

IL-17A in Asthma - A Question of Severity

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Abstract

Allergic asthma is a chronic inflammatory disease of the lung driven by aberrant responses to normally innocuous environmental allergens. Disease is characterized by excessive IgE synthesis, eosinophilic pulmonary inflammation, mucus hypersecretion, and airway remodeling, and airway hyperresponsiveness - all leading to the clinical features of disease - reversible episodes of coughing, shortness of breath and wheezing. While the excessive production of cytokines like IL-4, IL-5 and IL-13 by allergen specific-Th2 cells is sufficient to explain most features of allergic asthma, increasing evidence suggests that the Th2 paradigm does not explain the full spectrum of disease severity. In particular, severe asthmatics represent a small subset of asthmatics, in which disease is associated with more severe airway reactivity, a mixed eosinophilic/neutrophilic infiltrate, and insensitivity to treatment with corticosteroids. Thus, severe asthmatics are not only more susceptible asthma-related complications; they are underserved by currently available therapies. Recent evidence suggests that the development of severe disease may be associated with the development of a mixed Th2/Th17 response. Herein, we will assess the data from human and animal models suggesting a link between a mixed Th2/Th17 response, and the development of severe asthma. A greater understanding of the mechanisms responsible for the development of severe asthma may allow the development of efficacious therapeutics for the treatment of this intractable form of disease.

Allergic Asthma

Allergic asthma is a chronic inflammatory disease of the lung caused by excessive immune responses to normally innocuous environmental allergens. Chronic pulmonary inflammation causes smooth muscle hypertrophy and hyperplasia, goblet cell hyperplasia, fibrosis and airway remodeling, all of which contribute to the hallmark symptoms of allergic asthma - reversible episodes of coughing, shortness of breath, and wheezing. Incidence of asthma continues to rise, particularly in developed nations. Current estimates suggest that in the United States, 8.2% of the population suffers from the disease, with a total health care costs exceeding 19 billion dollars in 2009 [1], demonstrating a great social and economic impetus for greater understanding of disease pathology.

Classically, asthma is thought of as T cell mediated disease. In particular, Th2 cells are thought to be responsible for many of the pathological changes observed in the lung, largely by virtue of the cytokines they produce. IL-4 drives class switch to IgE [2], explaining elevated serum IgE levels observed in atopic asthmatics, and also supports differentiation of naive T cells into Th2 cells [3,4], promoting the further expansion of pathogenic Th2 cells. IL-5 promotes eosinophil development, maturation and recruitment to the airways, explaining the prominent eosinophilia noted in mouse and human models of disease [5]. IL-13 is thought to be a central mediator of disease pathology, as IL-13 is sufficient to induce most pathophysiological features of disease including mucus hypersecretion, airway remodeling, and airway hyperresponsiveness [6]. Indeed, allergen-induced asthma is completely abrogated in IL-13 deficient mice [7]. Collectively, these data support an important role for Th2-derived cytokines in the development of allergic asthma.

However, recent evidence suggests that the Th2 paradigm is not sufficient to fully explain the complete spectrum of disease. While individuals with mild or moderate disease do appear to mount a Th2-dominated immune response, in those with severe disease a number of observations suggest that other mechanisms may be involved. Rather than the predominant eosinophilia observed in patients with mild asthma, allergic inflammation in severe asthmatics is often neutrophilic in nature, and the degree of neutrophilia correlates with

disease severity [8-11]. Indeed, neutrophils represent the predominant cell types present in sudden onset, fatal asthma [12] suggesting that different mechanisms are driving the development of mild and severe asthma. Moreover, while steroids represent the most effective therapeutic option for individuals with milder asthma, individuals with severe asthma are frequently refractory to therapeutic interventions using glucocorticosteroids (GC) [13], and GCs can actually prevent neutrophil apoptosis, thereby enhancing neutrophil recruitment [14]. This again suggests that the disease processes that drive severe and mild disease are fundamentally different. A greater understanding of these processes may lead to better treatments for this intractable form of disease.

Severe asthma - A Mixed Th2/Th17 disease

Recent evidence suggests that the severity of disease is correlates with the expression of IL-17A and IL-17F. Compared to healthy controls, a significantly higher proportion of individuals with severe disease had high levels of IL-17A message in induced sputum, and these levels correlated strongly with the degree of neutrophilia [15]. Moreover, expression of these cytokines was low in lung biopsies taken from individuals with mild disease, while those with severe disease had significantly higher levels [16]. Similarly, elevated serum IL-17A levels were also identified as a risk factor for the development of severe asthma [17]. Furthermore, single nucleotide polymorphisms (SNPs) in the *IL17A* or *IL17F* promoters have been associated with development of allergic asthma [18,19]. Interestingly, one SNP associated with

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protection from asthma was found to encode an amino acid change (H161R) in IL-17F resulting in a mutant protein that was unable to induce the expression of genes normally driven by IL-17F, and was able to block the effects of IL-17A *in vitro* [20]. This suggests that mutant IL-17F binds to the IL-17R complex and, acting as a competitive inhibitor, prevents IL-17A and IL-17F signaling. However, homozygosity at this SNP was required for protection, suggesting that expressing one copy of the protective mutant is insufficient to effectively block IL-17A signaling [20]. Collectively, these studies suggest an association between IL-17A and IL-17F in the pathogenesis of severe asthma in humans.

