

Anti–interleukin-5 therapy for asthma and hypereosinophilic syndrome

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With the advent of monoclonal antibody therapy, it has been possible to target specific components of the immune system and to examine the effects of their neutralization on the clinical manifestations of disease in humans. Interleukin-5 (IL-5) is a key cytokine in eosinophil differentiation [1], maturation, recruitment, and activation at sites of inflammation [2]. It stimulates the expansion and differentiation of eosinophil precursors [3] and up-regulates expression of its own specific receptor α chain during human eosinophil development [4]. IL-5 is produced primarily by activated T cells, although secretion by CD8⁺ T cells [5], natural killer cells [6], mast cells [7], eosinophils [8], and airway epithelial cells [9] has been reported. In addition, it is chemotactic for eosinophils, stimulates their activation [10], prolongs their survival [11], and facilitates their degranulation in response to specific stimuli. In contrast to the broad range of biologic activities of the other cytokines known to have a role in eosinophilia (ie, IL-3, granulocyte macrophage colony-stimulating factor [GM-CSF]), the action of IL-5 is specific for eosinophils in humans. This specificity likely is conferred by the restriction of expression of the IL-5 receptor α (IL-5R α) chain to eosinophils and basophils [12].

Two monoclonal antibodies to human IL-5, SCH55700 (Schering-Plough Research Institute) and mepolizumab (GlaxoSmithKline), have been used in clinical trials for the treatment of asthma and hypereosinophilic syndromes. SCH55700 is a humanized monoclonal antibody of the IgG4/ κ subtype that is

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Table 1

Comparison of mepolizumab and SCH55700

Characteristic	Mepolizumab	SCH55700
Parent antibody	2B6 (mouse)	39D10 (rat IgG2a)
Antibody subclass	IgG1/ κ	IgG4/ κ
Kd for human IL-5	4.2 pM	81 pM
Mean half life (d)	Not available	24.5–30.1 ^a
Dose range in human trials	2.5–10 ^b mg/kg	0.03–3 mg/kg
References	[15,27,28,99,100]	[13,32,101]

^a 1-mg/kg single dose in patients with mild asthma.^b In some studies, patients received mepolizumab at a dose of 250 or 750 mg without regard to weight.

derived from the rat antihuman antibody 39D10. Complementarity-derived regions were grafted onto human frameworks, cloned into a cytomegalovirus promoter-based mammalian expression vector, and transfected into NS0 murine myeloma cells for antibody production [13]. The purified humanized 39D10 antibody, designated SCH55700, was shown to neutralize IL-5 by binding to amino acids 89–92 (ERRRV), the same amino acids that are bound by the parental rat molecule [14]. Mepolizumab (SB-240563) is a humanized monoclonal antibody of the IgG1/ κ subtype that is derived in a similar fashion from the murine monoclonal antihuman IL-5 antibody 2B6 and expressed in Chinese hamster ovary cells [15].

This article discusses the rationale for the use of anti-IL-5 therapy in asthma and hypereosinophilic syndrome and summarizes the available clinical data on the use of anti-IL-5 in these disorders. Although mepolizumab and SCH55700 share a number of features (Table 1), including excellent preclinical and clinical safety profiles, profound and prolonged effects on eosinophilia in animal models, and long terminal half-lives, the affinity of mepolizumab for human IL-5 is approximately 20-fold greater than that of SCH55700. In view of this difference in affinity and the fact that mepolizumab dosing in the clinical trials has been approximately 10-fold higher than that of SCH55700, comparisons among clinical trials of the two drugs need to be interpreted cautiously.

Asthma

Asthma, eosinophils, and interleukin-5

Asthma is a chronic inflammatory condition of the airways that is characterized by a prominent eosinophilic inflammatory infiltrate in the bronchial mucosa. For many years, it has been recognized that eosinophils can damage the airway mucosa and associated nerves through the release of granule-associated basic proteins; lipid mediators; reactive oxygen species; and a variety of other agents, including cytokines and chemokines. These observations and the broad correlation between activated eosinophils and severity of asthma have led to the

hypothesis that the eosinophil is the central effector cell in ongoing airway inflammation [16].

