Targeting Interleukin-17A – An Orchestrator of Neutrophil Mobilisation in the Lungs

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Abstract: This presentation briefly summarizes the immunology and the pathology of the T cell cytokine interleukin-17A (IL-17A) in the lungs and addresses the potential of IL-17A as a pharmacotherapeutical target. Accumulating experimental and clinical evidence suggests that IL-17A is of importance for coordinating the adaptive and the innate components of pulmonary host defence in mammals. This evidence also suggests that IL-17 is produced by several subsets of T cells, including the T helper-17 (Th-17) subset. Until now, IL-17A has emerged mainly as an orchestrator of the local accumulation and activity of neutrophils; a role that IL-17A plays by inducing the release of C-X-C chemokines, colony-stimulating factors and IL-6. Even though its true role may be more diverse, the proposed role of IL-17A is relevant not only for pulmonary host defence against bacteria but also for inflammatory conditions in the lungs, such as severe asthma, chronic obstructive pulmonary disease, cystic fibrosis, and lung allograft rejection. From an immunological point-of-view, IL-17A's position at the interface of adaptive and innate immunity, is intriguing. It forwards the possibility that intervention targeting IL-17A can provide new therapy against inflammatory lung disorders related to poor endogenous control of local neutrophils.

Keywords: Lung disease, therapy, neutrophil, IL-17, T cell.

INTRODUCTION

The protein that was to be named interleukin-17A (IL-17A) was initially named cytotoxic T lymphocyte associated antigen 8 (CTLA8) in the original publication by Rouvier and co-workers in 1993 [1]. Since then, IL-17A has become the focus of more than 1,500 scientific publications and is now the best known member of the interleukin-17 family of cytokines [2-6]. Interestingly, IL-17A is likely to coordinate the adaptive and the innate components of host defence in mammals and it may play its main role in several organs by orchestrating the mobilisation of neutrophils [2-7]. Moreover, the discovery of IL-17A has led to the identification of a previously unknown subset of IL-17-producing T helper cells in 2005; the Th-17 subset [4,5,8,9]. Notably, the characterisation of this Th-17 subset has challenged the classic "Th-1/Th-2 paradigm" and complicated the view of mammalian host defence. More recent studies on the Th-17 subset in animal models and in patients now implicate that IL-17A from Th-17 cells can play an important role in inflammatory conditions in the lungs, even though clinical trials of this concept are still lacking [4,5,8-10]. This review article summarizes the current knowledge about the immunology, the pathology and the potential utility of therapeutically targeting IL-17A in the lungs.

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IMMUNOLOGY

Molecular Characteristics

The human homodimeric protein IL-17A has a molecular size of approximately 35 kDa; with each monomer including 155 amino acids in humans [1,2,11,12]. Notably, IL-17A was first identified as a rodent cDNA transcript, obtained from a T-lymphocyte hybridoma [1]. The molecular characteristics of the IL-17 family of cytokines includes a canonical cystein knot fold of β-strands, disulphide linkages and a conserved amino acid sequence close to the C-terminal [13]. As indicated from mice, rats and humans, IL-17A is very well conserved between species, in particular around the glucosylation site of the cytokine. Currently, IL-17B, -C, -D, -F and the heterodimer IL-17A/F are all classified as IL-17 family members [2,13-19]. In contrast, the cytokine that was previously named IL-17E has now been re-named IL-25 because this cytokine has a more "esoinophilic" functional profile [2,13-19]. Among the IL-17 cytokines, IL-17F is believed to be the one most similar to IL-17A and this monomer can actually form a heterodimer with the IL-17A monomer [2,9,13-19].

Receptor Signalling

The IL-17 receptor subtypes display a unique structure and no homology with previously identified cytokine receptor families [20,21]. Interleukin-17 RA, the archetype IL-17 receptor protein is a type I membrane protein, consisting of a 293 amino acid extracellular domain, a 21 amino acid trans-membrane domain and a 521 amino acid cytoplasmic tail. This particular receptor protein is expressed in many different cell types in humans and mice [20,21]. So

2210-2892/10

far, four additional IL-17 receptor protein subtypes have been added to IL-17RA and these are named IL17RB, -C, -D and E [12,16,22]. More recently, it has been suggested that IL-17RB protein can serve as a receptor not only for IL-17B but also for IL-17E when formed as a heteromeric receptor complex with IL-17RA [2,12,16,22,23]. Just like IL-17RA, all known IL-17 receptors proteins are type I trans-membrane proteins, displaying significant alternative splicing [12].