The IL-17 family

IL-17A and IL-17F are closely related cytokines that are members of the IL-17 family of cytokines. This family also includes IL-17B, IL-17C, IL-17D, and IL-25 (IL-17E). Both IL-17A and IL-17F are secreted as homodimers, and have a well described role in host defense through their capacity to induce the production of anti-microbial peptides such as serum amyloid-A, lipocalin-2, C-reactive protein, β -defensins, and s100 proteins [21]. IL-17A and IL-17F also exhibit synergy with TNF α in the induction of pro-inflammatory mediators such as IL-6, and IL-8 [21]. Germane to the development of severe neutrophilic asthma, both IL-17A and IL-17F trigger the expression of a number of factors that drive neutrophil granulopoiesis and recruitment, such as G-CSF, GM-CSF, CXCL1, CXCL2, CXCL5 and CXCL8 [21]. Thus, it is possible that the increased recruitment of neutrophils observed in individuals with severe asthma, may be driven by the increased production of IL-17A and/or IL-17F observed in these patients. Although, the production of IL-17A/IL-17F heterodimers has also been reported [22-23], the precise biological role of such heterodimers has not been elucidated.

In contrast to IL-17A and IL-17F, IL-17E (IL-25) is increasingly being recognized as an important driver of Th2 immunity, and thus has been proposed as an important factor promoting the development of allergic disease [24]. Expression of IL-17B is rather broad, being expressed in the gut, prostate, ovary, testes and spinal cord, while IL-17C expression is more restricted, being expressed only in fetal kidney and prostate [25]. Finally, IL-17D is expressed in muscle, adipose tissue, brain lung and pancreas [26]. The biological roles of IL-17B, IL-17C and IL-17D are not known.

IL-17R family

IL-17 family members signal through a unique family of cytokine receptors, consisting of 5 known receptors: IL-17RA, IL-17RB, IL-17RC, IL-17RD, and IL-17RE. Both IL-17A and IL-17F bind to IL-17RA with low affinity, although the affinity of IL-17RA for IL-17A is higher than that for IL-17F [27]. To enhance IL-17A or IL-17F affinity and promote effective signaling, the IL-17RA pairs with IL-17RC, which markedly enhances affinity of the two cytokines for the IL-17R complex [27]. In contrast to IL-17A and IL-17F, IL-25 signals through a complex consisting of both IL-17RB and IL-17RA [28]. Thus, mice lacking IL-17RA have limited ability to respond to IL-17A, IL-17F, and IL-25. The roles for the other IL-17R family members (IL-17RD, IL-17RE) are unknown, however some reports do suggest that these receptors can also pair with IL-17RA, and thus enhance IL-17A or IL-17F signaling [29,30].

Sources of IL-17A: Th17 cells

The best characterized source of IL-17A and IL-17F is the CD4⁺ Th17 cell, which, in addition to producing high levels of IL-17A and IL-17F, also secretes IL-21 and IL-22. *In vitro*, Th17 cell differentiation is

optimally driven by a combination of IL-6, IL-1 β and TGF- β , although the presence of IL-21 can further support Th17 differentiation [31-33]. These cytokines induce activation of STAT3 (IL-6, IL-21) and SMAD2 and SMAD3 (TGF β) that in turn facilitate the expression of transcription factors that promote the development of Th17-lineage cells (ROR α , ROR γ t and IRF4), while suppressing the development of transcription factors involved in skewing towards the development of Th1 (Tbet, IRF1) and Treg (Foxp3) cells [34-36]. Exposure to Th17-driving cytokines also induces the expression of IL-23R on developing Th17 cells, allowing the cells to respond to IL-23, a critical survival and proliferative factor for Th17 cells [37]. However, recent studies have demonstrated that the type of cytokine used to differentiate Th17 cells can have a profound influence on the types of transcription factors and cytokines produced by skewed Th17 cells, raising the possibility that Th17 cells that develop under different conditions (or in different hosts) may have differential capacity to cause disease [38,39]. In support of this, Ghoreschi et al. have demonstrated that skewing Th17 cells in the presence of IL-1 β , IL-6 and TGF- β , induced the development of Th17 cells that produced very high levels of IL-17A and IL-17F, but co-expressed IL-10, and thus failed to induce disease in a model of EAE [38]. In contrast, differentiation in the presence of IL-1 β , IL-6 and IL-23 induced the development of a population of Th17 cells that produced lower levels of IL-17A and IL-17F, and no IL-10, but high levels of Tbet and IFN γ , and were capable of inducing the development of EAE [38]. Whether or not the cytokines or transcription factors produced by Th17 cells in individuals with severe asthma differ from those produced by Th17 cells from individuals with less severe forms of disease is unknown.

While the production of Th17 cytokines is associated with the development of severe asthma, there do appear to be mechanisms whereby Th2 cytokines can limit the development of Th17 responses. Newcomb et al. has recently demonstrated that Th17 cells from both mice and humans express IL-13R α 1 - a component of both the IL-13 receptor, and the type 2 IL-4 receptor [40,41]. Interestingly, polarization of naive T cells towards the Th17 lineage in the presence of IL-13, or re-stimulation of Th17 cells in the presence of IL-13 diminished their capacity to produce IL-17A and IL-21 [40,41]. This suggests that effector Th2 cytokines can directly antagonize Th17 cells, thus possibly preventing the development of more severe disease. It also raises the interesting possibility that defects in this pathway may predispose to the development of severe asthma. Thus, it would be interesting to assess IL-13 responsiveness in Th17 cells from individuals with severe versus mild asthma.