IL-5 transgenic mice exhibit a marked blood and tissue eosinophilia [17], and IL-5 gene disruption abolished eosinophilia, airway hyperreactivity, and lung damage in a mouse model of asthma [18]. IL-5 also was shown to be a basophilopoietic factor. Culture of leukocyte precursors with IL-5 yields mixed eosinophil and basophil colonies [19]. Basophils are less numerous in the airway infiltrate in asthma [20], but through interaction of allergen with surface high-affinity IgE, receptor-bound IgE may be an important contributor to allergen-induced asthma symptoms [21].

Studies have shown that the expression of IL-5 in bronchoalveolar lavage fluid (BALF) and bronchial biopsies is increased in asthma [22]. The level of IL-5 correlates with clinical features [23], and IL-5 mRNA in the bronchial mucosa was up-regulated after allergen-provoked asthma [24], which decreased after successful treatment with corticosteroids [25]. In an *Ascaris* model of allergen challenge in monkeys, anti-IL-5 almost completely abrogated eosinophilia and airways hyperresponsiveness [26].

Interleukin-5 blockade in asthma

For many years, it has been speculated that IL-5 blockade using an anti-IL-5 or an anti-IL-5R α antibody would improve symptoms in asthma by depleting eosinophils from the tissues; however, a small study of the effect of a humanized monoclonal antibody against IL-5 (mepolizumab) in mild atopic asthmatics found that a single intravenous infusion effectively depleted eosinophils from blood and induced sputum, but had no effect on airways hyperresponsiveness and the late asthmatic reaction after inhaled allergen challenge [27].

The study's conclusion that eosinophils were unlikely to be important in asthma pathogenesis was premature, however, because the study was underpowered. Furthermore, there was no information on the effectiveness of mepolizumab in the depletion of eosinophils from the bronchial mucosa. This factor was critical, because other cytokines, such as GM-CSF and IL-3, are involved in eosinophil maturation [2]. Eosinophil depletion from the tissue cannot be assumed from sputum and blood data. The effect of IL-5 blockade on basophil infiltration of the airway was not reported, and the selectivity of mepolizumab for the eosinophil was not established.

The authors examined the effect of anti-IL-5 on eosinophils and basophils at the site of disease (airway tissue) and the bone marrow [28]. A total of 24 mild asthmatics received three intravenous doses of 750 mg of mepolizumab or placebo over 12 weeks in a randomized, double-blind, parallel-group study (Fig. 1). Blood, bone marrow aspirate, and endobronchial biopsies were obtained before and after treatment, and airway hyperresponsiveness (AHR) and forced expiratory volume in 1 second (FEV₁) were recorded together with peak flow diaries. The authors confirmed the earlier study by showing that mepolizumab produced a median 100% decrease (interquartile range, 67%–100%) in blood

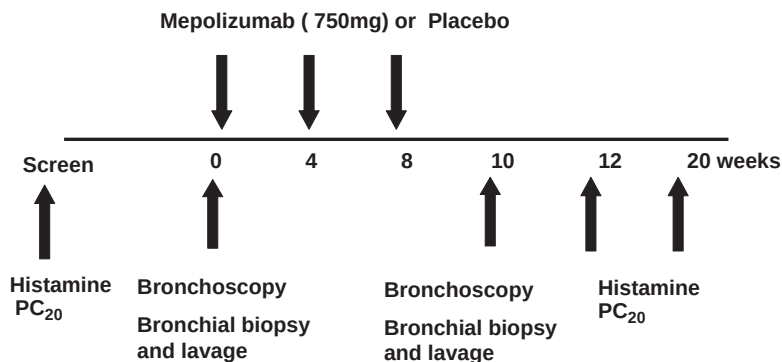


Fig. 1. The effect of mepolizumab on blood and tissue eosinophils: study design. (From Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199–204; with permission.)

