

Immunomodulation and reversal of airway remodeling in asthma

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Abbreviations

AHR airway hyperreactivity
ISS immunostimulatory DNA sequences
LAP latency-associated peptide
TGF transforming growth factor

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Introduction

Asthma is a common chronic disease affecting approximately 7% of the population in the USA [1,2]. Despite improved therapy for asthma, with more potent inhaled corticosteroids and long acting bronchodilators, patients with asthma continue to have a significant number of acute asthma exacerbations each year in the USA (10–11 million in 1998) that result in 13.9 million outpatient visits, two million requests for urgent care, and 423 000 hospitalizations [3]. The failure of current treatments to improve asthma morbidity is partly caused by a lack of adherence to therapy by patients, as well as by physicians not always following guidelines on instituting antiinflammatory therapy in asthma [1,2]. However, even if full adherence were achieved, none of the currently available pharmacological interventions are known to alter the course of the disease or induce remission in asthma. Thus, administration of allergen specific immunotherapy has been used in an attempt to induce long-term tolerance to airborne allergens in patients with allergic asthma. Insights into the feasibility of tolerance induction by immunotherapy in asthma can be derived from studies of allergen immunotherapy in allergic rhinitis and asthma. Allergen immunotherapy with airborne allergens is effective in reducing symptoms in patients with allergic rhinitis and is the only currently known therapy that may alter the natural history of allergic rhinitis by inducing tolerance to inhaled allergens [4]. In patients with grass-pollen allergy, immunotherapy for 3–4 years induces subsequent prolonged clinical remission accompanied by a persistent alteration in immunologic reactivity suggesting that immunotherapy can induce tolerance to inhaled allergens [5]. A meta-analysis in 2002 of 43 placebo-controlled, double-

blind studies of subcutaneous specific allergen immunotherapy treatment in patients with allergic rhinitis demonstrated that specific immunotherapy resulted in a mean reduction in symptoms of 45%, compared with placebo [6]. This is equivalent to, or even better than, the efficacy obtained with most medications used to treat allergic rhinitis. Specific immunotherapy leads to a down regulation of Th2 cytokines in nasal mucous membranes and induction of IL-10, which may account for specific immunotherapy preventing the development of new sensitizations to inhaled allergens [7], and reducing the progression of allergic rhinitis to asthma [8]. Based on the clinical effectiveness of allergen immunotherapy in patients with allergic rhinitis, studies have been performed with allergen immunotherapy in subjects with allergic asthma. These studies have produced conflicting results, with proponents pointing to the benefits of this approach [9] and opponents pointing to the limitations of this approach in asthma [10]. A recent (2003) Cochrane evidence-based review [11] of 75 randomized controlled clinical trials of immunotherapy in over 3000 asthma patients demonstrated that immunotherapy reduces asthma symptoms, as well as the use of asthma medications, and improves airway hyperreactivity (AHR). Overall the meta-analysis concluded that one would need to treat four asthmatics with immunotherapy to prevent one from having asthma symptoms, and treat five asthmatics with immunotherapy to avoid one requiring increased asthma medication. Allergen immunotherapy also significantly reduced allergen-specific AHR, with some reduction in non-specific AHR. There was no consistent effect on lung function. One trial found that the size of the benefit was possibly comparable to that of inhaled steroids. Thus far no studies of immunotherapy have investigated whether it inhibits airway remodeling in asthma. However, recent studies in mice and humans with asthma have investigated whether novel immunomodulators can prevent or reverse airway remodeling. These initial studies have focused on anti-IL-5 [12,13] and CpG DNA [14–17], demonstrating that both these agents inhibit transforming growth factor (TGF)- β expression and airway remodeling.

Immunomodulation and airway remodeling in asthma: the role of transforming growth factor- β

Recent studies [12,13] with agents that deplete eosinophils in mice and humans (anti-IL-5) have suggested an