Th2/Th17 cells

Recent studies in human subjects highlight a novel T cell population that may contribute to the development of severe asthma. Cosmi et al, demonstrated that human CD4⁺CD161⁺ T cells isolated from human PBMCs could develop into clones with the capacity to simultaneously produce Th2 (IL-4, IL-5, IL-9), Th17 (IL-17A, IL-21, IL-22) and Th1 (IFN γ) cytokines [42]. The authors also demonstrate that a small subset of circulating CD4⁺CD161⁺ could simultaneously produce both IL-4 and IL-17A after stimulation, and that the frequency of these "Th2/Th17 cells" was significantly higher in individuals with asthma [42]. Interestingly, *in vitro* stimulation of PBMCs from Der p1-sensitized donors with Der p1 increased the frequency of both conventional Th2 cells, and Th2/Th17 cells, suggesting that both Th2 and Th2/Th17 cells in asthmatic individuals were antigen specific [42]. A similar, IL-4⁺IL-17A⁺CD4⁺ subset was also observed by Wang et al., who identified a subset of CD4⁺ T cells that co-expressed CCR2 (a Th2 cell marker)

and CCR6 (a Th17 cell marker) [43]. The CD4⁺CRTH2⁺CCR6⁺ T cells produced IL-17A, IL-22, IL-4, IL-5 and IL-13 after stimulation with α CD3/ α CD28, and expressed transcription factors associated with both Th17 (ROR γ t) and Th2 (GATA3, c-MAF) differentiation [43]. The frequency of these cells was increased in asthmatic individuals, and in a murine model of allergic asthma [43]. While the mechanisms by which these cells develop *in vivo* are unclear, they also that stimulation of CCR6^{neg}CRTH2⁺CD4⁺ cells to either IL-6, IL-21 or IL-1 β , (but not IL-23), was sufficient to trigger the expression of IL-17A *in vitro* [43]. These studies highlight the plasticity of Th2 cells *in vivo* and suggest an additional mechanism whereby through the induced production of Th17 cytokines, Th2 cells may promote the development of severe asthma.

Alternative sources of IL-17A

In addition to the classical Th17 cells, and the newly described Th2/Th17 cells, a number of additional sources of IL-17A and/or IL-17F have been described. In both humans [16] and mice [44] epithelial cells have been demonstrated to have the capacity to produce IL-17F. As the number of epithelial cells in the lung is large, it is likely that they may represent a significant source of IL-17F, however, the triggers required to induce IL-17F expression in epithelial cells remain undefined. iNKT cells - T cells expressing a limited repertoire of TCRs specific for lipid molecules presented in the context of CD1d - have been demonstrated to be a significant source of IL-17A following inhalation of ozone [45]. While the specific ligand responsible for the induction of IL-17A from iNKT cells was unclear, the authors speculate that inhalation of ozone caused the lipid oxidation, and that these oxidized lipids were recognized by the iNKT cells. In support of this, the authors found no induction of IL-17A in iNKT cells in mice exposed to OVA [45]. CD3^{neg}CD11b⁺F4/80⁺ alveolar macrophages have been reported to produce IL-17A following systemic OVA alum sensitization, followed by OVA inhalation [46]. Furthermore, administration of aerosolized 2-chloroadenosine (a reagent used to deplete macrophages [47]) abrogated production of IL-17A [46]. While the factors responsible for inducing IL-17A in macrophages *in vivo* remains unclear, culture of isolated alveolar macrophages with supernatants from cultures of activated mast cells upregulated IL-17A production *in vitro*, suggesting an important role for mast cells in this process. Thus T cells are likely not the only cell capable of producing cytokines that can drive the development of severe asthma.

IL-17A production in murine models of allergic asthma

Given the association between IL-17A expression and severe asthma in humans, a number of studies have attempted to dissect the role of this cytokine in the development of allergen-induced AHR in mice. Following systemic sensitization with OVA in alum, or OVA + LPS (0.1 μ g) followed by airway challenge with OVA in both BALB/c and C57Bl/6 mice, increased expression of IL-17A can be detected in the lungs [44,46,48-52], confirming that IL-17A can be produced in murine models of allergic asthma.

However, given that the magnitude of the Th17 response appears to be associated with the severity of disease, identification of factors that regulate how much IL-17A is produced is clearly important. Interestingly, while intraperitoneal sensitization with OVA + LPS (0.1 μ g) induces some IL-17A expression, sensitization with OVA + LPS (0.1 μ g) via the airway, followed by intranasal challenge with LPS-free OVA induced substantially more IL-17A production in lung T cells [52], suggesting that the route of sensitization can influence the magnitude of the IL-17A response. Not surprisingly, the genetic background of

the host also plays an important role in the development of an IL-17A response. We have demonstrated that following inhalation of house dust mite (HDM), a common human aeroallergen, we observe differential development of Th17 responses, (as measured by the frequency of pulmonary CD4⁺IL-17A⁺ T cells), in mice on different genetic backgrounds [53]. While the lack of complement component C5 is a major genetic determinant promoting the development of robust IL-17A responses [compared to naïve mice, a 2.5 fold increase in frequency of pulmonary CD4⁺IL-17A⁺ T cells was observed in the lungs of HDM-treated C5 deficient mice (AKR/J, DBA2/J, FVBN/J and A/J)], even complement sufficient strains mount variable IL-17A responses following allergen exposure. Thus, despite similar sensitization protocols, the increase in the frequency of pulmonary CD4⁺ IL-17A⁺ T cells ranges from limited in BALB/c mice (~1.1 fold), to moderate in C57Bl/6 mice (~1.5 fold), and high in DBA/1J mice (~2.1 fold) [53]. Thus, the magnitude of the IL-17A response observed after allergen exposure is controlled by a number of factors, including host genetics, route of allergen sensitization and the nature of additional signals present at initial allergen sensitization.

