic cells to vaccination with trehalose dimycolate (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 [94]. In vaccine studies it has been shown that delivery of an IA^b-restricted peptide in the presence of TDM and the detoxified monophosphoryl lipid A (MPL) results in early expression of IL-6, TGFβ, and IL-23 in dendritic cells and IL-17 but not IFN-γ in the CD4 T cells [16]. This early IL-17 corresponds with early expression of the IL-23 receptor but not the IL-12 receptor in the CD4 cells [16]. These data suggest that Th17 cells, while not requiring IL-23 to become polarized [18], are able to respond to IL-23 very early during activation. Indeed in the absence of IL-23, there is a small but reproducible reduction in very early proliferation of CD4 T cells [16]. 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 TGFβ [19] as well as that between IL-1β and IFN during activation of naïve T cells will define the relative induction of a Th17 or Treg population. The importance of this balance is highlighted by the observed ability of Treg cells to limit bacterial control [4, 95, 96]. The receptor for TDM was recently identified as the macrophage-inducible C-type lectin (Mincle) [97, 98], and this

molecule acts through the FcRγ and Syk-CARD9 signaling pathway to promote inflammation as well as both IFN and IL-17 responses to mycobacteria [98]. In human studies, antigen-experienced cells make IL-17 in response to Mtb-activated antigen-presenting cells in an IL-23-dependent manner; it appears also that the production of IL-23 can be induced by NK cells recognizing the infected antigen-presenting cells [99].

9 Conclusions

This chapter does not answer the question raised in the title, as the animal model evidence suggests both positive and negative impacts in the TB disease process and the human data is still equivocal. We know however that this cytokine is induced in and expressed by 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 counterregulation of IL-1β, IL-12, and IL-23 at the dendritic cell level, and this may be related to the level of cytokine-inducing moieties (such as β-glucans) on the mycobacterial surface. There is conflicting evidence on the role of IL-17 in control of bacterial growth during mycobacterial infection, and this conflict may be resolved with careful analysis of the role of dose and concurrent inflammation during initial infection. There are several areas that should be investigated in order 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, NK and NKT 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 antimicrobial protein expression in epithelium. Although IL-17RA has a limited role in controlling the primary response to intracellular pathogens such as *Listeria monocytogenes* or *Mycobacterium tuberculosis*, other intracellular bacteria such as *Francisella tularensis* require IL-17 to mediate Th1 immunity. Furthermore, 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.

1 Introduction

The critical role of CD4+ T cells in host defense against variety of pathogens became clearly evident by the acquired immunodeficiency syndrome (AIDS) epidemic. It has been demonstrated that the risk of AIDS-defining illnesses such as those caused by *Mycobacterium tuberculosis*, *Pneumocystis*, and *Toxoplasma gondii* shows clear

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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 interferon-gamma 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 cytokine interferon-gamma results in susceptibility to *Mycobacterium tuberculosis* infection and an increase in susceptibility to *Toxoplasma* infection [3–7]. Alternatively, defective Th2 response predisposes to infection with helminths [8, 9]. In addition to these specific susceptibilities in CD4-deficient hosts, data in humans also suggested that Th1 and Th2 immune responses could be associated with specific tissue pathologies in the setting of infection. For example, a strong Th1 response was associated with the tuberculoid form of leprosy, an infection caused by *Mycobacterium leprae*, whereas a Th2 response was associated with the less controlled variant, lepromatous leprosy [10].

Although the Th1 and Th2 paradigm explained much of the risk of opportunistic infections (OIs) associated with HIV infection, not all AIDS-associated infections fell into intracellular or helminthic pathogens. Specifically, it remained unclear the mechanism by which CD4 deficiency predisposed patients to extracellular pathogens such as bacterial pneumonia. This particularly became 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 [1, 11]. 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 [1, 11]. 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 necessarily explain susceptibility to primary infection.

IL-17 was cloned in 1993, and its expression was largely restricted to CD4+ memory T cells [12]. IL-17 receptor was cloned in 1995 [13] and is a type I transmembrane protein. The initial biological activity of IL-17 was that it supported the differentiation of granulocyte progenitors from CD4+ T cells by stimulating G-CSF and IL-3 from bone marrow stromal cells [14]. Thus, we hypothesized that IL-17 and IL-17R signaling may regulate granulopoiesis in vivo and this differentiation and recruitment of neutrophils by IL-17-producing T cells that may in part explain the susceptibility of CD4+ T-cell-deficient host to extracellular pathogens.

2 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 [15]. The expansion of neutrophil progenitors by IL-17 is mediated

by the induction of granulocyte colony-stimulating factor and the transmembrane form of stem cell factor (SCF) [15, 16]. 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 [17]. 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 WT mice [18]. Wild-type mice show a nearly 100 % increase in CFU-GM 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 [18], demonstrating that IL-17RA signaling is critical for this response. This defect is likely due to defective production of G-CSF, IL-6, and scf [16, 18]. It has been well known that mice and humans with leukocyte adhesion defects such as CD18 deficiency show elevated levels of G-CSF and circulating neutrophils. Ley and colleagues have shown that this expansion of neutrophils is regulated by IL-17 and G-CSF [19, 20]. This G-CSF response is ultimately regulated by gut-derived IL-23 [21] which regulates IL-17 production by T cells. Stark et al. have proposed a model by which the normal uptake of apoptotic neutrophils in the lamina propria of the gut antagonizes IL-23p19 expression and thereby negatively regulating IL-17 production by gut T cells, thereby allowing precise physiological regulation of circulating neutrophil numbers [21]. 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 [20].

In addition to regulation of granulopoiesis, IL-17RA shows reduced PMN emigration in to the lung after bacterial challenge with *K. pneumoniae* which is associated with reduced levels of CXCL2 [18]. 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 [22–24]. IL-17A stimulation of human bronchial epithelial and venous endothelial cells and human synoviocytes results in the production and release of CXCL8 (IL-8) [24–26]. IL-17A also induces CXCL6 in human bronchial epithelial cells [24] and CXCL5 in murine in osteoblasts in vitro [27]. 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 [24]. Induction of CXCL8 by IL-17A in human synoviocytes in vitro requires NF-kB and the PI-3 kinase/Akt pathway [28]. 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 [29, 30]. These CXC chemokine responses to both IL-17A and IL-17F have shown marked synergy with TNF-alpha [24]. 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 [31]. Other potential transcription factors responsible for

IL-17-induced CXCL1 gene expression include CEBPδ which has been shown to mediate some of the synergy of gene expression observed with IL-17A and TNF-alpha dual stimulation [32].

In addition to defective recruitment of neutrophils in response to Gram-negative bacterial infection [18], 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 [24, 33]. This neutrophil emigration in the context of the lung is blocked by a neutralizing and specific anti-IL-17 antibody or pretreatment with glucocorticoids [25]. This effect is also observed with IL-17C, IL-17E, and IL-17F; however, IL-17E also results in significant emigration of eosinophils [34] 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 [35].

It has been shown that IL-17A and IL-17F are produced by a specific subset of CD4+ T cells, Th17 cells, [36–39] in addition to γδ T cells and NKT cells. This subset of T cells also produces IL-22 [40] and, in humans, both IL-22 and IL-26 [41]. Although, IL-17RA KO mice are not more susceptible to *Listeria monocytogenes* or Mycobacterium tuberculosis infection [42], IL-17 is required for immunity against other intracellular bacteria such as *Francisella tularensis* [43] and *Chlamydia muridarum* [44]. Moreover, although Th1 immunity as assessed by IFNγ recall responses to *L. monocytogenes* is not impaired in IL-17RA KO mice, IL-17 is required to drive Th1 immunity in *Francisella* and Chlamydial infections [43, 44]. Thus, it appears the lack of IL-17R signaling results in selective defects to most extracellular bacteria [18, 42] and some intracellular bacteria. In support of this, mice deficient in IL-23, a key survival factor for Th17 cells [45–47], 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 [47]. Interestingly, although IL-23 plays a limited role in primary early *M. tuberculosis* infection [48], it is required for long-term protection against *M. tuberculosis* [49]. Further evidence supporting a role of IL-17 in host defense against extracellular pathogens is data from Chung et al. that demonstrates that intra-abdominal abscess formation due to the anaerobic bacterium *Bacteroides fragilis* requires IL-17A [50]. 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 natural-occurring pathogen in mice [51]. Interestingly, in this model, IL-17A was produced in both IL-23p19 deficient and wild type, suggesting that the susceptibility of IL-23p19 KO mice is independent of IL-17A [51]. In addition to extracellular bacteria, Huang et al. have shown that IL-17RA signaling is also required for optimal host defense against systemic *Candida albicans* infection [52] and antibodies against IL-23p19 antagonize pulmonary IL-17 production, neutrophil emigration, and host defense against *Mycoplasma pulmonis* [53]. Conti et al. have shown that IL-17RA signaling is critical for oropharyngeal *C. albicans* infection [54]. Moreover, Puel et al. have described a patient with chronic mucocutaneous candidiasis due to a missense mutation in the

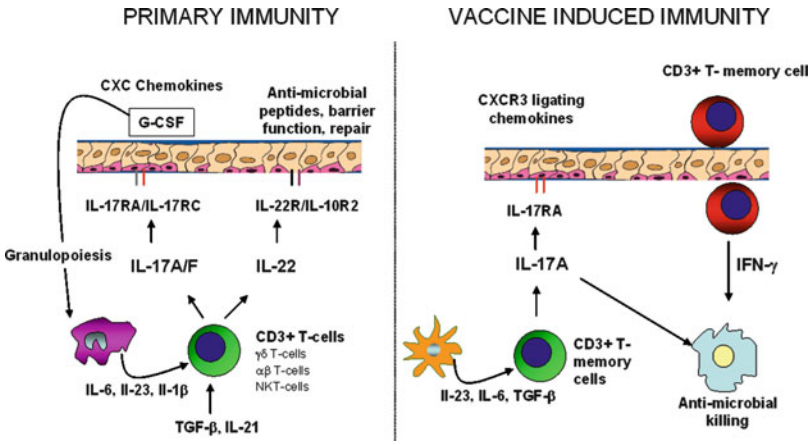

Fig. 1 Model of IL-17-producing cells in primary and vaccine-induced immunity. In primary immunity, IL-17 and Il-22 can be synthesized by several T-cell subsets. A likely critical target of IL-17 and Il22 is mucosal epithelium where IL-17 augments G-CSF and CXC chemokine production, resulting in recruitment of neutrophils. IL-22 along with Il-17 also augments antimicrobial peptides important for control of extracellular pathogens. In vaccine-induced immunity, there is an expansion of memory T cells which can also upregulate CXCR3 ligands through an IL-17-dependent mechanism, resulting in augmented recruitment of Th1 effectors leading to control of both extracellular and intracellular pathogens

extracellular domain of the IL-17R which prevents signaling in a patient with a chronic mucocutaneous candidiasis [55].

In addition to regulating CXCR1/2 ligands and G-CSF, IL-17 regulates antimicrobial peptide production of beta defensins [56] 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 antimicrobial proteins in human keratinocytes which lends further support of the T-cell lineage in mucosal immunity (Fig. 1) [40]. Due to the fact that there is redundancy in the beta defensin and calgranulins gene family, it will require further experimentation to determine the individual contributions of these proteins in Th-17-mediated mucosal immunity.

3 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 are becoming much more clear with contributions of TGF-β, IL-6, and IL-23 [38, 39, 51], the regulation of IL-17 by T cells in vivo is much more complex. In response to stimulation by lipopeptides from *Borrelia burgdorferi*, IL-17A localizes to a subset of T lymphocytes expressing tumor necrosis factor TNF-alpha and granulocyte-macrophage colony-stimulating factor (GM-CSF) [57]. Moreover, CD8+ T cells have been shown to produce IL-17 in response to *K. pneumoniae* in vitro [58], and depletion of CD8+ cells partially abrogates lung IL-17 responses in vivo to the same infection [58]. 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 [26, 58]. Recently, Michel et al. have demonstrated that a critical source of IL-17 in the airway is a novel NK1.1 negative, α-gal-ser-positive invariant NKT-cell population, and this population is critical for airway neutrophilia in response to endotoxin [59]. Lockhart et al. have shown that γδ T cells are the predominant source of IL-17 in *M. tuberculosis* infection in mice [60]. In response to the extracellular pathogen *E. coli*, Shibata et al. have shown that the Vγ1 subset of γδ T cells is critical for IL-17 production in an intraperitoneal model of infection [61]. γδ T-cell IL-17 is tightly regulated by both IL-23 and IL-1β [62, 63], and these cells can also be a source of IL-21 which can shape the subsequent Th17 response [63]. These data suggest that innate as well as adaptive immune cells are potent producers of IL-17 [64], projecting IL-17 to be a link between innate and adaptive immunity.

4 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 [65] 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 IFNγ and IL-17 production [66–68]. Following challenge with *M. tuberculosis*, protection is clearly associated with the recruitment of IFNγ-producing cells to the lung, resulting in activation of macrophages and mycobacterial killing [69]. 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 IFNγ CD4 T cells to the lung by upregulating CXCR3-ligating chemokines (CXCL9, CXCL10, and CXCL-11) [66]. The generation of the IL-17 vaccine-induced response is dependent on IL-23, since the vaccine-induced protection is lost in IL-23KO mice [66]. 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 [70]. 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 [70]. The only available vaccine against tuberculosis *M. bovis* BCG is not very effective at protecting against pulmonary tuberculosis [71] and has prompted the search for more effective vaccines against tuberculosis. This information about 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 [72]. IL-17-producing cells are induced by vaccination with whole cell pertussis vaccines (Pw), 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 [72]. IL-17A was shown to be critical in a CD4 T-cell-dependent, antibody-independent model of vaccine-induced protection following *S. pneumoniae* challenge [73]. 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. In a follow-up study, Chen et al. showed that vaccine-induced Th17 cells could recognize outer membrane proteins in the Gram-negative bacteria *K. pneumoniae*. These cells could mediate serotype-independent immunity, and thus, Th17 cells may be attractive targets to achieve boarder immunity than antibody approaches [74].

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 IFNγ and IL-17 in the intestine [75–77]. In this model, it is thought that CD4 T-cell-derived IFNγ is the major protective mechanism [78]. 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.

5 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 compromised responses to some vaccines. However, the risk-benefit of IL-17 neutralization has to be considered against exiting immunosuppression which may carry similar if not greater risk due to the relative lack of specificity of many of these drugs such as steroids and calcineurin inhibitors.

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Role of IL-17 and IL-17 Family Cytokines on Tumor Development

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Abstract Anticancer immune responses restrain tumor progression, but persistent immune activation can induce chronic inflammation that contributes to tumor development. The IL-17 family of cytokines comprises six family members (IL-17A to IL-17F) that all contribute to tissue inflammation. Interleukin-17A (IL-17A), the original member of this family, is also the signature cytokine of the recently identified proinflammatory T helper 17 (Th17) cell subset that has been shown to contribute to autoimmunity. Despite their well-characterized proinflammatory functions in vivo, the role of IL-17 cytokine family members on tumor progression remains highly controversial. In this chapter, we review the latest findings on the functions of IL-17 family members in cancer and discuss the potential of these molecules as therapeutic targets.

Keywords Inflammation • Cancer • Interleukin-17 • Immunomodulation • Anticancer immunity • Dendritic cells • T cells • Innate immunity • Adaptive immunity • Chemotherapy

1 Introduction: Inflammation, Cancer, and IL-17 Cytokine Family Members

Early oncogenesis results from genetic mutations leading to altered protein signaling pathways within cells that trigger their transformation. Cancer cells provide their own growth signals, are insensitive to growth-inhibitory signals, evade cell

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death, have unlimited replicative potential, promote angiogenesis, and metastasize to other tissues, ultimately resulting in tumor development [1]. While cell-intrinsic properties determine tumor cell aggressiveness [2, 3], the tumor microenvironment has also been shown to critically contribute to tumor progression [4–6]. Cancers are indeed composed of multiple cell types, including innate and adaptive immune cells that have a profound impact on tumor progression [7]. However, immune activation during tumor progression can lead to contrasting outcomes. Chronic activation of immune effector cells by tumor cells triggers the release of proinflammatory cytokines, chemokines, and other bioactive mediators, thereby creating an inflammatory milieu that favors tumor progression [8, 9]. In contrast, the immunosurveillance concept initially brought up by Burnet in 1957 and later substantiated by mouse and human findings states the immune system is capable of limiting tumor development [10, 11]. Immune effectors involved in the elimination of tumor cells include gamma delta T cells [12], natural killer (NK) cells [13], dendritic cells (DCs) [14], and natural killer T (NKT) cells [15], as well as members of adaptive immune responses (B and T cells) [16]. The contribution of innate and adaptive immune responses to the success of anticancer therapies has also been studied in mice and humans suffering from cancer [17, 18]. Investigations revealed that the efficacy of radiotherapeutic and chemotherapeutic regimens does not only rely on their direct cytotoxic effects on tumor cells but also on their ability to trigger the release of danger signals from tumor cells that in turn induce antitumor immune responses [17, 19]. These observations suggest paradoxical roles of the immune system during tumor progression and provide impetus to study the series of events set off by inflammatory responses in cancer.

Interleukin-17A (IL-17A), also commonly called IL-17, is produced by a recently discovered CD4+ T cell subset named Th17 cells. In addition to IL-17A, Th17 cells are characterized by their secretion of IL-17F, IL-21, and IL-22 and their expression of the IL-23 receptor (IL-23R) [20]. Th17 development is induced by IL-6 and TGF-β, and the IL-12 family member IL-23 maintains Th17 cell activity [21]. Increased understanding of Th17 cell functions has unraveled important roles for IL-17A in the development of autoimmune diseases as well as in protective mechanisms against bacterial or fungal infections, functions that were previously ascribed to Th1 or Th2 cells [22]. Th17 cells have emerged as key participants in a wide range of autoimmune disorders, including inflammatory bowel disease, psoriasis, multiple sclerosis, and ankylosing spondylitis [23]. Accordingly, elevated amounts of IL-17A and IL-17F are detected in several autoimmune diseases including (MS) [24], rheumatoid arthritis (RA) [25], and psoriasis [26], and the involvement of Th17 cells in tissue inflammation was confirmed in mouse models where IL-17-neutralizing antibodies alleviate disease [27, 28].

IL-17A is the original member of a group of 6 cytokines IL-17A, IL-17B, IL-17C, IL-17D, IL-17E (also called IL-25), and IL-17F that share homology in amino acid sequences with highly conserved cysteine residues essential for their 3-dimensional structure. The IL-17R family comprises five members (IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE) that share sequence homology to the earliest identified member, IL-17RA. Signaling specificity is determined by homo-/

heterodimerization of IL-17 receptors such as homodimers and heterodimers of IL-17A and IL-17F that bind to IL-17RA and IL-17RC heterodimers, while IL-17E signals through the IL-17RA and IL-17RB heterodimers. Reported functions for IL-17A, IL-17B, IL-17C, and IL-17F are the induction of proinflammatory cytokines from fibroblasts and peritoneal exudate cells and the promotion of neutrophil migration. By contrast, IL-17E seems to support Th2 responses, suggesting that IL-17 family members could have distinct functions in pathological situations. In a cancer setting, the functional roles of IL-17 family members are beginning to be understood but remain highly controversial. In this chapter, we review the recent studies that addressed the impact of IL-17 family cytokines on tumor development.

2 A Controversial Role of IL-17A/F in Tumor Progression

2.1 IL-17A/F and Tumor Angiogenesis

The effects of IL-17A on cancer progression have been explored in seminal studies from Tartour et al. [29]. Using cervical cancer cells, the authors showed that neither direct addition of recombinant IL-17A nor IL-17A gene transduction altered the growth of cervical tumors in vitro. However, IL-17-expressing cervical tumors grew faster upon transplantation into nude mice, suggesting that IL-17A has indirect tumor-promoting effects in vivo. The authors further noted that increased expression of IL-17A in vivo was associated with macrophage recruitment at the tumor site. Since macrophages are a cellular source of IL-6, which can act as an in vivo paracrine growth factor, this study indicated that the IL-17A tumor-promoting effects could be partly accounted for by IL-6 [29]. The tumor-promoting effects of IL-17A were later reported in additional tumor models such as MCA205 fibrosarcoma and MC38 colon carcinoma [30]. In addition, IL-17A was identified as a CD4 T cell-derived angiogenic factor that favors neovascularization in vivo [30]. While IL-17A had no direct effect on the in vitro proliferation of vascular endothelial cells, it promoted angiogenesis through stimulation of vascular endothelial cell migration and cord formation. Moreover, IL-17A was shown to increase vascular endothelial growth factor (VEGF), keratinocyte chemoattractant (KC), macrophage inflammatory protein 2 (MIP-2), prostaglandins, and nitric oxide (NO) production by fibroblasts, suggesting that IL-17 functions as a CD4 T cell-derived angiogenic mediator involved in inflammatory angiogenesis. CD4 IL-17-expressing T cells infiltrating into tumor tissues may stimulate tumor cells and stromal fibroblasts to secrete proinflammatory and proangiogenic factors such as VEGF, which inhibits dendritic cell development [31], resulting in enhanced tumor growth. Since neovascularization is required for solid tumor growth, these findings illustrate the contribution of IL-17A in promoting tumor growth through enhanced angiogenesis.

The molecular mechanisms accounting for the cancer-promoting activity of IL-17A have been investigated. In the B16 melanoma and MB49 bladder carcinoma tumor models, IL-17A was shown to support tumor outgrowth not only through direct effects on tumor cells but also by affecting tumor-associated stromal cells, which bear IL-17 receptors [32]. In line with previous work of Tartour et al. [29], IL-17 was shown to induce IL-6 production. Subsequent IL-6 release in the tumor microenvironment triggers the activation of signal transducer and activator of transcription (Stat) 3, which in turn drives the expression of prosurvival and proangiogenic genes. IL-17A was also shown to enhance de novo carcinogenesis in the DMBA/TPA-induced skin carcinogenesis model [33]. IL-17A can therefore support tumor progression by activating the IL-6/Stat3 signaling pathway.

The tumor-promoting functions of IL-17A have also been reported in spontaneous mouse models of cancer. IL-17A was indeed shown to participate in the development of colonic cancer in multiple intestinal neoplasia (Min) mice, a model of familial adenomatous polyposis. Ablation of IL-17A hampered tumor growth in mice bearing a heterozygote mutation in the adenomatous polyposis coli (Apc) gene (Apc(Min/+) mice) [34]. Decreased tumor progression was accompanied by reduced inflammatory cytokine secretion and T cell infiltration. In addition, the absence of IL-17A in Apc(Min/+) CD4 T cells abrogated their capacity to induce colitis in immunodeficient mice upon adoptive transfer. Therefore, in the Min cancer model, CD4 T cell-derived IL-17A promotes spontaneous intestinal tumorigenesis.

As discussed above, Th17 cells, beyond their robust secretion of IL-17A, produce IL-17F. The respective roles of IL-17A and IL-17F in inducing cancer progression have been explored. In an elegant study, Leppkes et al. explored in a mouse model of chronic intestinal inflammation the role of Th17 cells as well as their master transcription factor retinoic acid-related orphan receptor (ROR)γ. In an adoptive transfer model of colitis where wild-type CD4 + CD25- T cells induce colitis in rag1−/− mice, the authors showed the critical involvement of RORγ in the pathogenicity of Th17 cells and the redundant biological effects of IL-17A and IL-17F in vivo [35]. Indeed, while the transfer of IL-17A- or IL-17F-deficient CD4 cells induced colitis, mice that received Rorc-deficient T cells or IL-17F-deficient T cells with a neutralizing anti-IL-17A antibody had diminished colitis [35]. The protumor functions of IL-17F have also been illustrated in intestinal tumorigenesis where ablation of IL-17F impeded intestinal tumorigenesis in the small intestine of Apc(Min/+) mice [36]. Altogether, these studies suggest that to alleviate gut inflammation, the targeting of both IL-17A and IL-17F might be required to achieve a marked therapeutic effect.

The roles of IL-17A and IL-17F in tumor progression in humans remain debated. By studying immune-related genes in 125 frozen colorectal tumor specimens, Tosolini et al. found that patients featuring high expression of the Th17 cluster (containing the Rorc and IL-17a genes) in tumor-infiltrating cells had a poor prognosis, possibly suggesting that IL-17A contributes to cancer progression in human colon cancer [37]. The previous findings are in line with the observations made in hepatocellular carcinoma patients, where increased intratumoral

IL-17-producing cells also correlate with poor survival [38]. However, in ovarian cancer, levels of tumor-infiltrating Th17 cells and the levels of ascites IL-17 are reduced in more advanced diseases and positively predict patient outcome [39]. It is likely that IL-17A and IL-17F may play different roles depending on the tumor type and stage, and further work is thus warranted to obtain a comprehensive understanding of the roles of IL-17A and IL-17F in cancer promotion in humans.

2.2 IL-17A Promotes Anticancer CD8 T Cell Responses

Despite the aforementioned findings suggesting that IL-17A fosters cancer development, observations that adoptive therapy with polarized Th17 cells limits B16F10 melanoma growth suggest that depending on the immunological context, Th17 cells can exert antitumor functions [40, 41]. Martin-Orozco et al. indeed reported an active role of IL-17 in tumor immunosurveillance. They found that interleukin-17A (IL-17A)-deficient mice were more susceptible to developing lung tumors and that adoptive T cell therapy with tumor-specific Th17 cells prevented tumor development. Interestingly, Th17 cells exhibited stronger therapeutic efficacy than Th1 cells. Th17 cells favored dendritic cell recruitment into the tumor tissues and in draining lymph nodes, allowing antitumor CD8+ T cell priming in the draining lymph nodes. The therapeutic effect of Th17 cells fully relied on their ability to activate tumor-specific CD8+ T cells, suggesting that Th17 cells induce protective inflammation that promotes the activation of tumor-specific CD8+ T cells.

The anticancer properties of Th17 cells were further shown using MHC class II-restricted, T cell receptor (TCR) transgenic mice that recognize a novel epitope in tyrosine-related protein 1 (TRP-1), an antigen expressed by normal melanocytes and B16 murine melanoma. In this mouse tumor model that mimics human disease, Th17-polarized cells were highly effective at eliminating large B16F10 melanoma tumors [41]. Surprisingly, however, the therapeutic effect of the adoptively transferred Th17 cells was not only dependent on IL-17A but also on IFN-γ. These observations are attributable to the ability of Th17 cells to self-renew and persist as IL-17A-secreting cells in vivo and to give rise to a Th1-like effector cell progeny [42]. These studies illustrate the discrepant findings regarding the role of IL-17A in immune surveillance and they both suggest that Th17 cells, at least in adoptive transfer settings, can stave off tumor progression. Taking into consideration all these findings will be essential to contemplate the putative use of Th17 cells as a cell therapy strategy for patients suffering from cancer.

