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Review

IL-17-expressing cells as a potential therapeutic target for treatment of immunological disorders

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Abstract:
IL-17 is a multifunctional cytokine produced by activated CD4+ and CD8+ lymphocytes as well as stimulated unconventional Tγδ and natural killer T cells. IL-17 induces expression of chemokines, proinflammatory cytokines and metalloproteinases, thereby stimulating the inflammation and chemotaxis of neutrophils. Elevation of proinflammatory cytokines is associated with asthma and autoimmune disorders, such as multiple sclerosis, rheumatoid arthritis and psoriasis. Although the role of IL-17 in these disorders is not always easy to define, extensive research has demonstrated an aggravating influence of IL-17 in some animal models. Thus, the development of therapeutics to reduce IL-17 levels is a promising strategy for ameliorating inflammatory diseases.
This review briefly summarizes recent knowledge about stimulants and intracellular signaling pathways that induce development and maturation of IL-17-expressing cells. Its positive and negative roles on disease progression and its importance in vaccine-induced memory are also discussed. Finally, recent literature describing potential therapeutic approaches for targeting IL-17 is presented.

Key words:
IL-17, T cells, autoimmunity, asthma, cancer, vaccination, therapy

Abbreviations: AHR – retinoid acid receptor, CD – cluster of differentiation, CHS – contact hypersensitivity, CIA – collagen induced arthritis, CNS – central nervous system, EAE – experimental autoimmune encephalomyelitis, EC – endothelial cells, ECM – extracellular matrix; GM-CSF – granulocyte-macrophage colony-stimulating factor, IBD – inflammatory bowel disease, IFN – interferon, IL – interleukin, LPS – lipopolysaccharide, MMP – matrix metalloproteinase, MS – multiple sclerosis, NF-κB – nuclear factor kappa B, NKT – natural killer T cells, NOD – nucleotide oligomerization domain, PAMP – pathogen associated molecular patterns, PGE – prostaglandin E, PMBC – peripheral mononuclear blood cells, PRR – pattern recognition receptors, RA – rheumatoid arthritis, RAR – retinoid acid receptor, RORγ – retinoid-related orphan receptor gamma; RRMS – relapsing reemitting multiple sclerosis, siRNA – small interfering RNA (ribonucleic acid), SOCS – suppressor of cytokine signaling, STAT-3 – signal transducer and activator of transcription 3, TCR – T cell receptor, TGF-β – transforming growth factor beta, TLR – toll-like receptor, TNBS – 2,4,6-trinitrobenzene sulfonic acid, TNCB – 2,4,6-trinitrochlorobenzene, TNF – tumor necrosis factor, Treg – regulatory T cells, VEGF – vascular endothelial growth factor

Introduction

IL-17 was cloned and characterized as a novel cytokine that bound a to newly described receptor in 1995 [109]. It has been reported that IL-17 is mainly produced by CD4+ T cells, predominantly by the activated CD4RO+ fraction. Additionally, IL-17 is involved in inflammatory processes, stimulating the

production of IL-6, IL-8 and PGE2, and is indirectly involved in hematopoesis by inducing expression of GM-CSF in stromal tissues [24]. Later IL-17 was found to play a role in rheumatoid arthritis (RA) [14] and multiple sclerosis (MS) [69]. Follow-up studies led to designation of IL-17-expressing CD4+ cells as a new T helper population, Th17, which led to the revaluation of the role of IFN-γ producing Th1 cells in autoimmunity.

Experiments employing IL-23p19 knockout mice showed that these mice were resistant to experimental autoimmune encephalomyelitis (EAE) [20]. Follow-up studies on IL-23p19 knockout mice, revealed that IL-23 is involved in propagation of IL-17-producing cells because their number was drastically reduced in these mice. Further, it was found that IL-17-producing cells are responsible for EAE pathology because the adoptive transfer of Th17 lymphocytes into naive mice caused these mice to develop EAE [54], whereas IL-17 deficient mice were protected from developing EAE [75]. Although the detrimental role of IL-17 has been confirmed in many animal models of autoimmunity and other pathological conditions, its mechanism of action remains unknown. However, IL-17 has been shown to participate in the host defense by stimulating the migration of neutrophils, which provides an adequate response against fungal and some bacterial infections. These data demonstrate that IL-17 and IL-17-producing cells are powerful therapeutic targets for the treatment of EAE and other autoimmune disorders.

IL-17 biology

IL-17, an 153 amino acid polypeptide now synonymous with IL-17A, is the oldest described member of IL-17 family. It was identified on the basis of its 58% homology with the open reading frame of herpesvirus saimiri. Since that time, five additional members of the IL-17 family, named IL-17B through IL17F, have been recognized. Extensive examination has been conducted to reveal the role of IL-17F, which shares the highest homology with IL-17A and IL-17E, the most divergent member of the IL-17 family. IL-17F was discovered to be important, acting in parallel with proinflammatory cytokine IL-17A. In contrast, IL-17E (IL-25) was shown to act as an anti-inflammatory agent by inducing Th2 response [46]. Much less is known about the actions of IL-17B and IL-17C. In contrast to IL-17A and IL-17F, these cytokines stimulate TNF-α and IL-1β production in human leukemic monocytic cell lines, but they do not enhance IL-6 release from fibroblasts [57]. All of the members of the IL-17 family, except IL-17B, exert their biological activity as dimers by binding to widely expressed IL-17 receptors [46]. The IL-17 receptor family consists of five members: IL-17RA, IL-17RB, IL-17RC, IL-17RD and IL-17RE. These designations are based on sequence homology because the ligands for IL-17RD and IL-17RE are unknown [46]. Widely expressed IL-17RA receptors are known to form homodimers and heterodimers with other IL-17 receptor subtypes. Heterodimers consisting of IL-17RA and IL-17RB receptors are expressed by macrophages and mast cells of mucosal tissues. These heterodimers are involved in IL-17E (IL-25) signal transduction, which plays a role in the development of the Th2 response and lung eosinophilia [56]. Multimerization of IL-17RA and IL-17RC enables signal transduction from proinflammatory cytokines IL-17A and IL-17F. IL-17A and IL-17F bind to their receptors with different affinities. It has been reported that both IL-17A and IL-17F bind to IL-17RC with similarly high affinity, whereas the affinity of IL-17A to IL-17RA is 1,000 times greater than IL-17F [37]. Binding of these cytokines to multimeric receptors results in activation of MAPK kinases and the NF-κB pathway, leading to induction of proinflammatory cytokines, chemokines and metalloproteinases.