Functional role of IL-17A in allergic asthma

While human studies have demonstrated a strong link between the development of severe asthma and the production of Th17 associated cytokines, a direct causal role for IL-17A in enhancing asthma severity cannot be established in these studies. Thus, to explore the role of IL-17 signaling in the development of allergic asthma, a number of investigators have made use of IL-17RA-deficient mice. Schnyder-Candrian et al. demonstrate that *Il17ra*^{-/-} C57Bl/6 mice develop reduced pulmonary inflammation, IL-5 production and IgE levels compared to WT controls following intraperitoneal sensitization with OVA in alum, and intranasal OVA challenge [49]. Similarly, compared to WT mice, *Il17ra*^{-/-} mice on the BALB/c background also demonstrated significantly reduced pulmonary inflammation, eosinophilia, Th2 cytokine production (IL-4, IL-5 and IL-13), AHR [51]. Collectively these studies argue for an important role for IL-17A in promoting allergen sensitization. However, these studies are complicated by the recent demonstration that IL-17RA is also critical for the IL-25 signaling [28]. As IL-25 is widely regarded as a potent Th2 inducing cytokine, it is unclear whether the failure to develop robust allergic inflammation in *Il17ra*^{-/-} mice is due to a lack of IL-17A/IL-17F responsiveness, or a lack of IL-25 responsiveness.

To better address the roles of IL-17A in the development of allergic asthma, some studies have compared the development of asthma in mice specifically lacking IL-17A. Both C57Bl/6 and BALB/c *Il17a*^{-/-} mice demonstrated significantly reduced pulmonary eosinophilia, less robust Th2 cytokine production by spleen or lung cells, and decreased IgE levels following OVA alum sensitization and intranasal OVA challenge [54-55]. Interestingly, despite decrease airway inflammation AHR was not affected in *Il17a*^{-/-} mice following OVA alum sensitization and intranasal challenge, likely due to the intense Th2 response induced in the presence of alum [56]. In contrast, *Il17a*^{-/-} DO11.10 mice (expressing an OVA-specific TCR transgene) sensitized via OVA inhalation did demonstrate reduced airway inflammation and AHR demonstrating that following mucosal sensitization, IL-17A plays an important role in inducing AHR [55]. Similarly, blockade of IL-17A by administering anti-IL-17A monoclonal antibodies to OVA alum sensitized and challenged mice limited the development of allergen induced AHR, Th2 cytokine production and eosinophilia [46,50], suggesting a pathogenic role for IL-17A. Furthermore, we have demonstrated that blocking IL-17A in mice that develop a mixed Th2/

Th17 response associated with robust AHR (A/J mice) significantly reduced HDM-induced AHR [53]. Administration of exogenous IL-17A at the time of HDM-challenge was sufficient to induce more severe AHR in C3H/HeJ mice [53]. Similarly, in mice sensitized via mucosal of OVA exposure, challenge with OVA + IL-17A enhanced AHR over that induced by challenge with OVA alone [52].

However, while these studies suggest that the presence of IL-17A is associated with more severe disease, a handful of studies argue have observed either a protective, or no role for IL-17A in allergen-induced AHR. For example, IL-17A blockade had no impact on AHR or BAL cellularity in OVA alum sensitized and intranasally challenged BALB/c mice, although this is likely due to undetectable levels of IL-17A induced in these studies [45]. In contrast blocking IL-17A was found to exacerbate eosinophilia, IL-5 production in BAL and serum while IL-17A administration decreased eosinophil recruitment, chemokine production and Th2 cytokine production [48,49] suggesting a protective role for IL-17A. While these studies are difficult to reconcile with the studies arguing for a pathogenic role for IL-17A, a recent report by Besnard et al. suggests that the presence of IL-22 (another Th17-derived cytokine) may influence the ability of IL-17A to promote protective versus pathogenic responses in the lung. Interestingly, they demonstrate that blockade of IL-17A in *Il22*^{-/-} mice significantly enhanced eosinophilic inflammation and Th2 cytokine production, while IL-17A blockade in control mice had the opposite effect [57]. Thus, the authors argued that while pathogenic in the presence of IL-22, in its absence, IL-17A plays a protective role, limiting the development of allergen-induced AHR and eosinophilic inflammation [57], although it is equally plausible that IL-17A regulates the pathogenicity of IL-22. In support of the latter, a recent report that examined the relationship between IL-17A and IL-22 in a bleomycin-induced model of acute lung injury demonstrated that in the presence of IL-17A, IL-22 increased lung damage, whereas in the absence of IL-17A, IL-22 was tissue protective [58]. The possibility that IL-22 may regulate the pathogenicity of IL-17A (or vice versa) is interesting given that both are products of Th17 cells. As the pattern of cytokines present during initial Th17 cell differentiation can have a profound effect on the panel of transcription factors and cytokines secreted upon subsequent activation [38], it is conceivable that exposure to different cytokine milieus present during Th17 cell differentiation leads to different ratios of IL-17A versus IL-22, and the development of protective, versus pathogenic Th17 cells. The relative production of IL-22 and IL-17A in these models and in humans with mild versus severe asthma is a question that warrants further investigation.

Mucin production

Mucus hypersecretion and goblet cell hyperplasia are hallmark features of allergic disease. While administration of IL-13 directly to the airways is sufficient to induce marked increases in mucus secretion [6], a recent report suggests that IL-17A may have a unique role in promoting mucus hypersecretion. Using primary human tracheobronchial epithelial cells (TBEs), Chen et al demonstrate that out of a panel of cytokines tested, only IL-6 and IL-17A (but not IL-4, IL-9 or IL-13) enhanced expression of both *Muc5AC* and *Muc5B* [59]. In similar experiments using mouse TBEs, IL-6 and IL-17A induced 223 and 97 fold increases in *Muc5b* promoter activity respectively [59]. While the failure of IL-13 to induce mucin gene expression was surprising, *in vivo* studies making use of transgenic animals support the possibility that Th17 cytokines can induce mucus production. Over-expression of IL-17A in pulmonary epithelial cells (under the control of the CC10 promoter) induced marked infiltration with monocytes, and CD4⁺ or

B220⁺ lymphocytes accompanied by increased PAS staining [60]. These changes were not associated with detectable upregulation of any Th2-related genes examined (IL-4, IL-5, IL-9, IL-13) [60]. The mechanisms responsible for the IL-17A mediated induction of mucus production remain unclear, however, IL-17A has been demonstrated to induce the production of TSLP [54], an innate promoter of Th2 responses. Indeed, lung-specific overexpression of TSLP induces monocytic/lymphocytic infiltration, mucus hypersecretion, and extensive airway remodeling [61]. Thus, IL-17A may also contribute to the development of more severe asthma by directly (or indirectly) enhancing mucus production in the inflamed lung.