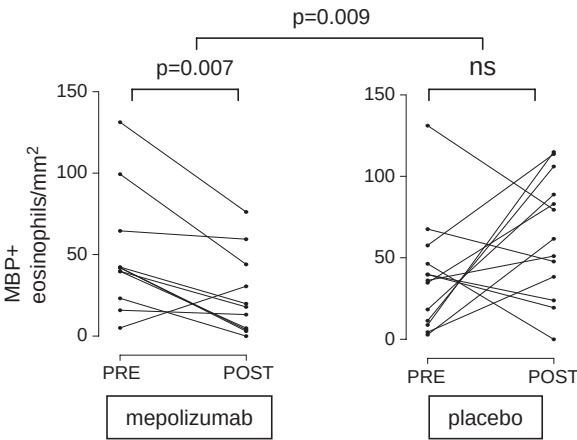
eosinophils from baseline, which was sustained for 12 weeks after treatment. Although significant reductions were seen in levels of bronchial mucosal and bone marrow eosinophils, the median reduction was only 55% for airway eosinophils (range, 29%–89%) and 52% for bone marrow eosinophils (range, 45%–76%) (Figs. 2 and 3). There were no significant changes in blood, airway tissue, or bone marrow basophil numbers. Clinical outcomes, including AHR, were unaffected by mepolizumab treatment (except for the median morning peak flow, which was increased significantly in patients receiving mepolizumab), and there were no changes in the other clinical measures of asthma.

Tissue staining with antibody to eosinophil major basic protein showed no significant reduction with anti-IL-5 treatment (Fig. 3). Despite high-dose anti-IL-5 treatment, there were residual airway eosinophils, which remained activated in asthmatic subjects. Tissue eosinophils were deleted selectively, and there was no significant alteration in the numbers of neutrophils, basophils, mast cells, macrophages, or lymphocytes. This finding was similar to the findings of Buttner et al [29], who found that mepolizumab had no effect on circulating T-cell subsets or circulating T-cell cytokine production. The dramatic depletion in blood eosinophils was associated with only a modest change in the bronchial mucosa and emphasized the importance of obtaining biopsy tissue from the target organ when evaluating eosinophil-depleting strategies.

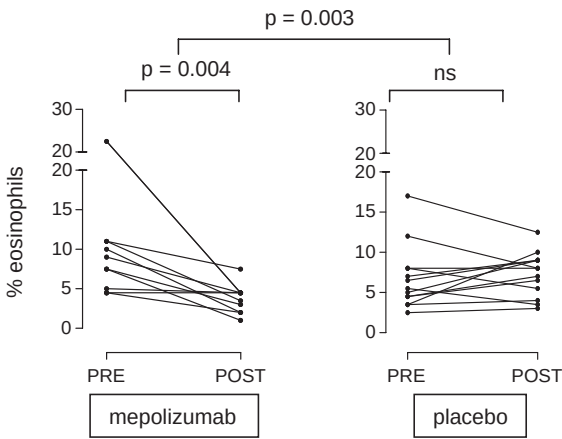
A previous study involving asthmatics of similar severity showed that oral corticosteroid therapy reduced mucosal eosinophil infiltration by a median of 81%

Fig. 2. Effect of mepolizumab or placebo treatment on eosinophils within the bronchial mucosa, bone marrow, and blood. MBP, major basic protein; ns, not significant. (From Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199–204; with permission.)