T-cells that secrete IL-17 and their differentiation

There are several T cell subtypes that secrete IL-17. In addition to antigen specific CD4+ (Th17) and CD8+ cells (Tc17) cells, which are the most potent producers of IL-17, an unconventional T cell subpopulation, which releases IL-17 and includes Tγδ and NKT cells, was recently described. Moreover, recent studies indicate that some subpopulations of IL-17-producing T lymphocytes, apart from traits characteristic of Th17 lineage, may also have specific features of distinct Th1 or Treg populations. Historically, scientists have mostly focused on CD4+ T cells, which secrete IL-17 at a much higher level than CD8+ T lymphocytes

[109]. CD4+ T cells that secrete IL-17 were shown to have unique features and were classified as a distinct T helper population called Th17. Development of Th17 cells from naive precursors is inhibited by IFN-γ and IL-4, which are released by Th1 and Th2 cells, respectively [28]; in contrast, IL-6 and TGF-β enhance the development of Th17 cells. Similar to Th1 and Th2 subtypes, the developmental program of Th17 cells is governed by a lineage-specific transcription factor known as RORγt [40]. The role of RORγt in the development of Th17 cells was confirmed by studies showing that RORγt knock-out mice lack Th17 cells and do not develop EAE [40]. These experimental data were supported by human studies showing that stimulated CD4+ T cell clones isolated from MS patients secreted more IL-17 than clones from healthy individuals [99]. Further studies showed that Th17 cells also play a role in animal models of psoriasis, inflammatory bowel disease and uveitis. The precise definition of the role of Th17 cells in different pathological conditions is hampered by the lack of specific surface markers to identify these cells. Th17 lymphocytes are mainly found among memory cells of peripheral mononuclear blood cells (PMBC), which belong to CCR4+CCR6+ or CCR2+CCR5 subpopulations.

The first report on IL-17-secreting CD8+ cells isolated from PMBCs indicated that these cells have a low potential for IL-17 secretion [109]. However, following assessment of IL-17 secretion by a stimulated fraction of CD8+ PMBCs, CD8+ T cells were found to be as potent as CD4+ T cells at production of IL-17 [90]. Both IL-6 and TGF-β, which promote expression of the Th17 lineage-specific transcription factor RORγt, are required for induction of Tc17 cells [59]. Additionally, development of Tc17 cells is supported by IL-23 and inhibited by IFN-γ [29].

The activities and functions of Tc17 cells are not well documented; however, they secrete other Th17-associated cytokines, and their cytotoxic potential is greatly reduced in comparison to Tc1 cells [36]. Tc17 cells are associated with conditions traditionally ascribed to Tc1 cells, including contact hypersensitivity (CHS). The role of IL-17 in CHS induction was demonstrated by the adoptive transfer of TNCB-sensitized T cells from IL-17$^{-/-}$ mice to wild-type mice, which significantly reduced CHS induction. This phenomenon was reversed when IL-17$^{-/-}$ mice were reconstituted with CD4+ T cells from wild-type mice before hapten immunization [74]. Moreover IL-17 takes part in elicitation phase, as IL-17R$^{-/-}$ mice receiving hapten-primed, wild-type CD8+ T cells showed a significant reduction in CHS compared to wild-type controls [30]. In humans CD8+ IL-17-secreting lymphocytes have been found in the skin of patients who suffer from CHS to nickel. In contrast to Th17, which are found in CCR2^{+}CCR5^{-} or CCR6^{+}CCR4^{+} subsets, Tc17 clones express CCR5 but not CCR4, suggesting a different path of circulation [48].

In a series of experiments conducted primarily in mice, RORγt-expressing, IL-17-producing clones were recognized as a distinct population in which differentiation is antagonized by Th1 or Th2 pathways. However, human T cell developmental pathways seem to be much more flexible because cells that co-express distinct cytokines, e.g., IL-4 and IFN-γ, are easily identified. Additionally, a high proportion of CD4+ T cells that simultaneously produce IL-17 and IFN-γ (Th17-1) have been detected in the intestine of Crohn's disease patients and in persons who suffer from uveitis [3, 95]. Additionally, these cells share phenotypic features with the Th17 lineage in their selective expression of CCR6, IL-23R and RORγt; Th17-1 clones are also characterized by the expression of the Th1-specific transcription factor T-bet [3]. Some results indicate that these double-positive cells are induced by IL-17-expressing clones in an environment that favors Th1, whereas factors that favor Th17 development are not sufficient to induce IL-17 expression in Th1 clones [89]. This is consistent with results showing inhibitory activity of Th1 or Th2 cytokines on Th17; however, the ability of IL-17 to suppress Th1 and Th2 is more delicate. Th17-1 cells have lower susceptibility to Treg-mediated regulation [3], and they preferentially cross the blood-brain barrier in MS patients [41], indicating their pathogenic potential.