IL-17A, neutrophils and asthma

As neutrophilia is observed in individuals with severe asthma, and IL-17A stimulation induces the production of a number of factors that directly increase neutrophil granulopoiesis and recruitment, it is reasonable to hypothesize that the ability of IL-17A to promote severe asthma may be dependent upon its ability to induce neutrophilia. Wilson et al demonstrate that the recruitment of neutrophils following OVA challenge in mice sensitized with OVA + LPS via the airways is dependent upon IL-17A, as significantly reduced neutrophilia and AHR was observed in *Il17ra*^{-/-} mice [52]. To directly test the importance of neutrophils in driving more severe AHR, they examined AHR in mice lacking CXCR2 (the receptor for the neutrophil chemoattractants CXCL1 and CXCL5) or treated with anti-Gr1 Abs to deplete neutrophils. Mice lacking neutrophils developed reduced AHR, suggesting that IL-17A mediated neutrophil recruitment can promote more severe allergen-induced AHR [52]. However, the mere presence of neutrophils in the an inflamed lung is not sufficient to induce more severe AHR, as mice sensitized with OVA in alum (inducing a strong Th2 response), and challenged with OVA plus CXCL1 or CXCL5 (to trigger neutrophil recruitment) demonstrated no increase in AHR [52]. Thus, while neutrophils can exacerbate AHR when recruited by IL-17A, their mere presence is insufficient to cause the development of more severe AHR. Thus, it is possible that in addition to inducing the recruitment of neutrophils to the airways, the production of IL-17A in severe asthma induces activation of the recruited neutrophils, thus promoting the development of more severe AHR.

IL-17A mediated enhancement of IL-13 signaling

In trying to dissect the mechanisms through which IL-17A may exacerbate AHR, we have recently demonstrated that while administration of IL-17A directly to the airways is not sufficient to increase AHR, administration of IL-17A + IL-13 increases AHR beyond that induced by IL-13 alone [53]. Similarly, while administration of IL-17A was not sufficient to induce expression of IL-13 driven genes (*Tff2*, *Arg1*, *C3*), the capacity of IL-13 to induce expression of these genes was significantly enhanced when IL-13 was administered with IL-17A [53]. These data suggest that IL-17A can synergize with IL-13 to enhance IL-13-driven gene transcription and AHR. Further supporting this, transfer of a combination of Th2 cells and Th17 [62], or of cells simultaneously expressing both Th2 and Th17 cytokines [43,63] induced more severe AHR than transfer of Th2 cells alone. Similar synergy between IL-17A and IL-13 has also been observed *in vitro*, as IL-17A was shown to enhance IL-13-driven secretion of IL-19 from primary human bronchial epithelial cells [64]. These studies are interesting, given the observations of Newcomb et al which showed antagonism between IL-13 and production of Th17 cytokines at the effector T cell level [40,41]. It should be noted however, that IL-13 responsiveness of epithelial cells, or airway smooth muscle has been demonstrated to be

sufficient to induce the development of AHR, suggesting that structural cells may be an important effector cell in the development of asthma [65,66]. Moreover, we have clearly demonstrated synergy between IL-13 and IL-17A in pulmonary fibroblasts [53], suggesting that the outcome of interactions between IL-13 and IL-17A may be cell specific. Collectively, these studies suggest that while IL-13 may limit IL-17A production initially, if both are present during the effector phase of an immune response, IL-17A can directly synergize with IL-13, enhancing its biological activities, and thus providing a mechanism responsible for IL-17A-mediated exacerbation of allergen-induced AHR.

IL-17A and steroid resistance

Steroids represent the gold standard in asthma care, and efficiently control symptoms in the majority of asthmatics. However, there is a subset of asthmatics with severe disease that are refractory to corticosteroids. Therefore, a greater understanding of the mechanisms that control the development of severe steroid resistant asthma is needed. McKinley et al. recently demonstrated a role for Th17 cells in the development of steroid-resistant asthma. They showed that while exposure to dexamethasone (DEX) significantly inhibited production of IL-5 and IL-13 from Th2 cells, IL-17A and IL-22 were completely insensitive to inhibition by DEX [63]. Moreover, AHR induced by transfer of *in vitro* Th2-skewed OVA specific T cells followed by OVA challenge was significantly decreased by *in vivo* treatment with DEX [63]. In contrast, transfer of Th17-skewed OVA specific T cells (which appeared to co-produce IL-4), followed by OVA challenge induced AHR that was completely insensitive to inhibition by DEX [63], suggesting that Th17 cells may be uniquely insensitive to the inhibitory effects of steroids, and promote the development of steroid resistant asthma.

There is also some evidence that IL-17A (and possibly IL-17F) can directly induce steroid insensitivity in epithelial cells. Vazquez-Tello et al. recently demonstrated that bronchial biopsies from individuals with asthma demonstrated increased expression of IL-17A, and decreased expression of glucocorticoid receptor α (GR α), the classical glucocorticoid receptor [67]. Moreover, exposure of epithelial cells to IL-17A (and IL-17F) *in vitro* significantly increased expression of GR β , an alternative steroid receptor that is thought to act as a dominant negative inhibitor of GR α [68], suggesting that steroid resistance in severe asthmatics may be mediated by reciprocal regulation of GR α and GR β by IL-17A. Interestingly, while steroids effectively blocked IL-17A-mediated production of IL-6 by epithelial cells from normal donors, steroids failed to inhibit IL-17A mediated production of IL-6 from asthmatic epithelial cells, demonstrating some level of steroid resistance [67]. Collectively these data suggest that steroid insensitivity observed in individuals with severe asthma may be linked to the development of mixed Th2/Th17 response.