important role for eosinophil-derived TGF- β in airway remodeling in asthma. Similarly, in mouse models of airway remodeling, immunostimulatory DNA sequences (ISS) containing a CpG motif prevent airway remodeling, airway eosinophilic inflammation and TGF- β expression [14–16], suggesting that a common mechanism to reduce airway remodeling in asthma is operative, by which therapies that inhibit TGF- β expression by eosinophils inhibit airway remodeling. Several other lines of evidence in animal models and human studies in asthma support this hypothesis. These include the ability of TGF- β to induce fibrosis [18], the expression of TGF- β in the asthmatic airway [19,20], the correlation of bronchoalveolar lavage and lung TGF- β expression with the degree of subepithelial fibrosis [19–21], and the significantly increased levels of TGF- β in patients with severe asthma who have prominent airway eosinophilic inflammation [19–21]. In addition, the importance of TGF- β to airway remodeling has been suggested from recent studies that showed that reductions in levels of eosinophil TGF- β expression are associated with significant reductions in levels of airway remodeling in studies using IL-5 deficient mice [12]. Similar results have also been noted in human asthmatic patients treated with anti-IL-5 in whom levels of eosinophil TGF- β expression and airway remodeling are significantly reduced [13]. These studies suggest that unless eosinophilic inflammation and expression of TGF- β in asthma are switched off, continued eosinophilic inflammation and TGF- β expression may lead to continued airway remodeling. Interestingly, recent studies have demonstrated that a 2-week course of prednisone in mild as well as moderate asthmatics does not reduce levels of airway TGF- β expression, or reduce the levels of expression of collagen I and collagen III in airway biopsies [22]. These observations suggest that acute exacerbations of asthma, even when treated with recommended doses of corticosteroids, may be associated with continued TGF- β expression in the airway.

Anti-IL-5 and airway remodeling

As IL-5 is a lineage specific eosinophil growth factor, studies have used IL-5 deficient mice or neutralizing antibodies to IL-5 to determine the role of IL-5 and eosinophils in airway remodeling in asthma. In a mouse model of allergen induced airway remodeling IL-5 deficient mice exhibited significantly less structural features associated with airway remodeling in asthma, namely reduced peribronchial fibrosis and reduced thickening of the peribronchial smooth muscle layer, compared with wild type mice [12]. The reduced peribronchial fibrosis in IL-5 deficient mice was evident from studies measuring lung collagen content, immunohistochemical staining of the airways for collagen III and V deposition, and peribronchial trichrome staining [12]. In humans, the basement membrane of airway epithe-

lium is composed of two layers, the basal lamina (referred to as the true basement membrane and of normal thickness in asthma) and the lamina reticularis (composed of collagen type I, III, V and fibronectin) which is thickened in asthma [21]. Immunohistochemical staining of the airways of wild type mice repetitively challenged with allergen demonstrated increased peribronchial deposition of collagens III and V, collagen types noted in the remodeled airway of human asthmatic patients [21]. Although IL-5 deficient mice had significantly reduced levels of airway remodeling, airway remodeling changes were not completely ablated in IL-5 deficient mice, suggesting that additional cytokines/mediators other than IL-5 are also involved in allergen induced airway remodeling. Repetitively allergen challenged IL-5 deficient mice had a significant reduction in the number of peribronchial cells staining positive for major basic protein, which was paralleled by a similar reduction in the number of cells staining positive for TGF- β , suggesting that IL-5, eosinophils, and TGF- β play an important role in airway remodeling.

The TGF- β 1 gene encodes a pro-cytokine consisting of a C-terminal TGF- β 1 sequence and a larger N-terminal region that after processing forms a protein called latency-associated peptide (LAP) [18]. LAP and TGF- β 1 remain non-covalently associated and in this configuration TGF- β 1 is latent as LAP blocks binding of TGF- β 1 to its receptor. Previous studies have demonstrated that the integrin $\alpha_v\beta_6$ binds and activates latent TGF- β 1 [23]. Interestingly IL-5 deficient mice challenged with allergen to induce airway remodeling had significantly reduced levels of airway epithelial expression of $\alpha_v\beta_6$ integrins compared with wild type mice [12]. As $\alpha_v\beta_6$ integrins activate TGF- β from a latent to a bioactive form, the reduced expression of $\alpha_v\beta_6$ integrins in IL-5 deficient mice could reduce the activation of latent TGF- β and contribute to the reduced functional effect of TGF- β in inducing airway remodeling in these mutant mice.

In studies in humans with asthma anti-IL-5 also inhibits airway remodeling. In a randomized, double-blind, placebo-controlled study bronchial biopsies were obtained before and after three infusions of a humanized, anti-IL-5 monoclonal antibody in 24 atopic asthmatic patients [13]. Treating asthmatics with an anti-IL-5 antibody, which specifically decreased airway eosinophil numbers, significantly reduced the expression of tenascin, lumican, and procollagen III in the bronchial mucosal reticular basement membrane when compared with placebo therapy. In addition, anti-IL-5 treatment was associated with a significant reduction in the numbers and percentage of airway eosinophils expressing messenger RNA for TGF- β 1 and the concentration of TGF- β 1 in bronchoalveolar lavage fluid. Therefore

eosinophils and TGF- β may contribute to tissue remodeling processes in asthma by regulating the deposition of extracellular matrix proteins.