Recently more attention has been given to IL-17-producing Tγδ cells, which are predominantly localized outside lymph nodes in mucosal tissues. In contrast to Tαβ cells, some naive Tγδ cells have features resembling the activated phenotype of Th17 lymphocytes, which is characterized by CCR6, RORγt, IL-23R and IL-17 production immediately after antigen encounter [93]. It has been suggested that IL-17 can be induced in Tγδ cells without TCR engagement, either by direct activation by PAMP acting through TLR2 and dectrin-1 receptors or by IL-1β and IL-23 released by activated dendritic cells [68, 93]. Thus, Tγδ cells may serve as the first line of defense against pathogens by acting immediately after the recognition

of stress and danger signals. IL-17 produced by Tγδ cells may stimulate the release of chemokines from epithelial cells or fibroblasts to attract neutrophils to eliminate the pathogen. This response was observed during *Mycobacterium tuberculosis* and *Escherichia coli* infections. Thus Tγδ cells appear to be the dominant and early source of IL-17 in response to infection. Subsequently, $CD4^+$ Tαβ Th17 cells may also develop and contribute to the immune response [84]. In addition, Tγδ cells seem to be involved in CIA [39, 84] and EAE where they work in an amplification loop to activate CD4+ IL-17-producing T cells.

Another population of innate T lymphocytes known as NKT cells was also shown to produce IL-17. These cells lack the NK1.1 antigen and constitutively express RORγt and the receptor for IL-23, which is characteristic of differentiated Th17 cells. Similar to Tγδ cells, IL-17 is induced in NKT cells by stimulation with IL-23 alone or after engagement with its TCR by polymorphic ligands. Both factors act synergistically; however, production of IL-17 is independent of IL-6, which is crucial for induction of antigen specific Th17 cells [83].

Factors that drive expansion of IL-17 producing cells

Naive unconventional T cell subtypes, e.g., Tγδ and NKT, are able to produce IL-17 immediately after antigen encounter. IL-17 production by CD4+ T cells is considerably more complex. Their differentiation into antigen-specific effector cells is under the control of cytokines at the site of induction. This specific milieu of cytokines is influenced by the activation status of the antigen presenting cell and is modulated by the engagement of pattern recognition receptors (PRRs), including membrane-bound TLRs (Toll-like receptors) [63] and cytoplasm-associated NODs (nucleotide oligomerization domains) (Fig. 1).

Cytokines that regulate IL-17 production by CD4+ T cells

Initial studies in mice indicated that IL-23 is crucial for driving the expansion of pathogenic CD4+ IL-17-expressing T cells [28, 54]. Following further investigation it was discovered that IL-23 is capable of inducing IL-17, but only in a fraction of activated cells. Stimulation of naive T cells with IL-23 did not lead to production of IL-17 because they do not express IL-23R [2].

Simultaneously, three independent groups discovered a combination of cytokines that induce development of Th17 cells in mice [6, 65, 101]. It was shown that supplementation of an anti-inflammatory TGF-β rich environment with proinflammatory cytokines, such as IL-6, was sufficient to induce IL-17 production by naive CD4+ T cells. It is widely accepted that TGF-β induces a population of FoxP3-expressing regulatory T cells (Treg), which suppress the inflammatory response. However, when in the presence of

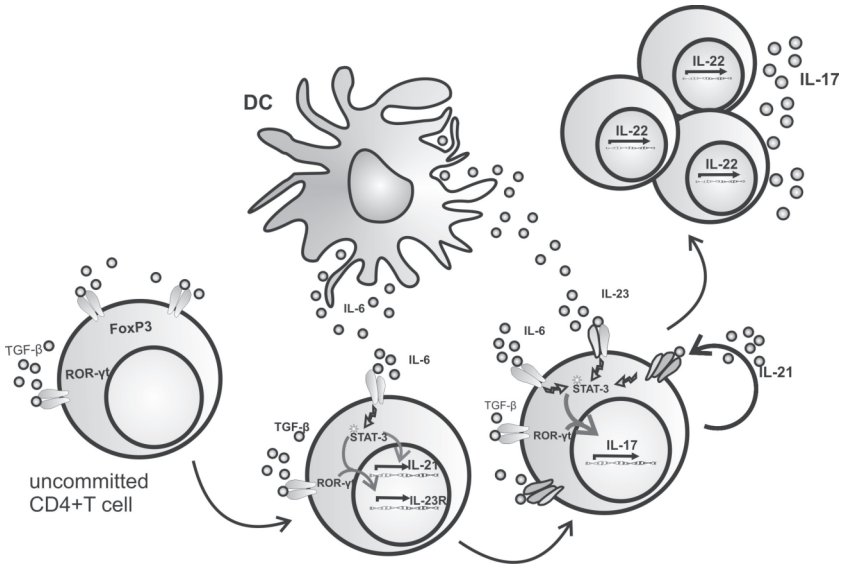

Fig. 1. Differentiation of the Th17 lineage. TGF-β induces expression of lineage-specific transcription factors FoxP3 and RORγt in uncommitted CD4+ cells. Dendritic cells (DC) sense microbial products through PRRs, promoting their cytokines production. IL-6 activates STAT-3, which is necessary and sufficient for induction of IL-21 expression, which increases precursor cell frequency. STAT-3 also liberates RORγt from FoxP3-mediated suppression. Active RORγt is sufficient for induction of IL-23R expression, which is essential for receiving stabilizing signals from IL-23. The combined activities of TGF-β, IL-6 and IL-23 induce expression of IL-17. Terminally differentiated Th17 cells produce IL-22