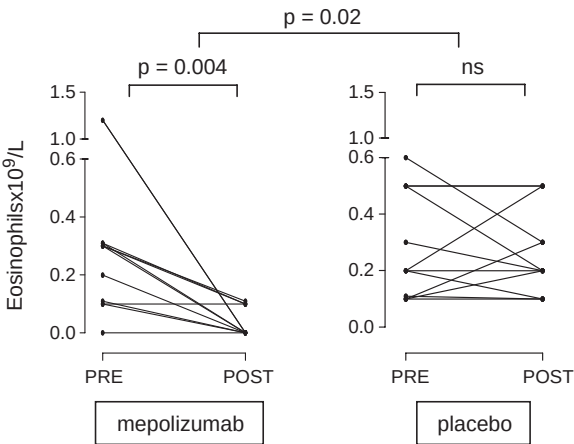
Bronchial mucosa



Bone marrow



Blood



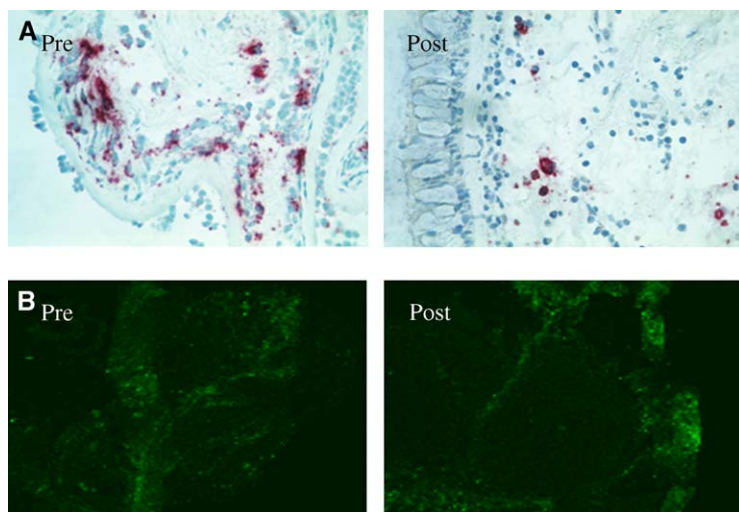


Fig. 3. (A) Bronchial mucosal biopsies stained with a monoclonal antibody against MBP before and after treatment with mepolizumab. Residual tissue eosinophils is seen after treatment (positive staining is developed with Fast Red). (B) Immunofluorescent staining with a polyclonal anti-MBP antibody of bronchial biopsies before and after mepolizumab treatment, demonstrating no significant change in the levels of extracellular MBP after anti-IL-5 treatment, despite a 55% decrease in intact tissue eosinophils. (From Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199–204; with permission.)

[30]. The authors interpret their findings as showing that mepolizumab does not deplete airway eosinophils sufficiently to impact the clinical features of asthma.

In agreement with this lack of clinical efficacy for mepolizumab, a larger study of the antibody in asthmatic patients taking inhaled corticosteroids (reported in abstract form) showed no effect on lung function, medication use, or quality-of-life measures of asthma [31]. Kips et al [32] described the effects of a second humanized anti-IL-5 monoclonal antibody (SCH55700). Their patients were severe asthmatics and persistently symptomatic despite treatment with high-dose inhaled corticosteroids or oral prednisolone. Volunteers received a single infusion of SCH55700 or placebo in a double-blind fashion, and blood and sputum eosinophils, spirometry, peak flow recordings, and symptoms were monitored for up to 30 days. In agreement with data obtained with mepolizumab [17,33], SCH55700 produced a significant decrease in blood eosinophil counts at the two highest doses used. Insufficient paired samples were obtained to determine whether SCH55700 had any appreciable effect on sputum eosinophils. The 0.3-mg/kg dose of SCH55700 produced a significant increase in FEV₁ at 24 hours, which persisted as a trend at subsequent time points. This improvement was not associated with changes in symptom scores, FEV₁–FVC ratio, or peak flow recordings. The rapid improvement in FEV₁ was difficult to interpret, and the fact that it was not sustained and only observed with the submaximal

dose was disappointing. This finding may have been related to the more acute bronchoconstricting effect of eosinophil-derived cysteinyl leukotrienes or to an eosinophil-independent property, because airway smooth muscle cells express the IL-5 receptor and because in vitro incubation with IL-5 selectively primes for hyperresponsiveness [34]. The rapid improvement in FEV₁ may have been caused by direct blockade of the actions of IL-5 on airway smooth muscle, which in turn improved airway caliber.

