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Immune dysregulation in asthma

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Allergic diseases and asthma are caused by dysregulated Th2-biased immune responses to environmental allergens in genetically predisposed individuals. Over the past several years there has been much progress in understanding the mechanisms by which Th2 responses are generated and the pathogenic role of natural killer T cells in asthma. In addition, there has been much progress in understanding the mechanisms of tolerance to allergens, the role of natural and adaptive allergen-specific regulatory T cells, and the strategies to prevent or to reverse allergic disease and asthma. Impaired expansion of regulatory T cells is hypothesized to lead to the development of allergy and asthma, and treatment to induce allergen-specific regulatory T cells could provide curative therapies for these problems.

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Introduction

Asthma and allergy are inflammatory diseases caused by dysregulated immune responses in the respiratory mucosa. It is believed that overzealous T helper (Th)2-driven responses result in the development of asthma. Thus, CD4⁺ T cells that produce Th2 cytokines play a prominent role in the lungs of asthmatic subjects [1]. Over the past year, we have learned a great deal more about specific mechanisms that result in asthma and allergy, which has led to new strategies to treat these medical problems.

In this review, we discuss recent reports on factors involved in the pathogenesis of asthma, and in the protection against asthma.

Factors involved in pathogenesis of asthma

Specific proinflammatory factors involved in the pathogenesis of asthma include thymic stromal lymphopoietin

(TSLP), interleukin 25 (IL-25), tumor necrosis factor α (TNF- α) and natural killer T cells (NKT cells).

Thymic stromal lymphopoietin

In this past year, there have been several reports regarding the role of TSLP in the pathogenesis of several atopic diseases. TSLP was first described in 1994 as a novel IL-7-like cytokine important in T cell and B cell differentiation [2]. TSLP, which is highly expressed in Hassall's corpuscles in the thymic medulla, is critically involved in positive selection of natural T regulatory (T_{Reg}) cells in the thymus [3]. However, TSLP appears to also be important in the periphery, where it enhances the capacity of dendritic cells (DCs) to induce the development of Th2 cells [4]. In particular, overexpression of TSLP in the lungs of mice results in the development of severe allergic airway inflammation [5^{**},6^{**}]. TSLP is, in fact, expressed at high levels in the lungs of patients who have asthma [7], which suggests that expression of TSLP by lung epithelial cells can indeed cause airway DCs to enhance the development of Th2-driven inflammation. Moreover, expression of TSLP by skin keratinocytes in the skin of mice has been shown to result in the development of a condition that has many of the features of atopic dermatitis [8].

Interleukin 25

Another proinflammatory factor in asthma is IL-25 (also known as IL-17E), which is produced by Th2 cells and mast cells. IL-25 was shown recently to be critically involved in the expulsion of helminths [9^{**},10^{**}]. IL-25 performs this function by inducing production of large quantities of Th2 cytokines from an unusual c-kit⁺, non-T/non-B, Fc ϵ R1⁻ cell. IL-25 might also enhance Th2 responses by actively inhibiting interferon γ (IFN- γ) and IL-17 production, suggesting that IL-25-producing cells might have a regulatory function, limiting pathologic (Th1-biased) inflammation at mucosal sites. By contrast, a related IL-17 family member, IL-17 (also known as IL-17A), produced by Th17 cells, is implicated in the exacerbation of autoimmune pathology [11,12]. Because IL-25 enhances Th2 cytokine production, however, it might also enhance the development of allergic inflammatory responses at mucosal sites by inducing eosinophilia, airway hyperreactivity and increased mucus production [13].

Tumor necrosis factor α

TNF- α is a cytokine produced by mast cells as well as by T cells, but its role in asthma has been controversial. However, a recent clinical study, in which patients who had refractory asthma were treated with the soluble TNF- α receptor 'etanercept', suggests that the TNF- α axis is upregulated and is proinflammatory in asthma [14^{*}]. Thus,

etanercept-treated patients had a significant improvement in pulmonary function and in asthma-related quality of life score. Whether the role of TNF- α is as important in mild-to-moderate asthma as it is in refractory asthma remains to be seen. Another TNF- α receptor family member, CD137 (also known as 4-1BB), might also be involved in the exacerbation of asthma, because blockade of CD137 (4-1BB) in mice greatly inhibited airway hyperreactivity, eosinophilic inflammation and IgE production [15,16].

Complement

The role of complement in allergic disease has been confusing and controversial, but two reports during the past year on C5 help resolve this issue. These studies suggest a dual role for C5a, in which C5a functions to inhibit airway inflammation during the initiation of sensitization, but might enhance airway inflammation and airway hyperreactivity in established disease [17,18]. It is clear from these studies, however, that innate immunity (including complement) has major effects on the development of asthma and allergy.