inflammatory signals, such as those generated by LPS, TGF-β supports formation of proinflammatory Th17 cells [101]. Thus IL-6, the major cytokine produced by LPS-activated dendritic cells, acts as a switch between Treg and Th17 lineages by suppressing induction of FoxP3 and favoring Th17 development [6]. These results were confirmed by studies showing that IL-$6^{-/-}$ mice had disequilibration between these two T cell populations, leading to an abundance of Treg cells and a reduction of IL-17-expressing lymphocytes, which resulted in resistance to EAE [86] and CIA [7]. However, depletion of Treg cells in IL-$6^{-/-}$ mice made them susceptible to both EAE and CIA, indicating that the development of Th17 lymphocytes can be controlled by factors other than IL-6. Indeed, it was found that IL-21, which belongs to the IL-2 family, acts similarly to IL-6 to regulate the balance of Treg and Th17 production [49]. Its inability to substitute for IL-6 in Th17 induction is a consequence of its low expression levels because it is mainly produced by activated Th17 cells. However, IL-21 acts like IL-2, creating a positive-feedback loop to increase Th17 precursor frequency [49].

Development of Th17, which is induced by the combination of IL-6 and TGF-β, is associated with induction of IL-23R. However, when the concentration of TGF-β is high, this process is repressed due to downregulation of IL-23R, which transmits signal that stabilize and sustain expansion of Th17. Additionally, it was found that IL-23p$19^{-/-}$ mice possess decreased frequency of Th17 cells, showing that IL-23 plays an essential role in the development of Th17 cells. Moreover, activation of IL-23R further stimulates maturation of Th17, leading to IL-22 production in terminally differentiated Th17 cells. IL-22 is a paracrine factor that participates in the remodeling of surrounding tissues, especially those of endothelial and epithelial barriers. Expression of IL-22 is reduced by high concentrations of TGF-β, which decreases cell responsiveness to IL-23 by diminishing expression of IL-23R [71, 115].

IL-17 production by human CD4+ T cells appears to be stimulated by similar factors found in mice. Initial data on the role of TGF-β in IL-17 production were inconsistent; some reports suggested that this cytokine was capable of inhibiting Th17 differentiation [1, 104] while others described its inessentiality in Th17 differentiation [17]. These divergent results were challenged when experiments carried out in serum-free medium demonstrated that TGF-β and IL-21 are necessary for Th17 induction [105]; without these cytokines, the human T cell profile was shifted towards Th1 [102]. It seems that interpretation of the initial results was confounded due to serum in the culture medium, which contains high concentration of TGF-β. TGF-β activity is known to be dose-dependent and has inhibitory activity at high concentrations. Further experiments showed that differentiation of the human Th17 lineage is regulated by proinfammatory cytokines such as IL-1, IL-6, IL-21 and IL-23 [102]. It was found that IL-1β is involved in activation of naive T cells isolated from peripheral blood, and this cytokine is the strongest inducer of IL-17. IL-6 and IL-21 further enhance IL-17 production [1, 104].

Signaling pathways involved in IL-17 induction in CD4+ cells

Discovery of a new Th17 lineage was associated with the identification of lineage-specific transcription factor RORγt. This orphan nuclear receptor with a ligand binding cavity was characterized as both necessary and sufficient for induction of IL-17 production. Naive CD4+ cells transfected with retroviruses harboring RORγt in the absence of lineage-inducing cytokines were shown to produce IL-17 [40]. Expression of RORγt is induced by TGF-β [64]. Concomitantly, TGF-β stimulates expression of the Treg cell inducer FoxP3, which interacts with RORγt to inhibit its transcriptional activity in the absence of additional cytokines. Proinflammatory cytokines such as IL-6 and IL-21 stimulate signal transducer and activator of transcription (STAT-3), which liberates RORγt from repression, leads to induction of IL-23R on naive T cells and promotes IL-17 expression [114, 115]. In memory T cell fractions, STAT-3 activation can also be induced by triggering IL-23R signaling.

Innate signals modulate Th17 generation

PRRs, which are predominantly expressed by innate immune cells, are pathogen sensors that recognize conserved microbial structures called pathogen-associated molecular patterns (PAMPs). Their triggering leads to activation of antigen presenting cells and production of cytokines, the patterns of which depend on pathogen type, allowing for the development of an appropriate adaptive immune response. It has been observed that activation of certain PRRs leads to sub-

sequent production of IL-17 [23, 38], which can influence disease severity and outcome [8].

Dendritic cells are major players in directing the inflammatory response due to their high potential for antigen processing and presentation as well as their ability to sense pathogen type *via* a variety of TLR receptors. Following stimulation by microbial products, TLRs activate IRF, MAPK and NF-κB signaling pathways, which leads to the production of cytokines (review in [94]). As mentioned above, IL-1β and IL-23 are involved in IL-17 induction. Production of IL-1β and IL-23 has been observed after activation of TLR2 by the fungal antigen zymosan. The microbes *Porphyromonas gingivalis*, *Helicobacter pylori*, *Haemophilus influenzae*, *Klebsiella pneumoniae* and *Mycobacterium tuberculosis* are capable of both inducing Th17 responses and activating TLR2 signaling. However, the ability of zymosan from yeast cell walls to induce Th17 lymphocytes is not restricted to TLR2. Zymosan is a complex molecule containing structures that are also capable of stimulating dectin-1 receptors, which synergize with TLR2 [25]. Stimulation of TLR2 by its specific ligand, PAM3CysSerLys4, induces the MyD88-dependent pathway and does not result in the production of high levels of IL-23 [13], indicating the importance of additional pathways for activating IL-23. Indeed, stimulation of dectin-1 receptors with β-glucan [10] or its linear form, culdran, induces Syk-dependent production of IL-23 and IL-1β [55] with simultaneous downregulation of IL-12 [26]. On the other hand, inhibition of the Syk-dependent pathway leads to suppression of IL-23 and IL-1β production and subsequent inhibition of Th17 development [91].