In early studies, it was demonstrated that IL-17 signals at its receptor at low concentrations, with relatively low affinity, and because of this, it was assumed that there are additional receptor molecules; molecules that are required to facilitate IL-17 signalling [13,20,21]. In support of this, there is now data suggesting that effective IL-17-signaling does require a heteromeric receptor complex consisting of IL-17RA and IL-17RC [24,25]. Collectively, published studies suggests that, after ligation of IL-17A to this receptor complex, a specific adaptor protein, Act1, is involved in the activation of down-stream signalling molecules, including the kinase TAK1 and the E3 ubiquitin ligase TRAF6, [9,24-27]. These signalling molecules, as well as some mitogen-activated protein [MAP] kinases, functionally link IL-17A to the down-stream activation of transcription factors, including nuclear factor-*k*B and CCAAT/enhancer binding Protein-β [25,26,28,29]. The receptor signalling for IL-17A may also involve janus-activated kinase [9,25,30].

Interestingly, it has been proposed that IL-17F as well as the heterodimer IL-17 A/F signal through the heteromeric receptor complex consisting of IL-17RA and IL-17RC [24,25,31-33]. Moreover, there might be a species difference between humans and mice for the receptor binding affinities [33,34]. In humans, IL-17A binds with comparable affinities to both IL-17RA and IL-17RC, but IL-17F binds to IL-17RC much stronger than to IL-17RA whereas, in mice, IL-17A binds to IL-17RA with much higher affinity than to IL-17RC.

Interleukin-17-Producing Cells in the Lungs

We still do not understand the full nature of IL-17A-producing cells in the lungs of humans and other mammals [9]. This means that it remains to be determined whether the majority of IL-17A-producing cells in humans lungs have the typical characteristics of "Th-17 cells", the postulated T helper cell subset that has been predicted to be critical for the production IL-17A [1,7-11]. Interestingly, Th-17 cells in humans display both similarities and differences, when compared with those in mice, but correspondingly conclusive studies in human lungs are lacking [35]. In fact, the current knowledge on IL-17-producing cells in human lungs is quite rudimentary.

Ivanov and co-workers have previously demonstrated immunoreactivity for IL-17A protein in a subset of relatively small, mononuclear cells from bronchoalveolar (BA) lavage samples harvested in healthy volunteers after exposure to organic dust [36]. The referred research group have also demonstrated that exposure to organic dust increase mRNA for IL-17A protein in BAL cells harvested under the referred conditions. In a preceding study on this model of severe neutrophilic inflammation, the same research group demonstrated a substantial increase in free, soluble IL-17 protein [37]. Thus, these results suggest that there are "lymphoid" cells within the human BA space that are capable of producing IL-17A protein. The fact that there is a correlation between messenger RNA for IL-17A and CD3γ in cells from induced sputum from patients with moderate to severe asthma, is compatible with these "lymphoid" cells in the BA space being T cells [38].

In contrast to the work on neutrophilic inflammation caused by organic dust, two previously published studies on patients with asthma have claimed that eosinophils account for the production of IL-17A protein in human airways [39,40]. Notably, though, none of these studies on asthma demonstrate the release of free, soluble IL-17A protein in isolated eosinophils *in vitro.*

There is interesting information from animal models as well. Systemic depletion of the CD4 and/or CD8 subsets of T cells prior to infection with the gram-negative bacterium *Klebsiella pneumoniae* in the lungs of mice *in vivo*, results in a marked decrease of IL-17 protein in BAL samples [41]. Moreover, when cultured *in vitro*, the CD4 but not the CD8 subset does respond to IL-23, an up-stream IL-17-stimulating cytokine, when stimulated *in vitro*, when using BAL cells from mice primed with endotoxin exposure in the airways *in vivo* [42]. The more specific characteristics of these CD4 cells remain unknown, however. Even more intriguing, mice that have been infected with *Mycobacteria* in the lungs display γδ T cells that produce IL-17 in the lungs [43,44]. Moreover, a particular subset of invariant natural killer cells, a subset lacking the NK1.1 marker, does produce IL-17A protein after stimulation with synthetic ligands or with endotoxin in the lungs *in vivo* [45]. Clearly, we need novel studies that explore IL-1β, IL-2, IL-6, IL-21, IL-23, IL-27 and tumor growth-transforming factor-β in the lung context; cytokines believed to be critical for the regulation of "Th-17 subset" [8-10].

