Role of Interferon-λ in Allergic Asthma

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Abstract
Type III interferons (IFNs), or IFN-λ, are known to have potent antiviral and antiproliferative activities. It inhibits viral replication and upregulates cytotoxic responses to virally infected cells. Besides these characteristics, IFN-λ also has additional activities in the immune system. In fact, it induces the proliferation of Foxp3-expressing regulatory T cells mediated in part by dendritic cells and inhibit the production of IL-5 and IL-13 in vitro. Regulatory T cells and the Th2 cytokines like IL-5 and IL-13 play important roles in the pathogenesis of allergic asthma. In humans, there seems to be an inverse link between IFN-λ and the severity of allergic asthma and allergic asthma exacerbations. Asthmatic patients, without a detectable viral infection show an inverse correlation between IL-28 and IL-29 mRNA levels and severity of allergic responses in the airways. These additional features of IFN-λ that affect the adaptive immune system make it a potential immunotherapeutic agent for the treatment of allergic asthma.

Introduction

Allergic asthma is a worldwide increasing chronic disease of the airways, which is characterized by airway hyperresponsiveness (AHR), airway inflammation, high levels of serum IgE, mucus hypersecretion and high levels of Th2 cytokines. Various types of cells are involved in the pathogenesis of this disease, such as dendritic cells (DCs), eosinophils, mast cells and T lymphocytes [1–3]. Allergic asthma is a very heterogeneous disease which is caused by sensitization and exposure via the airways to a number of innocuous allergens including proteins from house dust mites, moulds, tree and grass pollens, and animals [4, 5].

Type III interferons (IFNs), or IFN-λ, are a newly identified antiviral family of cytokines that are related to type I IFNs and IL-10 family members [6–8]. They consist of three members in humans, denoted IL-28A (IFN-λ2), IL-28B (IFN-λ3) and IL-29 (IFN-λ1), and two members in mice (IL-28A and IL-28B) [9]. IFN-λ is produced by various cells including antigen-presenting cells upon viral infection or Toll-like receptor ligation [10]. Besides its antiviral and antiproliferative activities, it exerts immunomodulatory effects that overlap type I IFNs in innate and adaptive immunity [11]. IL-29 seems to be an inhibitor of human Th2 responses because of its inhibitory effect on IL-13 production by T cells. Furthermore, it is able to decrease IL-4 and IL-5 production [12]. Recently, we reported that IL-28A modulates lung DC function to promote Th1 cell differentiation and suppress Th2-mediated responses [13].

These effects of IFN-λ are of direct relevance to the pathogenesis of allergic asthma and IFN-λ might therefore represent a new approach to treatment or prevention of the disease.
IFN-λ and Its Signaling Pathway

IFNs are produced in response to pathogen-associated molecular patterns binding to pattern recognition receptors. The subclassification of IFNs into types I, II and III is based on its receptors. The type III IFN family consists of three members in humans [IL-28A (IFN-λ2), IL-28B (IFN-λ3) and IL-29 (IFN-λ1)] and two members in mice (IL-28A and IL-28B) [8, 9]. IFN-λ expression can be detected in human blood, brain, lung, ovary, pancreas, pituitary, placenta, prostate and testis [7]. It can be induced by dsRNA and after infection with different viruses. Although virtually any cell type following viral infection can express IFN-λ, PBMCs and monocyte-derived DCs as well as bronchial epithelial cells appear to be the major source of IFN-λ [6, 7, 14–16]. Antigen-presenting cells, like DCs and macrophages, produce and secrete IFN-λ after stimulation with Toll-like receptor agonists [14, 17]. The IL-29 gene is regulated by virus-activated IFN regulatory factor (IRF) 3 and IRF7 as well as NF-κB, whereas IL-28A and IL-28B gene expression is mainly controlled by IRF7 and NF-κB [18, 19].

Receptors of type I IFNs are present on most cell types and those of type II IFNs are present mostly on hematopoietic cells, while receptors of type III IFNs are highly expressed on cells of epithelial derivation including hepatocytes and myeloid lineage cells such as DCs and macrophages [20–22]. IFN-λR comprises two subunits, IFN-λR1 (also designated IL-28Rα), which is specific for the IFN-λ, and IL-10R2 chain (also termed IL-10Rβ), which is also part of the receptors for IL-10, IL-22 and IL-26 [6, 7, 23]. IFN-λ is able to activate signal transducer and activator of transcription (STAT)-dependent and -independent pathways (fig. 1). Binding of IFN-λ to its receptor leads to the activation of the two tyrosine kinases, Janus kinase 1 and tyrosine kinase 2. This activation is followed by phosphorylation of STAT1 and STAT2 to a lesser extent STAT3, STAT4 and STAT5 proteins [9, 24, 25]. Phosphorylated forms of STAT1 and STAT2 further associate with IRF9 to form a heterodimeric complex called IFN-stimulated gene factor 3. This complex translocates to the nucleus to bind to the sequence of ISREs in the promoter of IFN-stimulated genes to upregulate their transcription. Jak1 = Janus kinase 1; Tyk2 = tyrosine kinase 2; ISGF3 = IFN-stimulated genes (ISG) factor 3; ISRE = IFN-stimulated response element; MAPK = mitogen-activated protein kinase; Erk = extracellular-signal regulated kinases; Jnk = c-Jun N-terminal kinase.