IL-17 pathways as targets for drug development

IL-17 is a complex molecule, which has both positive and negative effects on disease. This cytokine is important for enhancing the host defense at epithelial surfaces. In contrast, many reports show a deleterious role of IL-17 in autoimmunity, tumorigenesis, lung pathology and graft rejection. Thus factors that modulate IL-17 production and availability might be good targets for drugs development.

Role of IL-17 in autoimmunity

Multiple sclerosis (MS) is a central nervous system (CNS) disease associated with destruction of myelin sheets, leading to impaired nerve signal transduction. It has been traditionally regarded as a Th1-mediated disease driven by IFN-γ activated macrophages, which further amplify disease pathology [5]. Similar to other proinflammatory cytokines, the concentration of IL-17 is increased in cerebrospinal fluid (CSF) [69] and in CNS lesions [60] of MS patients, which correlates with neutrophil infiltration of the CNS. Furthermore, IL-17 neutralization with monoclonal antibodies [34] or loss of IL-17 in IL-17$^{-/-}$ mice [47] significantly, but not completely, suppressed EAE development. Undeniably, IL-17 is an important factor directing disease progression in CNS; however, its role can vary depending on the underlying pathological mechanism. For example, neutralization of IL-17 was not able to suppress EAE induced by IL-12 expanded T cells [51].

The role of IL-17 in rheumatoid arthritis (RA) is more complex. It has been shown that RA patients have elevated levels of IL-17 in synovial fluids [50], which correlates with the production of matrix metalloproteinase (MMP-1) and the destruction of synovial tissue [15] as well as enhanced osteoclastogenesis, which leads to bone erosion [50]. These processes are mostly attenuated in mice with collagen-induced arthritis (CIA) following treatment with anti-IL-17 antibodies [62], and their induction is effectively suppressed in IL-17 deficient mice [75]. The pathological role of IL-17 in arthritic joints is associated with its stimulation of MPP, VEGF and proinflammatory cytokine production and increased recruitment of T lymphocytes and innate immune cells. Surprisingly, these processes are not attenuated when arthritis is induced by proteoglican in IL-17$^{-/-}$ mice [22]. These results indicate the substitutive role of other factors and demonstrate the complexity of pathological mechanisms in patients with rheumatoid arthritis.

IL-17 is also produced by T cells in skin lesions of patients with psoriasis [96], a chronic skin disease characterized by hyperproliferation of keratinocytes and acantosis, production of proinflammatory molecules favoring angiogenesis and T cell infiltration; these pathological events result in the appearance of red skin with scales. It has been reported that IL-17 and Th17-associated cytokines, such as IL-23 [81] and IL-22 [113], work synergistically with IFN-γ to promote these pathological changes. Diminished lev-

els of IL-17 and Th17-associated cytokines correlate with reduced psoriatic lesions in patients treated with enantercept (tumor necrosis factor (TNF) receptor-immunoglobulin fusion protein).

Additionally, IL-23 and IL-17 have been linked to the pathogenesis of inflammatory bowel disease (IBD), including Crohn's disease and colitis ulcerosa. However, the role of IL-17A in intestinal inflammation has remained controversial. It might depend on the IL-17 isoform involved; IL-17A seems to be protective whereas IL-17F is involved in dextran sodium sulfate-induced colitis (DSS-induced colitis) [106]. Additionally, IL-17A was found to play a protective role in the CD45RBhi transfer model of colitis [79], whereas the same cytokine may aggravate trinitrobenzenesulfonic acid-induced colitis (TNBS-induced colitis) [112].

Thus targeting specific Th17 cells to promote proper production of IL-17 might be useful in the treatment of autoimmune diseases.

Contribution of IL-17 to pathogenesis of asthma

Asthma induces many irreversible changes in airway tissues. The bronchial lumen is narrowed through hyperplasia of smooth muscle cells and deposition of extracellular matrix (ECM) produced by hyperplasic myofibroblasts. Additionally, epithelial cells and mucus glands produce high amounts of mucus. All of these processes contribute to airway hyperresponsiveness in response to nonspecific stimuli, which manifests as wheezing and obstruction of bronchioles [19]. These processes are induced by inflammatory mediators produced by lung-infiltrating eosinophiles and neutrophils. Patients with persistent and chronic asthma who have low responsiveness to corticosteroids [27], which have been traditionally used for treatment of eosinophilic asthma, have increased neutrophil counts. Because elevated levels of neutrophil chemotaxis-inducing IL-17 have been detected in sputum samples and in bronchoalveolar lavage fluids of asthmatic patients in comparison to healthy controls [72], targeting IL-17 may prove useful for the treatment of asthma. Indeed, neutralization of IL-17 with a mAb reduced neutrophil counts in bronchial lavage fluids [31]. This approach may act directly on pathological processes in asthma because IL-17 was shown to play a role in expression of mucins [16] and profibrotic molecules, such as IL-6 and IL-11, by fibroblasts [72].