Natural killer T cells

Another pro-allergic pro-asthmatic factor that is part of the innate immune system and that has received much attention during the past year is the invariant T-cell receptor (TCR) natural killer T (*i*NKT) cell compartment. Several years ago, *i*NKT cells were shown to be required for the development of allergen-induced airway hyperreactivity (AHR) in mouse models of asthma [19]. More recently, the activation of *i*NKT cells has been shown to be sufficient to induce AHR [20]. In these experiments, *i*NKT cells were directly activated with glycolipid antigens, such as α -galactosyl-ceramide (α -GalCer) or glycolipids from the membranes of lipopolysaccharide-negative *Sphingomonas paucimobilis* bacteria. Activation of *i*NKT cells in this way resulted in severe AHR and airway inflammation. The induction of AHR occurred in the absence of eosinophils or B cells, and even in the absence of MHC class II-restricted CD4⁺ T cells [20], indicating that *i*NKT cell-driven AHR can occur in the complete absence of adaptive immunity.

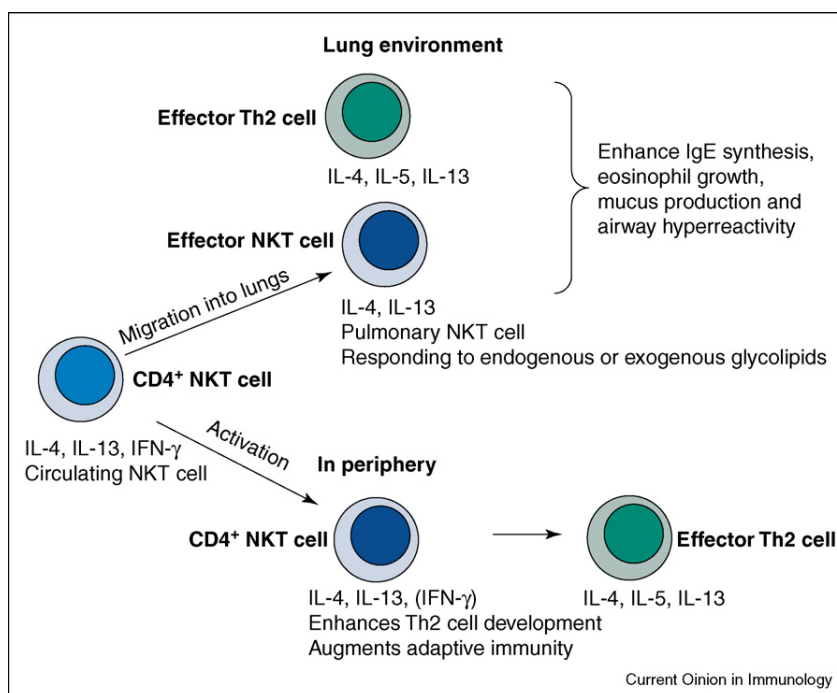
In addition, these results suggest that glycolipids from respiratory pathogens might activate *i*NKT cells and directly cause wheezing and AHR. Moreover, lipids from cypress tree pollen have been shown to directly activate human *i*NKT cells [21]. The cypress tree pollen-derived lipids were recognized in the context of the MHC class I-like molecules CD1a and CD1d as restriction elements, and induced the proliferation of CD4⁺ $\alpha\beta$ TCR⁺, $\gamma\delta$ TCR⁺ and some V α 24⁺ *i*NKT cells. In addition, the cypress tree pollen-responsive T cells provided help for IgE production and were more evident in the peripheral blood of allergic subjects during the pollinating season. Finally, injection of the glycolipids induced immediate Type 1 skin test reactions (wheal and flare reactions), indicating

that anti-cypress lipid IgE antibodies were present in cypress tree pollen-allergic patients. These results suggest that plant pollen might be an important and previously unrecognized source of lipid antigens that can directly activate *i*NKT cells. They also indicate that pollen lipids might play an important role in the development of some forms of asthma and allergy. The important role of *i*NKT cells in asthma in mice is supported by the observation that administration of α -GalCer 24 h before challenge with ovalbumin inhibited allergen-induced AHR [22–24], presumably because strong *i*NKT cell agonists such as α -GalCer induce *i*NKT cell anergy [25]. Given evidence that *i*NKT cells are both necessary and sufficient for AHR, future studies examining the capacity of glycolipids from respiratory pathogens and plant pollens in activating *i*NKT cells and in inducing AHR could greatly alter our understanding of respiratory pathobiology.