Cellular Effects of IL-17A in the Lungs

Based upon the published evidence, it seems most likely that IL-17A produces its neutrophil-accumulating effect in an indirect manner, by inducing the production and release of neutrophil mobilizing factors from stromal cells [2,5-7,9,10]. Thus, *in vitro* data on chemotaxis of human blood neutrophils demonstrates that IL-17A *per se* does not cause chemotaxis for these cells [45]. In contrast, stimulation of human bronchial epithelial cells with IL-17A induces production and release of the neutrophil-recruiting C-X-C chemokines, including IL-8, granulocyte chemotactic protein-2 and growth-related oncogene-α *in vitro* and macrophage inflammatory protein-2 *in vivo* [46-51]. Moreover, conditioned medium from IL-17-stimulated human bronchial epithelial cells increases chemotaxis of human blood neutrophils and this particular effect is blocked after pre-treatment of the conditioned medium with a specific neutralizing IL-8 antibody [47]. Interestingly, several studies forward evidence that mitogen-activated protein [MAP] kinases are involved in mediating these chemokine responses to IL-17A [48,49,51]. Of potential therapeutic interest, the IL-17-induced release of GRO-α and GCP-2 is attenuated by glucocorticosteroids [49], whereas there is conflicting data on this for IL-8 [49,50]. In analogy with the case in bronchial epithelial cells, venous endothelial

cells, lung fibroblasts and airway smooth muscle cells from humans and other mammals respond to IL-17A stimulation by releasing C-X-C chemokines [41,46,52-55].

Interleukin-6, a neutrophil-activating cytokine, is also released by structural cells such as bronchial epithelial cells and fibroblast in response to stimulation with IL-17A *in vitro* and probably also *in vivo* [47-50,56,57]. This is also true for the colony-stimulating factors, granulocyte colony-stimulating factor and granulocyte macrophage colony-stimulating factor [50,58].

Several experimental studies on mice and rats demonstrate that local stimulation with IL-17A causes a local accumulation of neutrophils in the BA space of these models *in vivo* [46,56,59-62]. It has also been demonstrated that the neutrophil-accumulating effect of IL-17A is selective, dose-dependent and long-lasting [46,58,59]. Interestingly, an induced over-expression of endogenous IL-17A protein causes neutrophil accumulation in the BA space of mice as well [63]. In line with this, mice lacking IL-17 receptors are less capable of accumulating neutrophils after stimulation with a bacterial pathogen in the BA space than the corresponding control mice [62]. Also, the fact that pre-treatment with a neutralizing anti-IL-17A antibody attenuates the IL-17A-induced accumulation of neutrophils in the BA space of rats is compatible with the effect being protein-specific [46].

In terms of chronic inflammatory lung disease and airway remodelling, it is of particular interest that IL-17A can cause local accumulation of proteases [2,6,7,9]. Indeed, it has been proven that local stimulation with IL-17A does increase the local activity of neutrophil elastase and matrix metalloproteinase-9 in the BA space *in vivo*, in rats and mice respectively [60,61]. Importantly, IL-17A can increase myeloperoxidase in the BA space as well, at least in rats *in vivo* [60]. However, the mechanisms may differ in sensitized airways, because when endogenous IL-17A is blocked prior to allergen challenge in mice *in vivo*, this blockade decreases the percentage of MMP-9-positive neutrophils in the BA space after allergen challenge [64].

Host Defence

Importantly, the involvement of IL-17A in host defence is yet to confirm in humans and this is also true for the lungs [7,9,10]. In contrast, the experimental evidence from studies on mouse models is solid and clearly indicates an important role of IL-17A in host defence of the lungs in this species [2,7,9,65]. Thus, it has repeatedly been shown that local administration of endotoxin from *E coli* increases the concentration of IL-17A protein in the BA space of mice *in vivo* [56,66,67]. Data from a study by Miyamoto and co-workers suggests that endogenous IL-17 is critical for the sustained but not for the early phase of the local neutrophil response [56]. Interestingly, local stimulation with endotoxin in the airways increases the archetype Th-17-stimulating cytokine IL-23 in inflammatory cells from the BA space and in lung tissue and IL-23 protein *per se* causes neutrophil accumulation in the BA space [42]. Notably, the latter effect is attenuated when endogenous IL-17 is blocked with a neutralizing antiobody. Of particular importance, Ye and co-workers have demonstrated that a lack of the receptor molecule IL-17RA leads to an increased mortality in pneumonia for mice after exposure to live *Klebsiella pneumonie* in the BA space [65]. This negative "clinical outcome" is associated with a concomitant reduction in local neutrophils, compared with control mice. There is now supportive evidence from mouse models of infection with bacterial species such as *Pseudomonas aureginosa*, *Bordetella pertussis*, *Mycoplasma pneumoniae* and *Streptococcus pneumonie* as well [9,68,69].