2.3 IL-17A Potentiates Anticancer Immune Responses Triggered by Chemotherapy

Cancer treatment mainly relies on surgery, radiotherapy, cytotoxic chemotherapy, and hormonotherapy. Most of these therapies are believed to directly attack and

eradicate tumor cells. This contention was however challenged by observations suggesting that optimal therapeutic effects require the immunoadjuvant effect of tumor cell death induced by cytotoxic anticancer agents [43]. We have subsequently reported that chemotherapy and radiotherapy antitumor effects rely on the immune system of the host. The interaction of the danger signal high-mobility group box 1 protein (HMGB1) released from dying tumor cells with Toll-like receptor 4 (TLR4) on dendritic cells (DCs) was required for the cross presentation of tumor antigens and the promotion of tumor-specific cytotoxic T cell responses and breast cancer patients harboring the loss-of-function Asp299Gly polymorphism of TLR4 relapsed earlier after receiving anthracycline-based chemotherapy [17]. Overall, these findings underscore the need to characterize anticancer immune responses that ensue following cancer treatment [17, 18].

Upon investigating the immune effector cells and molecules that mediate anticancer immune responses in response to chemotherapeutic treatment, the group led by Zitvogel identified a key role of γδ T cells, particularly the Vγ4- and Vγ6-expressing subsets that produce IL-17A, in the antitumor immune response induced by the cytotoxic agents anthracyclines and oxaliplatin [44]. Chemotherapy induced an early infiltration of interleukin (IL)-17-producing γδ (Vγ4(+) and Vγ6(+)) T lymphocytes (γδ T17 cells) that preceded the accumulation of CD8 T cells within the tumor bed. The IL-17A–IL-17RA signaling pathway was required for the priming of IFN-γ-secreting, antigen-specific T cells by tumor cells exposed to chemotherapy and mice in which the IL-17 signaling pathway was disrupted failed to respond to chemotherapeutic treatment. Importantly, in the authors' settings, IL-17A-producing cells that accumulated in tumors were mostly positive for γδ T markers. A contribution of Th17 cells as a source of IL-17A was ruled out by demonstrating that IL-6 and TGF-β, two key regulatory cytokines essential for the differentiation of Th17 cells [45, 46], were dispensable for the efficacy of chemotherapy or vaccination with dying tumor cells. Since the authors further reported that IL-17A did not directly induce IFN-γ production or enhance proliferation of CD8+ T cells, their results suggest that γδ T cells producing IL-17A contribute to the recruitment of antitumor effector CD8 T cells into tumor beds.

The role of IL-17A in staving off tumor growth following chemotherapeutic treatment was further exemplified in mammary adenocarcinoma and fibrosarcoma tumor models where the IL-1β/IL-1R signaling is essential to the therapeutic outcome of doxorubicin treatment. Using IL-17A−/− mice and an anti-IL-17Rα mAb, Mattarollo et al. demonstrated the key role of IL-17A in doxorubicin treatment of transplantable mammary tumors and fibrosarcomas generated de novo by MCA [47]. Interestingly, in contrast to the studies of Wang et al. [32], lack of IL-17A did not affect tumor growth in untreated mice, suggesting that IL-17A exerts contrasting effects depending on the tumor model used. Upon testing the role of lymphocytes in doxorubicin therapy of established AT3 mammary tumors and MCA205 fibrosarcomas, Mattarollo et al. found that doxorubicin efficacy in both AT3 and MCA205 tumors was compromised in mice lacking γδ T cells. γδ T cells that lacked IL-1R lost the capacity to amplify the action of chemotherapy, confirming the causal relationship with IL-1β and IL-17 production from γδ T

cells, resulting in IFN-γ production from tumor-specific CD8 T cells. Since IL-17 production from γδ T cells is dependent on IL-1R signaling and increases CD8 T cell IFN-γ responses [48], these findings are in line with previous reports documenting an essential role of IL-1β and CD8 T cells for the efficacy of chemotherapy [49]. Whether IL-17 is a major downstream response cytokine to IL-1β secretion remains to be demonstrated, but these two studies provide important additional data in mammary tumor transplant and de novo fibrosarcoma models to support a role for IL-17A in doxorubicin mechanism of action.

3 IL-17E Inhibits Tumor Progression

Like IL-17A, IL-17E has potent inflammatory effects in vitro and in vivo. Overexpression of IL-17E in mice skews the immune response toward a Th2 pattern and favors eosinophil expansion through IL-5 production. Benatar et al. have investigated the role of IL-17E in tumor progression. For this, the antitumor efficacy of IL-17E was examined in a variety of human tumor xenograft models, including melanoma, breast, lung, colon, and pancreatic cancers. The authors found that recombinant mouse or human IL-17E administration promoted anticancer responses [50]. Using human tumor xenograft models in mice, they further showed that the combination of IL-17E with chemotherapy or immunotherapy agents has a superior ability to prevent tumor outgrowth as compared to either agent alone. IL-17E induced a marked increase in eosinophils in the blood of tumor-bearing mice and the in vivo anticancer effects of IL-17E correlated with the splenic eosinophil numbers. In addition, B cells were found to be necessary for IL-17E-mediated antitumor activity [50]. It is noteworthy that the same authors demonstrated that the anticancer effects of Virulizin, an anticancer agent that prevents tumor growth in human tumor xenograft models, relies, at least in part, through its ability to induce IL-17E by B cells [51]. Overall, these findings suggest that the immunomodulatory effects of IL-17E contribute to impede tumor development.

While the previously mentioned findings referred to the immunomodulatory activity of IL-17E, the direct effect of IL-17E on cancer cells remained elusive. In an elegant work, Furuta et al. have investigated the effect of IL-17E on mammary cancer cells [52]. They found that nonmalignant mammary epithelial cells (MECs) secreted IL-17E, which triggers apoptotic cell death of breast cancer cells. Importantly, IL-17E did not affect nonmalignant MECs. In mice, IL-25 treatment was effective at inhibiting tumor growth without any significant side effect to the normal tissues. These findings were further substantiated with the differential expression of the IL-25R, which was expressed in high amounts in tumors from patients with poor prognoses but was low in nonmalignant breast tissue. The IL-25/IL-25R pathway triggered caspase-mediated apoptosis in breast cancer cells [52]. These results provide impetus to the design of novel, effective, and safe breast cancer therapeutics. Indeed, unlike IL-2, which has pleotropic effects on the immune response, administration of IL-25 would only induce apoptosis specifically

in cancer cells that express IL-25R. Since IL-25R is expressed by 19 % of invasive ductal carcinomas in the study from Furuta et al. (13/56), these results may have clinical relevance given the breast cancer prevalence. Thus, the selection of patients with IL-25R overexpressing tumors will be essential to evaluate the putative benefit of IL-17E in the clinical management of breast cancer.

4 An Elusive Contribution of IL-17B, IL-17C, and IL-17D in Tumor Progression

While IL-17B has been implicated in inflammatory response and autoimmune diseases such as rheumatoid arthritis, its role in cancer has not been extensively studied. Furuta et al. propose that IL-17B, which binds IL-25R with a lower affinity than that of IL-25, could endow breast cancer cells with a growth advantage [52]. The authors reported that IL-17B was expressed in most breast cancer cell lines that expressed high amounts of IL-25R, whereas IL-17B was absent from nonmalignant MCF10A cells. Consistently, IL-17B was overexpressed in 12 out of 40 breast cancer specimens examined but undetectable in normal tissues. ShRNA-mediated reduction of IL-17B amounts in breast cancer cells impaired their growth and invasive potentials, while recombinant IL-17B protein did the opposite. Altogether, this study indicates that IL-17B may favor the tumorigenicity of breast cancer cells in an autocrine manner. However, Sanders et al. found that recombinant human IL-17B inhibits endothelial cell–matrix adhesion and cellular migration, suggesting that IL-17B might harbor antiangiogenic properties [53].

The functions of IL-17C and IL-17D have not been explored in a cancer setting. However, it is noteworthy that IL-17C was recently shown to bind to IL-17RE, thereby inducing the expression of a nuclear IkappaB family member, IκBζ, in Th17 cells to potentiate the Th17 cell response [54]. Given the importance of Th17 cells in tumor immunosurveillance as previously discussed in this chapter, we can speculate that IL-17C has the potential ability to shape anticancer immune responses through modulating Th17 cell activities.

5 Concluding Remarks

IL-17 family members exert contrasting effects on tumor development. While IL-17E seems to be endowed with anticancer properties through the modulation of cancer cell growth and immune activation, IL-17A and IL-17F favor or repress tumor growth according to the tumor type and immune environment. The other members of the IL-17 cytokine family IL-17B, IL-17C, and IL-17D have not yet been fully characterized, and their role in cancer remains unclear. The reported effects of the IL-17 family members are summarized in Table 1.

Table 1 Proposed pro- and antitumor functions of IL-17 family members during tumor development

Cytokine	Protumor functions	Antitumor functions
IL-17A	Supports angiogenesis [30, 32]	Favors CD8 T cell priming [40]
	Promotes the release of IL-6 [29, 32]	Potentiates the anticancer immune response following chemotherapy [44, 47]
	Drives intratumor Stat3 activation [32, 33]	
	Promotes gut inflammation and carcinogenesis [34, 35]	
IL-17B	Might support cancer cell growth in an autocrine manner [52]	Might harbor antiangiogenic properties [53]
IL-17C	Unknown	Unknown
IL-17D	Unknown	Unknown
IL-17E	None reported	Synergizes with chemotherapy to prevent tumor outgrowth [50]
		Activates anticancer B cells [50, 51]
		Triggers apoptotic cell death of malignant breast cancer cells [52]
IL-17F	Promotes gut inflammation and carcinogenesis [35, 36]	None reported

Clinical studies have shown that anti-IL-17A therapies are effective to treat inflammatory diseases such as psoriasis. Thus, the therapeutic use of anti-IL-17A neutralizing antibodies can be contemplated in cancers where IL-17A promotes cancer growth. This therapeutic strategy would have however to be implemented with caution since IL-17A is crucial for the defense against fungal infections. Indeed, treatments with anti-IL-17A might increase the risk of opportunistic infections. It is noteworthy that the studies examining the role of IL-17 in cancer are mainly restricted to studying the role of IL-17A. The neutralization of other IL-17 cytokine members in vivo will be determinant to assess their functions in anticancer responses in humans. An understanding of the functions of these cytokines will assist the design of more effective treatments for not only cancer but also autoimmune diseases.

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Part IV
IL-17 in Clinical Autoimmune Disease

IL-17 and Th17 Cells in Rheumatoid Arthritis and Other Inflammatory Conditions

Pierre Miossec

Abstract IL-17 was identified in 1995/1996 as a T cell-derived cytokine with effects on inflammation and neutrophil activation. Rheumatoid arthritis (RA) 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. Clinical results are started to be obtained in RA and other inflammatory conditions.

Keywords IL-17 • Th17 • Cytokines • Inflammation • Destruction

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 will review these new findings in light of the previous knowledge [1, 2]. We will focus on rheumatoid

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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.

1 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 [3]. 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 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 (PMNs). 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 [3], 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 higher in RA synovial fluids when compared to osteoarthritis (OA) fluids [4]. 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 [5]. 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.

2 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 to F. IL-17F has a 50 % sequence homology with IL-17A [6]. When used alone on synoviocytes, IL-17F has similar effects as IL-17A but to a lower extend [7]. Sometimes even IL-17F has minimal or even no effect when used alone. However, when combined with TNFα, a synergistic effect is often observed, almost as potent as with IL-17A and TNF combination.

The structure of the IL-17 receptor (IL-17R) has been clarified recently. The first described IL-17R had a rather low affinity, suggesting the presence of additional chains [8]. At least two members are now taken into account [9]. The first receptor is the original IL-R renamed IL-17RA and belongs to a new family of cytokine receptors. The second receptor is IL-17RC. The physical association of the two receptors has been shown to compose two chains of a single receptor complex. 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 small interference RNA studies, with inhibition of the cell surface expression of one or the two receptors. As expected inhibition of the expression of IL-17RA inhibits the response to IL-17A used alone in synoviocytes [7]. However, inhibition of IL-17RC gives the same result. It looks like 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 RA blood, mRNA levels of IL-17RA and RC are increased [7].

Contrasting with the limitation of IL-17 production to T cells and a few other cell types, its receptor complex is widely expressed by many tissue cell types. Following receptor interaction, IL-17 shares transcriptional pathways with IL-1 and TNFα. In particular, the MAP kinase p38 and NFkB are the key transcriptional factors for IL-17 function [10, 11]. These pathways have been identified in synoviocytes, chondrocytes, and osteoblasts.

3 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 [12]. Although we focus here on RA, some of these results apply to the long list of conditions of inflammatory conditions such as ankylosing spondylitis (AS), psoriasis and psoriatic arthritis, and poly- and dermatomyositis.

We will consider the different cell types present in the RA joint and we will summarize the key properties of IL-17 on these targets.

On synoviocytes, IL-1ß, TNFα, and IL-17 induce cytokine and chemokine production, IL-1 being much more potent than IL-17 or TNFα [13, 14]. Using low concentrations of IL-17 and IL-1ß in combination, a synergistic effect is often observed. As an example, such synergy was observed between IL-1, TNFα, 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 [15, 16]. These findings indicate that low levels of cytokines produced by monocytes (IL-1 and TNFα) and T cells (IL-17) can act together on synoviocytes leading to the formation of an immune-mediated infiltrate.

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 and a specific overexpression of the p75 type II TNF receptor [17, 18].

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 upregulation of ELR + CXC chemokines such as IL-8 is observed in IL-17A-treated synoviocytes [7]. In contrast, CC chemokines such as Rantes and MCP-1 are increased with TNF alone but addition of IL-17 leads to an inhibition [7].

On human chondrocytes, IL-17 induces prostaglandin E2 and nitric oxide production by cartilage explants in an IL-1 independent way [19, 20]. 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 [4]. IL-17 and other cytokines stimulating osteoclastogenesis, such as IL-1β and TNFα, increase the expression of RANKL with a decrease of osteoprotegerin expression in osteoblasts/stromal cells [21]. IL-17-producing T cells express the membrane form and secrete the soluble form of RANKL [22]. These functions make IL-17 a new cytokine involved in bone resorption [23]. In the context of RA, T cells in juxta-articular bone are the source of IL-17, which acts then locally leading to destruction [24]. In these conditions, addition of IL-1 or TNF 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 [5, 25]. 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 [25, 26].

4 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 [27, 28]. IFNγ 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 next step was the discovery of IL-23, another monocyte

product, shown to be a key cytokine for the Th17 pathway [29]. As shown previously for the Th1 and Th2 balance, Th1 and Th17 pathways are regulated in opposite directions: IFNγ inhibits IL-17 and IL-17 inhibits IFNγ.

This effect results from changes in cytokine receptor expression. The IL-12 specific IL-12R β2 chain is a marker of Th1 cells, and the IL-23 specific IL-23R specific chain, 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 the IL-12 effects on IFNγ production [30].

In synovium, IL-17-producing cells represent a minor subset of lymphocytes found in lymphocytic infiltrates around vessels [31]. These T cells are there in contact with synoviocytes, monocytes, and dendritic cells. In bone, the same interactions also occur, whereas 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 fragment release in supernatants, demonstrating the direct contribution of IL-17 to destruction [32]. From these results, IL-17 and its producing T cells appear to contribute to the RA inflammatory destructive process. Other cell types such as mast cells are now seen as a major source of IL-17 [33].

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 [34]. In 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 [35]. 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 IFNγ but never with IL-4 [34]. These double positive cells are also isolated from normal blood [36]. However, in situ immunostaining of the RA synovium shows two isolated populations of T cells producing either IFNγ 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. Of interest 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 [31]. As for B cells, the plasma cell morphology of the IFNγ and IL-17-producing cells strongly suggests that this is a fixed pattern related to a final stage of differentiation.

5 The Th17/Regulatory T Cell Balance

A key issue is the interaction between Th17 and regulatory T cells [37]. 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, in order to let the defense mechanisms be activated.

Chronic inflammation activates the Th17 pathway. At the same time, the regulatory T cell pathway is inhibited by the production of proinflammatory cytokines such as IL-6 and IL-1 [38]. 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 thus contributing to chronicity [13]. 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.

6 IL-17 Targeting for Treatment

The demonstration of the role of Th17 cytokines in inflammation has justified 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-17A and IL-17RA. Preclinical studies have demonstrated their efficacy in mouse and human models. As a rule, the combination of IL-17 and TNFα inhibitors is more efficacious in these models [39].

The inhibition of IL-17 was obtained for the first time in the clinic with Secukinumab (AIN-457), an anti-IL-17A mAb developed by Novartis in a phase I trial in patients with psoriasis. A single injection of an anti-IL-17 antibody was able to reduce skin lesions. The first published results in a peer-reviewed journal were those of a phase II trial with LY2439821, a humanized anti-IL-17 antibody developed by Lilly in patients with RA. These results in RA are in line with the results obtained with AIN457 [40]. The second published paper reported on the effects of AIN457 in three conditions RA, psoriasis, and uveitis [41]. Interesting preliminary results have been obtained but not yet published in AS.

The first administration of AMG 827 developed by Amgen to target IL-17RA was performed in patients with psoriasis. The phase II trial has been completed but the results which look promising have not been published. Other conditions are being tested. Presence of IL-17 positive cells and in vitro effect of IL-17 on myoblast could suggest IL-17 targeting in myositis but this has not been tested yet [42].

More recent issues include the need to target other IL-17 family members, such as IL-17F, or IL-17RC or the two IL-17 receptor chains. Other options include the use of small molecules to target transcription factors implicated in the Th17 pathway such as Rorγt/Rorc, Rorα, and Stat3.

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 but

apparently not in RA [43]. 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 could be used as therapeutic tools. IL-4 inhibits the production and functions of IL-17 on RA targets [13, 15]. IL-25 also named IL-17E, a member of the IL-17 family, downregulates Th17 function in autoimmune inflammation [44]. 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 pro-inflammatory cytokines and also their effects on the function of T-reg cells [37]. Since IL-17 can induce tissue cells to produce TNFα, IL-6, and IL-1, these cytokines may not only promote inflammation but also inhibit T-reg functions at the tissue sites. Currently, disease recurs when treatment with inhibitors of TNFα 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 TNFα inhibitors [45]. In active RA, T-reg cells preferentially accumulate in the affected joints but are not able to regulate tissue inflammation. TNFα inhibitors may control disease not only by inhibiting effector TNFα function and reducing inflammation but also by correcting at least in part, the defect in regulatory T cell functions [45]. 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 infections, first 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 inflammation 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 [39]. Accordingly, primary or secondary lack of response to TNF inhibitors may represent a possible indication. Anti-TNF nonresponders 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.

7 Conclusion

The story of IL-17 has started 10 years ago and this is the time it took to become a cytokine in fashion [28]. 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 in place to verify if these concepts are indeed correct.

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Targeting Th17 Cells for Therapy of Multiple Sclerosis

Aaron J. Martin and Stephen D. Miller

Abstract Multiple sclerosis is a demyelinating disease of the central nervous system mediated by autoreactive T lymphocytes. The Th17 lineage of effectors has been implicated in the inflammatory response against CNS autoantigens. Findings in experimental autoimmune encephalomyelitis (EAE, the animal model for multiple sclerosis) suggest that targeting the Th17 response may have a beneficial outcome for patients suffering from MS. Several existing and emerging therapeutic strategies will be 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 specifically required for Th17 development and by the administration of cytokines or other drugs that interfere with differentiation; however, these strategies may also lead to enhanced rates of certain infectious diseases. Prevention of functional Th17 responses can be accomplished by inhibiting leukocyte trafficking or by neutralizing Th17 cytokines (IL-17 and/or GM-CSF). While several promising therapeutic candidates have been identified employing the EAE model, both the risks of immunomodulation and the efficacy of such candidates in human patients need to be completely characterized and carefully considered.

Keywords Multiple sclerosis • Experimental autoimmune encephalomyelitis • Th17 • Autoimmunity

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1 Introduction

Multiple sclerosis (MS) is an inflammatory 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 Treg lineages, depending on the conditions under which they first become activated. It is popularly believed that the Th17 lineage, with further contribution from Th1 cells, is responsible for much of the autoimmune damage to the CNS in MS [3, 28].

Lineages of Th cells are defined by their profile of secreted cytokines, as well as the cytokines, signaling molecules, and transcription factors required for their differentiation. Th lineages typically have exclusive differentiation pathways that proceed at the cost of other lineages. Th1 cells mediate inflammation and are most noted for secreting interferon-γ, while the Th2 lineage directs humoral responses via the secretion of IL-4, IL-5, IL-9, IL-10, and IL-13 [16]. The Th1/Th2 paradigm previously dominated the landscape of immunology. However, these two subtypes of activated Th cells are now joined by several subsets of immunosuppressive Tregs and the potently proinflammatory Th17 lineage [35, 39]. As was described in more detail in preceding chapters, Th17 cells are defined by the secretion of IL-17 and represent a lineage of Th cells distinct from the Th1 and Th2 populations. Th17 cells have been reported to secrete two isoforms of IL-17 (IL-17 A and IL-17 F), as well as IL-9, IL-21, IL-22, and GM-CSF [40]. Th17 cells derive from naïve $CD4^+$ precursors in a process dependent on the cytokines TGF-β and IL-6. Two additional cytokines contribute to the development and function of Th17 cells: IL-21, produced by Th17 cells themselves, induces IL-17 secretion in a positive feedback loop, while IL-23, of antigen presenting cell origin, serves to stabilize nascent Th17 populations into committed effectors.

The differentiation of Th17 cells is inhibited by cytokines associated with the differentiation of other T effector lineages: IFNγ and IL-4 as well as IL-2, IL-25, type-I interferons, and the IL-10 family member IL-27 [35]. 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 [35]. STAT-3 transduces signals associated with IL-6 and IL-23 receptors and thus has an obligatory role in Th17 development 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 chapter) and secrete cytokines in situ, which elicits inflammation by recruiting neutrophils and otherwise mediating tissue destruction [3].

A pathologic 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 support in basic and clinical research as the major immunopathologic population in CNS autoimmunity [3, 13, 35, 39]. EAE is the animal model for multiple sclerosis 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 [3, 16, 28, 35]. In support of this hypothesis, elevated levels of IFNγ and IL-12 (required for the development of Th1) in the serum or cerebrospinal fluid or the infiltration of IFNγ-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 [16, 35]), while genetic targeting of T-bet, the Th1 master regulator, renders mice resistant to EAE induction [14]. The initial evidence suggesting that a distinct lineage initiated EAE was the illuminating experiments by Cua et al. and Langrish et al., demonstrating 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 [9, 25]. The presence of copious IL-17 in the cerebrospinal fluid of MS patients and large numbers of $IL\text{-}17^+$ 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 has taken place in MS where links between IL-12 and disease have been reevaluated due to this subunit commonality [16, 28, 35, 38]. While Th17s unquestionably contribute to CNS immune pathology, the Th1 lineage also contributes importantly to CNS autoimmunity. Multiple studies have demonstrated that in vitro-polarized Th1 and Th17 populations are sufficient to induce EAE when transferred into recipient mice, although the disease manifests with different symptoms with distinct regions of the CNS affected. Th17-dominated transfers cause a cerebellar infiltrate of primarily neutrophils resulting in atypical EAE (ataxia, spasms), while Th1-dominated transfers mediate a monocytic infiltrate into the spinal cord resulting in classical EAE symptoms (flaccid, ascending hind limb paralysis) [24, 36].

As mentioned above, Th17 cells are defined by their ability to synthesize IL-17A and F and have been shown to be highly encephalitogenic; however, it does not appear that their role in EAE is dependent on IL-17 production [18]. Targeting IL-17 genetically or with a neutralizing antibody reduces EAE symptoms, but does not completely protect mice from disease. This could reflect that by inhibiting IL-17, which functions in EAE to compromise the blood–brain barrier and recruit neutrophils to the inflammatory site, these general inflammatory processes are blunted, but other Th17 or even Th1 cytokines are more critical to CNS pathology. Th17 cells have also been shown to produce IL-21, IL-22, and IL-9, all of which appear to contribute to EAE and the generation or function of Th17s, although none of these are absolutely required nor are effective intervention targets (discussed in

detail in [7]). Genetic targeting or blockade of IL-9, IL-21, IL-22, or associated receptors does not ablate Th17 differentiation nor protect animals from EAE. GM-CSF had previously been associated with Th17 function, but this molecule was recently demonstrated to be secreted by Th17 cells and to play a critical role in EAE progression. The loss of GM-CSF synthesis in T cells was shown to totally protect mice from EAE. Interestingly, both transferred Th1 cells and Th17 cells required GM-CSF for encephalitogenic activity. GM-CSF-deficient mice were able to mount a Th17 response to neuroantigen immunizations, and displayed T cell-containing CNS infiltrates, indicating that GM-CSF potentiates tissue destruction but not invasion by Th17s in EAE [7].

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 [28, 34]. 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 [34]. As discussed above, studies of EAE have definitively demonstrated a causative role of Th17 lymphocytes in disease, and the current literature espouses three main strategies to prevent this inflammatory population of lymphocytes from exerting immunopathologic 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 (αCD20 and αCD52). 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 chapter will discuss *limiting Th17 autoimmune response* by targeting effector cytokines and lymphocyte trafficking. These strategies are represented graphically in Fig. 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 [3]]. Likewise, humans with mutations to the IL-23/IL-17 axis are at higher risk of contracting respiratory tract infections. Regardless, as will be discussed below, this risk is generally manageable and should not offset the potential benefit of Th17 targeting in CNS autoimmunity.