The different levels of depletion of blood eosinophils compared with depletion of bone marrow and bronchial mucosal eosinophils by anti-IL-5 treatment could be explained in several ways. The monoclonal antibody may not penetrate tissues or act systemically to reach bone marrow. Previous data from other studies of antibody treatments and pharmacodynamic studies of the distribution of anti-IL-5 in animals make this explanation unlikely [35].

A second possibility is that other cytokines can sustain tissue eosinophilia in the absence of IL-5. Obvious candidates are IL-3 and GM-CSF, and their levels have been shown to be increased in the airways of asthmatics compared with the airways of controls [36]. Liu et al [37] showed that expression of the IL-5 receptor on airway eosinophils is down-regulated in vivo after inhaled allergen challenge and that this finding was associated with a loss of IL-5 responsiveness. Gregory et al [38] demonstrated that exposure of blood eosinophils to IL-3, IL-5, or GM-CSF in vitro leads to sustained down-regulation of surface IL-5R α chain expression, reduced responsiveness to IL-5, but no sustained changes in C-C chemokine receptor 3 (CCR3) expression.

IL-5, IL-3, and GM-CSF share a common receptor β subunit, and mice with gene deletion of this common signaling component have residual eosinophils [39]. In these mice, eosinophils can be recruited to the airway after inhaled allergen challenge of sensitized animals, and this recruitment seems to depend on the chemokine eotaxin [40]. The authors previously showed that the expression of eotaxin is increased in the airways of asthmatic subjects [41], and blockade of its receptor, CCR3, may be required to deplete airway eosinophils in humans. Although IL-5 gene deletion or antibody blockade abolished inhaled antigen-induced AHR in some mouse models, this finding was not seen in all mouse strains [42]. These experiments suggest that there are non-IL-5 mechanisms of airway eosinophilia and noneosinophil mechanisms for inducing AHR.

Eosinophils and remodeling

Although the potential for eosinophils as pro-inflammatory cells is well known, less attention has been given to their possible role in repair and remodeling processes, despite the well-documented association of tissue eosinophilia and eosinophil degranulation with several fibrotic syndromes [43–46]. This remodeled phenotype, which may be the consequence of excessive repair processes after repeated airway injury, includes increased deposition of extracellular matrix (ECM) proteins in the reticular basement membrane (RBM) [47] and in the bronchial submucosa [48]. These ECM proteins include procollagen III (the ma-

ture precursor for collagen III) and the proteoglycans tenascin and lumican [47,49–51]. In addition to their structural role, ECM proteins may influence cellular function, including adhesion, differentiation, and survival [52,53]. Other features of remodeling include increased smooth muscle mass [54], goblet cell hyperplasia [55], and new blood vessel formation [56].

Previous evidence suggesting that the eosinophil may be important in the pathophysiology of airways remodeling included the observation that the thickening of the subepithelial basement membrane was associated with increased levels of bronchial mucosal eosinophils in severe asthmatics [57]. Eosinophils are a source of several molecules implicated in tissue remodeling processes, such as transforming growth factor α (TGF- α) [46], TGF- β [58], vascular endothelial growth factor [59], matrix metalloproteinase 9 [60], tissue inhibitor of metalloproteinase 1 [61], and IL-13 [62]. TGF- β is a potent regulator of fibroblast and myofibroblast function with the production of several ECM proteins, including collagens, proteoglycans, and tenascin [63,64].