PATHOLOGY

We still do not know whether IL-17A really plays a causative role in inflammatory lung disease of humans [4,9,10]. However, there is accumulating evidence for an involvement of IL-17A in human lung disease. Currently, the strongest case is that for asthma.

Asthma

Important early work on IL-17A in patients with asthma was published by Chakir and co-workers [40]. Their study demonstrates a marked, relative increase in the number of cells that are immunorectivity for IL-17A protein in bronchial biopsies from patients with moderate to severe asthma, compared with mild asthma. In line with this, there are now studies demonstrating that IL-17-protein is increased in BA lavage fluid, sputum, and blood from patients with asthma [39,40,70]. It is of particular interest that, collectively, the referred studies suggest that there is no major increase in the concentration of IL-17A in patients with mild to moderate asthma, compared with healthy control subjects, because this implicates that the local increase in IL-17A is liked to a special phenotype of severe asthma; possibly associated with local accumulation of neutrophils [6,7,9,10]. On the other hand, a recent study comparing the "principal component analysis" of patients with chronic airflow obstruction with those that have intermittent airflow obstruction, actually claims that IL-17A is among the dominant variables in the latter group [71]. Clearly, this claim warrants further investigation. In further support for the involvement of IL-17A and neutrophils in asthma, the messenger RNA for IL-17A and the chemokine IL-8, respectively, is increased in cells from induced sputum of human patients with asthma [38]. Moreover, in the same patients, the level of IL-17 mRNA does correlates with the percentage of neutrophils in sputum samples [38].

In line with what has been documented in human patients with asthma, the results from two independent studies on mouse models of airway allergy support a role for endogenous IL-17A in mediating neutrophil accumulation [61,65]. In principle, both studies show that the blocking of endogenous IL-17A with a neutralizing antibody prior to allergen challenge attenuates the early neutrophil accumulation in the BA space following after the same challenge. In addition, one of these studies shows that the referred blockade of endogenous IL-17A prior to allergen challenge does reduce the relative number of accumulated MMP-9-expressing neutrophils in the BA space. The latter may have pathogenic bearing, because it suggests that IL-17A has an impact on neutrophil activity in acute, allergic airway inflammation.

There is also indirect evidence for the involvement of IL-17A in asthma and airway allergy; evidence for an

involvement of IL-17A in bronchial hyperreactivity. It is well-known that bronchial hyperreactivity is linked to asthma, even though not synonymous to it. It is therefore of interest that there is evidence that the local concentration of IL-17A protein in sputum samples does correlate with airway reactivity to methacholine in human patients with either asthma or chronic bronchitis [72]. In line with this finding in humans, allergen-induced airway hyperreactivity to methacholine in mice is less pronounced in a mouse strain lacking endogenous IL-17A protein [73]. Notably, a recent experimental study has focused on this issue and addressed it in relation to the importance of Th-17 cells and neutrophils in a mouse model of airway allergy [74]. The study does suggest that local allergen sensitisation primes more of a Th-17 than a Th-2 response, in contrast to systemic sensitisation *via* the peritoneal cavity. According to the same study, Th-17 cells actually home to the BA space where they release IL-17A and when endogenous IL-17A is lacking, there is less allergen-induced increase in airway reactivity to metacholine [74]. Notably, the same study also indicates that even though neutrophils are required but not sufficient for the allergen-induced increase in airway reactivity.

Chronic Obstructive Pulmonary Disease

Currently, there is only one published study assessing IL-17A in chronic obstructive pulmonary disease [75]. This study forwards data that shows an increased immuno-reactivity for IL-17A protein in the bronchial submucosa in smokers with and without airflow obstruction [75]. In support of this, there are two independent studies on mice that include data suggesting an increase of IL-17A protein in the lungs after exposure to tobacco smoke *in vivo* [76,77].

Cystic Fibrosis

There are two published studies on human patients suggesting the involvement of IL-17A in the pathogenesis of cystic fibrosis [78,79]. These two studies show an increase in the concentration of IL-17A, as well as in its up-stream regulating cytokine IL-23, in BA lavage and/or in sputum samples harvested during exacerbations of cystic fibrosis. However, the samples were harvested from patients colonised with *Pseudomonas aureginosa* and the controls were healthy subjects, so there is uncertainty with reference to what the conditions are like in patients with cystic fibrosis during stable clinical conditions.