2 Targeting Th17 Responses

2.1 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

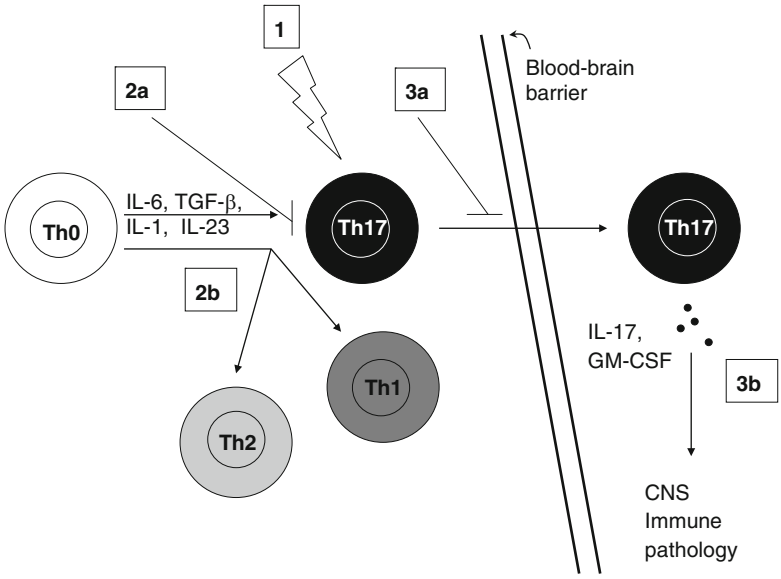

Fig. 1 Current and emerging strategies for preventing CNS autoimmunity. Naïve, autoreactive T cell clones responding to self-antigens in the presence of IL-6, IL-23, IL-1, and TGF-β develop into inflammatory Th17 cells, cross the blood–brain barrier into the CNS, and exert their effector function. The 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), and (3) prevention of Th17 function (sequestration with fingolimod, natalizumab, or antibody neutralization of IL-17 and/or GM-CSF). Some current and proposed therapeutic agents target multiple processes simultaneously

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 TH17s, 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 Fig. 1.

2.1.1 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 chapter. Anti-CD52 (alemtuzumab or Campath-1 H) targets both the T cell and B cell compartments (reviewed in [8, 28]). 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 (2.2–0.14 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-1 H suffer from bacterial sepsis and cytomegalovirus reactivation. An unexpected side effect to Campath-1 H 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 [8, 20].

2.1.2 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 [15, 20]. In addition to lymphocytotoxicity, 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 IFNγ, 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 upregulated by IL-17) [3, 15]. 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 [20]. As is to be expected following a large-scale loss of lymphocytes, these three drugs carry risks of opportunistic infections and leukemia. Antimicrobial prophylaxis is commonly required for patients receiving cladribine or cyclophosphamide. There is also an increased incidence of leukemia (usually acute promyelocytic or myelocytic leukemia—APL or AML) and cardiotoxicity associated with mitoxantrone use [15]. These adverse effects make these drugs suitable only for treatment of aggressive forms of MS if non-ablative forms of immunomodulation are ineffective.

2.2 *Prevention of Th17 Differentiation*

Despite the efficacy and availability of immunoablative agents, there are serious risks associated with such approaches. A more attractive strategy would be to 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 the last several years, and this interest has provided optimism that targeting lineage-specific cytokines, transcription factors, or signaling pathways is of potential value in a clinical setting. Targeting IL-23 as well as Th17 lineage antagonism by IL-25, IL-27, and IFN-β will be discussed. These molecular targets comprise strategies 2a and 2b in Fig. 1.

2.2.1 IL-23 Neutralization

IL-23 was first thought to be required for Th17 development, as the addition of IL-23 to T cell cultures increased IL-17 production, and IL-23-deficient animals are completely protected from EAE [33]. Closer examination revealed that IL-23 does not stimulate naïve T cells, but potentiates the synthesis of IL-17, IL-22, and GM-CSF in previously activated T_H cells [7, 29]. However, because IL-23 does not exhibit pleiotropic activity to the same degree as TGF-β or IL-6, it was thought to be an attractive therapeutic target for preventing Th17-mediated CNS damage. Following the rationale that IL-23p19-deficient mice are completely resistant to EAE [9, 25], Chen et al. showed that administration of a monoclonal anti-IL-23p19 antibody significantly improves the clinical outcome of EAE [6]. 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 [6]. Despite a promising preclinical profile and successful trials in Crohn's disease (phase IIa) and psoriasis (PHOENIX—phase III), antibody neutralization of IL-23 (using ustekinumab) afforded no benefit to newly diagnosed relapsing–remitting MS patients [11]. This could be attributed to several factors. Th17 cells were observed in EAE-primed IL-23-deficient mice, but they failed to infiltrate the CNS, highlighting IL-23's role not in the initiation of Th17 responses, but rather the initiation of neuroinflammation [17]. Possibly, ustekinumab administration to patients after the onset of MS is insufficient to stop the response of already-established effector/memory populations or to prevent the de novo generation of Th17 cells in the inflamed CNS. The blood–brain barrier is already compromised during active MS/EAE, and there may be less of a dependence on IL-23 for tissue entry at this phase of disease [11]. Alternatively, other developmentally important cytokines could compensate for the loss of IL-23 and allow Th17s to mediate CNS tissue damage in its absence.

2.2.2 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 showed that T cells activated in the presence of IL-27 generally fail to transfer disease [12]. 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 [23]. 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 pathologic 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 dendritic cell regulation. Further research on this subject would be of immense interest, due to the profound influence CNS dendritic cells exert on MS/EAE [2, 28]. 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 [23].

IFN-β is a first-line therapy for relapsing–remitting MS patients and is known to exert potent anti-inflammatory effects [22]. Despite its long-standing use and efficacy in slowing the relapse rate and lesion burden in a subset of patients, it is not 100 % effective, its mechanism is incompletely understood, and best results are usually obtained when IFN-β is used in conjunction with another form of therapy [20]. Nonetheless, it exhibits a diversity of anti-inflammatory effects and appears to disrupt Th17 function in MS via several mechanisms [27]. 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 matrix metalloproteinases are decreased in patients receiving IFN-β, as the MMP activity is a known mechanism of IL-17-mediated

tissue destruction [28]. 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 [27]. More recent reports indicate that IFN-β inhibits the differentiation and function of Th17 cells more extensively than originally thought. In vitro exposure of dendritic cells taken from human MS patients to IFN-β reduces their production of IL-23 and increases their production of IL-27, which would theoretically decrease the stability of autoreactive Th17 lineages (reviewed in [33]). A similar study has determined that IFN-β can exert anti-Th17 effects directly on human T cells, as IFN-β exposure was shown to result in decreased STAT-3 activation and diminished IL-17 and IL-21 productions [5]. The same associations were observed in mice and correlated with a decrease in CNS infiltration. While these results indicate that IFN-β may be effective in preventing the establishment of Th17s, and/or early events associated with CNS autoimmunity (i.e., tissue entry), there remains the problem of halting an established Th17 response. To that point, Th17s in MS patients have been shown to express higher levels of the IFN-β receptor than Th1 cells [10]. Furthermore, upon in vitro restimulation, of whole PBMCs, Th17 cells exhibited a higher apoptosis rate than Th1s in the presence of IFN-β. While these findings suggest that fully mature Th17 lineages from patients with active MS may be sensitive to IFN-β-induced apoptosis, evidence to the contrary exists. Axtell and colleagues found that the severity of EAE induced by adoptive transfer of Th1 cells could be decreased by IFN-β treatment, but that EAE induced by Th17 transfer was exacerbated by IFN-β treatment [1]. Furthermore, this group reported that high serum levels of IL-17 were associated with a resistance to IFN-β treatment in MS patients. Together, current data suggest that IFN-β may have limited efficacy in treating established, Th17-dominated forms of MS and may be more effective when Th1 cells are predominant or during the development of a Th17 response.

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 [16]. The disappointing performance of IFN-γ in animal 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 herein 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 [6]. 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 [14]. Due to the close relationship between Th1 and Th17 cells in disease as well as their respective roles in EAE, it seems prudent to avoid Th1 skewing when targeting the Th17 lineage in MS/EAE.

2.3 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 will discuss the antibody neutralization of IL-17 as well as the sequestration of leukocytes by $S1P_1$ antagonists and the monoclonal antibody natalizumab (αVLA4). These approaches are designated 3a and 3b in Fig. 1.

A 2005 study by Hofstetter and colleagues illustrated the feasibility of IL-17 neutralization as a method to reduce CNS immune pathology in animals [19]. Although reductions in clinical score were observed in anti-IL-17-treated animals, symptoms were still observed. An exciting series of papers appearing in the late 2006 detailed the immunization of mice with self-IL-17 using either a protein carrier (ovalbumin) or an IL-17-conjugated viruslike particle [31, 37]. 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, prophylactic OVA-IL-17 immunization resulted in zero incidence as evidenced by a lack of neurological deficit or weight loss. 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. Th17 cells are clearly able to initiate CNS autoimmunity and cause tissue destruction; however, based on the work of Becher et al., CNS tissue damage does not appear to be predicated on production of IL-17 [7, 18]. IL-17 nonetheless has been shown to contribute to the breakdown in blood–brain barrier permeability and to recruit neutrophils to the CNS [33]. These observations combined with the apparent efficacy of IL-17 neutralization in EAE form the rational basis for a clinical trial, which is currently enrolling relapsing–remitting MS patients to test the efficacy of AIN457, a fully humanized anti-IL-17A antibody. AIN457 demonstrated a favorable safety profile in phase I trials in psoriasis and RA; there was no significant difference in the frequency of adverse events, such as opportunistic infections [21]. AIN457 as well as both immunization methods employed in the animal studies described above involves highly specific IL-17A antibodies which do not react against any of the other IL-17 isoforms. As discussed in Rohn et al., this may have important implications pertaining to the increased susceptibility of IL-$17^{-/-}$ mice to certain bacterial infections [31]. It was noted that IL-17A and IL-17 F partially overlap in function, especially with regard to neutrophil recruitment. Thus, specifically targeting IL-17A may not impair IL-17 F-mediated neutrophil recruitment and may not increase susceptibility to bacterial infections. AIN457 was found to significantly improve psoriasis and rheumatoid arthritis scores in early clinical trials; however, MS trials will be conducted with caution based on the failure of ustekinumab (anti-IL-23) and on recent data suggesting a reduced role of IL-17 in CNS immune pathology.

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 α4β1 integrin (VLA4) [8, 20, 28]. 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, the 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.

2.4 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, for which phase III (FREEDOMS and TRANSFORMS) MS trials have been completed, targets the sphingosine 1-phosphate receptor expressed on T lymphocytes [4, 30]. It is known to sequester lymphocytes in secondary lymphoid organs, thereby preventing their entry into the CNS and the resulting tissue damage. Fingolimod treatment resulted in a decrease in CNS lesions and in the relapse rate of MS patients. Adverse effects occurred with similar frequency and severity to a standard treatment (IFN-β). Recent data collected from the trial indicate that fingolimod treatment reduces the number of circulating Th17 cells in MS patients by 95 % [30]. Accompanying animal studies support this finding, as reduced Th17 cells were observed in lesions of rats and mice with EAE. The number of lesions was also reduced. While these results are very promising, given the contributions of Th1 cells to EAE and MS and given the encouraging efficacy in disease burden in MS patients, it seems unlikely that the effects of fingolimod on CNS autoimmunity are restricted to only the Th17 lineage. A second mechanism by which fingolimod suppresses autoimmunity has been proposed. Liao and colleagues showed that the addition of S1P (an $S1P_1$ ligand) to T cell cultures supportive of Th17 differentiation (containing a TCR stimulus, TGFβ, and IL-6) resulted in a high degree of conversion to the Th17 lineage, accompanied by IL-17 secretion [26]. 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 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 suggested 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.

Finally, glatiramer acetate (GA) is an FDA-approved treatment for MS that has several immunomodulatory effects [32]. GA is a mixture of synthetic amino acid polymers consisting of glutamic acid, lysine, alanine, and tyrosine. 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 proinflammatory conditions to an anti-inflammatory Th2 state. The latter of GA's three mechanisms 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.

3 Summary

This chapter has discussed the immunomodulatory agents which 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 Fig. 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, which specifically antagonize Th17 cells and preserve immune competence. Interfering with Th17 development can be accomplished either by blocking the process by targeting cytokines required for the lineage's differentiation or by immune deviation using exogenous cytokines antagonistic to Th17 cells but supportive of other lineages. Despite promising results in animal studies and in vitro studies using human cells, attempts to prevent or divert the differentiation of Th17 cells have yielded disappointing results in the clinic, as IL-23 neutralization failed to reduce MS disease burden and the success of IFN-β therapy is inversely correlated with IL-17 expression at the time of treatment. These findings illustrate the importance of the timing of treatment and may suggest that agents targeting the differentiation of Th17 cells following the initiation of disease may have limited clinical success, although due the complexity of Th1–Th17 interplay in MS, at least IFN-β remains valuable. The final strategy discussed above, the prevention of Th17

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	Phase III	2
IL-25 (IL-17E)	Th17 → Th2 shift	None	2
IL-27	Th17 → Th2 shift	None	2
IFNβ	Th17 → Th2 shift	IV/approved	2
Anti-IL-17	IL-17	II	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 [36])

function, is accomplished by interfering with leukocyte trafficking or by neutralizing IL-17. Despite recent observations that IL-17 is dispensable for Th17-mediated CNS autoimmunity, the upcoming clinical trial of anti-IL-17A will be of great interest. 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. Infections secondary to anti-IL-17A administration in humans do not appear to be a major cause for concern [21]. If AIN457 fails to afford any clinical benefit to MS patients, it would be of interest to follow up the study of Codarri, et al. [7], in which GM-CSF, rather than IL-17, was shown to be the primary Th17 product responsible for tissue damage. A safety trial for anti-GM-CSF in RA patients is currently ongoing (www.clinicaltrials.gov identifier: NCT00995449).

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 relapsing–remitting MS (reducing the relapse rate by 94 %), but have limited benefit for severe-progressive MS patients (whose condition deteriorates over the long term) [8]. Likewise, the use of strong immunosuppressive agents is advisable only against aggressive forms of MS [15]. 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 [28]. 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.

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Involvement of Interleukin-17 Cytokines in Human Asthma

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Abstract Several clinical phenotypes of asthma constitute a clinical health problem globally and current therapy is still insufficient to meet clinical need. This chapter scrutinises the accumulated evidence that interleukin (IL)-17A and other members of the IL-17 cytokine family are involved and play a pathogenic role in human asthma. Whereas there is now substantial evidence from patients with asthma arguing for an involvement of IL-17A locally as well as systemically, there is still a lack of conclusive evidence that IL-17A plays a causative role. However, the evidence for the involvement of IL-17A-producing T helper (Th) cells in human lungs and blood is growing. Moreover, evidence for an involvement of additional members of the IL-17 cytokine family, such as IL-17F and IL-25, is emerging. Thus, there is a rationale and a need for new and interventional studies on well-characterised human patients with asthma, targeting IL-17 cytokines specifically. This type of studies may more firmly establish the causative role and potential as therapeutic target for IL-17 cytokines as well as Th17 cells in various phenotypes of human asthma.

Keywords Allergy • B cell • Cytokine • Interleukin-17A • IL-17E • IL-17F • IL-25 • Macrophage • Neutrophil • T cell

1 Introduction

The idea that the archetype T helper (Th) 17 cell cytokine interleukin (IL)-17A (originally known as IL-17) is involved in obstructive airway disease including asthma was forwarded already in the very beginning of the new millennium [1].

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More than 10 years later, we still do not know for sure whether this cytokine does play a causative role in asthma. The original suggestion was based on plain observations from three experimental studies documenting the neutrophil-mobilising effects of recombinant IL-17A protein in an animal airway model in vivo and in human bronchial epithelial cells in vitro [2–4]. Briefly, these early experimental studies indicated that IL-17A induces the transcription and release of neutrophil-mobilising cytokines from bronchial epithelial cells. Thereby IL-17A indirectly accumulates neutrophils and eventually also activates these innate effector cells locally in the airways. *Molet* and co-workers subsequently published supportive clinical evidence for an involvement of IL-17A in human asthma, obtained from the airways of patients with mild disease [5]; evidence that more recently has been substantiated by additional studies on human patients [6, 7]. This chapter is focused on critically scrutinising the evidence from human subjects that IL-17A, as well as more recently discovered members of the IL-17 cytokine family, are involved in and may play a causative role in the pathogenesis of asthma.

2 Interleukin-17A in the Airways of Patients with Asthma

2.1 Data on Bronchoalveolar Lavage Samples

In their early study, *Molet* and co-workers reported that the average concentration of soluble IL-17A protein in bronchoalveolar lavage (BAL) samples is more than 50 % higher in patients with mild asthma than in healthy control subjects [5]. A substantial fraction of these patients displayed a positive skin prick test, indicating allergen sensibilisation, and some were ex-smokers. However, none had a history of long-term and heavy smoking and no patient was treated with glucocorticoids. Likewise, it was claimed that no patient had a respiratory infection during the 6 weeks preceding the study inclusion, so it seems unlikely that the finding could be attributed to mere microbial activation of pulmonary host defence. *Molet* and co-workers also demonstrated that the immunoreactive signal for IL-17A protein is approximately three times more frequent among BAL cells from patients with mild asthma than among BAL cells from control subjects. Even though it was not statistically proven, *Molet* and co-workers also reported a trend towards an increase in the relative number of sputum and BAL neutrophils, respectively, paralleling the increase in IL-17A protein in the patients with asthma. *Molet* and co-workers also confirmed that the relative number of eosinophils is moderately increased in patients with asthma.

Fully compatible with the fundamental work by *Molet* and co-workers, *Glader* and co-workers recently demonstrated an approximately 100 % average increase in extracellular IL-17A protein in BAL samples after bronchial exposure to endotoxin in healthy human volunteers [8]. Notably, this modest but reproducible increase in IL-17A was accompanied by a corresponding increase in IL-23 and IL-21 protein,

two additional "Th17-driving" cytokines. Thus, the study by *Glader* et al. provides a rationale for a more in-depth characterisation of the IL-17A-producing cells in the airways of patients with asthma and other types of chronic inflammatory airway disease.

2.2 Data on Sputum Samples

In line with their original findings in BAL fluid and cells, *Molet* and co-workers also showed that the immunoreactive signal for IL-17A protein is close to three-fold more frequent among sputum cells from patients with mild asthma, compared with healthy control subjects [5]. This data also confirmed that the relative number of eosinophils is substantially increased in sputum samples from patients with asthma. In contrast, there was a trend only towards a corresponding increase for neutrophils.

Barczyk and co-workers published a study indicating an association between an increased concentration of IL-17A protein in induced sputum and bronchial hyperreactivity to metacholine in a mixed group of patients [9]. This patient group consisted of patients with asthma or chronic bronchitis [9]. In their study, *Barczyk* and co-workers showed that the increase in sputum IL-17 among the patients with bronchial hyperreactivity is less than 50 %, compared with control subject without bronchial hyperreactivity. It is of interest that *Barzyck* and co-workers also forwarded evidence that the sputum concentration of IL-17A protein does correlate with the sensitivity index (PC20) for metacholine challenge. Notably, the patients with asthma had mild to moderate disease and some patients were treated with inhaled glucocorticoids. The particular study was relatively small and it was probably because of this and the inconsistent treatment with glucocorticoids, that the trend towards a difference in sputum IL-17A between asthma patients and control subjects did not reach statistical significance. This may also be true for the difference in IL-17A between patients with chronic bronchitis and control subjects.

Compatible with the study by *Barczyk* and co-workers, *Sun* and co-workers detected a close to four-fold concentration of IL-17A protein in induced sputum from patients with severe asthma, compared with healthy control subjects [10]. This increase in IL-17A was accompanied by a close to 100 % increase in the relative number of neutrophils, compared with control subjects. *Sun* and co-workers also included patients with mild to moderate asthma in their study. Interestingly, when analysing IL-17A protein in all stages of disease, *Sun* and co-workers showed that the concentration of sputum IL-17A corresponds to the severity of disease. The same was true for the concentrations of MPO and IL-8 protein, respectively. None of the patients with asthma was treated with a glucocorticoid, none was recently infected and no patient was a smoker. However, when a mixed group consisting of all three degrees of disease severity was treated with an inhaled glucocorticoid during 1 week, this treatment modestly reduced the IL-17A protein concentration, suggesting a certain degree of sensitivity to glucocorticoids in human airways. The utilised glucocorticoid did not markedly affect relative number of neutrophils in

sputum, however, pointing towards a possible dissociation between these two outcomes in human patients.

Bullens and co-workers published important evidence on IL-17A in patients with asthma in 2006. These investigators demonstrated that there is an increase in the mRNA for IL-17A in sputum cells from patients with asthma and that he increase in mRNA is of a similar magnitude in mild and moderate to severe asthma, respectively [11]. They found no pronounced difference in this respect for patients with allergic and non-allergic asthma, either. In spite of investigating a limited study material, *Bullens* and co-workers were also able to demonstrate a positive and statistically significant correlation between the relative number of neutrophils in sputum and the mRNA for IL-17A, thereby providing evidence for IL-17A being associated with neutrophil accumulation in patients with asthma, even though a causative relationship still remains to be proven in asthma.

Assessing airway remodelling outcomes versus cytokine content in induced sputum, *Kaminska* and co-workers published a study in 2009 that compared these parameters for patients with chronic and reversible phenotypes of severe asthma, respectively [12]. Unexpectedly, there was an approximately three-fold higher average concentration of IL-17A protein in sputum among patients with reversible severe rather than with chronic severe asthma [12]. Thus, the results of this particular study did not substantiate the notion that there is a much more pronounced accumulation of airway neutrophils in chronic than in reversible asthma, even though there was a trend towards somewhat higher concentration of sputum neutrophils in the patients with chronic severe compared with reversible severe asthma.

In a recent study, *Doe* and co-workers compared the concentration of IL-17A protein in the sputum of patients with asthma with that observed in patients with COPD [6]. Unexpectedly, these investigators detected a somewhat higher average concentration of IL-17A in patients with COPD than in those with asthma, even though the highest individual concentrations were actually detected in patients with asthma [6].

2.3 Data on Exhaled Breath Condensate

In 2006, *Matsunaga* and co-workers published an innovative study on exhaled breath condensate in patients and healthy control subjects who were non-smokers and without any glucocorticoid treatment [13]. In essence, the results of this study indicates that the relative concentration of "exhaled" IL-17A protein is increased by approximately 50 % in patients with mild asthma, compared with control subjects. Interestingly, the increase in IL-17A among these patients with asthma seemed to be associated with a similar increase in the chemokine IL-8 and in TNF-α and TGF-β, respectively; cytokines that can be linked to neutrophilic inflammation and Th17 development [14–16]. Notably, this particular study demonstrated a trend towards a correlation between the concentration of "exhaled" IL-17A protein and the degree of airway reactivity and airway obstruction in the patients with asthma.

2.4 Data on Bronchial Tissue

Important clinical evidence for an increase of IL-17A protein in patients with asthma was published in a study by *Chakir* and co-workers in 2003 [17]. The study showed that patients with moderate to severe asthma have an immunoreactivity signal for IL-17A protein in the subepithelial bronchial layer that involves almost a six-fold higher number of positive cells, compared with healthy control subjects. There were no smokers among the patients or control subjects and none of the patients was treated with glucocorticoids [17]. A relative increase in IL-17A signal was obtained when analysing the intraepithelial bronchial layer as well. Likewise, among the patients with severe asthma, there was a substantial increase in TGF-β and type I collagen as well, pointing out plausible associations with Th17 mobilisation and remodelling, respectively. The major part of the immunoreactivity signal for IL-17A protein proved to be sensitive to 2 weeks' of treatment with a systemic glucocorticoid in this study, again pointing out that there may be a certain degree of sensitivity to glucocorticoids in this setting.

In a short report published in 2009, *Al-ramli* and co-workers forwarded data showing an increase in subepithelial cells immunoreactive for IL-17A protein in patients with asthma [7]. In severe asthma, the magnitude of this increase in IL-17A protein was approximately three-fold that in control subjects and paralleled disease severity. The pattern was similar for the corresponding messenger RNA as well, even though the relative increase was then more pronounced. Unfortunately, the referred publication included insufficient information on medication including glucocorticoids.

When analysing the number of submucosal cells immunoreactive for IL-17A protein, *Doe* and co-workers recently forwarded evidence that the most pronounced increase may actually be present in patients with mild to moderate asthma [6]. In this case, the relative increase was approximately five-fold, compared with control subjects. There was no statistically significant difference for the two asthma groups, however, possibly due to lack of power in the material. It is unclear whether there were differences in medication between groups, since no specific information on treatment with glucocorticoids was provided. Moreover, in this study, an inconsistent number of tobacco smokers were included in each respective subject group; thereby making smoking an uncontrolled, confounding factor.

3 Interleukin-17A in the Blood of Patients with Asthma

The very first study specifically addressing IL-17A protein in patients with asthma was published by *Wong* and co-workers in 2001 [18]; actually a little earlier than the study on IL-17A in the airways by *Molet* and co-workers [5]. The study by *Wong* and co-workers was conducted on blood cells and plasma and the results revealed a trend towards an approximately 100 % increase in the concentration of

soluble IL-17A protein in the plasma of patients with allergic asthma, compared with healthy control subjects without allergy [18]. However, this particular trend did not reach statistical significance, possibly due to the limited size of the study material. Subsequently, the preliminary finding by *Wong* and co-workers was more definitively confirmed by data published in the seminal study by *Molet* and co-workers [5], who thus demonstrated a statistically significant increase in the number of blood eosinophils immunoreactive for IL-17A in patients with mild asthma, compared with healthy control subjects.

Of particular interest is a recent study by *Agache* and co-workers; one that forward evidence for high serum levels of IL-17A constituting an independent risk factor for severe asthma [19]. Comparing patients with mild, moderate and severe asthma, these investigators found that patients with severe asthma have approximately a 100 % increase in serum IL-17A protein, compared with patients with mild to moderate asthma. Unfortunately, the study did not separately address patients with or without atopy. However, *Agache* and co-workers also demonstrated that a serum level exceeding 20 pg/mL is associated with a more than three-fold increased risk for severe asthma. Notably, the study contained evidence that level of serum IL-17A is negatively correlated with the level of blood neutrophils, as well as with spirometry indicators of airflow in the small airways. Since these findings are compatible with, even though not definitive evidence for, local IL-17A recruiting neutrophils from the blood and contributing to small airway obstruction, the study lends support to the notion that IL-17A is involved in the pathogenesis leading to severe asthma.

More evidence for an involvement of IL-17A in blood was recently provided by *Zhao* and co-workers [20]. When analysing plasma from patients with allergic asthma, these investigators obtained evidence that the concentration IL-17A protein is increased by a five-fold for the entire group of patients with allergic asthma and with a ten-fold for the patients with severe asthma, compared with healthy control subjects. Interestingly, the concentration of IL-17A protein correlated with IL-23 protein in the group of patients with asthma, thus pointing out a putative link to Th17 mobilisation.