Although the studies of allergen-induced AHR in mice strongly suggested that *i*NKT cells might be important in human asthma, direct assessment of the role of *i*NKT cells in human asthma was necessary to establish this possibility. Using CD1d tetramers loaded with α -GalCer, monoclonal antibody specific for the invariant TCR of NKT cells, and RT-PCR analysis to detect the invariant TCR of *i*NKT cells, the frequency and distribution of *i*NKT cells in the lungs and in the circulating blood of patients with moderate-to-severe persistent asthma was assessed [26^{••}]. Surprisingly, ~60% of the pulmonary CD4⁺CD3⁺ cells in the lungs of these patients who had asthma were not class II MHC-restricted CD4⁺ T cells, but rather *i*NKT cells. The *i*NKT cells expressed the invariant V α 24⁺ TCR and produced IL-4 and IL-13, but not IFN- γ . By contrast, the CD4⁺ T cells found in the lungs of patients with sarcoidosis, an inflammatory disease in which large numbers of CD4⁺ Th1 cells are found in the lungs, were conventional CD4⁺ CD3⁺ T cells and not *i*NKT cells. These studies strongly suggest that *i*NKT cells play a prominent pathogenic role in human asthma [26^{••}] (Figure 1).

The presence of large numbers of *i*NKT cells in the lungs of patients with asthma is surprising, and suggests that these cells might have been mistakenly identified in the past as conventional CD4⁺ Th2 cells. Most of the *i*NKT cells in the lungs of patients with asthma expressed CD4 and produced IL-4 and IL-13, but not IFN- γ , suggesting that a Th2-like subset of *i*NKT cells was recruited or expanded in the lungs of patients with asthma [26^{••}]. In this and another study, *i*NKT cells were not increased in the peripheral blood of patients with asthma, nor did circulating *i*NKT cells show any change in functionality [27]; this indicates that the immunology of asthma must be studied not by the examination of peripheral blood alone but rather by the evaluation of cells from within the lung. The specific mechanisms by which the Th2-like

Figure 1



The precise role of NKT cells in asthma is not yet clear, but might include a role as an effector cell in the airways and/or a role in amplifying the function of allergen-specific Th2 cells in asthma.

subset of *i*NKT cells enters or expands in the lungs, and whether the number of *i*NKT cells in the lungs correlates with disease severity, are not yet clear.

Oxidative stress

The innate immune system is also affected by the redox state in the lungs. Therefore, allergens that induce oxidative stress can enhance airway immune responses [28^{••}], perhaps providing a mechanism for the capacity of some inhaled allergens to induce significant immune responses. Similarly, disruption of antioxidant pathways, for example those regulated by the Nrf2 transcription factor, leads to severe allergen-driven AHR in mice [29]. The exact pathways that link oxidative stress and enhanced immunity, however, are not yet clear.

Protective immunity

If allergy and asthma are caused by enhanced Th2 responses, then therapy for these problems must focus on reducing Th2-driven inflammation. Several recent studies have focused on enhancing inhibitory pathways, which can be induced, for example, with antigen-specific immunotherapies (Box 1). Strategies include administration of allergen orally, or sublingually, immunization with an adjuvant such as *Listeria monocytogenes* [30–32], or immunization with peptides of the allergen [33] to induce protective immune responses. Immunotherapy with

antigen peptides or with *Listeria* as an adjuvant has been shown to induce antigen-specific adaptive T_{Reg} cells that produce IL-10 [31,33]. T_{Reg} cells could also be induced by respiratory mucosal exposure to antigen [34]. These T_{Reg} cells expressed membrane-bound TGF- β and activated the Notch 1–hairy and enhancer of split 1 (Notch 1–HES1) axis in target cells, suggesting that TGF- β and Notch 1 pathways are crucial in the regulation of tolerance in the lungs.

Natural CD25⁺ T_{Reg} cells

Whereas antigen-specific adaptive T_{Reg} cells are induced by immunization with antigen or by exposure to the environment, natural CD25⁺ T_{Reg} cells develop in the thymus, although they might expand in the periphery

Box 1 Allergen immunotherapies for asthma and allergy.

Asthma and allergy are thought to be caused by either a deficiency of T_{Reg} cells or an overabundance of allergen-specific Th2 cells. Although treatments with medications can limit the symptoms of asthma and allergy, the most intellectually appealing therapy is allergen immunotherapy, which reduces Th2 cytokine production and enhances T_{Reg} cell development. There are many forms of allergen immunotherapy (subcutaneous, sublingual/oral, peptide based), and new methods involving adjuvants (e.g. TLR agonists) to enhance the efficiency of these therapies are being developed.

upon antigen exposure. Natural CD25⁺ cells, however, can inhibit allergen-induced AHR by way of IL-10-dependent mechanisms [35] or by inhibiting antigen presentation by DCs [36]. Both natural CD25⁺ T_{Reg} cells and adaptive antigen-specific T_{Reg} cells express high levels of the transcription factor Foxp3 [31,34], which acts as a T_{Reg} cell lineage specification factor because it correlates closely with suppressor activity, irrespective of CD25 expression [37**]. The number of Foxp3⁺ natural CD25⁺ T_{Reg} cells that inhibit allergen-induced lung pathology in an antigen non-specific manner increases greatly during gastrointestinal nematode infection (*Heligmosomoides polygyrus*) [38,39], which suggests a mechanism by which infection might inhibit the development of asthma and allergy. CD25⁺ T_{Reg} cells from both wild-type and IL-10-deficient mice infected with *Heligmosomoides polygyrus* could inhibit immune responses when adoptively transferred into naïve recipients.