Immunostimulatory CpG DNA and airway remodeling

The immunostimulatory activity of CpG DNA is contained in a six base pair DNA sequence which follows the formula, 5'-purine-purine-CpG-pyrimidine-pyrimidine-3' [24]. This sequence of DNA does not code for a protein, but rather exerts its biologic function by binding to Toll-like-receptor-9 (TLR-9) which is expressed on cells of the innate immune system [24]. Several studies have demonstrated that CpG DNA inhibits acute Th2 responses, eosinophilic inflammation, and mucus expression in mouse models of asthma induced with allergen [25,26] or respiratory syncytial virus challenge [27]. More recent studies [14–16] have demonstrated that CpG DNA can prevent as well as reverse airway remodeling in mice exposed to repetitive airway challenge with allergen. In a preventive model of asthma, CpG DNA reduces the thickness of the peribronchial smooth muscle layer, accumulation of peribronchial myofibroblasts, and reduces peribronchial fibrosis (peribronchial collagen III and V deposition). In addition, CpG DNA significantly reduces bronchoalveolar lavage and lung levels of the pro-fibrotic cytokine TGF- β , suggesting that inhibition of TGF- β expression may account for the reduction in airway remodeling. In addition to preventing airway remodeling in mice, ISS can also reverse features of airway remodeling that have already been induced. In particular ISS reverses peribronchial collagen deposition as assessed by measuring total lung collagen, peribronchial trichrome staining, as well as peribronchial collagen III and V immunostaining [16]. ISS indirectly inhibits fibroblast collagen synthesis through its effects on macrophages (reducing TGF- β 1 expression by macrophages), as well as by reducing the number of TGF- β 1 positive eosinophils and mononuclear cells recruited to the airway [16]. In addition, ISS significantly reduces levels of expression of the chemokine TARC, as well as the number of peribronchial CD4+ lymphocytes [16]. CD4+ lymphocytes are likely to play an important role in orchestrating the airway remodeling response by generating Th2 cytokines such as IL-5 [12] and IL-13 [21] which have been implicated in airway remodeling. ISS induced reduction in the number of peribronchial CD4+ lymphocytes is therefore likely to contribute to reducing peribronchial fibrosis by reducing levels of cytokines such as IL-5 and IL-13 which contribute to fibrosis. Overall, these studies suggest that CpG DNA not only prevents Th2 mediated airway inflammation in response to acute allergen challenge, but also inhibits TGF- β expression and airway remodeling associated with chronic allergen challenge.

In studies in primates, ISS also inhibits airway remodeling [17]. In these studies, house dust mite allergic and exposed primates were treated with inhaled ISS for approximately 8 months. Treatment with ISS reduced airway responsiveness approximately twofold. In addition, the ISS treated primates had airways with thinner reticular basement membranes, fewer mucus cells, fewer eosinophils, and fewer mast cells than sham treated allergic primates. The ability of ISS to inhibit airway inflammation as well as airway remodeling in mouse and primate models of asthma suggests the potential utility of this approach as a therapy in human asthma.

Conclusion

According to current international asthma guidelines, the level and adjustment of antiinflammatory therapy for asthma are based on evaluation of asthma symptoms and lung function. Interestingly, studies have suggested that a treatment strategy in asthmatics aimed at reducing airway hyperresponsiveness led to more effective control of asthma, and greater improvement of chronic airway inflammation than a treatment strategy solely based on asthma guidelines [28]. In addition, the treatment strategy aimed at reducing airway responsiveness was more effective in reducing the thickness of the subepithelial reticular basement membrane layer when comparing bronchial biopsies obtained at entry and after 2 years of treatment [28]. Airway remodeling as an end point in therapeutic trials in asthma has been less frequently studied than more common clinical endpoints such as symptom scores, medication usage, asthma exacerbations, spirometry, or AHR to methacholine. The need for invasive bronchoscopy to obtain a biopsy is one of the practical limitations in performing such studies evaluating changes in airway remodeling in human asthmatics. However, as novel therapies with immunomodulating properties are developed, measurement of their potential impact on airway remodeling should be considered as an additional end-point in selected clinical trials. Further studies are also needed to determine the clinical significance of reductions in airway remodeling induced by immunomodulators such as anti-IL-5 and CpG DNA.

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