Conducting a genome wide association (GWA) study, *Moffat* and co-workers in 2007 showed that genetic variants of the chromosome 17q21 locus are associated with the occurrence of childhood asthma [21]. Now, the same research group recently showed that the magnitude of the release of IL-17A protein in unstimulated and PHA-stimulated mononuclear cells from the umbilical cord of newborns is linked to the occurrence of single nucleotide polymorphisms (SNPs) at the 17q21 locus [22]. Thus, these two studies indicate a link between the systemic release of IL-17A and occurrence of childhood asthma via a defined chromosome locus, even though the clinical significance of this link needs further revaluation.

4 Cellular Sources of IL-17A in Patients with Asthma

4.1 Data on Sources in the Lungs

The first documentation of IL-17A in BAL and sputum samples that was published by *Molet* and co-workers consisted of data obtained by using three different techniques: immunocytochemistry, Western blot and in situ hybridisation [5]. Given the collective evidence from experimental studies on cellular sources of IL-17A, the findings published by *Molet* and co-workers are in need of functional evaluation [1, 14–16, 23–25]. This is because there is no published study demonstrating that live human eosinophils actually can release IL-17A protein in response to any stimulus.

In contrast to what *Molet* and co-workers reported, most published evidence from animal in vivo and human cell models, respectively, indicates that IL-17A originates from studies of peripheral blood mononuclear cells that are CD3-positive mainly [1, 14–16, 23–25]. Examining BAL cells from healthy volunteers exposed to organic dust, *Ivanov* and co-workers reported in 2005 that there is immunoreactivity for IL-17A in human BAL cells with a "lymphoid" morphology, as well as a substantial increase in detectable mRNA for IL-17A [26]. Clearly, there is substantial published evidence compatible with the notion that T lymphocytes constitute a source for IL-17A protein in human airways.

Two recent studies on humans have forwarded important evidence that there is a subset of Th cells producing IL-17A protein in different compartments of human lungs. In 2010, *Glader a*nd co-workers published the first of these two studies [8]. This particular study demonstrated the presence and increase of CD3- and CD4-positive cells containing IL-17A in BAL samples harvested after intrabronchial exposure to endotoxin in healthy human volunteers. These IL-17A-containing Th cells were detected among BAL cells that contained mRNA for the archetype Th17 transcription factor RORCvariant2 as well; the latter correlating with mRNA for IL-17A after endotoxin exposure [8]. The findings are clearly compatible with the presence of "Th17-like" cells in human airways.

The second and more recent study demonstrating T helper cells producing IL-17A protein in human lungs was published in 2011 by *Purwar* and co-workers [27]. Interestingly, *Purwar* and co-workers demonstrated that approximately 1 % of all CD4-positive cells contain IL-17A protein in lung tissue from patients undergoing surgery for lung cancer. Correspondingly, the same study demonstrated that approximately 0.8 % of all CD8-positive cells contain IL-17A protein in lung tissue from patients undergoing surgery for lung cancer. These findings illustrate that there may be more than one subset of T lymphocytes producing IL-17A in the lung tissue of human subjects.

In terms of the specific characteristics of T lymphocytes producing IL-17A in the airways of patients with asthma, there is very limited data published so far. *Bullens* and co-workers demonstrated in 2006 that there is a positive and strong correlation between the levels of mRNA for IL-17A and CD3γ in sputum from patients with

moderate to severe asthma [11]. In this context, it is interesting that *Al-ramli* and co-workers described in their study on patients with asthma that immunoreactivity for IL-17A protein was detected in mononuclear, inflammatory cells mainly, residing in the submucosa [7]. Tentatively, it also remains to be confirmed whether IL-17A-producing Th cells in the lungs of patients with asthma comprise all the typical "Th17 characteristics" previously documented in experimental models of asthma [15, 16, 28–34].

4.2 Data on Sources in the Blood

There is an increasing body of evidence for blood cells producing IL-17A in patients with asthma as well. In 2005, *Hashimoto* and co-workers published data showing a reproducible IL-17A response in isolated mononuclear cells to stimulation with anti-CD3 plus anti-CD28 antibodies and house dust mite extract, respectively, using blood cells from patients with allergic asthma [35]. Interestingly, three recent studies take this topic further.

Zhao and co-workers showed in 2010 that there is a subset of CD3-positive and CD8-negative cells containing signal for IL-17A among peripheral blood mononuclear cells in patients with allergic asthma [20]. The data suggest that the percentage of these cells is approximately 50 % higher in patients with allergic asthma, compared with control subjects and, notably, it increases with disease severity. By generating T-cell clones from the CCR6- and CD161-positive fraction of CD4- and CD3-positive peripheral blood cells and examining their cytokine content, *Cosmi* and co-workers generated evidence that IL-17A and IL-4 as well as other archetype Th17 and Th2 cytokines can be produced by the very same cells [36]. Similar findings have been reported by *Wang* and co-workers, who recently claimed that the subset of CD4-positive cells that contain IL-17A as well as the Th2 cytokines IL-4, IL-5 and IL-13 are CD45-, CCR6- and CRTH2-positive and contain the two archetype transcription factors for Th17 (RORCvar2) and Th2 (GATA-3) as well [37]. Consequently, the existence of a Th17/Th2 subset of memory T helper cells has been proposed. According to *Cosmi* and co-workers' study, these Th17/Th2 cells are capable of inducing the secretion of IgE from autologous human B lymphocytes in vitro, thus illustrating a functional link to allergy. In an even more recent study, *Zhao* and co-workers forwarded evidence of an increased frequency of CD4-positive cells containing IL-17A protein in the blood of patients with allergic asthma, compared with control subject but reported no other characteristics of these blood cells [38].

5 Additional IL-17 Cytokines Investigated in Patients with Asthma

There are two additional members of the IL-17 cytokine family that currently can be linked to asthma and allergy, namely, IL-17F and IL-25 [14–16, 25]. The data on these IL-17 cytokines is interesting but still very limited.

5.1 Interleukin-17F

Interleukin-17F is potentially relevant in the context of acute and severe asthma in humans [14–16, 25], and the first evidence for this was published by *Kawaguchi* and co-workers in 2001 [39]. In essence, *Kawaguchi* and co-workers demonstrated two critical aspects of IL-17F: Recombinant IL-17F stimulates the production and release of neutrophil-mobilising cytokines in human bronchial epithelial cells, and allergen challenge of patients with allergic asthma increases the signal for IL-17F mRNA among BAL cells [39]. Yet another interesting aspect of IL-17F is that this cytokine may be produced and released by several, principally different cell types: structural cells as well as immune cells [14–16, 25, 39]. The evidence for this includes data from epithelial cells, basophils, mast cells, monocytes and T lymphocytes [14–16, 25, 39].

Kawaguchi and co-workers has published an intriguing finding on a genetic mutation that makes the IL-17F molecule biologically inactive in vitro [39]. These investigators have also shown that patients with asthma or COPD lack this mutation, whereas the same mutation can be present in healthy control subjects. However, the frequency of this mutation is fairly low among healthy control subjects and contradictive data exists, so this particular issue is in need of further evaluation [39, 40].

The study by *Al-ramli* and co-workers that was published in 2009 included data showing an increase in subepithelial immunoreactivity for IL-17F protein in patients with asthma [7]. The magnitude of this increase in IL-17F protein-paralleled disease severity and this was the case for the corresponding messenger RNA as well. Interestingly, *Al-ramli* and co-workers described that IL-17F protein was detected in the epithelial layer and even in epithelial cells in the very same patients that expressed IL-17A in the submucosa [7].

Additional support for IL-17F being a relevant cytokine was forwarded in the recent study by *Doe* and co-workers [6]. These investigators reported more signal for IL-17F protein in the submucosa of bronchial tissue harvested from patients with asthma, compared with healthy control subjects [6]. Even though it was not statistically significant, *Doe* and co-workers demonstrated a trend towards a higher tissue expression of IL-17F in severe asthma compared with mild to moderate asthma, IL-17 thus displaying an expression pattern partially different from that of IL-17A [6].

5.2 *Interleukin-25*

In 2001, *Fort* and co-workers published data indicating that interleukin-25 (formerly known as IL-17E) is functionally different from IL-17A, IL-17F and the heterodimer IL-17A/F [14–16, 41]. *Fort* and co-workers showed that IL-25 has an eosinophil-mobilising capacity, rather than a neutrophil-mobilising one, via its ability to induce IL-4, IL-5 and IL-13 in the airways [14–16, 41]. Recently, *Corrigan* and co-workers demonstrated that allergen challenge causes a modest but reproducible increase in the bronchial mucosal as well as submucosal cells immunoreactive for IL-25 in patients with allergic asthma [42]. Notably, the number of IL-25-positive cells in the submucosa correlated with the late phase decrease in ventilatory capacity (FEV_1). Collectively, the limited number of studies conducted until date have forwarded evidence that IL-25 can be produced by eosinophils, mast cells, endothelial cells, T lymphocytes as well as alveolar macrophages (14–16, 42, 43). Critical functional evidence on the cellular sources is lacking, though.

6 Should We Target Signalling via IL-17 Cytokines in Asthma?

Asthma is a disease currently believed to be driven at least in part by Th cells and there are clinically important phenotypes of asthma with a neutrophilic inflammatory component [15, 16]. This and the fact that there may be increased concentrations of the neutrophil-mobilising cytokines IL-17A and -17F locally and/or systemically in severe asthma may be viewed as a therapeutic rationale for blocking the signalling these two IL-17 cytokines. However, it is important to also consider the growing body of evidence that IL-17A and -F constitutes critical signalling molecules in host defence against bacteria, fungi and, possibly, viruses [14–16, 25]. The mere idea that specific inhibition of IL-17 cytokines can cause increased susceptibility to infections constitutes a strong argument for targeting these cytokines with caution. For these reasons, it seems feasible that local inhibition is a safer approach than systemic inhibition. Given the limited knowledge on IL-25, even more so on IL-17B, IL-17C and IL-17D, more basic studies are needed to establish a solid therapeutic rationale for targeting these particular IL-17 cytokines.

7 Conclusions

Clearly, the published evidence from small-scale studies on patients with severe asthma is compatible with IL-17A and IL-17F being both involved in and contributing to the local mobilisation of neutrophils, possibly in the reversible

type of severe asthma primarily. One interesting issue with potential clinical implications is to clinically evaluate to what extent allergic and non-allergic asthma differs in terms of the involvement of individual IL-17 cytokines [16, 44–47]. Moreover, before pursuing large-scale clinical trials on IL-17A and IL-17F as therapeutic targets in various phenotypes of asthma, it may be worthwhile to more critically evaluate to what extent the pathways mediated by IL-17 and IL-17F are sensitive to drugs that are already in clinical practice. Also, given the emerging complexity of endogenous signalling via IL-17A and IL-17F, it remains an open question whether it is the IL-17 cytokine *per se* or up- or downstream signalling pathways that should be targeted to achieve clinical efficacy in severe asthma. In particular, given what is now known about the signalling of IL-17A and IL-17F via the IL-17A/C receptor complex from experimental models, it seems important to characterise downstream signalling in asthma patients with different stages of disease severity [15, 16, 48, 49]. Also, when planning and conducting large-scale clinical trials on drugs inhibiting IL-17A and IL-17F, immunosuppressive effects need to be carefully monitored.

Finally, the pathogenic and therapeutic rationale for focusing on IL-25 remains more uncertain than that of IL-17A and IL-17F. However, given the potential role of IL-25 in eosinophilic inflammation, targeting this cytokine in the most common phenotypes of asthma may actually prove to be more clinically useful than targeting IL-17A and IL-17F.

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IL17 and/or IL22 as Potential Target(s) for Crohn's Disease

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Abstract The interleukin (IL)17A-producing T cells (Th17) play an essential role in maintaining tissue homeostasis and in repelling enteropathogenic infections. Aberrant IL17A-dependent signalling has been linked to the pathogenesis of Crohn's disease (CD), thereby prioritising IL17A as a potential therapeutical target in such common and incurable inflammatory bowel disease. Despite preliminary encouraging results in mice, blocking of either IL17A or IL17 receptor (IL17R) was inefficient in induction and maintenance of response or remission in CD patients. Herein, we shall review current paradigms on the regulation of pathogenic and protective Th17 functions, through the innate lymphoid cell (ILC)-derived IL22 and/or the NKG2D regulatory pathway.

Keywords Innate lymphoid cells • Crohn's disease • IL17 • IL22 • IL23

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Abbreviations

CD	Crohn's disease
IBD	Inflammatory bowel disease
IFN	Interferon
IL	Interleukin
TCR	T cell receptor
TNF	Tumour necrosis factor
UC	Ulcerative colitis

1 Introduction

Crohn's disease (CD) is a common relapsing-remitting intestinal illness, defined as the major clinical form of inflammatory bowel disease (IBD). CD 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 natural history of CD is unpredictable through the influence of multiple genetic and environmental factors. The CD patient's quality of life is profoundly affected by severe diarrhoea, abdominal pain, fever, weight loss, malnutrition and bleeding. CD is associated with transmural inflammatory lesions which can potentially concern any part of the gastrointestinal tract. Some CD patients can also experience additional complications, such as fistulae, abscesses, bowel strictures and/or extra-intestinal manifestations (mostly involving eyes, skin, joints and liver) [1]. However, an overall 25–30 % therapy refractoriness is seen, regardless of the drug, resulting in accumulation of adverse events and indicating that the treatment of CD is, at present, far from optimal [2].

Although the aetiology of CD remains unclear, clinical and experimental evidences suggested that CD might result from defective innate and adaptive immune responses within the gut mucosa of genetically predisposed individuals, leading to an overly aggressive inflammatory and stress response to microbial antigens [2]. Notably, mutations in the IL23 receptor (IL23R) have been recently linked to the predisposition to CD [3]. In addition, IL23 is over-expressed in the mucosa of CD patients which is also characterised by enhanced responsiveness to IL23 [4]. IL23 is a heterodimeric cytokine composed of the p40 subunit of IL12 paired with a specific p19 subunit. IL23 plays an essential role in the development of the Th17 cell lineage and in the production of the lineage-defining cytokines IL17 and IL22 [5, 6]. Both IL17 and IL22 are over-expressed in inflamed intestinal mucosa of CD patients [7]. Consistently, genome-wide association studies revealed additional inborn errors in several regulatory factors involved in the innate and adaptive maintenance of cells that secrete IL17 and/or IL22, prioritising human IL17/22-based therapy in CD [8]. Herein, we shall review both the concept and the applications of cytokine-based therapy in CD before discussing the most recent clinical advances using IL17/22-targeting molecules.

1.1 What Have We Learned from TNFα-Neutralising Therapy in CD?

To correct the vulnerability of CD patient's mucosa to persistent inflammatory responses, pharmacological blockade of cytokines and/or of their receptors might be achieved by the use of monoclonal neutralising antibodies. The pathophysiological role of a large set of inflammatory cytokines has been thereby evaluated in clinical trials (Table 1). Until now, tumour necrosis factor-alpha (TNFα) is the only cytokine used to treat IBD, reinforcing its pathophysiological role in such chronic inflammatory disorders (Fig. 1). TNFα is a 17-kDa pleiotropic cytokine produced by macrophages, monocytes and T lymphocytes. TNFα signalling influences multiple cellular processes, such as 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 for TNFα. 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 responses [9] and in maintaining clinical remission [10] in CD and in ulcerative colitis (UC) [11]. Scheduled maintenance of infliximab infusions (at a dose of 5 mg/kg every 8 weeks) is now preferred to episodic treatments, in regard to both efficacy and immunogenicity. More recently, another anti-TNFα antibody has been approved for the treatment of CD (referred to as adalimumab). Adalimumab is a fully human recombinant IgG1 with an increased half-life than infliximab (10–20 days), which binds with high affinity to both soluble and membrane-bound TNFα. Subcutaneous administration of adalimumab was efficient in increasing the clinical response rate and in maintaining clinical remission in CD and is currently assessed in UC [12–14]. Besides infliximab and adalimumab, certolizumab was efficacious in inducing a clinical response and in maintaining remission in CD [15, 16]. Certolizumab (also referred to as CDP-870) is a poly(ethylene glycol) (PEG)ylated humanised Fab fragment, which does not induce apoptosis of T cells or monocytes. In addition, certolizumab shows a higher affinity and increased half-life when compared to infliximab (14 days). Additional molecules targeting soluble, but not membrane-bound, TNFα were also tested, but showed no efficacy for induction and maintenance of response or remission in CD (CDP-571, etanercept and onercept). Together, the use of certain anti-TNFα in the management of CD is effective for maintenance of remission, steroid sparing and mucosal healing. However, important concerns still remain. Notably, serious therapeutic complications have 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) [17, 18]. Furthermore, among patients who initially respond to the induction regimen of infliximab, up to 40 % will subsequently lose responsiveness over time [19]. Additional development of biotherapies is thereby of great priority in CD patients that fail to respond to TNFα-neutralising therapy.

Table 1 Cytokine-based therapeutics evaluated in IBD

Targeted cytokine	Generic/brand name	Alias	Manufacturer	Compound class	Mode of injection	Half-life (days)	Phase study (ClinicalTrials.govIdentifier)	
							CD	UC
G-CSF	Filgrastim	Neupogen®	NIAID	Recombinant cytokine	sc	0.1	I (NCT00025805)	N/A
GM-CSF	Sargramostim	Leukine®	Bayer HealthCare Pharmaceuticals, Inc.	Recombinant cytokine	sc	0.1	III	N/A
IL2	Daclizumab	Zenapax®	PDL BioPharma, Inc.	Humanised mAb	iv	20	N/A	II (NCT00073047)
IL2	Basiliximab	Simulect®	Cerimon Pharmaceuticals	Chimeric mAb	iv	7	N/A	II (NCT00430898)
IL6	C326	–	Avidia	Avimer	iv	7	I (NCT00353756)	N/A
IL6	Tocilizumab	MRA, R-1569, atlizumab, Actemra®	Chugai Pharmaceuticals	Humanised mAb	iv	4	II	N/A
IL10	Tenovil®	–	Schering-Plough	Recombinant cytokine	sc	0.1	III	N/A
IL11	Oprelvekin	Neumega®, rhIL-11	Wyeth	Recombinant cytokine	Oral	0.1	II (NCT00040521)	I (NCT00038922)
IL12/IL23p40	ABT-874	J695, briakinumab	Abbott	Humanised mAb	iv	9	II (NCT00562887)	N/A
IL12/IL23p40	Ustekinumab	CNT0 1275	Centocor, Inc.	Fully human mAb	iv, sc	20	III	N/A
IL12/IL23	Apilimod mesylate	STA-5326	Synta Pharmaceuticals Corp.	Small molecule	Oral	N/A	II (NCT00234741)	N/A
IL15	AMG-714	HuMax IL15™	Genmab, Amgen, Immunex	Fully human mAb	sc	N/A	N/A	N/A
IL17A	AIN457	Secukinumab	Novartis Pharma GmbH	Fully human mAb	iv	N/A	II	N/A

Interferon-β	r-IFN-beta 1a	Rebif®	EMD Serono	Recombinant cytokine	sc	3	N/A	II (NCT00303381)
Interferon-β	r-IFN-beta 1b	Betaseron®	Bayer HealthCare Pharmaceuticals, Inc.	Recombinant cytokine	sc	0.1	N/A	II (NCT00303381)
Interferon-γ	Fontolizumab	HuZAF™	PDL BioPharma, Inc.	Humanised mAb	sc	80	II	N/A
TNFα	Etanercept	Enbrel®	Wyeth	Recombinant cytokine receptor	sc	0.6	II	N/A
TNFα	Onercept	r-TBP-1	EMD Serono	Recombinant cytokine-binding molecule	iv	14	N/A	N/A
TNFα	CDP-571	Humicade®	UCB Pharma	Chimeric mAb	iv	18	II (NCT00072943)	N/A
TNFα	Infliximab	Remicade®	Schering-Plough	Chimeric mAb	iv	7	Approved	Approved
TNFα	Adalimumab	D2E7, Humira®	Abbott	Fully human mAb	sc	15	Approved	III (NCT00573794, NCT00385736, NCT00408629)
TNFα	Certolizumab pegol	CDP-870	UCB Pharma	Humanised Fab' fragment	sc	14	III	N/A
TNFα	Golimumab	CNTO 148	Centocor, Inc.	Fully human mAb	iv, sc	7	N/A	III (NCT00488631, NCT00488774)

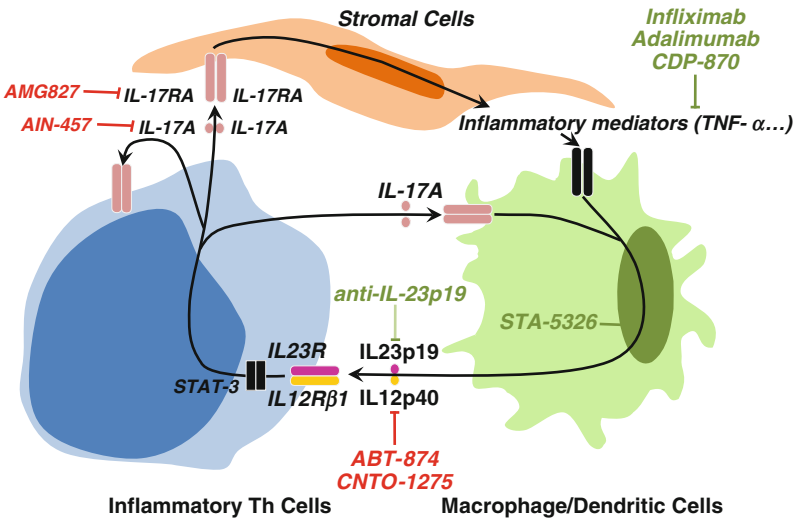

Fig. 1 Overview of effective (in *green*) and ineffective (in *red*) biologic agents targeting IL17, IL22, IL23 and TNFα in CD

1.2 How to Treat CD Patients Resistant to TNFα-Neutralising Therapy?

Other molecules are currently evaluated that directly target T-cell-derived factors, such as anti-interferon-γ (IFNγ) (Table 1). Fontolizumab is a humanised monoclonal IgG1 antibody which binds and blocks IFNγ. IFNγ plays a pivotal role in the pathogenesis of IBD. Two European clinical trials evaluated independently the safety and efficacy of fontolizumab in moderate-to-severe adult CD patients [20, 21]. In both studies, treatment was generally well tolerated, and a significant induction of the remission rate was observed in about 60 % of treated patients when compared to placebo group. Consistently, subcutaneous administration of fontolizumab triggered biological response, as shown by the decrease of serum C-reactive protein (CRP) level [20, 21] and/or the median CD endoscopic index of severity [21]. In the most recent study including a total of 201 patients with active CD who were randomised to receive fontolizumab or placebo, clinical response rates were similar in both treatment groups. Nevertheless, fontolizumab was well tolerated, and a significant decrease in CRP levels was also observed in fontolizumab-treated patients when compared to controls, confirming a biological response [22]. Further studies are still warranted to assess its clinical efficacy.

More recently, several small molecules inhibiting IL12-related cytokines have been evaluated, such as apilimod mesylate that is a unique morpholinopyrimidine derivative (Synta Pharmaceuticals Corp.). Following selection from an 80,000-compound library and optimisation, this compound, also known as STA-5326, was found efficient in selectively downregulating the IFNγ/*Staphylococcus*

aureus-induced expression of both IL12 p35 and IL12/IL23 p40 subunits in human peripheral blood monocytes without affecting their viability [23] (Fig. 1). Oral administration of STA-5326 at a dose of 10 mg/kg was also effective in improving the severity of T-cell-mediated colitis by inhibiting the secretion of IFNγ by *lamina propria* mononuclear cells in mice [23]. Despite the observation of dizziness, nausea, headache and fatigue as the most common STA-5326-induced side effects, an open-label trial has reported encouraging preliminary results in regard to the safety and effectiveness of oral daily administration of STA-5326 [24]. However, a randomised, double-blinded and placebo-controlled phase IIb clinical trial failed to demonstrate efficacy of a 4-week oral daily administration of 50–100 mg of STA-5326 to adult patients with moderate-to-severe, active CD (CDAI score 220–450) [25].

A human recombinant full-length IgG1 λ genetically modified to recognise IL12 p40 subunit (ABT-874 or also referred as J695 or briakinumab; Wyeth Research and Abbott Laboratories in collaboration with Cambridge Antibody Technology) has been evaluated in a multicentre, randomised, placebo-controlled, double-blind phase II clinical trial [26] (Fig. 1). Despite decreased expression of IL12, IFNγ, TNFα and IL17 in colonic *lamina propria* mononuclear cells [26, 27], 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 weeks of follow-up, it failed to be effective in inducing clinical response and remission [26], suggesting a pharmacodynamic effect. The efficacy (defined as a CDAI score $<$150 points after 6 weeks), safety (at 12- and 24-week post-administration of placebo or ABT-874) and pharmacokinetics of intravenous infusions of 400 and 700 mg every 4 weeks have been evaluated by a phase II clinical trial for the treatment of moderate-to-severe CD in adults, but the conclusions have not yet been released (NCT00562887).

Additional biologics remain in the pipeline and might appear promising. Notably, a multisite, randomised, placebo-controlled phase II/III clinical study evaluated the safety and efficacy of intravenous and subcutaneous infusion of CNTO 1275 in patients with moderate-to-severe CD, over a 54-week period (Centocor Inc. in collaboration with Medarex). CNTO 1275, also referred as ustekinumab, is a fully human IgG1 κ monoclonal antibody, which binds to the IL12/23 p40 subunit with high affinity, thereby neutralising subsequent activation of the IL12/23-dependent signalling by blocking its binding to the IL12Rβ1 in activated human peripheral blood mononuclear cells [28] (Fig. 1). At 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 vs. 30.2 % of placebo-receiving patients, $p = 0.05$). In patients previously treated with infliximab, CNTO 1275 showed greater efficiency ($p = 0.02$) [29]. Consistently, short-term intravenous treatment with CNTO 1275 was sufficient to trigger biological response in moderate-to-severe CD patients despite conventional therapy, as shown by reduced serological expression of inflammatory mediators [30]. Nevertheless, long-term follow-up of the safety and efficacy of CNTO 1275 maintenance therapy is now eagerly awaited. On the basis of what we have learned

from the above-mentioned clinical trials using pharmacological compounds targeting IFNγ or IL12, novel biologic therapy targeting either IL17 or 22 shall be a more efficacious cytokine-based therapeutic strategy.