Lung Allograft Rejection

Vanaudenaerde and co-workers forwarded early evidence that IL-17A is involved in acute rejection of lung allografts in humans [80]. Their study demonstrated that IL-17A in BA lavage samples can be increased at the messenger RNA and the protein level, during acute allograft rejection. There is also one experimental study on rats *in vivo* indicating that collagen type V-specific lymphocytes mediate lung allograft rejection, with a concomitant increase in messenger RNA for IL-17A locally at the site of rejection [81].

STRATEGIES FOR THERAPEUTICALLY TARGETING IL-17A

The current understanding of mammalian immunology suggests that "up-stream" cytokines such as IL-6, IL-15, IL-23, tumor-growth-transforming factor-β and tumor necrosis factor bear the potential to influence IL-17A production in T cells in the lungs and in other organs [2-5,10]. In addition, there is autocrine influence of IL-21 [2-5,10]. The induced IL-17A production, in turn, stimulates the subsequent production of "down-stream" neutrophil-mobilising cytokines including IL-6, IL-8 and colony-stimulating factors; factors that all exert direct actions on neutrophils [2-5,10]. This implicates that there are three principal strategies for therapeutically targeting IL-17A: 1) up-stream of it; 2) at the level of the cytokine *per se*; and 3) down-stream of it [10]. An overview of these strategies is presented in Fig. (**1**); where drugs of key interest are listed.

It should be noted that none of the therapeutic strategies addresses in Fig. (**1**) have yet been tested in inflammatory lung disease of human patients. However, targeting up-stream cytokines, such as IL-23 and tumor necrosis factor,

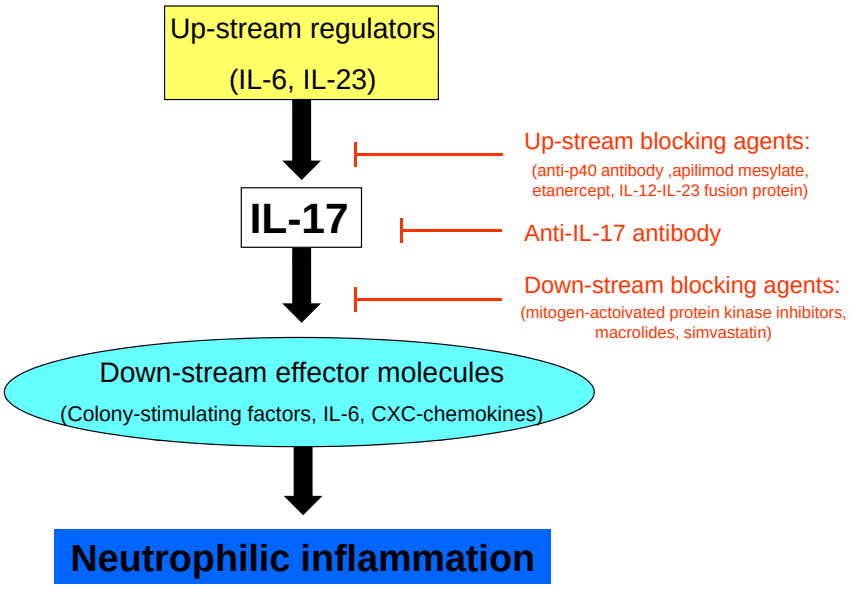

Fig. (1). Principal levels of targeting IL-17 signalling. Modified, with permission, from [10].

seems very feasible, since a monoclonal, neutralizing antibody against the p40 subunit of IL-23 and IL-12 is currently being evaluated in clinical trials on patients with *Morbus Chron* [10,81,82]. As an alternative to this antibody, there is at least one drug inhibiting the transcription of IL-23 and IL-12 (apilimod mesylate) as well as an IL-12 – IL-23 fusion protein that reduces IL-23-signalling [10]. Hypothetically, targeting IL-17A per se may be the most specific strategy, though, and targeting down-stream events may be the least specific strategy.

CONCLUSIONS

A solid clinical rationale for therapeutically targeting IL-17A in human lung disease is not yet established. The main reason for this is the relatively small number of published studies on the immunology and pathology of IL-17A in human patients; studies that until now have been conducted on relatively small patient materials. However, given what we now know about the experimental immunology and pathology of IL-17A in animal models, it can be speculated that targeting the neutrophil-orchestrating cytokine IL-17A will be therapeutically beneficial in lung diseases characterized by T helper cell activity and an excess accumulation and activation of neutrophils. Whether this should include subgroups of patients with acute or chronic lung allograft rejection, asthma, chronic bronchitis, chronic obstructive pulmonary disease, cystic fibrosis acute or chronic lung allograft rejection is in need of further study.

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ABBREVIATIONS

BA = bronchoalveolar

IL = interleukin

RNA = ribonucleic acid

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