Role of IFN-λ in Allergic Asthma

Allergic asthma is a very heterogeneous disease of the airways that is usually caused by sensitization and exposure to a number of otherwise innocuous allergens, including proteins from house dust mites and animals. Other proteins from grass and tree pollens, which seasonally induce allergic rhinitis, might also cause allergic asthma. The disease is characterized, for example, by airway hyperreactivity, mucus production, elevated IgE serum levels and airway inflammation, all of which are caused by aberrant Th2 cytokine (IL-5, IL-13 and IL-4) production [1–3]. Th17 cells also play an important role in the
pathogenesis of asthma. They induce neutrophil recruitment into the lungs, increase AHR and mucus production of goblet cells, and impair Th2-mediated recruitment of eosinophils to the airways [28, 29]. Another explanation for the inappropriate immune response to allergens observed in patients with asthma is a reduced or altered function of regulatory T cells. They exert multiple suppressive functions, e.g. through secretion of the anti-inflammatory cytokines IL-10 and TGF-β. Regulatory T cells are able to ameliorate airway inflammation and AHR [30, 31].

Several studies have demonstrated that IFN-λ has additional immunomodulatory properties in innate and adaptive immunity, in addition to its anti-viral capacities, that influence the outcome of allergic asthma (fig. 2). Recently, we have shown in a murine model of allergic asthma that administration of recombinant IL-28A to the airways results in reduction of the Th2 cytokines IL-5 and IL-13 in CD4+ T cells as well as lung-draining mediastinal lymph nodes, while it induced IFN-γ production from mediastinal lymph nodes. Similar to the inhibition of Th2 responses, IL-28A also downregulated Th17 cell...
responses in this murine model of allergic asthma. Administration of IL-28A also resulted in reduction of AHR, goblet cell metaplasia, and suppression of eosinophil and neutrophil numbers in the bronchoalveolar lavage fluid [13]. We also demonstrated by using IL-28Rα–/– mice that endogenously produced IL-28 cytokines are involved in dampening the allergic airway inflammation and mucus secretion in sensitized and allergen-challenged mice [13]. Another research group demonstrated that human IL-29 is able to inhibit the production of IL-13 by T cells, but in an IFN-γ-independent manner, which is mediated partly via monocyte-derived DCs [32]. Furthermore, IL-29 preferentially inhibited IL-13 production, but also significantly decreased IL-4 and IL-5, so it generally affects Th2 responses [12]. These results were supported by another group which administered a human IL-29-expressing adenovirus to sensitized and challenged mice, which led to the downregulation of IL-4, IL-5 and IL-13 [33]. Those results were accompanied by a reduction in the number of eosinophils, mucus production, serum IgE levels and airway inflammation. Additionally, Li et al. [33] found a significant induction of CD4+CD25+Foxp3+ regulatory T cells after administration of IL-29. Regulatory T cells play an anti-inflammatory role in allergic asthma because they are able to suppress Th17 and Th2 responses [34–36].

**IFN-λ and Regulatory T cells**

Mennechet and Uze [22] showed that treatment of monocyte-derived DCs with IL-29 led to induction of the proliferation of regulatory T cells. Signals such as Toll-like receptor stimulation or type I IFNs induce DCs to mature in that they acquire the ability to migrate to lymphoid organs and to present antigens to naïve T cells through major histocompatibility complex classes I and II. IFN-λ promotes the generation of partially mature DCs displaying a tolerogenic phenotype, so that they express high levels of major histocompatibility complex class I and II but low levels of costimulatory molecules, and they acquire the ability to migrate to lymph nodes. IFN-λ-matured DCs specifically induced IL-2-dependent proliferation of a CD4+CD25+Foxp3+ T cell population with contact-dependent suppressive activity on T cell proliferation [22]. Thus, IFN-λ drives immature DCs into a maturation stage that promotes regulatory T cell expansion. We have also demonstrated that IL-28A treatment could induce IL-12 production upon challenge with lipopolysaccharide by lung CD11c+ DCs as well as bone marrow-derived DCs, which express IL-28Rα [13]. IL-28A reprograms CD11c+ DCs to promote Th1 responses and inhibit Th2 and Th17 cell development and the associated pathology of allergic asthma, like airway inflammation, goblet cell metaplasia and AHR [13]. Additionally, the two Th1-inducing factors, IFN-γ and IL-12, were found to be critical mediators for the immunosuppressive functions of IL-28A in allergic asthma. These findings are of importance as they indicate that IL-28 exhibits powerful immunomodulatory effects on DCs, suggesting a wider role for IL-28 in T cell immunoregulation.