1.3 IL17A: A Potential Magic Bullet in CD?

Besides Th1 cells and their related cytokines (e.g. TNFα, IFNγ or IL12), several lines of evidences suggest that IL17A plays a predominant role in the pathogenesis of CD. Primarily secreted by $CD4^+$ and $CD8^+$ lymphocytes, the isoform IL17A 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 IL17R, IL17A exerts a synergistic effect on the cellular response to additional cytokines [31]. In CD, IL17A-secreting cells are more abundant within the inflamed mucosa of patients [7]. Interestingly, commitment to Th17 development is potentiated by the bacterial muramyl dipeptide, which is a cognate agonist of the CD-associated molecule NOD2 [32]. Similarly, Th17 differentiation and IL17A secretion are dampened in germ-free mice [33], as well as in animals deficient for MyD88—an essential adaptor of Toll-like receptor signalling [34]. In mice, IL17A is also found to be elevated in spontaneous models of IBD [35]. This pro-inflammatory role of Th17 cells has been confirmed in several mice models of colitis including the SCID transfer model. Notably, infusion of Th17 cells to SCID mice induced a more severe disease than did Th1 cells, further suggesting the predominant role of Th17 over Th1 response in CD [36]. Conversely, resistance to colitis in IL23 knockout mice correlated well with the absence of IL17A-producing $CD4^+$ T lymphocytes despite normal presence of antigen-specific IFNγ-producing Th1 cells [6]. Importantly, a recent report showed an enhanced accumulation of IL23-responsive innate lymphoid cells (ILCs) producing IL17A in the intestine of CD patients [4]. Consistently, responsiveness to IL23 is promoted by the transforming growth factor-β that is found to be over-expressed in CD [37]. Expressing IL23R, Th17 cells exhibit certain functional features of Th1 cells, such as their low cytotoxicity, ability to help B cells and poor potential to control regulatory T cells [38]. Besides its pro-inflammatory role, IL23-dependent signalling events have been shown to protect against enteropathogens, such as *Citrobacter rodentium* [37] and *Helicobacter hepaticus* [39]. In addition, mice lacking $IL23p19^{-/-}$ failed to develop colitis in the absence of IL-10 while knockout mice for the Th1 cytokine $IL12p35^{-/-}$ develop a strong IL10-dependent [6], alpha TCR-dependent colitis in mice [40]. Furthermore, inhibition of IL23p19 by a neutralising monoclonal antibody was effective in both prevention and treatment of active colitis in mice [36], but induction of colitis by TNBS in IL23p19-deficient mice led to more severe inflammation than in wild-type controls [41]. The above-mentioned data collectively showed that IL23 plays a major role in CD that is consistent with previous genome-wide association studies. Noteworthy, a rare

coding variant of IL23R has been reported to confer disease protection by impairing IL23-induced Th17 function, but not differentiation *per se* [42]. Taken together, IL17 represents a relevant therapeutical target in IBD.

Similarly to monoclonal antibodies targeting the p40 subunit of IL12/IL23, pharmacological blocking using an IL17R IgG fusion protein as well as genetic inactivation of IL17R improved hapten-induced colitis in mice [43]. Recent clinical trials in asthma and autoimmune diseases prompted a clinical evaluation in IBD. A multicentre, randomised, double-blind, placebo-controlled, parallel-group study (NCT00584740) evaluated the efficacy, safety and tolerability of two intravenous infusions of a fully human anti-IL17A monoclonal IgG1k (referred as AIN457 or secukinumab; 10 mg/kg; Novartis Pharma GmbH) in patients with moderate-to-severe active CD from North America (Fig. 1). Isolated from a hybridoma, the recombinant AIN457 presents an affinity of 0.227 nM for human IL17 and a dissociation equilibrium constant K_D for binding to IL17 of about 0.188 $\pm$ 0.036 nM, as defined by BIAcore measurements (WO/2007/117749). Surprisingly, a double-blind, placebo-controlled clinical trial on a cohort of 59 CD patients revealed that blockade of IL17A was ineffective in CD. Moreover, a post hoc analysis showed response rates in favour of placebo, suggesting that inhibition of IL17A would rather exacerbate CD [44]. Consistently, transfer of T cell populations from IL17A-deficient mice to *Rag1*$^{-/-}$ mice accelerated the onset of disease coincident with higher IFNγ levels [45]. Finally, a fully human anti-IL17RA IgG2 antibody (AMG827; Amgen) that blocks signalling of IL17A, IL17F and IL25 was also inefficient in phase II for the treatment of moderate-to-severe CD patients (NCT01150890). Taken as a whole, the use of IL17A/IL17RA blocking antibodies was disappointing in the treatment of human CD but challenged our understanding of the regulatory factors of pathogenic Th17 cells during the course of the disease.

1.4 What Is Beyond IL17: Targeting Innate Lymphoid Cells?

Why anti-IL17A monoclonal antibody failed to be effective in CD but not in other autoimmune disorders? Recent findings may give preliminary answers. Over-expressed in CD [46], IL22 is thought to be involved in wound healing and in host defence [47]. IL22 is primarily produced by certain subsets of Th17 cells but also by ILCs, referred as ILC22 [48]. Interestingly, IL17A antibody neutralisation decreased airway inflammation in mice deficient for IL22, whereas IL17A antibody blockade during the antigen challenges partially restored inflammation in immuno-competent host [49]. In the colon, we thereby suspect that IL17A functions more likely as a negative regulator of inflammation when innate sources of IL22 are limited upon injury. Further investigations are now warranted to determine whether a defective secretion of innate cell-derived IL22 is found in CD.

Another therapeutic perspective would be to act on additional pathways involved in the secretion of pro-inflammatory Th17 cytokines. A striking

characteristic of effector T lymphocytes in CD is the presence of NK receptors on their surface [50, 51]. We have identified a subset of effector $CD4^+$ T cells mediating inflammatory response in CD and expressing the NK-activating receptor NKG2D [50]. $CD4^+NKG2D^+$ T cells produced high levels of IL17 and IL22 and expressed the surface markers CCR6, IL23R, CD161 and the master Th17 transcription factor RORC in CD [52]. These results suggest that the NKG2D pathway could represent a specific therapeutic target to correct Th17 function in CD. Consistently, a neutralising anti-NKG2D antibody has already shown its efficacy to reduce the severity of colitis in mice [53].

2 Concluding Remarks

Cytokines play a key role in orchestrating gut mucosal immune dysfunction in CD and thus become an important target for the development of therapeutic modalities for the disease. Both experimental and clinical evidences showed that IL17A, IL22 and IL23 are involved in CD pathogenesis. Nevertheless, targeting the IL17A/IL17RA axis is still challenging in humans, in that CD patient may be characterised by impaired innate and/or adaptive secretion of IL22 [49]. Advances in our understanding of the regulation of IL17 and IL22 function might provide crucial clues to the development of more rational therapeutic ways directed towards the restoration of the integrity of the mucosal immune system in CD [7]. In the near future, the question on whether IL22 is a reliable therapeutic target for IBD patients will be answered, hopefully.

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Role of IL-23, IL-17, and IL-22 in Psoriasis

Robert Sabat and Kerstin Wolk

Abstract Psoriasis is a chronic, relapsing skin disease that affects at least 2 % of the Caucasian population. The characteristic skin alterations usually appear in early adulthood and markedly stress the patients for the rest of their life. Additionally, psoriasis is often associated with arthritis and metabolic alterations leading to shortened life expectancy. The results from numerous in vitro tests, mice experiments, and clinical sample investigations strongly suggest that IL-23, IL-17, and IL-22 play important roles in psoriasis pathogenesis. This chapter provides an overview about the significance of these mediators in this process and the benefit of targeting their action.

1 Psoriasis: A Common Chronic Disease with Characteristic Skin Alterations

Psoriasis is a chronic, relapsing skin disorder [1, 2]. With a prevalence of about 2 % in the Caucasian population, it is a very common disease in Europe and North America. In other ethnic groups, the prevalence of psoriasis is much lower. Men and women are affected at the same rate. The first manifestation of the disorder frequently occurs around the age of 20 (type I or early onset psoriasis) or between 50 and 60 (type II or late onset psoriasis). Type I psoriasis, which accounts for approximately 75 % of all psoriasis patients, is associated with a more severe course of disease and a limited success of treatment [3].

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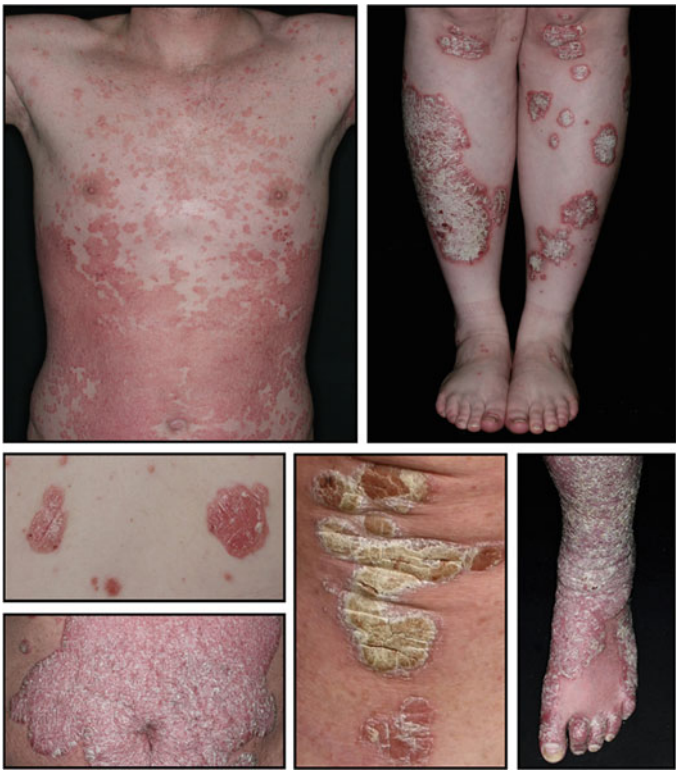

Fig. 1 Typical clinical pictures of patients with psoriasis vulgaris

Psoriatic skin lesions are variform, sharply demarcated, red, and slightly raised with silver-whitish scales (Fig. 1). Frequent locations for these lesions include the extensor side of the extremities, the sacral region, and the head [1–3]. The lesions often have a small point form at the onset. During the course of the disease, they grow and can develop a geographic shape or, in very severe cases, cover the whole body. It is also common to observe nail changes in psoriasis. Additionally, more than 10 % of psoriasis patients have arthritis. Importantly, the prevalence of the metabolic syndrome is clearly higher among psoriasis patients than among controls, and patients with severe psoriasis die 3.5–4.4 years younger [4, 5].

The etiology of psoriasis comprises genetic, environmental, and lifestyle factors [1, 2, 6, 7]. Genetic disposition appears to play an important role especially for type I psoriasis. It is long known that type I psoriasis is associated with an increased prevalence of certain HLA types, such as HLA-Cw6, HLA-B13, HLA-B17, HLA-Bw57, and HLA-DR4. People with HLA-Cw6, for example, have a tenfold higher risk of disease. In addition to the HLA alleles (i.e., molecules crucial for antigen presentation to CD8+ T cells), several psoriasis susceptibility loci and gene polymorphisms/deletions/copy number variations have been described to be

associated with the disease risk [8, 9]. They concern genes associated with keratinocyte differentiation [*PSORS4* (chr. 1q21, e.g., deletion of *LCE3B* and *LCE3C*) and *PSORS1* (e.g., *CDSN*)], innate immunity (SNPs in *IL 20*, *DEFB4*, *IL 28RA*, copy number variation for *DEFB* genes), and the Th17 pathway (SNPs in, e.g., *IL 23R*, *IL 12B*, *IL 23A*, and *TRAP3IP2*) [9, 10]. Therefore, psoriasis genetics seems to be complex and concerns numerous genes, whose polymorphisms are relatively common in population and individually confer only limited contribution to disease risk. This means that environmental and lifestyle factors play an important role in the initiation and maintenance of psoriasis. In fact, several observations have been made regarding external trigger factors. An infection with β-hemolytic streptococci often precedes the first manifestation of psoriasis, in particular of psoriasis guttata [11]. Consecutive new exacerbations can be triggered by mechanical irritation (so-called Koebner's reaction; new lesions emerge at locations that are mechanically irritated), medications (e.g., β-receptor blockers, lithium, chloroquine, nonsteroidal anti-inflammatory agents, tetracyclines, and interferons), and infections (viral and bacterial infections) [6].

Already at the onset of a psoriatic lesion, histological alterations can be observed. They include dermal edema, dilatation of vessels of the papilla in the dermis, and perivascular cell infiltration composed of T cells, dendritic cells (*DC*), and monocytes/macrophages [6, 12]. Later, the density of infiltrates increases, and CD8+ T cells and neutrophilic granulocytes are found particularly in the epidermis. Neutrophilic granulocytes form very characteristic Munro's microabscesses in the epidermis. Other prominent changes are found in the epidermis: acanthosis (raised number of keratinocytes and the thickening of the spinous layer), loss of the granular layer, parakeratosis (dysfunction of the cornification process with nucleus-containing keratinocytes in the cornified layer), and hyperkeratosis (thickening of the cornified layer). In the chronic stage, the epidermal changes come to the fore (Fig. 2). At the same time, an increasing amount and dilatation of capillaries in the dermal papillae, surface vessels that facilitate a renewed immigration of the immune cells, are observed [1, 6].

In many aspects, the psoriasis pathogenesis resembles an adaptive immune reaction which leads to an overshot regeneration-like reaction of the skin tissue cells [6]. Adaptive immune reactions proceed in two main phases: the sensitization and the effector phase (Fig. 3). During the sensitization phase, professional antigen-presenting cells (*APCs*) like myeloid DCs (*mDCs*) capture locally present antigens (*Ags*) and are activated. Subsequently, they mature and migrate to the local lymph nodes (*LN*), where they present fragments of this Ag on special proteins at their surface. These mature activated APCs then induce the development of Ag-specific, skin-directed effector and memory Th17, T22, and T1 cells. The sensitization phase can be followed by a silent phase of variable length. The subsequent effector phase is provoked by exogenous triggers that activate skin resident immune cells, such as macrophages or mast cells. As a result of this activation, these cells secrete small proteins like chemokines and other inflammatory cytokines that lead to the infiltration of various immune cells (neutrophilic granulocytes, various subpopulations of

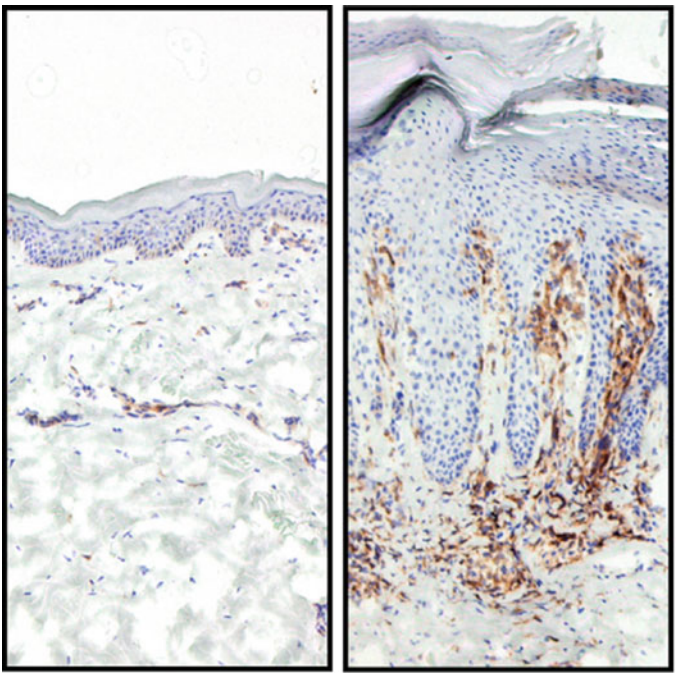

Fig. 2 Microscopic alterations of psoriatic skin

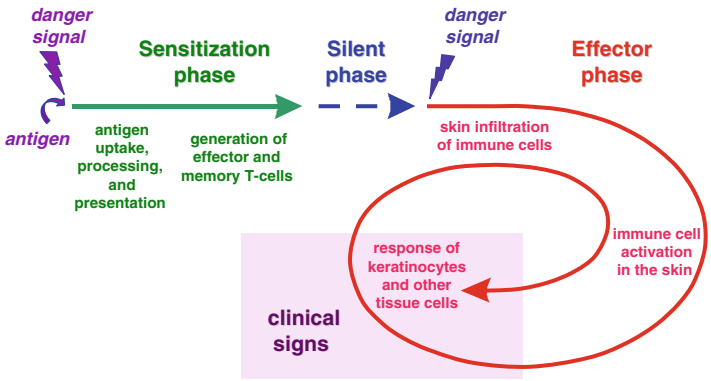

Fig. 3 Phases of the psoriasis pathogenesis

T cells, monocytes/macrophages, and various subpopulations of DCs) into the skin. Immigrated immune cells are enabled to produce further cytokines that activate local cells to strengthen their secretion of chemokines and other inflammatory mediators that, in turn, further support the infiltration process. Over time, the simultaneously happening processes, including immune cell infiltration, immune cell activation, and tissue cell activation, amplify each other, making the effector phase becoming self-employed. Finally, the cocktail of cytokines present in the skin

alters the biology of keratinocytes and endothelial cells, resulting in a reaction that reminds an overshot regeneration process (like wound healing). Importantly, only the last part of effector phase creates the visible psoriasis-typical skin alterations. After a successful treatment, the effector phase transposes into the silent phase. However, a weak exogenous trigger can again initiate the effector phase.

The numerous results from in vitro tests, mice experiments, and clinical trials strongly suggest that IL-23, IL-22, and IL-17 play important roles in psoriasis pathogenesis. In the following chapters, we shortly described the biology and significance of these mediators in this situation.

2 Target Cells of IL-23, IL-17, and IL-22 in Psoriasis

IL-23, IL-17, and IL-22 are small proteins that influence cells by binding to the extracellular part of their specific transmembrane receptors. This interaction changes the conformations of the receptors and leads to phosphorylation of kinases associated with the intracellular receptor part. The activated kinases induce numerous intracellular changes concerning among others protein modifications, gene expression, protein synthesis and secretion, vesicle transport, and cytoskeleton state. In the majority of cases, the most reliable way to identify the target cells for cytokines is the analysis of cellular expression of respective receptor complexes and specific signaling events.

IL-23 is a member of the IL-12 family of cytokines, a part of the IL-6 superfamily. It acts via a receptor complex composed of IL-23 receptor (*IL-23R*) and IL-12Rβ1 [13, 14]. IL-23 itself is a disulfide-linked heterodimer consisting of two different subunits: proteins with molecular weights of 19,000 (p19) and 40,000 (p40) Dalton [15]. During the binding of IL-12 to its receptor complex, p40 interacts with IL-12Rβ1 and p19 with IL-23R1. IL-23R and IL-12Rβ1 are highly expressed on memory T, NKT, and NK cells, and on naïve T cells upon activation by TGF-β and IL-6. Engagement of the IL-23 receptor complex activates Janus kinase 2 (*Jak2*, associated with IL-12Rβ1) and tyrosine kinase 2 (*Tyk2*, associated with IL-23R) [13, 14]. These kinases subsequently phosphorylate the intracellular part of the receptor complex and thereby create docking sites for signal transducer and activator of transcription 3 (*STAT3*) and STAT4. The STATs are subsequently phosphorylated, and dimerize and translocate into the nucleus to activate target genes.

The receptor complex for both IL-17A and IL-17F is composed of IL-17RA and IL-17RC [16–20]. IL-17RC interacts with IL-17A and IL-17F with comparably high affinity (KD ~1 nM). In contrast, IL-17RA binds IL-17A (KD ca. 2 nM) but not IL-17F (~1,000-fold lower affinity) effectively [21]. However, the absence of either chain prevents the cytokine-induced response [22]. Whereas IL-17RA is broadly expressed in hematopoietic and nonhematopoietic cells, IL-17RC is thought to be mainly expressed by nonhematopoietic cells and macrophages [17, 23, 24]. Following interaction with its receptor complex, IL-17A/F preferentially activates the NF-κB and MAP kinase (p38 kinase, JNK, and ERK1/2) pathways [20, 24, 25].

IL-22 belongs to IL-10 family of cytokines, a part of IL-10-IFN superfamily [26]. The IL-22 receptor complex is composed of IL-22R1 and IL-10R2 [27–29]. IL-10R2 is ubiquitously expressed [30], which can be explained by its function as part of several cytokine receptors [26]. Therefore, the expression of the IL-22R1 subunit determines whether a cell is an IL-22 target or not. Interestingly, several studies demonstrated lacking IL-22R1 expression in bone marrow, blood mononuclear cells, thymus, and spleen, as well as in resting and activated primary immune cells including monocytes, B cells, T cells, NK cells, macrophages, and DC populations [30–33]. In contrast to immune cells, a few organs express IL-22R1 and therefore contain putative target cells of IL-22 [30]. 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 skin and pancreas. Interestingly, most IL-22R1-expressing tissues form outer body barriers and contain epithelial cells. Regarding isolated cell populations, IL-22R1 is expressed on keratinocytes, bronchial and intestinal epithelial cells, intestinal subepithelial myofibroblasts, hepatocytes, and pancreas acinar cells [34]. Like IL-23, IL-22 activates a signaling cascade via the Jak/STAT pathway in its target cells. IL-10R2 is associated with Tyk2, and IL-22R1 appears to be associated with Jak1. The main STAT molecule activated by IL-22 is STAT3 [34]. Additionally to STAT3 tyrosine phosphorylation, IL-22 sometimes leads to activation of STAT1 and/or STAT5. Furthermore, serine phosphorylation of STAT3 and an activation of the three major MAP kinase pathways (p38 kinase, JNK, and ERK1/2) by IL-22 were reported [35, 36].

3 Effects of IL-23, IL-17, and IL-22 with Relevance for Psoriasis

IL-23, IL-17, and IL-22 might play important roles in psoriasis pathogenesis. However, it seems that each of these mediators decisively contributes to different steps of pathogenic cascade, namely:

- IL-23 to the generation of effector and memory T cells during the sensitization phase and to the T effector cell activation/survival during the effector phase
- IL-17 to the immune cell infiltration and tissue cell activation during the effector phase
- IL-22 to the immune cell infiltration and tissue cell activation during the effector phase

3.1 Sensitization Phase: Generation of Effector and Memory T cells

In secondary lymphoid organs, incoming matured, Ag fragment-presenting mDCs induce and govern the development of effector and long-living memory T cells

from naïve T cells. This evolution is necessary for the T cells to obtain substantial functions and the ability to immigrate into peripheral tissues.

The naïve [L-selectin (CD62L)- and CCL7-expressing] T cells continuously circulate between blood and secondary lymphoid organs, such as lymph nodes, spleen, and tonsils. In T cell areas of these organs, they foregather with mature mDCs. If the TCR of a naïve CD4+ T cell has an adequately high affinity for binding a MHC II molecule-Ag peptide complex, this T cell sticks to the respective mDC. Importantly, this interaction ensures the specificity of the developing effector T cells because (1) the structure of the portion of the MHC II–Ag peptide complex is recognized by only a specific TCR, (2) only one type of TCR is expressed by a naïve T cell, and (3) this TCR type is transmitted to its offspring.

The interaction between TCR and MHC II–Ag peptide complex is stabilized by the T cell transmembrane molecule named CD4 that binds to a nonpolymorphic region of the MHC II molecule.

The specific contact area between a T cell and mDC is named immunological synapsis and represents a unique arrangement of molecules within it [37]. The initial interaction between the T cell and the mDC is stabilized by nonspecific binding through adhesion molecules that actively form a ring around the clustered MHC II molecule–Ag peptide–TCR complexes. Further important components of the immunological synapsis include costimulatory molecules, such as the members of the B7 family: CD86 (B7-2), CD80 (B7-1), B7h, PD-L1, and PD-L2 [38]. For example, CD86 and CD80 interact with the T cell molecules CD28 and CD152 (cytotoxic T-lymphocyte-associated protein 4, *CTLA-4*). Costimulatory signals are necessary for full activation of T cells, their survival, and development of functioning memory cells [38, 39].

A third signal is essential for the proliferating T cells to obtain their effector function (polarization). According to the current knowledge, naïve CD4+ T cells can be polarized into six different directions: Th1, Th2, Th17, Th22 cells, inducible regulatory T cells (*iTreg*), and type 1 regulatory T cells (Tr1). Th1, Th17, and Th22 cells appear to be mainly important for the psoriasis pathogenesis.

If IL-12 (p35/p40 heterodimer) is present, the T cells are polarized into Th1 cells through activation of the transcription factors STAT4 and T-bet [40–43]. The generation of Th1 cells is further supported by interferon (*IFN*)-γ, which increases the expression of IL-12Rβ2. During repeated activation, Th1 cells primarily secrete IFN-γ, IL-22, and TNF-α.

The activation of naïve T cells in the presence of transforming growth factor (TGF)-β, IL-6, and IL-1β induces Th17 cell development and IL-23R expression [41, 44]. IL-23 plays an essential role in the maintenance and survival of these cells. The Th17 development is dependent on endogenous expression of retinoic acid-related orphan receptor (*ROR*)γt, RORα, STAT3, and IRF4. Th17 cells primarily produce IL-17, IL-26, and GM-CSF. STAT3 phosphorylation has been shown to be required for IL-17A and IL-17F production.

In the presence of TNF-α, activators of the hydrocarbon receptor (*AHR*), and IL-6 or IL-23 (Sabat unpublished), naïve T cells are polarized toward Th22 cells [45, 46]. These cells primarily secrete IL-22 during repeated activation.

Interestingly, the different Th cell subsets display distinct patterns of chemokine receptors on their surface. CXCR3 and CCR5 expression characterizes Th1 cells. Th17 cells express CCR4 and CCR6 but no CXCR3, and Th22 cells bear CCR4, CCR6, and CCR10 [45–47].

3.2 Effector Phase: Infiltration of Immune Cells into the Skin

The superficial perivascular infiltrates of lymphocytes and monocytic cells and the dilatation of the blood vessels in the dermal papillae appear as the first microscopically visible events during the development of psoriatic plaques. The migration of immune cells out of the blood vessels into the surrounding tissue might be induced by inflammatory cytokines released from activated resident macrophages, mast cells, or DCs. Such cytokine release may occur upon minimal injury due to the entry of small numbers of microbes from the body surface into the skin and/or the destruction of few keratinocytes. In fact, via Toll-like receptors, microbial products activate Langerhans cells, macrophages, and mast cells to secrete TNF-α and IL-1β. Damaged keratinocytes release self-RNA, which upon association with the antimicrobial peptide cathelicidin (*LL37*) is able to induce TNF-α and IL-6 production by mDCs in a Toll-like receptor 8-dependent manner.