Conclusions

IL-17A is a cytokine with a well described protective role in anti-microbial and anti-fungal responses, as well as a pathogenic role in autoimmune diseases. In contrast, allergic diseases such as asthma have long been associated with the development of a Th2-dominated immune response. Recent evidence suggests however, that through its ability to induce neutrophilia, enhance IL-13-driven signaling, and promote steroid insensitivity, IL-17A is likely to play a role in the development of severe asthma in humans. However, while IL-17A may represent an important therapeutic target for treatment of asthma, it is likely only to be effective in a subset of asthmatics on the severe end of the spectrum in which disease is caused by a mixed Th2/Th17 response. However, as this population is presently refractory to treatments

with existing medications (i.e. corticosteroids), such a therapeutic intervention is likely to have significant impact on the quality of life of these patients.

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References

- American Lung Association. Epidemiology & Statistics Unit (2007) Trends in Asthma Morbidity and Mortality.
- Finkelman FD, Katona IM, Urban JF Jr., Holmes J, Ohara J, et al. (1988) IL-4 is required to generate and sustain *in vivo* IgE responses. *J Immunol* 141: 2335-2341.
- Le Gros G, Ben-Sasson SZ, Seder R, Finkelman FD, Paul WE (1990) Generation of interleukin 4 (IL-4)-producing cells *in vivo* and *in vitro*: IL-2 and IL-4 are required for *in vitro* generation of IL-4-producing cells. *J Exp Med* 172: 921-929.
- Swain SL, Weinberg AD, English M and Huston G (1990) IL-4 directs the development of Th2-like helper effectors. *J Immunol* 145: 3796-3806.
- Hogan SP, Mould AW, Young JM, Rothenberg ME, Ramsay AJ, et al. (1998) Cellular and molecular regulation of eosinophil trafficking to the lung. *Immunol Cell Biol* 76: 454-460.
- Wills-Karp M, Luyimbazi J, Xu X, Schofield B, Neben TY, et al. (1998) Interleukin-13: central mediator of allergic asthma. *Science* 282: 2258-2261.
- Leigh R, Ellis R, Wattie JN, Hirota JA, Matthaei KI, et al. (2004) Type 2 cytokines in the pathogenesis of sustained airway dysfunction and airway remodeling in mice. *Am J Respir Crit Care Med* 169: 860-867.
- Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, et al. (1999) Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 160: 1001-1008.
- Louis R, Lau LC, Bron AO, Roldaan AC, Radermecker M, et al. (2000) The relationship between airways inflammation and asthma severity. *Am J Respir Crit Care Med* 161: 9-16.
- Wenzel SE, Szefer SJ, Leung DY, Sloan SI, Rex MD, et al. (1997) Bronchoscopic evaluation of severe asthma. Persistent inflammation associated with high dose glucocorticoids. *Am J Respir Crit Care Med* 156: 737-743.
- Gibson PG, Simpson JL, Saltos N (2001) Heterogeneity of airway inflammation in persistent asthma: evidence of neutrophilic inflammation and increased sputum interleukin-8. *Chest* 119: 1329-1336.
- Lamblin C, Gosset P, Tillie-Leblond I, Saulnier F, Marquette CH, et al. (1998) Bronchial neutrophilia in patients with noninfectious status asthmaticus. *Am J Respir Crit Care Med* 157: 394-402.
- Barnes PJ and Adcock IM (2009) Glucocorticoid resistance in inflammatory diseases. *Lancet* 373: 1905-1917.
- Cox G (1995) Glucocorticoid treatment inhibits apoptosis in human neutrophils. Separation of survival and activation outcomes. *J Immunol* 154: 4719-4725.
- Bullens DM, Truyen E, Coteur L, Dilissen E, Hellings PW, et al. (2006) IL-17 mRNA in sputum of asthmatic patients: linking T cell driven inflammation and granulocytic influx? *Respir Res* 7: 135.
- Al-Ramli W, Prefontaine D, Chouiali F, Martin JG, Olivenstein R, et al. (2009) T(H)17-associated cytokines (IL-17A and IL-17F) in severe asthma. *J Allergy Clin Immunol* 123: 1185-1187.
- Agache I, Ciobanu C, Agache C, Anghel M (2010) Increased serum IL-17 is an independent risk factor for severe asthma. *Respir Med* 104: 1131-1137.
- Wang JY, Shyr SD, Wang WH, Liou YH, Lin CG, et al. (2009) The polymorphisms of interleukin 17A (IL17A) gene and its association with pediatric asthma in Taiwanese population. *Allergy* 64: 1056-1060.
- Jin EH, Choi EY, Yang JY, Chung HT and Yang YS (2010) Significant Association between IL-17F Promoter Region Polymorphism and Susceptibility to Asthma in a Korean Population. *Int Arch Allergy Immunol* 155: 106-110.
- Kawaguchi M, Takahashi D, Hizawa N, Suzuki S, Matsukura S, et al. (2006) IL-17F sequence variant (His161Arg) is associated with protection against asthma and antagonizes wild-type IL-17F activity. *J Allergy Clin Immunol* 117: 795-801.