**IFN-λ as a Therapeutic Agent for Allergic Asthma**

Recent studies have demonstrated that asthmatic adults have increased sputum IL-28A/B mRNA, but similar IL-29 mRNA expression compared to healthy subjects [37]. IL-28 mRNA correlated with the relative and absolute numbers of eosinophils present in the sputum sample of the patients. IL-29 mRNA, however, correlated negatively with asthma symptoms in steroid-naïve patients and was significantly higher in steroid-treated patients. Additionally, both IL-28 and IL-29 mRNA levels were higher in asthmatic children than in asthmatic adults. Those results suggest that asthmatic patients have substantial IFN-λ mRNA levels in their airways. Whether the presence of IFN-λ in the airways of asthmatic subjects causes inflammation or is caused by viral infection or bacterial colonization needs to be further investigated. Nevertheless, there is evidence that IL-29 could have an immunoprotective role [37]. In fact, IL-29 was shown to inhibit Th2 cytokine production and led to amelioration of asthmatic features [12, 32, 33]. Furthermore, a protective role of IL-28 in allergic diseases like asthma has been supported by a study showing deficient IFN-λ induction (IL-29 and IL-28A/B) by viral and bacterial stimuli associated with severity of asthma exacerbations in allergic asthma patients [38]. In this study, the authors showed that primary bronchial epithelial cells from atopic asthmatics are deficient in IFN-λ production. When these atopic asthmatics were experimentally infected with rhinovirus, they showed a strong correlation between the severity of asthma exacerbation, viral load and IFN-λ deficiency [38]. Asthma exacerbations are the main cause of hospitalization in children. They occur in association with respiratory viral infections, and human rhinoviruses (HRVs) have been postulated to trigger this crisis [39, 40]. A re-

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cent study demonstrated that baseline production of IL-29 in children with asthma without respiratory symptoms was lower than in healthy control subjects [41]. Moreover, in this study the authors could show that, consistent with previous observations in adults [38], in vitro analysis of IL-29 production in primary nasal epithelial cells derived from children with asthma after HRV infection was suppressed compared with this cytokine production from nasal epithelial cells of healthy children. Additionally, it has been described that wheezing children infected with HRV had higher levels of type III IFN-λ1 than nonwheezing children infected with HRV with asthma [41]. Thus, HRV infections are associated with pediatric asthma exacerbations and IL-29 levels increase with worsening acute asthma symptoms during HRV infections. Therefore, it might be possible that type III IFN-λ1 is involved in different aspects of asthma pathogenesis which need to be further investigated.

We further discovered that IL-28 could also act as an immunoregulatory cytokine that affects the differentiation of T cells. This is an important aspect for the therapy of allergic asthma. In fact, we showed that, IL-28 had the ability to suppress Th2 responses by induction of the Th1 cytokines IFN-γ and IL-12, which antagonize the production of Th2 cell differentiation (fig. 2) [13]. The current approach in anti-Th2 cytokine therapy is clinically not as beneficial as expected. Targeting IL-4 with monoclonal antibodies or a recombinant human soluble IL-4 receptor, for example, did not result in significant improvements in asthma symptoms or exacerbations [42]. Because IL-4 and IL-13 both signal through the common IL-4Ra chain, therapeutic interventions that block both cytokine pathways could be successful. For example, a mutated IL-4 protein and a monoclonal antibody against the IL-4Ra have been developed and are in clinical trial. These therapies are promising as they showed reduced allergen responses in asthmatic patients [43, 44].

Another monoclonal antibody, lebrikizumab, which selectively blocks IL-13, showed significant improvement during a phase II clinical trial in a group of patients who expressed high levels of periostin, a protein induced by IL-13 and released by bronchial epithelial cells. Periostin induces AHR as well as proliferation of fibroblasts, thus inducing fibrosis and remodeling in the lungs of asthmatic subjects [45]. We previously reported that blockade of IL-13 and not IL-4 in the lungs of T-bet-deficient mice reduced AHR and remodeling in the lungs of treated mice [46].

Treatment with monoclonal antibodies to IL-5 indeed reduced serum eosinophils in subjects with severe asthma, but did not have an effect on clinical symptoms of asthma like AHR, forced expiratory volume in one second (FEV₁) and peak flow measures [47, 48]. Nevertheless, in patients with prominent sputum eosinophilia and severe asthma, treatment with IL-5 monoclonal antibody significantly reduced asthma exacerbations [49].

IFN-λ as a therapeutic agent may be of interest for the treatment of asthma because it induces Th1 cytokines, thereby antagonizing the effects of all Th2 cytokines. IFN-λ may be sufficient to tip the balance from Th2 to Th1 by modulating DCs that polarize T cells towards Th1, thereby suppressing Th2 responses. Administration of IFN-λ may also be an approach of treatment and prevention of asthma exacerbations, which are driven by viral infections and influenced by impaired expression of type III IFNs. Because IFN-λ deficiency is correlated with viral load and the severity of asthma exacerbation, treatment with IFN-λ might therefore boost the clearance of viral infections and thereby reduce asthma exacerbations. These findings of IFN-λ in allergic asthma support the idea that it might be beneficial for future therapies of this disease.

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