The mechanisms by which TNF-α provokes skin infiltration of immune cells include (1) the activation of the endothelia enabling them to interact with blood immune cells and (2) the induction of a broad chemokine production in the skin tissue cells (keratinocytes, fibroblasts, and endothelial cells).

In the endothelial cells, TNF-α causes the transport of so-called Weibel–Palade bodies containing P-selectin to their cell surface and increases the synthesis of P- and E-selectin [6]. As a consequence, P- and E-selectin molecules are highly expressed on the endothelial cell surface and interact with their ligands on immune cells. Additionally, TNF-α induces the production of vasodilator substances (e.g., prostaglandins). These changes enable the rolling of immune along endothelial cells of cutaneous postcapillary venules, representing the initial step in the extravasation process [6].

During the next step, the chemokines via their transmembrane G-protein-coupled receptors activate immune cells and induce a conformational change in the integrin molecules and their consequent increased affinity to their endothelial ligands. The numerous chemokines whose secretion is induced by TNF-α in keratinocytes, fibroblasts, and endothelial cells include CXCL11, CCL20, CCL2, CXCL8, and CCL17 (the latter only in dermal endothelial cells) [48–53]. The interaction between CCL17 and CCR4 expressed by T cells might be relevant for the Th17 and Th22 cell migration into the psoriatic skin. In line, in psoriasis lesions, the majority of CD4+ T cells and about half of the CD8+ T cells express CCR4. CXCR3 ligands (CXCL9, CXCL10, CXCL11) may be particularly important for the migration of Th1- and CD8+ T cells into the epidermis. Another chemokine/chemokine receptor pair is composed of CCL20 and CCR6 and is responsible for

the skin infiltration of Th17 and Th22 cells and mDCs. Via CCR2, CCL2 attracts macrophages and mDC precursors. Importantly, TNF-α is not the only cytokine with great chemokine induction capability. IL-17 induces chemokines in keratinocytes, fibroblasts, and endothelial cells as well; these include CXCL1, CXCL5, CXCL8 (attracting neutrophilic granulocytes), and CCL20 [48, 49, 54–56]. Importantly, IL-17A and IL-22 enhance the upregulating effect of TNF-α on some chemokines (e.g., CXCL1, CXCL8, and CCL20) [48, 49, 54, 57].

As mentioned above, immune cells after activation by chemokines change the conformation of integrin molecules that lets an affine binding between integrins and their ligands on endothelial cells and in consequence a tight adhesion of immune on endothelial cells. CD11a/CD18 seems to be the most important integrin for skin homing. It binds to endothelial CD54 (*ICAM-1*) and CD102 (*ICAM-2*). Interestingly, TNF-α induces the expression of endothelial CD54 (ICAM-1) and CD102 (ICAM-2).

The following passage of immune cells through the endothelial wall is called diapedesis. It is also dependent on integrins and takes place probably via gaps formed between endothelial cells. TNF-α, IFN-γ, and IL-22 can cause endothelial cells to remodel the basal membrane [36]. As a consequence of the infiltration, massive immune cell infiltrations are found in psoriatic lesions. The numbers of T cells, macrophages, and DCs are very similar in that situation. Among T cells, CD4+ cells are mainly present in the dermis, while CD8+ cells primarily home into the epidermis.

3.3 Effector Phase: Immune Cell Activation in the Skin

After their migration into the skin, the immune cells are activated. Immigrated DCs might be stimulated by means of self-RNA–LL37 complexes as well as by neutrophilic granulocytes and NK cells. In the dermis and epidermis, different APC populations such as macrophages and various types of DCs activate T cells, and vice versa. T cells arriving in the epidermis may additionally be stimulated by keratinocytes. As a consequence of activation, the T cells proliferate. Psoriatic lesions even demonstrate some characteristics of lymph nodes. The T cell mediators with the highest expression in psoriatic lesion and the greatest difference in the expression between nonlesional and lesional skin are IL-17A, IL-17F, and IL-22 [30, 58, 59]. IL-17A and IL-17F are apparently secreted by effector/memory Th17 cells [58]. However, also mast cells and neutrophilic granulocytes have very recently been described to produce IL-17 in psoriasis lesions [60]. The main source of IL-22 in psoriatic lesions should be T22 and T1 cells [31, 45, 46, 59]. The expressions of IL-17A, IL-17F, and IL-22 are approximately ten times higher than that of IFN-γ [59]. Further cytokines present in psoriatic lesions include IL-26, with levels similar to IFN-γ, and IL-21, IL-13, and IL-4, with rather low levels.

As far as it is known, IL-22 is the only T cell cytokine that is present in the blood of psoriasis patients at detectable levels [36]. Interestingly, the IL-22 blood levels correlate with the severity of disease [36].

As mentioned above, T cells in psoriatic lesions are apparently activated by APCs. An important question for the understanding of the psoriasis pathogenesis is how monocytes/macrophages and DCs activate these immigrated T cells. We assume that in this process, APC-derived IL-23 plays a dominant role. Indeed, the number of IL-23-expressing myeloid cells and the IL-23 expression levels are increased in psoriatic skin. In contrast to IL-23p19, IL-12p35 levels are not upregulated. Another question is about the Ags that are involved in the stimulation of T cells in the skin. So far, this question cannot be answered.

3.4 Effector Phase: Tissue Cell Response

The most striking signs of the tissue cell response in psoriatic lesions include the profoundly altered epidermis structure and the extension of the blood vessels in the upper dermis. Upon the initiation of psoriatic lesions, keratinocytes are mainly activated by mediators produced by Th1 cells (IFN-γ, IL-22) and by Th17 and Th22 cells (IL-17, IL-26, IL-22). Over time, however, the cytokines produced by macrophages and DCs (TNF-α, IL-6, IL-24) and lastly by stromal cells in the dermis (keratinocyte growth factor (*KGF*), insulin-like growth factor 1, and fibroblast growth factor 10 (*FGF-10*)) might become increasingly important. Activated keratinocytes secrete chemokines (e.g., CXCL8, CCL20, and CXCL9) and show enhanced CD56, CD1d, and HLA I and II expression. Together, this strengthens the influx of immune cells into the lesions and facilitates the immune cell activation, respectively. Furthermore, keratinocytes produce numerous mediators such as IL-19, IL-20, TGF-α, and nerve growth factor (*NGF*) that activate stromal cells in the dermis and, in autocrine self-amplifying loops, add to their own activation. Finally, the stimulation of keratinocytes by the cytokine mix present in established psoriatic lesions leads to (1) increased proliferation of these cells in the basal layers, (2) alteration of their maturation, and (3) their elevated production of antimicrobial proteins and cytokines.

The considerably elevated keratinocyte proliferation in psoriatic lesions is caused by an increase of the interfollicular epidermal stem cell number and by an approximately fivefold shortening of their cell cycle duration. This hyperproliferation significantly contributes to characteristic thickening of epidermis (acanthosis). Until today, the mediator(s) that is/are responsible for this fast proliferation could not be identified [7].

In normal skin, the maturation of keratinocytes from the epidermis basal layer to the cornified layer takes approximately 28 days. This process is abbreviated in psoriatic lesion to approximately 5–7 days. The last step of the maturation process is called the terminal differentiation. This is a particular apoptotic process that encompasses the stepwise transition of vital keratinocytes of the upper epidermis layers to corneocytes and results in the formation of the mechanically resistant stratum corneum. This process starts in the stratum spinosum with the synthesis of necessary proteins. The diverse alterations in terminal differentiation include the

aggregation of keratin-(K)1 and K10 chains in macrofibrils, the formation of the cornified envelope of the keratinocytes by cross-linking of newly synthesized structural proteins, the synthesis of extracellular lipids, and the dissolution of the cell nucleus and other cell organelles.

The massively inhibited keratinocyte terminal differentiation in psoriasis contributes to the epidermal acanthosis as well. It additionally leads to hyperkeratosis, parakeratosis, and hypogranularity [6]. Unlike the mediators of hyperproliferation, the mediators that are very likely to be responsible for the altered keratinocyte differentiation in psoriasis lesions have been identified. These include IL-22 and IL-20 [61]. IL-22 and IL-20 inhibit the expression of proteins that are crucially involved in the terminal differentiation of keratinocytes: profilaggrin, K1, K10, calmodulin-like-5, keratinocyte differentiation-associated protein, cornified envelope protein 1B, desmocollin-1, and kallikrein 7 [36, 48]. By reducing the levels of these proteins, IL-22 and IL-20 affect all the steps of the terminal differentiation and lead to the appearance of corneocytes with remaining nuclei and cytoplasm rests that are not continuously exfoliated because of alterations in the corneodesmosomal degradation. Importantly, TNF-α, IFN-γ, and IL-17 may amplify some effects of IL-22 and IL-20 by enhancing the expression of their transmembrane receptors and/or by influencing their signal transduction [36, 48]. Like IL-22, IL-20 exclusively affects nonhematopoietic cells [31, 32, 62]. Whereas IL-22 mediates its effects through a receptor complex composed of IL-22R1 and IL-10R2, IL-20 can affect keratinocytes via two different receptor complexes: IL-20R1/IL-20R2 and IL-22R1/IL-20R2 [63]. It seems that the IL-22R1 receptor chain is responsible for the psoriasis-relevant effects of these two mediators. In the skin, IL-22R1 is preferentially expressed by keratinocytes and only to a minimum extent by fibroblasts; it is absent in resting and activated immune cells [30, 32, 48].

Interestingly, IL-22 provokes mechanisms that may enhance its own action, in the sense of a positive feedback regulation. An example is the induction of STAT3 that is the major transcription factor activated upon IL-22 receptor signaling and that is essential for the IL-22 effects [48]. Interestingly, mice, which transgenically express a constitutively active STAT3 variant in keratinocytes, develop psoriasis-like skin alterations [64]. IL-22 also induces the keratinocyte production of IL-20 [65] that, as mentioned above, demonstrates IL-22-like effects on keratinocytes [48, 66].

The expressions of diverse antimicrobial proteins, including β-defensin 2 (*BD2*), BD3, S100A7 (*psoriasin*), S100A8, S100A9, and LCN2, are strongly enhanced in lesional skin of psoriasis patients [6]. This elevation is probably responsible for the low frequency of bacterial skin infections that, despite their altered skin barrier function, is observed in these patients. The reason for the increased production of the antimicrobial proteins is the simultaneous action of specific cytokines, of which IL-17 and IL-22 play the most important role [30, 67]. In fact, leaving out of IL-17 or IL-22 from a mix of several inflammatory mediators had profound and unique impact on the antimicrobial protein expression in in vitro epidermis models [59]. Interestingly, the IL-17- and IL-22-induced antimicrobial proteins kill a range of different microbes. BD2 acts against gram-negative bacteria, whereas BD3 destroys gram-positive bacteria. S100A7 acts against *Escherichia coli*, and S100A8/A9

inhibits gram-negative and gram-positive bacteria and some fungi. Besides producing antimicrobial proteins, activated keratinocytes produce numerous chemokines (see above) and cytokines. In fact, IL-17-stimulated keratinocytes in particular from psoriasis patients secrete, for example, IL-36α and IL-36γ [68].

Other characteristics of psoriatic lesions include the dilation and increased length of upper dermal blood vessels. Complement products and, as mentioned above, TNF-α might be responsible for the dilation. The strengthened angiogenesis, which is responsible of the strong vessel extension in the diseased skin, is probably dependent on vascular endothelial growth factor (*VEGF*) [69–71].

4 Targeting of IL-23 Pathway Members for Psoriasis Treatment

The most convincing evidences that IL-23 and IL-17 play important roles in psoriasis pathogenesis have been provided by clinical trials using anti-p40 and anti-IL-17/anti-IL-17RA antibodies, respectively. The antibodies against p40 were developed for psoriasis treatment by two different pharmaceutical companies: Centocor (ustekinumab) and Abbott Laboratories (briakinumab). Respective phase III clinical trials demonstrated that approximately 45 % (ustekinumab) [72, 73] to 55 % (briakinumab) [74–76] of psoriasis patients achieved at least 90 % reduction of their cutaneous symptoms after 12 weeks of therapy. This is about three times better than with classical antipsoriatic therapies (e.g., methotrexate) [74]. Furthermore, the biologics demonstrate a generally less systemic toxicity compared to traditional agents. However, these clinical results induce a lot of further questions. Why only a portion of patients show very good response to these therapies? What is the consequence of the IL-23/IL-12 inhibition regarding the development of the protective adaptive immunity especially in young patients? Do these therapies give serious adverse events during long treatment? Regarding the last point, Tzellos et al. described that the odds ratio for major adverse cardiovascular events (MACE) is approximately 4.2 higher in patients receiving anti-p40 antibodies compared with those receiving placebo [77]. Importantly, these results were based on clinical trials with very short observation periods (mostly 12 weeks) that include relatively young patients. That means that in real situations, the risk for myocardial infarction, cerebrovascular accident, or cardiovascular death as a consequence of anti-p40 therapy might even be higher. Interestingly, the increase in MACE was not observed in clinical trials with TNF-α-blocking biologics [78]. Furthermore, the long-lasting application of anti-p40 antibodies and consequent attenuation of T1 and T17 immunity might enhance the risk for serious infections and immune cell-controlled cancers. Regarding anti-IL-17 and IL-17RA antibodies, only results from phase II clinical trials were reported so far [79, 80]. They demonstrate very good responses to this treatment. However, studies with large numbers of patients are necessary for final judgment of the efficacy and

adverse events of these treatments. Therapies focusing on the inhibition of IL-22 effects are under development. The great advantage of such treatments would especially be a lack of "classical" immunosuppression. Especially for younger patients in whom beneficial, protective memory T1 but also Th17 cells must develop, long-term inhibition of the effects of IL-22/IL-20 would be distinctly more sensible.

5 Conclusions

The results from numerous scientific studies strongly suggest important roles of IL-23, IL-17, and IL-22 in the psoriasis pathogenesis. In the case of IL-23 and IL-17, these roles were recently proved by the outcome of clinical trials with anti-p40 and anti-IL-17/anti-IL-17RA antibodies, respectively. However, these trials revealed a profound improvement of skin symptoms by these therapies in only a portion of psoriasis patients. Furthermore, they implicate that anti-p40 therapy enhances risk at least for major adverse cardiovascular events. In consideration of these facts and the costs of such therapies, there is a great need for biomarkers that before treatment identify patients with high benefit from these therapies. In the event that the inhibition of IL-22 action will be effective in cutaneous skin diseases, this inhibition is not supposed to induce major adverse events.

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Contribution of IL-22 to Experimental Skin Inflammation

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Abstract The skin represents the first line defense against various stresses including pathogens and injuries. Cutaneous homeostasis and defenses are maintained by permanent cross talk among dermal fibroblasts, keratinocytes, and cells of the immune system residing in or recruited to skin, through the production of cytokines and chemokines. In this chapter, we discuss the biological effects of IL-22 on keratinocytes and more particularly its position in skin inflammation and in the development of inflammatory skin diseases such as psoriasis.

Focused on in vitro human models, we present the mechanisms of action of IL-22 as well as its involvement in structure, metabolism, differentiation, chemotaxis, antibacterial activity, innate immunity, and tissue remodeling of epidermis. Pertinence of these models is debated by confrontation to structural and molecular characteristic of psoriatic skin. IL-22 belongs to cytokine milieu impregnating inflammatory skin. We attempt to delineate more precisely its unique position among other proinflammatory cytokines and in the synergistic effect of these cytokines. Finally, we consider the local cell sources of IL-22 in the psoriatic skin.

We question potential new therapeutic strategies for psoriasis focused on more specific and downstream targets, i.e., keratinocyte-targeting cytokines, rather than on modulating producing cells by immunosuppression.

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1 Introduction

Skin represents a very attractive tissue that is a paradigm for studying the cross talk between the inflammatory immune system and an organ. Indeed, skin constitutes the largest physical and chemical barrier against various stress including pathogens, constituting the first line of defense. Skin is now considered as a multitasking immune organ involved in promoting health [1]. To be effective for protection against a large panel of injuries, this system requires sensitivity and selectivity to detect a signal of danger, a strong reactivity to develop a rapid response, and efficiency. These requirements need sophisticated interactions between keratinocytes, the epithelial cells of epidermis (the outer skin layer in contact with environment), and "sentinel" immune cells infiltrating normal epidermis, i.e., T lymphocytes and Langerhans cells. These interactions are conducted by cytokines, maintaining the homeostasis of skin; if required, a coordinated inflammatory response is triggered, relayed by specific cytokines. Troubles in the resolution of this phenomenon could generate a cytokine-mediated vicious circle, promoting chronic inflammation and autoimmunity, characterized by the accumulation of resident/infiltrating immune cells in the epidermis, altered differentiation of keratinocytes, and epidermal thickening.

Numerous convenient in vitro or in vivo models are available to study normal and inflammatory skin. Keratinocytes can be isolated from surgical samples from plastic surgery and cultured in liquid medium to obtain an adherent monolayer cell, known as NHEK (normal human epidermal keratinocytes), or at the air-medium interface to obtain multilayered epidermis that exhibits morphological and growth characteristics similar to human skin, known as RHE (reconstituted human epidermis) [2]. This chapter is focused on these models. In addition, mouse models are available to study skin inflammation, which however never exactly mimic human diseases [3].

During the past few years, an increasing number of reports demonstrated that keratinocytes are direct targets for a specific set of cytokines, conducting dramatic changes in their biological properties such as inducing the secretion of chemokines and antimicrobial peptides, or modulating the differentiation states or migration capacities. The intensity of the responsiveness highlighted that keratinocytes, beside their role as physical protectors, are also main actors in skin immunology. In the 1990s, functional receptors for the classical inflammatory cytokines interleukin (IL)-1 and tumor necrosis factor (TNF) or the Th1-derived interferon (IFN)-γ have been described on keratinocytes. More recently, IL-17, IL-22, and oncostatin M (OSM) have also been described as important regulators of epidermis functions, and their receptors have been detected on keratinocytes. Some of these cytokines are able to induce skin inflammation in animal models, as do IL-12 or IL-23. However, keratinocytes do not display functional receptors for IL-12 and IL-23 [4], suggesting that other epidermal cells are the targets and that the effect on keratinocytes is indirect. In addition, receptors for the Th2 cytokines IL-4 and IL-13

have been also described on keratinocytes, but the induced biological activities appear to be antagonistic to inflammation [5].

The use of transcriptomic technologies to analyze the keratinocyte gene expression profile induced by cytokines demonstrates that most of them have overlapping activities, sometimes displaying completely redundant biological properties. The use of shared molecules in intracellular signaling pathways, such as STAT3 in OSM or IL-22 signaling, could explain redundancy [6, 7]. In another hand, powerful synergistic effects on keratinocytes can be also evidenced when cytokines of different families are associated. These points are discussed below.

2 IL-22 and Keratinocytes

IL-22 is one of the cytokines of the IL-10 family. IL-10 was identified in 1989 as a "cytokine synthesis inhibitory factor" [8]. Mainly produced by immuno-hematopoietic cells, IL-10 inhibits the antigen-presentation capacity of macrophages and dendritic cells as well as IL-2 production by Th1 T cells and proinflammatory cytokines production by monocytes/macrophages. IL-10 also activates B-cell proliferation and differentiation [9].

Since the end of 1990s, five cytokines structurally related to IL-10 have been described and presently form this family of cytokines: IL-19, IL-20, IL-22, IL-24, and IL-26 (AK-155) [8, 10–15]. In contrast to the anti-inflammatory functions of IL-10, other IL-10-related cytokines have proinflammatory activities.

IL-10-related cytokines act through heterodimeric receptors belonging to the type II cytokine receptor family and induce the JAK/STAT pathway, in particular STAT3. IL-10 binds the IL-10R1/IL-10R2 receptor [16, 17]. The IL-10R2 chain is also a component of the IL-22 receptor, together with the IL-22R1 subunit [12, 18], and of the IL-26 receptor, in association with the IL-20R1 chain [19, 20]. IL-19, IL-20, and IL-24 act through the IL-20R1/IL-20R2 receptor [11, 21]. IL-20 and IL-24 also bind the IL-22R1/IL-20R2 complex [21–23]. In contrast to the ubiquitous expression of the IL-10R2 chain, the IL-10R1 subunit is mainly detected in lymphoid tissues and immune cells [9, 24]. During wound healing, IL-10 inhibits the infiltration of neutrophils and macrophages in the injured tissue and downregulates the expression of proinflammatory cytokines (IL-1β, IL-6, TNF-α) and chemokines [CCL2, CCL3] [25]. The lack of IL-10R1 expression and STAT3 phosphorylation in response to IL-10 suggests an indirect effect on keratinocytes. This is reinforced by the absence of any biological activities of IL-10 on primary keratinocytes or on the HaCat keratinocyte cell line [6, 26].

Whereas the IL-22R1, IL-20R1, and IL-20R2 chains are all expressed in skin, pancreas, and liver, the IL-20R2 subunit is the only one detected in immune cells [6, 24, 27, 28], suggesting that the spectrum of action of the IL-10-related cytokines, except for IL-10 itself, is restricted to non-immuno-hematopoietic cells. A growing number of studies now report the implication of IL-22 in the biology and function of the skin, as well as its possible involvement in the

development and/or maintenance of inflammatory cutaneous diseases such as psoriasis.

2.1 Among the Cytokines of the IL-10 Family, IL-22 Is the Strongest Activator of Keratinocytes

Keratinocytes express low levels of IL-20R2 and high levels of the IL-10R2, IL-20R1, and IL-22R subunits, rendering them susceptible to the action of IL-19, IL-20, IL-22, IL-24, and IL-26 [6, 24, 29].

Regarding in vitro studies, our group and Ouyang's group compared the effects of the IL-10-related cytokines respectively on NHEK and on RHE [30, 31]. IL-19, IL-20, IL-22, IL-24, and IL-26 all induced STAT3 phosphorylation in NHEK, IL-22 and IL-24 being the most potent activators of this transcription factor, in accordance with the receptor expression pattern [30]. Accordingly, analysis of the gene expression profile induced by these cytokines on NHEK showed that IL-22 and to a lesser extent IL-24 and IL-20 are the most potent keratinocyte activators. These three cytokines upregulate the expression of genes associated with inflammation and innate immunity, such as S100A7-psoriasin and β-defensin 2 (BD-2); IL-22 and IL-24 also downregulate differentiation-associated genes, including CK10 [30]. In addition, IL-19, IL-20, IL-22, and IL-24 induce hyperplasia of RHE, IL-22 and IL-24 having the major hyperplastic effects [31]. These effects are unlikely due to hyperproliferation of keratinocytes from the basal layer since the expression of basal keratinocyte proliferation marker Ki-67 is not altered. On the other hand, IL-19, IL-20, IL-22, and IL-24 upregulated CK16 (associated with suprabasal keratinocyte proliferation). Interestingly, IL-22 was the only cytokine to induce hypogranulosis in RHE. No biological activities of IL-26 have been observed so far on keratinocytes. The comparison of IL-10-related cytokines activities on keratinocytes showed that IL-22 has the most potent effects, followed by IL-24, then IL-20, and finally IL-19.

2.2 IL-22 Induces an Inflammatory Phenotype of Cultured Keratinocyte

IL-22 is a key factor in keratinocyte activation, chemotaxis, antimicrobial defense, and differentiation; primary keratinocytes, as well as the keratinocyte cell lines HaCat and SVK14, express the IL-10R2 and IL-22R1 subunits [6, 29], whose expression is upregulated by IFN-γ. The binding of IL-22 to its receptor induces the phosphorylation of STAT3 and to a lower extent STAT1 in keratinocytes [6, 32]. IL-22 upregulates, in a dose- and time-dependent manner, the expression of S100A7-psoriasin, S100A8 and S100A9 transcripts and protein, known for their proinflammatory activities, and the matrix metallo-proteases. Moreover, IL-22

strongly induces the hyperplasia of RHE, resulting mostly from an inhibition of keratinocyte differentiation, with a thickening of the spinous layer and a disappearance of the granular layer. Indeed, IL-22 downregulates the expression of CK10, involucrin, loricrin, and filaggrin, all associated with keratinocyte differentiation, whereas it has no detectable effect on keratinocyte proliferation. Finally, IL-22 induces keratinocyte migration in an in vitro wound-healing model. IL-22 also upregulates the expression of the antimicrobial peptides human BD-2 and BD-3, but not BD-1. This induction depends on the keratinocyte differentiation state since an elevated calcium concentration promotes the IL-22-induced BD-2 expression [32]. High levels of IL-22 are associated with strongly upregulated BD-2 expression in skin from patients with psoriasis and atopic dermatitis [32].

2.3 IL-22 Belongs to a Set of Cytokines Displaying a Massive Synergistic Effect on Epidermal Inflammation

In addition to IL-22, numerous cytokines of the IL-1 (IL-1α and β, IL-18), IL-2 (IL-4, IL-13, IL-21), IL-6 (IL-6, OSM, IL-31), IL-17 (IL-17A and IL-17F), IFN (IFN-α, IFN-γ), or TNF (TNF-α, TNF-β) families have been described to directly target keratinocytes [33]. Except for IL-4 and IL-13, these cytokines modified the expression of genes associated with inflammation, innate immunity, and differentiation. However, comparative and quantitative analysis demonstrates huge differences of expression induced by specific cytokines. For example, IL-22, as oncostatin M, both via the STAT3 activation pathway induced a strong inhibition of keratinocyte differentiation, as shown by decreased expression of filaggrin, loricrin, and involucrin and increase in the overall thickness of RHE [6, 7]. In contrast, the induction of chemokines, S100A7, or BD-2 by IL-22 and OSM is moderate. While IL-17 and the NFκB-recruiting cytokines IL-1 and TNF-α strongly induced chemokines, S100A7, or BD-2 expression, they have no significant effect on keratinocyte differentiation [34, 35]. Nevertheless, it appears that single-cytokine stimulation generates a rather limited effect on keratinocytes, namely, a limited number and/or modulation of expression of targeted genes.

Since in physiological or physiopathological conditions, tissues are surrounded not by one cytokine but a complex milieu, study of the biological activities of cytokine combinations is of great interest. For example, combination of IL-17A and IFN-γ or IL-17A and TNF-α results in a synergistic effect on CXCL8 production by keratinocytes [36, 37]. IL-17A and IL-22 synergize in the upregulation of BD-2 and S100A9 production [38, 39].