21. Reynolds JM, Angkasekwinai P, Dong C (2010) IL-17 family member cytokines: regulation and function in innate immunity. *Cytokine Growth Factor Rev* 21: 413-423.
22. Liang SC, Long AJ, Bennett F, Whitters MJ, Karim R, et al. (2007) An IL-17F/A heterodimer protein is produced by mouse Th17 cells and induces airway neutrophil recruitment. *J Immunol* 179: 7791-7799.
23. Wright JF, Guo Y, Quazi A, Luxenberg DP, Bennett F, et al. (2007) Identification of an interleukin 17F/17A heterodimer in activated human CD4+ T cells. *J Biol Chem* 282: 13447-13455.
24. Barlow JL, McKenzie AN (2009) IL-25: a key requirement for the regulation of type-2 immunity. *Biofactors* 35: 178-182.
25. Li H, Chen J, Huang A, Stinson J, Heldens S, et al. (2000) Cloning and characterization of IL-17B and IL-17C, two new members of the IL-17 cytokine family. *Proc Natl Acad Sci U S A* 97: 773-778.
26. Starnes T, Broxmeyer HE, Robertson MJ, Hromas R (2002) Cutting edge: IL-17D, a novel member of the IL-17 family, stimulates cytokine production and inhibits hemopoiesis. *J Immunol* 169: 642-646.
27. Shen F, Gaffen SL (2008) Structure-function relationships in the IL-17 receptor: implications for signal transduction and therapy. *Cytokine* 41: 92-104.
28. Rickel EA, Siegel LA, Yoon BR, Rottman JB, Kugler DG, et al. (2008) Identification of functional roles for both IL-17RB and IL-17RA in mediating IL-25-induced activities. *J Immunol* 181: 4299-4310.
29. Rong Z, Wang A, Li Z, Ren Y, Cheng L, et al. (2009) IL-17RD (Sef or IL-17RLM) interacts with IL-17 receptor and mediates IL-17 signaling. *Cell Res* 19: 208-215.
30. Li TS, Li XN, Chang ZJ, Fu XY, Liu L (2006) Identification and functional characterization of a novel interleukin 17 receptor: a possible mitogenic activation through ras/mitogen-activated protein kinase signaling pathway. *Cell Signal* 18: 1287-1298.
31. Bettelli E, Carrier Y, Gao W, Korn T, Strom TB, et al. (2006) Reciprocal developmental pathways for the generation of pathogenic effector TH17 and regulatory T cells. *Nature* 441: 235-238.
32. Veldhoen M, Hocking RJ, Atkins CJ, Locksley RM, Stockinger B (2006) TGF-beta in the context of an inflammatory cytokine milieu supports de novo differentiation of IL-17-producing T cells. *Immunity* 24: 179-189.
33. Korn T, Bettelli E, Gao W, Awasthi A, Jager A, et al. (2007) IL-21 initiates an alternative pathway to induce proinflammatory T(H)17 cells. *Nature* 448: 484-487.
34. Mathur AN, Chang HC, Zisoulis DG, Stritesky GL, Yu Q, et al. (2007) Stat3 and Stat4 direct development of IL-17-secreting Th cells. *J Immunol* 178: 4901-4907.
35. Manel N, Unutmaz D, Littman DR (2008) The differentiation of human T(H)-17 cells requires transforming growth factor-beta and induction of the nuclear receptor RORgamma. *Nat Immunol* 9: 641-649.
36. Yang XO, Pappu BP, Nurieva R, Akimzhanov A, Kang HS, et al. (2008) T helper 17 lineage differentiation is programmed by orphan nuclear receptors ROR alpha and ROR gamma. *Immunity* 28: 29-39.
37. McGeachy MJ, Chen Y, Tato CM, Laurence A, Joyce-Shaikh B, et al. (2009) The interleukin 23 receptor is essential for the terminal differentiation of interleukin 17-producing effector T helper cells in vivo. *Nat Immunol* 10: 314-324.
38. Ghoreschi K, Laurence A, Yang XP, Tato CM, McGeachy MJ, et al. (2010) Generation of pathogenic T(H)17 cells in the absence of TGF-beta signalling. *Nature* 467: 967-971.
39. McGeachy MJ, Bak-Jensen KS, Chen Y, Tato CM, Blumenschein W, et al. (2007) TGF-beta and IL-6 drive the production of IL-17 and IL-10 by T cells and restrain T(H)-17 cell-mediated pathology. *Nat Immunol* 8: 1390-1397.
40. Newcomb DC, Zhou W, Moore ML, Goleniewska K, Hershey GK, et al. (2009) A functional IL-13 receptor is expressed on polarized murine CD4+ Th17 cells and IL-13 signaling attenuates Th17 cytokine production. *J Immunol* 182: 5317-5321.
41. Newcomb DC, Boswell MG, Zhou W, Huckabee MM, Goleniewska K, et al. (2011) Human T(H)17 cells express a functional IL-13 receptor and IL-13 attenuates IL-17A production. *J Allergy Clin Immunol* 127: 1006-1013.
42. Cosmi L, Maggi L, Santarlasci V, Capone M, Cardilicchia E, et al. (2010) Identification of a novel subset of human circulating memory CD4(+) T cells that produce both IL-17A and IL-4. *J Allergy Clin Immunol* 125: 222-230.
43. Wang YH, Voo KS, Liu B, Chen CY, Uygungil B, et al. (2010) A novel subset of CD4(+) T(H)2 memory/effector cells that produce inflammatory IL-17 cytokine and promote the exacerbation of chronic allergic asthma. *J Exp Med* 207: 2479-2491.
44. Suzuki S, Kokubu F, Kawaguchi M, Homma T, Odaka M, et al. (2007) Expression of interleukin-17F in a mouse model of allergic asthma. *Int Arch Allergy Immunol* 143 Suppl 1: 89-94.
45. Pichavant M, Goya S, Meyer EH, Johnston RA, Kim HY, et al. (2008) Ozone exposure in a mouse model induces airway hyperreactivity that requires the presence of natural killer T cells and IL-17. *J Exp Med* 205: 385-393.