IL-17 and tumor growth

Recent reports demonstrate the complex role of IL-17 in tumor development. Some results indicate its pathogenic role in malignancy while others demonstrate a role for IL-17 in defense against tumors. The net effects of IL-17 on tumor development depend on tumor type.

IL-17-expressing CD4+ and CD8+ T cells are increased in mice with tumors, and their numbers gradually increase during tumor development, reaching their highest levels in advanced cancer states. Although CD4+ T cells have been recognized as the main producers of IL-17, CD8+ T cells are an equally important source of this cytokine in tumor tissues [52]. IL-6 and TGF-β, which are abundant cytokines in tumor tissue, were shown to induce CD8+ IL-17-producing T cells. These cells have a lower cytotoxic potential due to inhibited granzyme expression and decreased production of IFN-γ; consequently, these cells are able to support tumorigenesis. [59].

Additionally IL-17 has pro-tumor activity due to its promotion of angiogenesis. Newly formed blood vessels supply growing tumors with nutrients and enable tumor spreading. They are formed by endothelial cells (EC), which acquire angiogenic potential after activation. IL-17 works as a proangiogenic factor and stimulates EC migration and cord formation [77]; however, IL-17 does not support EC proliferation. Moreover, IL-17 indirectly enhances EC proliferation *via* induction of proangiogenic VEGF and IL-8 in fibroblasts [78]. IL-17 can also induce chemokine production and subsequently enhance recruitment of endothelial progenitor cells to support angiogenesis.

However, recent findings indicate substantial eradication of large established tumors after adoptive transfer of exogenously expanded CD8+ IL-17-expressing T lymphocytes, which are converted to IFN-γ producing cells in mice [33]. Moreover, the anti-tumor activity of CD8+ T cells was supported by adoptive transfer of antigen-specific Th17 cells, leading to inhibition of lung carcinoma development [67].

Vaccine induced-immunological memory relies on IL-17

As mentioned previously, IL-17 often plays a detrimental role in pathology; thus its elimination is regarded as a potential treatment. In contrast, some reports suggest that IL-17-producing T cells are critical

components of vaccine-induced immunological memory, indicating that compounds that induce Th17-associated immune responses may have potential application as adjuvants. Encounter with live pathogen leads to development of proper immunological responses that comprise both humoral and cellular components. Vaccination has been developed as a medical procedure that enables individuals to gain immunological memory against infectious agents, without the hazard resulting from infection with live pathogens. However, elimination of virulence from pathogens often leads to diminished cell-mediated immune responses. Thus to induce effective immunity, antigens from specific pathogens are mixed with adjuvants, which are substances that increase and modulate immunological responses. Good adjuvants should favor the induction of pathogen-specific responses efficient for eliminating infectious agents. Thus the type of response developed during vaccination depends on the specific adjuvant used.

Recent reports suggest that IL-17-producing T cells induced during vaccination play a role in the responses to extracellular and intracellular pathogens and viruses [58]. IL-17-expressing cells induced during vaccination participate in the recruitment of neutrophils, which control responses to extracellular bacteria and have been shown to provide protection from *Helicobacter pylori* and *Streptococcus* in mouse models [21, 61]. Moreover, IL-17 mediates recruitment of INF-γ producing Th1 cells [21], which potentiate the killing activity of macrophages, suggesting a potential role for vaccine-induced IL-17-producing cells in the elimination of intracellular pathogens, such as *Mycobacterium tuberculosis*. Indeed, Th1 responses that control *M. tuberculosis* infection seem to be dependent on IL-17-producing cells, which are induced during vaccination; neutralization of IL-17 reduces Th1 cell accumulation in mice after challenge [42]. Direct evidence showing that IL-17 producing cells are essential for the efficient induction of immunity to *Mycobacterium* have been provided by animal studies [85]. Similarly, vaccine-induced IL-17 and IFN-γ appear to be required for sufficient protection from *Bordetella pertussis* [32].

In sum, Th17 cells are crucial players in the generation of vaccine-induced protective responses against various pathogens. Therefore, understanding the mechanism of the induction and maintenance of IL-17 vaccine-induced responses might have a significant impact on vaccine strategies against infectious diseases.

Targeting development of IL-17 producing cells

IL-17 has been demonstrated to be involved in several pathological conditions. However, more research is necessary to establish the precise role of this cytokine and translate findings from animal models to humans because some approaches, which have been promising in animal studies, often fail in clinical trials. The methods for directly reducing IL-17 levels are limited to antibodies; however, targeting IL-17-inducing factors, such as cytokines, transcription factors or molecules responsible for propagation and signaling, may also be helpful (Fig. 2).