IL-22, in association to IL-1α, IL-17, OSM, and TNF-α, demonstrated a very strong synergy in increasing the expression of inflammatory molecules such as psoriasin/S100A7 or BD-2, or IL-8 in vitro by NHEK (approximately 10,000 times the spontaneous expression for BD-2) [34]. When IL-22 is removed of the cytokine mixture, CXCL8 and BD-2 expression is reduced of 30 %, whereas the decrease is

about 70 % after IL-17 removal. Using Affymetrix microarray analysis of NHEK treated by this cytokine mixture, about 1,400 genes are overexpressed, among others associated to innate immunity, and 1,560 downregulated, including 120 dealing with differentiation, and unexpected ones, whose relationship with skin inflammation remains to be established.

In addition, ex vivo studies on human skin explants demonstrated upregulation of BD-2, S100A7, and CXCL8 expression in response to the same combination of cytokines. In vivo intradermal injection of these five cytokines in mouse increased CXCL1, CXCL2, CXCL3, S100A9, and BD3 expression, associated with neutrophil infiltration and an early epidermal acanthosis [34](and manuscript in preparation).

3 IL-22 and Psoriasis

Affecting 2.5 % of the world's population, psoriasis is one of the best-studied chronic inflammatory disorders, characterized by a thickened epidermis, hyperproliferation, and abnormal differentiation of keratinocytes, accompanied by vascular hyperplasia and inflammatory immune cell infiltrates at the lesion site. The involvement of cytokines in the cross talk between immune cells and keratinocytes was postulated over 15 years ago for the cause of psoriasis [40], and psoriasis has been classically considered as a Th1 disease. Recent data showing that immune cells infiltrating the psoriatic skin secrete large amounts of inflammatory cytokines, such as IFN-γ, TNF-α, OSM, IL-22, IL-17, and IL-23, which play an important role in the pathogenesis of psoriasis, strongly modified this classification. Psoriasis is becoming the paradigm of an inflammatory disease involving the Th17 subset, complementing rather than replacing the Th1 theory.

3.1 IL-22 Induced a Psoriasis-Like Phenotype and Is Overexpressed in Psoriatic Lesions

As soon IL-22 has been described to target keratinocytes, it has been observed that the cytokine induces a psoriasis-like phenotype on RHE, i.e., hyperplasia with a thickening of the spinous layer and a disappearance of the granular layer [6, 30], and that the expression and secretory patterns of IL-22-treated keratinocytes resembled most of the features of psoriatic lesions [41]. Indeed, IL-22 is overexpressed in psoriatic lesions, whereas IL-22R1 and IL-10R2 are expressed at a similar level in psoriatic and healthy skin [32, 42]. IL-22 mRNA expression is upregulated in psoriatic skin lesions, whereas IL-22 mRNA levels in peripheral blood mononuclear cells from psoriatic patients and normal subjects are similar. The local production is confirmed by the presence of high IL-22 levels in culture

supernatants of psoriatic lesional skin. In addition, circulating IL-22 levels are significantly higher in psoriatic patients than in normal subjects [32, 42]. Interestingly, we can notice that OSM, the other STAT3 involving cytokine able to target keratinocytes with a quasi-redundant effect than IL-22, is also overexpressed in psoriatic lesions [7, 42], as well as the other Th17 cytokines IL-17A and IL-17F [43]. While the respective contribution of IL-17 and IL-22 in the physiopathology of psoriasis is now established, that of IL-22 and OSM remains to be elucidated.

Regarding in vivo reports, IL-22, as well as IL-20 or IL-24, overexpression in transgenic mice causes neonatal lethality with skin abnormalities resembling psoriatic skin, including a thickened epidermis and abnormal epidermal differentiation [11, 44]. On another hand, the epidermal thickness as well as STAT3 phosphorylation is highly reduced in IL-23-treated mice in the presence of IL-22-neutralizing antibodies or in IL-22-deficient mice [45]. Using an imiquimod-induced model of psoriasis in mice [46], Van Belle et al. recently demonstrated that IL-22-deficient mice are almost totally protected from psoriasis-like lesions [47].

At last, the role of IL-23 and Th17 cytokines in the pathophysiology of psoriasis has been highlighted by the demonstration that IL12/23p40 antibodies have therapeutic efficacy [48].

3.2 IL-22 Is Produced by Psoriatic Skin-Infiltrating Th17 and Th22 Lymphocytes

High levels of IL-22 are produced in psoriatic skin. CD4 and CD8 T cells infiltrating psoriatic lesions are an important source of IL-22, higher than peripheral T cells from psoriatic patients or controls [42]. These infiltrating T cells were also able to produce high IL-17 levels [43]. Interestingly, supernatants from T cells isolated from psoriasis skin are able to induce a “psoriasis-like” phenotype in cultured normal keratinocytes [42]. Further analysis of T cell infiltrating psoriatic skin suggested that Th17 lymphocytes produced IL-22 [49]. Studied at the clonal level, a correlation was observed between IL-22 and IL-17 production by T cells isolated from chronically inflamed tissue, including psoriatic skin, suggesting the coproduction of IL-17 and IL-22 by Th17 lymphocytes [50]. Besides the Th17 cells, a so-called Th22 subpopulation producing IL-22 but not IL-17 or IFN-γ has been recently described in circulation and in normal human dermis, expressing CCR6 and the skin-homing receptors CCR4 and CCR10 [35, 51, 52]. Th22 clones derived from psoriatic lesional skin have been further described, mostly in the epidermis compartment of the skin [53]. Not specific of psoriasis, Th22 is also increased in lesional skin of atopic dermatitis [54]. Th22 subset is induced by Langerhans cells in skin [55]. More recently, it has been demonstrated that the majority of IL-22-producing lymphocytes in psoriatic skin are T cytotoxic (Tc)22 more than Th22 [56]. Interestingly, these authors also suggested the plasticity of the cytokine production in skin between Th17 and Th22 and Tc17 and Tc22 cells.

Otherwise, in both mice and man, it has been suspected that non-T cells in skin produce IL-22, such as dendritic cells, NK cells, and macrophages [47, 56].

3.3 Correlation of the Innate Immunity Profile of Psoriatic Skin Lesions and In Vitro Inflammatory Keratinocyte

We described above that IL-22 in vitro induces a RHE morphology resembling with the one of lesional psoriatic skin. We also reported that the combined activity of IL-22 with IL-1α, IL-17A, OSM, and TNF-α led to high levels of chemokines and host defense proteins expression and inhibition of genes associated with keratinocyte differentiation [34] (unpublished results), generating an in vitro model of skin inflammation recapitulating some features of lesional psoriatic skin. The pathophysiological relevance of this model was assessed by comparison of its transcriptional profile with that of psoriatic skin [34]. Overall, increased expression of IL-22 associated to IL-17A, IL-1, IL-23, and OSM was reported in psoriatic skin. A correlation was found between in vitro and in vivo quantitative transcriptional profiles, demonstrating that a part of the characteristic "signature" for psoriasis was obtained in the in vitro model of keratinocyte inflammation obtained in the presence of these cytokines.

4 Concluding Remarks

In recent years, an increasing number of reports have led to defining a panel of proinflammatory cytokines able to play a central role in the induction and maintenance of chronic skin inflammation. Among them, IL-22 plays a key role. Figure 1 resumes the synergistic proinflammatory biological activities of IL-22 associated to IL-17, IL-1, TNF-α, and OSM on keratinocytes.

Taken together, it appears that IL-22 is a cytokine mainly produced by Th22 skin-infiltrating lymphocytes implicated in the initiation and/or maintenance of the pathogenesis of psoriasis. IL-22 is especially involved in the development of the epidermal hyperplasia and hypogranulosis and in combination with other proinflammatory cytokines and chemokines, in the protection against microbial infection. The hyperplastic effect of IL-22 on keratinocytes in psoriasis suggests a deleterious effect, although this question is not necessarily so clear-cut, as the question of its redundancy with OSM in the disease.

Numerous studies focused on psoriasis as the paradigm of skin inflammatory diseases, albeit parallels between animals or in vitro models to a specific human pathology remained sometimes unsubstantiated. These studies open the way to new therapeutic strategies focusing on more specific and downstream targets,

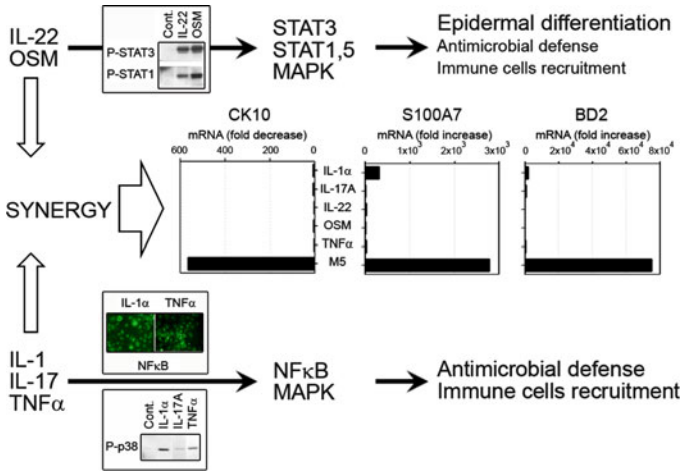

Fig. 1 Characterization of the cytokine expression profile in psoriatic skin lesions, cell signaling pathways involved, and synergy of biological activities for epidermal differentiation and innate immunity (*CK10* cytokeratin 10, *BD2* β-defensin 2, *M5* combination of IL-1α, IL-17A, IL-22, OSM, and TNF-α)

i.e., keratinocyte-targeting cytokines rather than on a systemic inhibition of T lymphocytes overproducing cytokines.

The specific blockade of new cytokines or their receptors is an alternative approach for the treatment of psoriasis. In any cases, cytokines are very potent factors, with the advantages (or the defects, depending on the case) to be pleiotropic and redundant and to be involved in cascade. Such treatment could reduce the biological effects of an overproduced cytokine and/or disrupt the vicious circle involving it. Targeting more than one cytokine might be an alternative strategy to assure a complete and sustained clinical improvement.

IL12/23p40 antibodies have been demonstrated to have a powerful therapeutic efficacy [48], and some clinical trials using anti-IL17 are currently underway. In the near future, we look forward to the therapeutic advantages of IL-22 inhibitors such as antibodies or receptor antagonists. Obviously, the in vitro models of inflammatory epidermis induced by a specific set of cytokines could be useful tools to screen new drugs.

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Part V
Novel Tools and Therapeutic Avenues

Genetic Mouse Models to Investigate IL-17 Responses

Andrew L. Croxford and Thorsten Buch

Abstract Th17 cells have recently acquired notoriety after their association with tissue inflammation. IL-17A secretion is considered the hallmark of Th17 function. It exhibits strong proinflammatory properties and is considered to be a major driving force in the pathogenesis of autoimmunity. A vast amount of data is now available, highlighting the complex role of IL-17 family cytokines and their functions in physiological processes. As a consequence of the burst of interest surrounding these cytokines, new mouse strains have been developed to study their roles in pathogenic processes. In this chapter, examples of the knowledge gained using mice designed to track IL-17-expressing cell types, alter IL-17A expression or disrupt IL-17 signalling are delineated. Also, current options available for generating such strains and their potential drawbacks are discussed.

Keywords IL-17A • IL-17F • Lineage tracing • Reporter strain • Transgenesis • Cre-loxP • Gene targeting

1 The Th17 Subset

Twenty years ago, Mosmann and Coffman put an immunological dogma into place by describing the stable differentiation of naive T helper cells into two distinct effector lineages, Th1 and Th2, defined by their 'signature' cytokines, IFN-γ and

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IL-4 [42]. These patterns were hypothesised to be mutually exclusive, with cytokines from one subset inhibiting differentiation of the other and supporting differentiation into its own lineage. Much of the current thinking surrounding T helper cell subsets is still derived from these landmark experiments. Naive T cells can differentiate into distinct lineages, and these lineages have a unique and transcriptionally stable gene expression signature. While the dogma of a Th1/Th2 dichotomy for $CD4^+$ T helper (Th) cell differentiation was stable for many years, the field has seen in recent years multiple additions to the types of differentiated Th cells. Interleukin-17 (IL-17)-expressing T cells, now termed Th17 cells, are now widely considered to have fulfilled the criteria as a distinct T helper cell subset as distinct transcription factors necessary for their differentiation were defined. They have been at the centre of a recent surge of interest within the immunology community, mostly because of their connection to a variety of immunological dysfunctions.

2 Classical Methods to Investigate Cytokine Biology

The two Th17 cytokines IL-17A and IL-17F are part of the communication system between cells of the immune system and other cells in the body. Such information exchange is essential for successful immune responses, maintenance of immune homeostasis and implementation of tolerance. The 'crosstalk' between the involved cell types is facilitated by direct interaction of cells through surface receptors as well as release and binding of soluble messengers. These soluble immune mediators, broadly termed 'cytokines', attract immune cells to sites of inflammation and direct the immune response by supporting differentiation of specialised subsets and by attraction as well as activation of specialised effector cells.

The development of enzyme-linked immunosorbent assay (ELISA) allowed the quantification of cytokines in liquids such as serum and cell culture supernatants. ELISA-mediated cytokine quantification is possible for IL-17A and F through commercial kits or antibody pairs. The enzyme-linked immunosorbent spot (ELISPOT) facilitates the counting of cultured cells secreting a particular cytokine and is also widely used to identify IL-17A-expressing cells [15]. Flow cytometry allows the easy analysis of cell surface differentiation markers in combination with various cytokines through the use of intracellular cytokine staining. However, because this method requires fixation, cells cannot be sorted, transferred or analysed by any assay requiring live material. To obtain living cytokine-secreting cells, a cytokine secretion assay, which is in essence a combination of ELISPOT and flow cytometry, overcomes this problem and allows the detection of secreted cytokine through catching by specific antibodies coupled to the cell surface [2, 3]. This technology allows the purification of viable cytokine-secreting cells and reflects physiological cytokine release. One drawback of this method is, however, that the cells have to be activated in vitro to facilitate secretion of the specific cytokines in amounts sufficient for detection.

3 Transgenic Methods for the Investigation of IL-17 Biology

Much of the knowledge we now possess describing in vivo cytokine function has been a direct result of experiments using transgenic cytokine-overexpressing mouse lines and mice with targeted gene disruptions [8]. In fact, some of the very first targeted genomic mutations were in genes encoding the cytokines IL-4 [31], IL-2 [51] and IL-10 [30]. Subsequently, to answer more intricate questions, cell type-specific gene deficiency and inducible gene deficiency, broadly termed 'conditional knockouts', were introduced. This method relies on the combination of expression of a recombinase (usually Cre) controlled in time or location, with a target allele modified in such a way that it contains target sites for this recombinase (usually loxP sites) [48]. The advantages of conditional knockouts can be exemplified using mice deficient for IL-17A. Due to numerous cellular sources of this cytokine [27, 45], complete IL-17A deficiency has not yet answered what role Th17 cells play in vivo. A different way to detect cytokine production, for example, IL-17A and IL-17F, is the use of transgenic expression of marker molecules under control of their specific promoters. Fluorescent proteins are nowadays the method of choice for such reporter transgenes. Molecules such as β-Gal, luciferase and surface proteins (e.g. Thy1.1, human CD2, human CD4 [4, 39, 50]) allow the generation of 'reporter transgenes' in a more specific sense as a direct correlate for ongoing gene transcription. Such 'reporter' mice allow the study of cytokine expression without modifying protein itself. They enable the rapid and easy investigation of development and differentiation, proliferation, survival and migration by indicating the localisation and phenotype of cytokine-secreting cells in an unaltered environment. Furthermore, they can also be combined with other genetic modifications to enhance the potential of previously existing models.

One problem of many commonly used reporter mice is that the half-life of the reporter protein is too long, thus masking rapid changes in transcriptional responses. To overcome this, a PEST degradation domain can be attached to the fluorescent protein [35]. This reduces the half-life of the reporter protein dramatically and thus links the detection closer to promoter activity. Nevertheless, this method cannot overcome the problem of distinct translation and folding kinetics of non-secreted reporter proteins compared with secreted cytokine proteins. Therefore, a further challenge in cytokine biology lies in monitoring not only expression but also protein stability, storage and release of a cytokine. Attempts to achieve this have only been made so far by fusion of IL-15 to EGFP [14, 52].

Reporters using surface molecules and not fluorescent proteins have distinct advantages because antibodies labelled with different fluorochromes can be used to identify marker-expressing cells. Thus, this approach may be a more versatile alternative in multicolour staining protocols. Also, such surface markers can be used to deplete mice of cytokine-expressing cells after administration of a specific antibody. Another strategy becoming ever more 'fashionable' is the use of multicistronic transgenes, combining a fluorescent protein, luciferase, Cre and possibly diphtheria toxin receptor (DTR) [53].

A distinct class of mice, sometimes also called reporters, are animals which combine expression of Cre recombinase with a fluorescent conditional reporter strain. Such animals are effectively tracing whether, in case of the use of IL-17A or IL-17F promoters, a cell is or has been expressing one of the cytokines at some point during its life, going as far back as its earliest developmental stage. While such tracer strains are very effective in lineage analysis experiments, they are not able to indicate acute and immediate cytokine expression. These strains are hampered by the kinetic of Cre expression, recombination of a conditional STOP cassette and final expression of the reporter molecule itself, which in all likelihood requires a longer time than expression of the protein being traced.

For the generation of reporter strains, conventional transgenic mouse strains and targeted insertions ('knock-ins'), bacterial artificial chromosome (BAC) transgenes can be used. In conventional transgenesis, pronuclear injection leads to random genomic integration of the transgene (tg), which contains all required expression elements. To rely solely on all endogenous elements, knock-in strains can be generated in which the marker gene is introduced into the locus of interest [49]. In this approach, internal ribosome entry sites (IRES) [43] or 2A sites can be used to avoid the generation of null alleles. BAC-mediated marker expression is an alternative method, which does not require promoter characterisation but maintains the speed of conventional transgenesis [23]. For the generation of reporter strains by either of these methods, fluorescent proteins such as EGFP, EYFP, RFP, mCherry and tdTomato are in most cases the molecules of choice, given their ease of use in microscopy and FACS analysis. As mentioned above, expression of foreign surface proteins such as Thy1.1 or human CD4 represents another option.

4 Basic Biology of IL-17A and IL-17F

As is so often the case in scientific discovery, the observation that TGF-β and IL-6 are sufficient to induce IL-17A expression from naive T cells was made somewhat unexpectedly. It was the result of trying to identify soluble factors capable of inhibiting Foxp3 expression, thus induction of regulatory T cells, upon activation in the presence of TGF-β. [6, 37, 38, 53]. Given that TGF-β and IL-6 are thought to have largely opposing functions, their apparent synergy during IL-17 expression from T cells was for many a curious observation. Indeed, disrupting IL-6 signalling, either through receptor or ligand knockouts, resulted in an inhibition of Th17 differentiation [29]. Depletion of regulatory T cells, however, was able to overcome the resistance to EAE observed in both IL-$6^{-/-}$ and $gp130^{-/-}$ T cells, which was credited to a partial restoration in Th17 differentiation even in the absence of IL-6 [28, 29]. These findings implied that another factor was capable of inducing Th17 cells in combination with TGF-β. IL-21 in combination with TGF-β was later identified as the alternative pathway to IL-6-mediated Th17 differentiation [28].

After initial differentiation of Th17 cells in the presence of TGF-β and IL-6 and/or IL-21, further events take place to ensure that the proinflammatory phenotype is maintained during an immune response. IL-23, an antigen-presenting cell

(APC)-derived cytokine of the IL-12 cytokine family, was demonstrated to promote the production of IL-17 from primed T lymphocytes [1]. It was further reported that IL-23 was key to expanding IL-17-expressing $CD4^+$ T cells. *Il23a*-deficient mice were found to be fully resistant to EAE and also lacked T cells capable of expressing IL-17 [11, 32, 44]. It was then proposed that IL-23 was a factor responsible for expansion of differentiated Th17 cells. It was shown in these same publications that IL-23-polarised cells express high levels of IL-17A, IL-17F and TNF-α. Further evidence comes from the finding that transgenic expression of RORγt in naive T cells is sufficient to upregulate *il23r* (IL-23R) expression [21], implying that IL-23R signalling is a critical event which takes place during Th17 differentiation.

Like Th1 and Th2 cells, Th17 cells are biologically defined by expression of a unique transcription factor. The RORγt isoform of RORγ, a member of the retinoic-acid-receptor-related orphan nuclear hormone receptor family [22] that had been described to be solely expressed in immune cells [12], was shown by Ivanov and colleagues to be the master regulator controlling Th17 differentiation [21]. In this study, RORγt was induced by TGF-β and IL-6 [34], and overexpression of RORγt was able to induce de novo Th17 differentiation if both Th1- and Th2-polarising cytokines were neutralised. Also, RORγt-deficient T cells are also almost completely incapacitated in IL-17A and IL-17F production in response to TGF-β, IL-6 and IL-21 [47, 60]. RORα, a close relative of RORγ, is considered to be a minor player in Th17 generation since RORα deficiency in T cells only resulted in a minimal decrease in IL-17A and IL-23R expression [57]. In addition to RORγt, interferon regulatory factor-4 (IRF-4) seems to be another factor crucial to the generation of Th17 cells as its deficiency completely abolishes Th17 differentiation. Such *IRF-4*$^{-/-}$ T cells are unable to upregulate RORγt expression in response to TGF-β and IL-6 [7], and as such IRF-4 function can be placed upstream of RORγt. Collectively, these publications identified RORγt and IRF-4 as key regulators of Th17 cell differentiation.

5 Genetically Manipulating the IL-17A Signalling Pathway

A new research niche focussing on IL-17 signalling and the Th17 cell subset has benefited greatly from the introduction of new mouse strains either reporting on IL-17 expression or carrying alterations of IL-17 expression or signalling capabilities. It seems to be generally accepted that IL-17A and IL-17F play a major role in coordinating local tissue inflammation via the induction of proinflammatory chemokines and neutrophil-mobilising cytokines. This is at least in part due to release of CXCL1, a potent attractant of neutrophils, in direct response to IL-17A signalling [25]. After their generation in the bone marrow, neutrophils, which are the most abundant nucleated cell in the blood, are rapidly recruited to sites of infection by a variety of chemoattractants.

IL-17RA is to date the most thoroughly characterised receptor of the IL-17R family [26]. Many studies aimed at elucidating the role of IL-17 signalling in host defence have relied on mice lacking the IL-17RA chain. With respect to defence

against bacteria, IL-17A was shown to be induced in a time- and dose-dependent fashion during an immune response to K*lebsiella pneumoniae* [17, 58]. Conversely, mice deficient for the IL-17R succumb to a pulmonary challenge with the same bacteria [59]. This report documented a dramatic decrease in neutrophil recruitment into the infected lung, reduced granulopoietic output from the bone marrow and significantly reduced production of local G-CSF. In support of this data, antibody-mediated neutralisation of IL-17A was able to reduce accumulation of neutrophils into the bronchoalveolar space after administration of lipopolysaccharide (LPS) [13, 41].

In addition to its role in host defence against extracellular bacteria, IL-17 signalling is now known to be essential during the immune response against fungi and parasites. Upon *Candida albicans* challenge, IL-17R-deficient mice were shown to have increased kidney fungal burden and decreased survival [20]. Infection with toxoplasma, a protozoan pathogen, in IL-17R-deficient mice resulted in a decreased survival rate and increased parasite burden due to reduced neutrophil recruitment [24]. Thus, genetic ablation of the IL-17 signalling cascade has revealed an important role for IL-17 signalling against a broad array of pathogenic organisms.

6 IL-17 Modulation in CNS Autoimmunity

IL-17A was also shown to be upregulated in CNS lesions of multiple sclerosis patients [36]. It is thought that IL-17A drives the inflammatory process and contributes to blood-brain barrier breakdown. While IL-17A was considered to be one of the main driving forces behind CNS inflammation in EAE, it was surprising that this opinion was largely based on correlative relationships between Th17 cells and their presence in an inflammatory lesion. Initial attempts to neutralise IL-17A in EAE had only yielded minor relief of disease severity. Blocking IL-17A with an antagonistic antibody or IL-17A and IL-17F with a soluble IL-17 receptor both failed to completely abrogate the progression of EAE [19]. Komiyama et al. addressed the role of IL-17A in EAE using a genetic model, generating an IL-17A-deficient mouse strain [27]. Unlike the deficiencies in IL-23 (p19) and IL-6, which render mice completely resistant to EAE, loss of IL-17A does not completely inhibit the development of EAE.

At this juncture, transgenically manipulated IL-17A expression was utilised to address the importance of IL-17A in the disease pathogenesis of MOG-induced EAE. Mice were generated in which T cells were forced to produce high levels of IL-17A. This was achieved by crossing the IL-17A^{ind} allele to the CD4-Cre transgene [16]. Surprisingly, highly increased levels of T cell-derived IL-17A did not accelerate or increase the severity of EAE or alter the quality of CNS inflammation. Furthermore, it was shown that IL-17A neutralisation in IL-17F-deficient mice, which are otherwise fully susceptible to EAE [55], did not ameliorate progression of paralysis in these mice [16]. Thus, using a combination of strains

designed to shut off or overexpress IL-17A, we can deduce that IL-17A is not a crucial cytokine for the pathogenesis observed in EAE. One could more likely conclude that the disruption of IL-17A and IL-17F production is merely a contributing factor, but not the main cause of the complete EAE resistance observed in IL-23- and IL-6-deficient mice [11, 29].

7 Fate Mapping of Th17 Cells Using Reporter and Tracer Mice

While T cells are able to express a plethora of cytokines, only few of these define terminally differentiated T cell (sub)lineages. Because stability lies at the heart of the definition as a new lineage, the topic of 'plasticity' is currently a major focus of Th17 research. The generation of Th17 reporters has helped greatly in revealing many facets of Th17 regulation and phenotypic plasticity. Interestingly, even though the hallmark cytokine of Th17 cells is IL-17A, the first three described reporter mice utilised the IL-17F promoter. These reporters expressed red fluorescent protein (RFP), Cre recombinase and Thy1.1 [10, 33, 56]. The problem with these reporters was, however, that expression of IL-17A and IL-17F, although largely overlapping, is not consistently linked in individual cells—an observation confirmed by use of these reporters.