46. Song C, Luo L, Lei Z, Li B, Liang Z, et al. (2008) IL-17-producing alveolar macrophages mediate allergic lung inflammation related to asthma. *J Immunol* 181: 6117-6124.
47. Takemura Y, Iwasaki Y, Nagata K, Yokomura I, Tando S, et al. (2005) Influence of depletion of alveolar macrophages on apoptosis in Candida-induced acute lung injury. *Exp Lung Res* 31: 307-321.
48. Hellings PW, Kasran A, Liu Z, Vandekerckhove P, Wuyts A, et al. (2003) Interleukin-17 orchestrates the granulocyte influx into airways after allergen inhalation in a mouse model of allergic asthma. *Am J Respir Cell Mol Biol* 28: 42-50.
49. Schnyder-Candrian S, Togbe D, Couillin I, Mercier I, Brombacher F, et al. (2006) Interleukin-17 is a negative regulator of established allergic asthma. *J Exp Med* 203: 2715-2725.
50. Park SJ, Lee KS, Kim SR, Min KH, Choe YH, et al. (2009) Peroxisome proliferator-activated receptor gamma agonist down-regulates IL-17 expression in a murine model of allergic airway inflammation. *J Immunol* 183: 3259-3267.
51. Durrant DM, Gaffen SL, Riesenfeld EP, Irvin CG and Metzger DW (2009) Development of allergen-induced airway inflammation in the absence of T-bet regulation is dependent on IL-17. *J Immunol* 183: 5293-5300.
52. Wilson RH, Whitehead GS, Nakano H, Free ME, Kolls JK, et al. (2009) Allergic sensitization through the airway primes Th17-dependent neutrophilia and airway hyperresponsiveness. *Am J Respir Crit Care Med* 180: 720-730.
53. Lajoie S, Lewkowich IP, Suzuki Y, Clark JR, Sproles AA, et al. (2010) Complement-mediated regulation of the IL-17A axis is a central genetic determinant of the severity of experimental allergic asthma. *Nat Immunol* 11: 928-935.
54. Yang XO, Chang SH, Park H, Nurieva R, Shah B, et al. (2008) Regulation of inflammatory responses by IL-17F. *J Exp Med* 205: 1063-1075.
55. Nakae S, Komiya Y, Nambu A, Sudo K, Iwase M, et al. (2002) Antigen-specific T cell sensitization is impaired in IL-17-deficient mice, causing suppression of allergic cellular and humoral responses. *Immunity* 17: 375-387.
56. Brewer JM, Conacher M, Hunter CA, Mohrs M, Brombacher F, et al. (1999) Aluminium hydroxide adjuvant initiates strong antigen-specific Th2 responses in the absence of IL-4- or IL-13-mediated signaling. *J Immunol* 163: 6448-6454.
57. Besnard AG, Sabat R, Dumoutier L, Renaud JC, Willart M, et al. (2011) Dual Role of IL-22 in Allergic Airway Inflammation and its Cross-talk with IL-17A. *Am J Respir Crit Care Med* 183: 1153-1163.
58. Sonnenberg GF, Nair MG, Kim TJ, Zaph C, Fouser LA, et al. (2010) Pathological versus protective functions of IL-22 in airway inflammation are regulated by IL-17A. *J Exp Med* 207: 1293-1305.
59. Chen Y, Thai P, Zhao YH, Ho YS, DeSouza MM, et al. (2003) Stimulation of airway mucin gene expression by interleukin (IL)-17 through IL-6 paracrine/autocrine loop. *J Biol Chem* 278: 17036-17043.
60. Park H, Li Z, Yang XO, Chang SH, Nurieva R, et al. (2005) A distinct lineage of CD4 T cells regulates tissue inflammation by producing interleukin 17. *Nat Immunol* 6: 1133-1141.
61. Zhou B, Comeau MR, De Smedt T, Liggitt HD, Dahl ME, et al. (2005) Thymic stromal lymphopoietin as a key initiator of allergic airway inflammation in mice. *Nat Immunol* 6: 1047-1053.
62. Wakashin H, Hirose K, Maezawa Y, Kagami S, Suto A, et al. (2008) IL-23 and Th17 cells enhance Th2-cell-mediated eosinophilic airway inflammation in mice. *Am J Respir Crit Care Med* 178: 1023-1032.
63. McKinley L, Alcorn JF, Peterson A, Dupont RB, Kapadia S, et al. (2008) TH17 cells mediate steroid-resistant airway inflammation and airway hyperresponsiveness in mice. *J Immunol* 181: 4089-4097.

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64. Huang F, Wachi S, Thai P, Loukoianov A, Tan KH, et al. (2008) Potentiation of IL-19 expression in airway epithelia by IL-17A and IL-4/IL-13: important implications in asthma. *J Allergy Clin Immunol* 121: 1415-1421.
65. Perkins C, Yanase N, Smulian G, Gildea L, Orekov T, et al. (2011) Selective stimulation of IL-4 receptor on smooth muscle induces airway hyperresponsiveness in mice. *J Exp Med* 208: 853-867.
66. Kuperman DA, Huang X, Koth LL, Chang GH, Dolganov GM, et al. (2002) Direct effects of interleukin-13 on epithelial cells cause airway hyperreactivity and mucus overproduction in asthma. *Nat Med* 8: 885-889.
67. Vazquez-Tello A, Semlali A, Chakir J, Martin JG, Leung DY, et al. (2010) Induction of glucocorticoid receptor-beta expression in epithelial cells of asthmatic airways by T-helper type 17 cytokines. *Clin Exp Allergy* 40: 1312-1322.
68. Oakley RH, Sar M and Cidlowski JA (1996) The human glucocorticoid receptor beta isoform. Expression, biochemical properties, and putative function. *J Biol Chem* 271: 9550-9559.