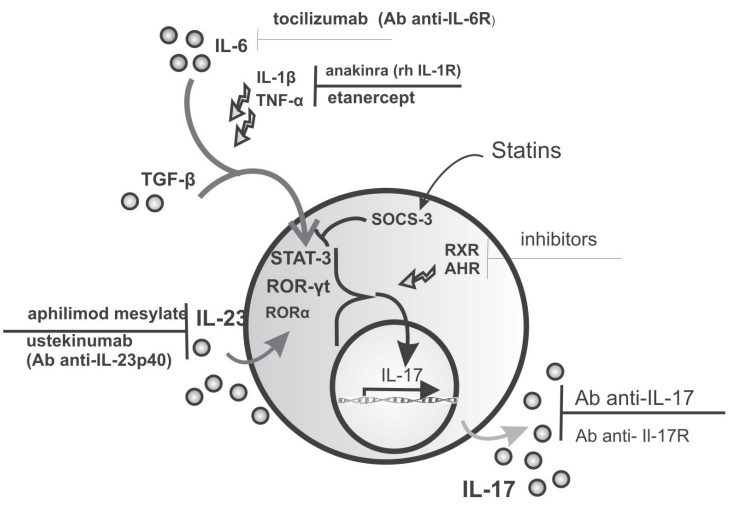

Fig. 2. Potential therapeutic targets in the IL-17 pathway. IL-23 is a cytokine necessary for driving expansion of Th17 cells. Its availability can be diminished by anti-IL23 antibodies or inhibitors of IL-23p40 transcription. The pleotropic IL-6 can be eliminated by targeted antibodies. Additionally, IL-1β and TNF-α, which support Th17 differentiation, can also be inhibited by targeted antibodies. The transcription factors RORc, RORα, RXR and AHR, which modulate expression of IL-17, can be blocked by specific inhibitors. Induction of SOCS-3 by statins or inhibitors of angiotensin converting enzyme influence STAT-3 activity and shift the balance towards the Treg phenotype. Effector functions of IL-17 are reduced by antibodies that bind IL-17 or its receptor, hampering activation of downstream pathways

Targeting cytokines that drive expansion of Th17

Several cytokines have been shown to induce or support development of the Th17 lineage. Their neutralization by antibodies, especially when the cytokine participates in disease progression, may have therapeutic potential.

Proinflammatory cytokines, such as IL-1β, IL-6 and IL-23, participate in induction of IL-17 expressing cells. IL-1β and IL-6 are multifunctional cytokines, which regulate many disease-associated processes. Their neutralization with antibodies has been useful in clinical settings even before the discovery that they are involved in the induction of the highly proinflammatory Th17 cell population. IL-1 signaling is disrupted by anakinra, a recombinant human IL-1R [18], while IL-6 activity is suppressed by tocilizumab, an anti-IL-6R blocking antibody [92]. Both of these antibodies have been approved for the treatment of RA. It has been speculated that depleting IL-23, which is a key molecule for Th17 propagation [2], may be more effective in downregulating IL-17. IL-23 is a heterodimeric cytokine that consists of a p19 chain and a common p40 chain, which is also found in IL-12. Antibodies against the p40 chain were shown to have therapeutic potential in psoriasis [35], colitis [76] and EAE in preclinical studies.

Ustekinumab (CNTO 1275; Centocor) is a fully human antibody against Il-23p40 that is safe and well tolerated. Despite strong evidence showing that this antibody was able to reduce ongoing disease in the clinic [9], it failed in phase II clinical trials in patients with relapsing reemitting multiple sclerosis (RRMS). After 19 weeks of treatment, reduction of sclerotic plaques measured by incorporation of gadolinium was not observed [88]. However, ustekinumab caused rapid and significant improvement in patients with moderate and severe psoriasis and was approved for their treatment [80]. Positive effects of ustekinumab application were also observed in patients with Crohn's disease, importantly in those who did not respond to infliximab (anti-TNF-α). Currently the effectiveness of ustekinumab is being tested in other conditions. Another anti-Il-23p40 antibody, ABT-874, was also effective in phase II clinical trials in patients suffering from moderate or severe psoriasis [43] and patients with Crohn's disease [66].

Supplementing a mixture of standard cytokines that drive expansion of Th17 cells with TNF-α can further amplify Th17 expansion in mice and humans; TNF-α does not induce Th17 development on its own. Several biological drugs targeting TNF-α have been approved for the treatment of autoimmune disorders associated with increased activity of the Th17 lineage. Etanercept, a fusion protein of TNF receptor 2 and IgG1, is used to treat RA and psoriasis. Although its mechanism of action in RA has been well-defined, it is unknown how etanercept exerts its therapeutic effect in psoriasis. Despite the marginal role of TNF-α in the development of Th17 cells, treatment of psoriasis with etanercept leads to diminished expression of Th17-associated genes in patients who respond to the drug but does not affect expression of Th17-associated genes in non-responders [110].

Targeting of IL-23 availability by downregulating its expression is another method that may aid in the treatment of Th17-dependent diseases. Nuclear factor κB (NF-κB) is a common transcription factor that induces expression of proinflammatory genes. NF-κB family member, c-Rel, selectively regulates expression of the p40 chain of IL-23 and IL-12 [87]. Its translocation to nucleus and the consequent expression of IL-12 and IL-23 are reduced after treatment with apilimod mesylate (STA-5326) [103]. Potential application of this strategy was confirmed in preclinical studies in a model of IBD [103] and in phase I/II clinical trials in patients with moderate or severe Crohn's disease [11].

Modulating intracellular pathways

Transcription factor RORγt and retinoid acid receptor (RAR) are involved in the differentiation and propagation of Th17 lymphocytes. Additionally, aryl hydrocarbon receptor (AHR) plays a role in Th17 expansion and is essential for IL-22 production. RORγt, RAR and AHR therefore represent potential therapeutic targets. Their targeting may not only diminish production of IL-17, but also shift the balance between Th17 and Treg cells, which are reciprocally connected. Although not all of these factors have characterized inhibitors, there are several molecules that regulate them indirectly.