Through the use of the IL-17F-RFP reporter in combination with a Foxp3-GFP strain, cells co-expressing CD4, GFP and RFP were found in cultures designed to polarise Th17 cells. This suggested that both Th17 and Treg cell developmental programmes are induced simultaneously and coexist for a period of time prior to complete differentiation of Th17 cells [56]. In addition to the fact that IL-6 inhibits differentiation of induced Treg cells, it was further shown that naturally occurring GFP^+ Tregs are able to 'redifferentiate' into RFP^+GFP Th17 cells in the presence of IL-6. We contributed to this unresolved issue when we showed that fully differentiated Th17 cells are resistant to TGF-β-mediated upregulation of Foxp3, implying that terminal differentiation of Th17 cells inhibits subsequent reversion to a Treg phenotype [10]. Also, we and others observed that upon adoptive transfer of live Th17 cells, their signature cytokine expression is unstable, and downregulation of IL-17A and IL-17F occurs in the absence of proinflammatory cytokines [5, 10, 46].

A common feature of 'Th17-mediated' inflammatory states is the presence of $IL\text{-}17A^+IFN\text{-}\gamma^+$ T cells in the inflamed organ. Using intracellular staining alone, it was not clear if these double-positive cells are derived from differentiated Th17 cells or Th1 cells. Lee et al. showed in a Thy1.1-expressing IL-17F reporter strain that purified Th17 cells exposed to different cytokine milieus respond to IL-12 with upregulation of IFN-γ [33]. Therefore, it seems that $IL\text{-}17^+IFN\text{-}\gamma^+$ cells are, at least in part, reprogrammed Th17 cells, but this point remains to be definitively proven using a fate mapping approach in vivo. Following the initial findings documented for Th17 cells using the above-mentioned strains targeting IL-17F expression, a new IL-17A-Cre strain was developed by Stockinger and colleagues and was used to highlight some fundamental aspects of Th17 biology [18]. For lineage tracing

they crossed the IL-17A-Cre and an EYFP conditional reporter. This marked all cells with EYFP that had previously expressed IL-17A by excision of a STOP cassette inhibiting expression of the EYFP reporter protein. Analysis of Th17 development in IL-23p19-deficient IL-17A tracer mice revealed, perhaps not surprisingly, less EYFP-expressing cells. However, a crucial observation was made, when the well-documented double-positive IL-17A^{+}IFN-γ^{+} population was not observed in the absence of IL-23. The authors suggested that IL-23 expression lies upstream of T-bet and may be necessary for its expression in vivo and that IL-23 signalling marks at least one stage of the observed plasticity of Th17 cells in their decision to upregulate IFN-γ. The authors also showed that GM-CSF was co-expressed by EYFP^{+} cells [18], opening the question of which molecular switch will result in GM-CSF expression from Th17 cells. Should this be found, it will again raise the issue of whether or not Th17 cells are in fact a stable cell lineage at all or whether an intrinsic plasticity exists within this cell population. Evidence gathered to date clearly points in the direction that Th17 cells can in fact upregulate expression of cytokines generally considered to be hallmark cytokines of other lineages, namely, IFN-γ and GM-CSF. Given the clear association and absolute requirement of GM-CSF for the pathogenesis of EAE [9, 40], this particular avenue of research will no doubt garner great interest. Potentially, the generation of new reporter strains designed to specifically follow GM-CSF expression in vivo will be required to answer more intricate questions relating to GM-CSF biology.

Thus, the question as to when a cell becomes terminally differentiated to stably produce IL-17 is still open, but many interesting observations were made using the genetic tools currently available to us and will no doubt continue to contribute to the pool of knowledge in the coming years.

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IL-17A as a Therapeutic Target for Autoimmune Diseases

Franco Di Padova and Jose Carballido

Abstract The identification of the IL-23-Th17-IL-17A axis and the definition of the CD3+ CD4+ Th17 cell subset have introduced new paradigms to explain the origin of autoimmune events in animal models, subverting the established Th1-Th2 paradigm.

In humans, IL-17A and Th17 cells have been implicated in a variety of inflammatory and autoimmune diseases. However, the picture is more complex as other IL-17A-producing cells are detected in several pathological conditions. In particular, CD8+ T cells, γ δT cells, astrocytes, macrophages, neutrophils, mast cells, and eventually innate lymphoid cells have been associated with IL-17A production in different diseases.

Antibodies targeting IL-17A, IL-17RA, the p40 common chain (shared by IL-12 and IL-23), and other biologics have been used for the treatment of different autoimmune diseases. These therapies are not only beneficial for the patients but are also contributing to define the cell types involved and the pathways causing the pathological events. In psoriasis, the clinical results seem to suggest a complex scenario in which, in various patients, different IL-17A-producing cells, such as CD4+ Th17 cells, CD8+ T cells, γ δT cells, neutrophils, and mast cells, might be variably involved. The majority of these subpopulations would respond to IL-23 and produce IL-17A. Subtle differences in disease pathogenesis are supported by treatment with other biologics. Even more complex scenarios can be present in other autoimmune disorders.

Keywords CD4+ T cell • CD8+ T cells • Th17 cell • γ δT cells • IL-17A • IL-17F • IL-22 • IL-17RA • Anti-IL-17A antibodies • Anti-IL-17RA antibodies

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1 Introduction

The origin of autoimmune events in several animal models has now been linked to the CD3+ CD4+ Th17 cell subset. The identification of the IL-23-TH17-IL-17A axis has introduced a new paradigm to explain many autoimmune events [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. IL-17A and Th17 cells are involved in the pathogenesis of several experimental autoimmune models of arthritis, colitis, uveitis, and encephalomyelitis. In these pathological conditions, Th17 cells are present and contribute to the inflammatory condition, neutralization of IL-17A ameliorates disease, and IL-17A-deficient animals are protected [2].

Also in humans, several studies support a role for IL-23, Th17 cells, and IL-17A in autoimmune disorders. However, the picture appears more complex as other IL-17A-producing cells have been detected. Several data suggest that CD8+ T cells [3], γδ T cells [4, 5], a fraction of natural killer (NK) cells [6], neutrophils [1, 3], macrophages [7], mast cells [8, 9], astrocytes, and oligodendrocytes [10] can variably contribute to pathology. More recent data suggest that also NKp44− innate lymphoid cells are able to secrete IL-17A and might be involved in causing autoimmune diseases [11, 12].

Several studies are now trying to define which IL-17A+ cells contribute to the different autoimmune disorders and to clarify the role of IL-17A and of the different cell types. 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 identified CD4+ T cells as the main source of IL-17A. On the other hand, the 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 which are more easily extracted or apt to culture conditions. The main limitation of the histochemistry approach is linked to the difficulty to identify precisely cell surface markers and often to the lack of specificity of the reagents. In conclusion, additional studies are required to identify more precisely the cells contributing to the production of IL-17A and to identify the cells which secrete IL-17F, IL-17A/F heterodimer, and IL-22. IL-17F and IL-22 are expressed at increased levels in several diseases [13, 14].

The introduction of anti-cytokine antibodies, such as those targeting IL-17A (secukinumab, ixekizumab) [15–17], IL-17RA (brodalumab) [18], which neutralizes signaling not only by IL-17A but also by IL-17F, IL-25, and IL-17C [19–22], and the p40 common chain (ustekinumab, briakinumab) [23–25], shared between IL-12 and IL-23, is helping to understand the contribution of these cytokines to autoimmune pathology [26, 27]. As an example, studies with neutralizing antibodies selective for IL-17A [15–17] and for the IL-17RA [18] may help to clarify the contributions of IL-17A and of IL-17A plus IL-17F in human autoimmune disorders. The analysis of

animals deficient in either of these two genes has revealed distinct functions of IL-17A and IL-17F in inflammatory responses [28]. In addition, it is not clear how the production of IL-17A and IL-17F is regulated at the single cell level, as IL-17A and IL-17F are not always coexpressed and IL-17A and IL-17F single-positive cells are detected at relatively high frequencies [28, 29]. In mice, CD4+ IL-17A+ T cells seem to be prevalent in the lamina propria and intestinal intraepithelium [28]. Whether this differential expression results from the existence of different cell types of various stages of maturation or of environmental factors has not been investigated. In addition, an IL-17A/F heterodimer has been described in both man and mice [29–31]. The role of the heterodimer in inflammatory diseases in mice and humans needs further investigations. The antibodies neutralizing IL-17A or blocking the IL-17RA inhibit also the effects of the IL-17A/F heterodimer.

2 Biologics and Human Autoimmune Diseases

The introduction of biologics for the treatment of autoimmune diseases has not only been beneficial to patients, but it is also showing how autoimmune diseases are complex and variegate. The analysis of the response to biologics might open the possibility to dissect this complexity. As several approaches are effective in psoriasis, this disease can be used as a model to clarify subtle differences in pathogenesis that might be common to other autoimmune diseases.

2.1 Psoriasis

Chronic plaque psoriasis is a common immune-mediated skin disease characterized by demarcated erythematous, squamous lesions, with an estimated worldwide prevalence of 2–3 % [32]. The role of genetic factors in psoriasis has been confirmed from family and twin studies [33]. The concordance of psoriasis in monozygotic twins is 35–72 %, while it is 12–30 % in dizygotic twins [34, 35]. Psoriasis heritability has been estimated at 60–90 %, which is among the highest of all multifactorial genetic diseases [36].

The clinical manifestations collectively known as psoriasis are the result of complex interactions between the receptors of different cell types with multiple environmental triggers such as physical trauma, drugs, infection, and stress [37]. It is more common in Caucasians but can affect any race with no sexual predilection [38, 39]. The age of onset shows a bimodal distribution with one peak at 20–30 years and another at 50–60 years [40]. Among different psoriasis susceptibility loci (PSORS), the strongest association is with the major histocompatibility complex (MHC) on chromosome 6p21 (PSORS1) which includes the HLA-Cw*0602 allele [41]. The HLA-Cw*0602 allele is also associated with the early-onset type of psoriasis [42–44]. In addition to having a lower age of onset, HLA-Cw*0602-positive patients present more severe clinical symptoms [45]. Moreover, polymorphisms in IL-23p19, IL-12/23p40, and IL-23R are associated with increased risk of psoriasis

[46–48]. A role for IL-17A and IL-23 is also confirmed by the detection of elevated levels of IL-17A messenger RNA (mRNA) [49, 50] and of IL-23R in the skin of psoriatic patients [51]. Increased expression of mRNA for IL-17A, IL-17F, TNF-α, IL-21, IL-22, and CCL20 is observed with disease activity and normalizes with cyclosporine therapy [52]. Thus, psoriasis has complex multifactorial features, genetic heterogeneity, high heritability, a broad range of onset ages, and different prevalence in various populations. Considerable overlap has been shown between the molecular pathways involved in psoriasis and those leading to other inflammatory or autoimmune diseases such as Crohn's disease, systemic lupus erythematosus, rheumatoid arthritis, and Behcet's disease [53].

CD45RO+ memory effector T cells, either CD4+ or CD8+, have been reported to have a role in the pathogenesis of psoriasis [54, 55]. In man, IL-23R+ CD4+ and CD8+ T cell subsets have been described [56, 57]. In the upper lesional dermis, CD4+ T cells are abundantly present, whereas in the lesional epidermis, CD8+ T cells are in the majority [58, 59]. More recently, Th17 cells and Th1 cells were recovered at increased levels in the lesional epidermal and dermal samples of psoriatic patients [3, 52]. Th17 cells were predominantly localized to the dermis [52]. In addition, Tregs of patients with severe psoriasis have an enhanced propensity to differentiate into IL-17A-producing cells on ex vivo stimulation [60]. CD8+ T cells (Tc17 cells) may also play a significant role in the pathogenesis of psoriasis, as they are increased in lesional psoriatic skin [3, 61]. Tc17 cells can produce TNF-α and IFN-γ (Th1-related cytokines) as well as IL-17A, IL-21, and IL-22 (Th17-related cytokines) [61]. Psoriatic skin disease has been reconstituted in the SCID mouse by engraftment of uninvolved human skin and CD4+ T cells from patients with psoriasis and appears dependent on resident CD8+ T cells contained in the skin graft [62, 63].

Moreover, γδ T cells might represent another T cell population involved in psoriasis. These T cells are increased in affected skin of psoriatic patients, express constitutively the IL-23R, are responsive to IL-23, and produce large amounts of IL-17A [5, 64]. A subset of γδ T cells express CD28, and most of them are CD2 positive [65]. A study with IL-23R green fluorescent protein reporter mice reveals that, under resting conditions, a large fraction of γδ T cells but also minor fractions of CD11b+ macrophages and of CD11c+ dendritic cells (DC) express IL-23R in lymph nodes [66]. Under these conditions, CD4+ cells have minimal expression of the IL-23R, which, however, is expressed under inflammatory conditions [66]. Moreover, it has been reported that in a psoriatic model in mice induced by imiquimod, IL-17A, IL-17F, and IL-22 are produced by a skin-invading population of γδ T cells and RORγt+ innate lymphoid cells [67].

In addition, IL-17A+ mast cells and neutrophils are found in psoriatic lesions, and frequently these cells release IL-17A in the process of forming specialized structures called extracellular traps [3, 9]. IL-23 can induce mast cell extracellular trap formation and degranulation [9]. Whether these cells are positive for IL-17A, IL-17F, or both is still a matter of debate [3]. While mast cells and neutrophils stain positive for IL-17A in skin sections, only occasional CD4+ or CD8+ T cells stain for IL-17A [3]. However, substantial percentages of skin-derived CD4+ and CD8+ cells are able to produce IL-17A when properly activated [3], suggesting that T cells might express cytokines only temporarily or that they might release them efficiently

into the extracellular milieu. Even if many factors might be contributing to the variability of the results, the percentage of dermal CD4+ and CD8+ T cells expressing IL-17A or other cytokines is widely distributed [3] suggesting heterogeneity in the composition of the cellular infiltrate of different patients. An additional challenge would be to define a potential association between the characteristics of the tissue infiltrate and genetic heterogeneity. It might be worth noting that the patient with a high proportion of IL-17A+ CD8+ dermal T cells had a HLA-Cw6 phenotype [3]. In this context, one could also speculate on the contribution of other IL-17A+ cells such as γδ T cells. In summary, psoriasis is a complex multifactorial disease, and available data indicate that different mechanisms are operative and that the IL-17A+ cell populations might not be equally represented in distinct patients and in various stages of the disease.

Even if the clinical studies with anti-IL-17A and anti-p40 antibodies would suggest a common pathogenesis in psoriasis, the heterogeneous outcome of several other clinical studies confirms that distinct pathogenic subtypes are present in psoriasis. As shown in Table 1, several studies have demonstrated that anti-IL-17A [15, 16, 18] and anti-p40 (anti-IL-12/23) [23–25, 68, 69] principles are highly effective in psoriatic patients. These findings confirm the centrality of the IL-23-IL-17A axis, as these levels of efficacy have not been previously seen in studies with other biologics. Safety data for these agents is limited but has been favorable to date [15, 16, 18, 23–25, 68, 69]. Anti-IL-17A antibodies or anti-p40 antibodies, which target both IL-12 and IL-23, demonstrate potent and rapid effects on cytokine and chemokine expression and reduction of CD3+ T cells in psoriatic skin [15, 70]. As similar levels of PASI responses are obtained with anti-IL-17A or anti-p40 antibodies, it appears that, in the majority of psoriatic patients, the contributions of the IL-12/IFN-γ axis and of IL-22 are limited or can be bypassed.

The majority of psoriatic patients respond also to anti-TNF principles [71–73] and to cyclosporin A (CsA) [74, 75]. It has also been proposed that the clinical response to anti-TNF therapy could involve the IL-23-IL-17A axis. TNF would activate CD11c+ inflammatory DC to produce IL-23 establishing a TNF-IL-23-IL-17A axis [76]. In support of this proposal, down-modulation of the myeloid DC genes and of the IL-17A pathway is detected in responders to an anti-TNF principle (etanercept) [77]. As discussed previously, different populations of IL-23R+ cells could contribute to pathology.

Moreover, IL-17A and TNF act synergistically together and with other cytokines to induce an upregulated expression of several target genes [78, 79]. By neutralizing one critical cytokine, the remaining pool of cytokines would not generate the required threshold of activation in the responding cells, normalizing the inflammatory response. In particular, TNF and IL-17A activate keratinocytes, promoting the release of innate inflammatory molecules such as DEFB4, S100A7, and IL-8 [79]. However, neutralization of IL-23, IL-17A, or TNF does not cure the disease as relapses do occur when treatment is suspended [73].

A different insight into the pathogenesis of psoriasis can be obtained from clinical studies which have been using different approaches including targeting cell population markers, growth factors, and molecules involved in homing and costimulation

Table 1 Clinical improvement of psoriatic patients treated with different biologics (selected clinical studies and results)

	PASI 50 (%)	PASI 75 (%)	PASI 90 (%)	Ref.
Anti-IL-17A (ixekizumab at week 12)				[16]
Placebo ($n = 26$)		8	0	
75 mg at week 0, 2, 4, 8, 12, 16 ($n = 29$)		83	59	
150 mg at week 0, 2, 4, 8, 12, 16 ($n = 28$)		82	71	
Anti-IL-17RA (brodalumab at week 12)				[18]
Placebo ($n = 38$)	16	0	0	
140 mg at week 0, 1, 2, 4, 6, 8, 10 ($n = 39$)	90	77	72	
210 mg at week 0, 1, 2, 4, 6, 8, 10 ($n = 40$)	90	82	75	
Anti-p40 (IL-12 + IL-23) (ustekinumab—PHOENIX II at week 12)				[23]
Placebo ($n = 410$)	10	3.7	0.7	
45 mg weeks 0 and 4, then every 12 weeks ($n = 409$)	83.6	66.7	42.3	
90 mg weeks 0 and 4, then every 12 weeks ($n = 411$)	89.3	75.7	50.9	
Anti-p40 (IL-12 + IL-23)(briakinumab at week 12)				[25]
Methotrexate 5–25 mg/week weekly ($n = 163$)		36.2		
200 mg weeks 0 and 4, then 100 mg every 4 weeks ($n = 154$)		76.6		
Anti-TNF α (adalimumab at week 16)				[72]
Placebo ($n = 398$)		7	2	
40 mg eow ($n = 814$)		71	45	
Cyclosporine (at week 16)				[75]
3 or 5 mg/kg/day ($n = 42$)		71	33	
Anti-CD11a (efalizumab at week 12)				[83]
Placebo ($n = 122$)	16	5	<1	
1 mg/kg/week ($n = 232$)	52	22	4	
2 mg/kg/week ($n = 243$)	57	28	6	
Human anti-CD2 LFA-3/IgG1 fusion protein (alefacept at week 12)				[82]
Placebo ($n = 59$)	32	11		
0.075 mg once a week for 12 weeks ($n = 55$)	63	31		
0.150 mg once a week for 12 weeks ($n = 58$)	42	19		
Denileukin diftitox (at week 4)				[80]
Placebo ($n = 12$)	0			
0.015 mg/kg/day (3 day week for 4 weeks) ($n = 8$)	25			
Anti-CD4 (HuMax-CD4 at week 7)				[84]
Placebo ($n = 17$)	6			
280 mg once weekly for 4 weeks ($n = 16$)	19			

[80–84]. The majority of these approaches have shown partial beneficial effects, as the percentage of responding patients and the entity of the response are not comparable to those achieved with anti-IL-17A, anti-P40, anti-TNF biologics, or with CsA (Table 1).

In this context, the response of psoriatic patients to efalizumab, a humanized monoclonal antibody to CD11a, is particularly interesting [83]. CD11a is the α-chain of lymphocyte function-associated antigen (LFA-1), which is composed by

CD11a and CD18. LFA-1 is the most widely expressed form of the leukocyte-specific β2 subfamily of integrins, interacts with intracellular adhesion molecules (ICAMs), and contributes to leukocyte migration into tissues, antigen presentation to T cells, and interaction with keratinocytes [83]. However, the most prominent therapeutic effect of efalizumab is blockade of lymphocyte trafficking [85]. This explains the peripheral leukocytosis that occurs in all patients taking adequate doses of efalizumab, and CD8+ T cells contribute significantly to the leukocytosis [85]. In some patients, when the drug is stopped, there is therefore a reservoir of CD11a+ CD8+ T cells in the circulation ready to pour into the skin resulting in relapse [86]. This study confirms that in skin lesions developing during relapse, there are abundant T cells and DCs. The authors propose that CD8+ T cells enter the skin first initiating a psoriatic inflammatory cascade via DC activation [86]. This study is relevant as it suggests that, in a subgroup of patients, CD8+ T cells and antigen-presenting cells are main determinants of psoriasis pathogenesis. In addition, efalizumab is usually effective in patients with stable psoriasis but is not very active in controlling disease flares [87] again suggesting that other cell populations might be responsible for reactivation of the disease. In the same direction, the partial clinical efficacy of several interventions targeting T cells, including CTLA4-Ig [81], anti-CD4 antibodies [84], and alefacept (LFA-3 TIP) [82, 88, 89], demonstrates a critical role for memory/effector T cells in a limited number of patients [82]. In particular, the modest response to anti-CD4 antibodies [84] seems to suggest that CD4 cells including Th17 and Th1 cells are poorly involved in psoriasis. However, some patients were clear or had marked long-lasting improvements after the treatment [84] suggesting that, in selected patients, CD4+ T cells are responsible for the clinical picture.

In conclusion, the clinical results seem to suggest a complex scenario in which, in selected patients, different percentages of IL-17A-producing cells, including CD4+ cells, CD8+ T cells, γδT cells, neutrophils, mast cells, and eventually innate lymphoid cells, might be involved. The majority of these subpopulations would respond to IL-23 and produce IL-17A, so explaining the critical role of the IL-23-IL-17A axis. Again potential contribution by neutrophils in a minority of patients seems to be supported by an initial positive outcome of an anti-IL-8 antibody, ABX-IL8 [90], which however was not validated in a subsequent clinical study [91]. Subtle differences in disease pathogenesis are therefore underscored by treatment with biologics. In summary, these data suggest the involvement of different IL-17A-producing cell populations. We would like to propose that this might also contribute to the different level of response to anti-IL-17A and anti-p40 principles.

2.2 Anti-IL-17A Blockade in Rheumatic Diseases

Anti-IL-17A approaches have also been tested in several other autoimmune diseases, and these principles have shown beneficial effects in patients with rheumatoid arthritis (RA), psoriatic arthritis (PsA), and ankylosing spondylitis (AS) [15, 17, 92–95]. Secukinumab has been reported to induce clinically relevant responses

of variable entity in patients with RA, PsA, and AS [15, 93–95], while ixekizumab has shown activity in RA [17, 92]. Ustekinumab significantly reduces signs and symptoms of PsA and diminishes skin lesions compared with placebo [96]. In this last study, a difference in the entity of the response of the arthritic and the skin components is evident with better response achieved for the skin and a faster response for the joint disease. These data and the partial resistance of the joint disease suggest that the joint disease and the skin component recognize slightly different pathogenic mechanisms and that additional pathways might be involved in the pathogenesis of the arthritic component. In PsA, a trend toward a beneficial clinical effect of secukinumab was seen and larger clinical trials are planned [93].

The variable response rates to anti-IL-17A or anti-p40 approaches might be linked to activation of additional cell types or to the fact that blockade of only one or few cytokines might become inadequate to influence the cytokine milieu that contributes to inflammation and pathological changes. Recent data in the human RA synovium SCID mouse model confirm that different mechanisms might be operative in RA, as secukinumab is effective only when CD3+ T cells are present in the synovial tissue, while anti-B cell approaches require the presence of B cells [97].

As a rule, in the clinical trials, less than 50 % of the patients achieve an ACR70, and it is not clear whether the magnitude of the response of a patient to the different treatments would be similar. In other terms, it is not known whether there are patients who would be resistant to all available treatments. In RA, some TNF inadequate responders can benefit from anti-IL-17A antibodies [92].

In patients with active AS, secukinumab substantially reduces spinal inflammation as detected by MRI [95]. MRI changes are seen as early as 6 weeks after start of treatment and are maintained up to week 28, supporting the use of anti-IL-17A principles for this disease.

As for psoriasis, also in these diseases, different IL-17A-producing cells have been identified including Th17 cells [98–100], KIR3DL2+ CD4 T cells [101], CD161++ CD8+ T cells [57], γδ T cells [4], mast cells [8, 102], and neutrophils [103, 104]. Moran et al. [104] reported that IL-17A expression is localized to several immune cell subtypes within the inflamed synovial tissue including neutrophils, mast cells, and CD3+ cells. As already discussed for psoriasis, it is possible that different cell types might variably be involved in different patients or at different stages of the disease, and this could be partially responsible for heterogeneity in pathogenesis and response to treatments. In particular, neutrophils appear to be a significant source of IL-17A in affected joints from patients with AS with extensive spinal fusion [103], while mast cells might be a major contributor in patients with short disease duration, prominent peripheral arthritis, and limited axial involvement [102].

2.3 Anti-IL-17A Blockade in Additional Diseases

Secukinumab has also shown positive effects in patients with uveitis [15], but is not beneficial in patients with Crohn's disease [105]. The lack of a response in Crohn's disease is remarkable as many of the genetic variations associated with psoriasis

and PsA are also present in immune disorders considered psoriatic comorbidities, such as Crohn's disease [106]. IL-17A expression is clearly detectable in CD3+ T cells or CD68+ monocytes/macrophages [7], and IL-17A+ cells have been isolated from patients with Crohn's disease [107, 108]. In addition, CD3− CD56− innate lymphoid cells positive for IL-17A and IL-17F are present in inflammatory bowel diseases, and a significant and selective increase in CD127+ CD56− innate lymphoid cells in the inflamed intestine in Crohn's disease patients but not in ulcerative colitis patients has been reported [109].

In this disease, ustekinumab shows a partial response [110, 111], suggesting that other IL-17A-related cytokines produced by T cells or the IL-12 pathway might be relevant. It would be interesting to know the reason for the termination of brodalumab study (NCT01150890) in Crohn's patients, as this could shed light on the potential contribution of IL-17F and other IL-17 family members to pathology of this disease.

3 Conclusions

Anti-IL-17A approaches and anti-p40 approaches have now entered the clinic, and they are effective in the treatment of patients with different unrelated autoimmune diseases. In addition, they are helping to dissect the pathogenesis of these diseases and the search for more appropriate and personalized treatments. Even if, in some clinical conditions such as psoriasis, the IL-23-IL-17A axis seems to be well established, in other immune disorders considered psoriatic comorbidities such as Crohn's disease, other pathogenetic mechanisms appear prevalent. In psoriasis, different cells responding to IL-23 and producing IL-17A might contribute to pathology. This complexity might be operative also in other autoimmune diseases.

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