Loss of tolerance in asthma and allergy

Allergic diseases and asthma might be caused by the lack of T_{Reg} cell development, the loss of tolerance to environmental allergens, or the over-development of allergen-specific Th2 cells. Tolerance to environmental allergens can be influenced by many events, including the factor TIM-1 (T cell, immunoglobulin and mucin) [40] — a cell surface molecule expressed preferentially by Th2 cells encoded by an important asthma susceptibility gene [41]. TIM-1 appears to be an important costimulatory molecule, and cross-linking of TIM-1 results in enhanced Th1 and Th2 cytokine production and loss of tolerance. In humans, the hepatitis virus binds to TIM-1, but the natural ligands of TIM-1 are not known (although they might include Tim4) [42].

Loss of T_{Reg} cells might be caused by use of corticosteroids. Although corticosteroids are effective in the treatment of patients with asthma and allergy, they appear to inhibit the development of T_{Reg} cells by blocking the development of tolerance and the function of DCs that induce antigen-specific T_{Reg} cells [43]. There is currently no information about whether corticosteroids also inhibit the development of CD25⁺ natural T_{Reg} cells, but results with steroids and antigen-specific T_{Reg} cells suggest that treatment with corticosteroids in patients with allergy and asthma could potentially enhance Th2 responses and could adversely affect the long-term course of allergic diseases and asthma.

Asthma and allergy might also be controlled by the deletion of allergen-specific T cells. The tracking of antigen-specific cells in humans is difficult because of the low frequency of these cells. The major antigenic epitope of rye grass allergen *Lol p 1* in HLA-DRB1*0401 individuals was identified using HLA-DR*0401 transgenic mice and peripheral blood cells from HLA-DR*0401 individuals. Using DRB1*0401 tetramers

loaded with this major epitope of *Lol p 1*, we detected allergen-specific CD4 T cells in the peripheral blood of DRB1*0401 rye grass allergic individuals following *ex vivo* expansion with allergen. These tetramer-positive cells produced IL-4 but little IFN- γ . By contrast, we were unable to detect rye grass tetramer-positive cells in cultures from HLA-DR*0401 non-allergic individuals, even after expansion with IL-2. These results suggest that rye grass allergen-specific T cells in DR*0401 non-allergic subjects are present at low levels (owing to deletion or suppression), differ in a fundamental way in their requirement for *ex vivo* expansion (e.g. they might be anergic), or utilize TCRs distinct from those of allergic individuals [44*].

The activation of innate immunity also appears to result in the inhibition of allergy and asthma. For example, TLR9 signaling with CpG oligonucleotides when administered into the lungs can inhibit Th2 cytokine production. This occurs first by inhibition of DC antigen presentation to Th2 cells, and second by inhibiting production of cytokines by mast cells and basophils [45]. TLR2 and TLR4 signaling by high molecular weight hyaluronan provides anti-inflammatory effects as well in the absence of infection, although low molecular weight degradation products of hyaluronan appear to promote inflammation by signaling through TLR2 and TLR4 [46*]. Commensal intestinal microflora might also provide anti-inflammatory effects and enhance oral tolerance by signaling through TLR4. Elimination of commensal bacteria with broad spectrum antibiotics prevents development of oral tolerance, and enhances allergic sensitization [47**] and susceptibility to intestinal inflammation [48**]. It is possible that the mucosa of patients with asthma and allergy is defective in the ability to respond to commensal flora and to develop mucosal tolerance. Thus, bronchial epithelial cells from asthmatic patients, when infected with rhinovirus, produced reduced amounts of IFN- β compared with bronchial epithelial cells from normal individuals [49]. The reduced amount of IFN- β production by bronchial epithelial cells from asthmatic individuals is thought to lead to more extensive infection with rhinovirus, which then causes worsening of asthma.

Conclusions

Much progress has been made in understanding specific mechanisms that result in allergic inflammation and asthma. It is now apparent that multiple mechanisms are involved in the development of allergy and asthma, including TSLP, IL-25, TNF- α , oxidative stress and NKT cells. By contrast, other mechanisms play important anti-inflammatory roles in preventing the development of these problems, including innate immunity and T_{Reg} cells. In the future, new therapies for asthma and allergy will be developed targeting these proinflammatory mechanisms and enhancing anti-inflammatory pathways.

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