RORc is a human homologue of RORγt, which is indispensable for the development of IL-17 producing cells [40]. However, it was found that IL-17 expression is not completely abolished in RORγt$^{-/-}$ mice, indicating the presence of another crucial transcription factor involved in Th17 cell development. Indeed,

RORα, a member of the nuclear orphan receptor subfamily, is abundantly expressed in T cells and stimulates T cell differentiation towards Th17. Forced expression of RORα stimulates IL-17 production in a dose-dependent manner while its deficiency reduces the number of IL-17 producing cells, which correlates with ameliorated EAE [108]. ROR subfamily structure is characteristic of nuclear receptors because it consists of a highly conserved DNA binding domain and a ligand binding domain, which is moderately conserved among members. This ligand pocket seems to be an ideal target for inhibiting IL-17 production.

The activity of RORc and RORγt is regulated by FoxP3. Overexpression of FoxP3 reduces production of Th17-associated cytokines without influencing ROR expression [107]. Factors that promote FoxP3-expressing Treg cells and therefore modulate the balance between Treg and Th17 may have potential therapeutic applications.

Simvastatin (3-hydroxy-3-methylglutaryl coenzyme A reductase inhibitor) is a cholesterol-lowering agent that inhibits protein isoprenylation, thereby interfering with signal transduction pathways. Mice with EAE treated with statins showed reduced plaque formation and delayed onset of disease. It was recently demonstrated that simvastatin enhances expression of FoxP3 and inhibits RORc, causing a reduction in IL-23 and IL-6 production [111]. Concomitantly, simvastatin-induced suppressor of cytokine signaling 3 (SOCS-3), represses induction of STAT-3 and following activation of RORc [17]. Treatment with statins was also shown to inhibit production of IL-17 [111].

The angiotensin system, which is involved in blood pressure regulation, is another target for diminishing Th17 responses [53]. Lisinopril, which blocks angiotensin converting enzyme and therefore inhibits the generation of biologically active angiotensin, and candesartan, which is an antagonist of the angiotensin receptor, are recently identified modulators of Treg cells. These drugs induce FoxP3-expressing cells and inhibit Th17 lymphocytes through induction of SOCS-3, which results in a diminished Th17 response and suppression of EAE [82].

Retinoic acids have been identified as modulators of the balance between Treg and Th17; they induce Treg cells and inhibit production of IL-17 cells [73]. Retinoic acids bind to retinoic acid receptors (RAR and RXR), which belong to the same family of nuclear receptors as RORγt. In preclinical studies, they reduced colon inflammation in biopsies from IBD patients and suppressed TNBS-induced colitis in mice [4]. They were also shown to ameliorate EAE by inhibition of Th17 cells [45].

For many years, the ligand-regulated transcription factor aryl hydrocarbon receptor (AHR) was linked to dioxin-mediated toxicity through induction of cancer-promoting CYP1. However, recent studies indicate its role in immunity and Th17 commitment. AHR is preferentially expressed by Th17 lymphocytes. Its stimulation leads to increased frequency of IL-17 producing cells, which also express IL-22 [100], whereas its deficiency in $AHR^{-/-}$ mice reduces generation of IL-17 producing cells [44]. The importance of AHR in induction of IL-17 producing cells was further supported in a model of EAE. $AHR^{-/-}$ mice have delayed onset of disease compared to wild-type mice, whereas AHR stimulation leads to accelerated EAE development [100]. However, AHR inhibition can be especially useful in psoriasis, a disease associated with enhanced levels of the Th17-associated cytokine, IL-22. It has been shown that a population of skin-homing Th17 cells that co-express CCR6, CCR4 and CCR10 downregulate IL-22 expression after AHR inhibition by siRNA without influencing IL-17 production [98].

Exclusive inhibition of IL-17 signaling

Specific inhibition of IL-17 signaling can be achieved by blocking the interaction of IL-17 with its receptor or by targeting IL-17 availability through deprivation of IL-17 by antibody treatment or use of an IL-17R fusion protein. An IL-17RC fusion protein that neutralizes both IL-17A and IL-17F may be useful in the treatment of CIA, EAE and psoriasis. Its application in IBD and asthma needs further examination due to the differential impact of IL-17 isoforms on the progression of these diseases. Potential application of compounds acting on IL-17RA is limited because they only sequester IL-17A or IL-17E, which bind to homodimers of IL-17RA and IL-17RB. Preclinical studies have demonstrated the efficiency of such therapy; the mIL-17R Fc fusion protein ameliorates adjuvant-induced arthritis [12]. This strategy is currently being applied to develop biological compounds.

Antibody neutralization of IL-17 is another method for inhibiting IL-17. Multiple preclinical studies indicate that this is an efficient tool for ameliorating disease. Currently two anti-IL-17 antibodies are under investigation in clinical trials. AIN457 is a human anti-IL-17A antibody that has been developed by

Novartis to treat psoriasis and RA. Initial results with this agent in RA patients have demonstrated its safety and efficacy. Another antibody, LY2439821 developed by Eli Lily, has shown improvement in disease in early phases of clinical trials.

Binding of IL-17A or IL-17F to their receptors induces signaling cascades that modulate the expression of proinflammatory genes. Compounds that target IL-17R may lead to its disrupted activation and the subsequent decline of proinflammatory gene expression. IL-17R is a multimeric complex consisting of IL-17RA and IL-17RC [97]. IL-17RC has much higher affinity for IL-17 than IL-17RA [37], and IL-17RA has a large intracellular domain that interacts with TRAF6 through Act-1, which is indispensable for signal transduction. Antibodies against IL-17RA were shown to suppress receptor activation by both IL-17A and IL-17F isoforms [70]. Thus potential drugs targeting IL-17RA, which are currently under investigation, might be useful in the treatment of inflammatory processes.

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