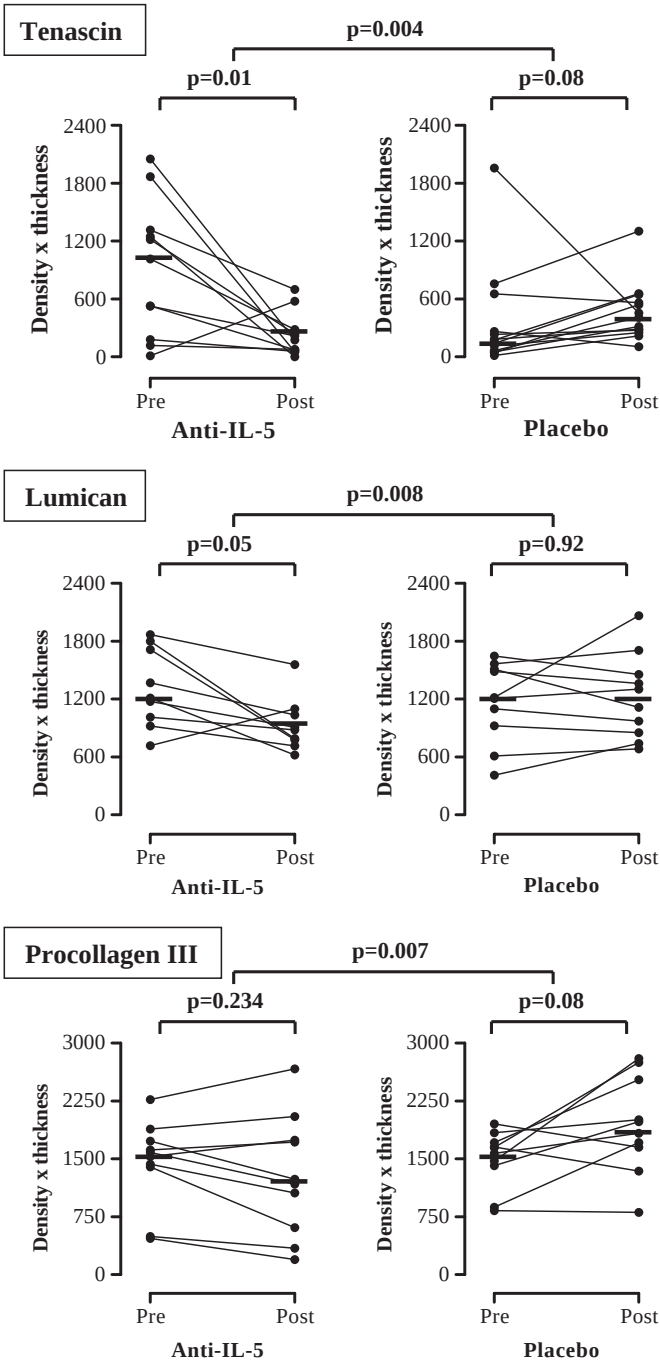
It was shown *in vivo* that eosinophil-derived TGF- β is associated temporally with myofibroblast formation and the expression of tenascin and procollagen I by fibroblasts in human atopic skin [65]. Direct evidence for the production of repair markers was obtained *in vitro* by co-culture of eosinophils and fibroblasts. Eosinophil-induced myofibroblast formation was associated with the generation of tenascin transcripts and protein product, which was inhibitable by neutralizing antibodies to TGF- β .

Anti-interleukin-5 and airway remodeling

The accumulating evidence suggested that that even a partial reduction of eosinophil levels in the airway wall may alter repair and fibrotic processes. The thickness and density of tenascin, lumican, and procollagen III immunoreactivity was measured in the RBM of bronchial biopsies from the asthmatics described earlier who had received three injections of mepolizumab [29,66]. The data were quantified from images collected using confocal microscopy and were related to eosinophil numbers. Expression of TGF- β 1 mRNA by airway eosinophils was assessed by *in situ* hybridization, and TGF- β 1 protein was measured in BALF by ELISA.

At baseline, airway eosinophil infiltration and deposition of extracellular matrix protein was increased in the RBM of asthmatics, compared with non-asthmatic controls. Treatment of asthmatics with anti-IL-5 antibody, which specifically decreased airway eosinophil numbers, significantly reduced the expression of tenascin, lumican, and procollagen III in the bronchial mucosal

Fig. 4. The effect of anti-IL-5 on the total expression (thickness $\times$ density) of tenascin, lumican, and procollagen III in the bronchial subepithelial RBM. The crossbars represent the median values. (From Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL5 treatment reduces deposition of extracellular matrix proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029–36; with permission.)



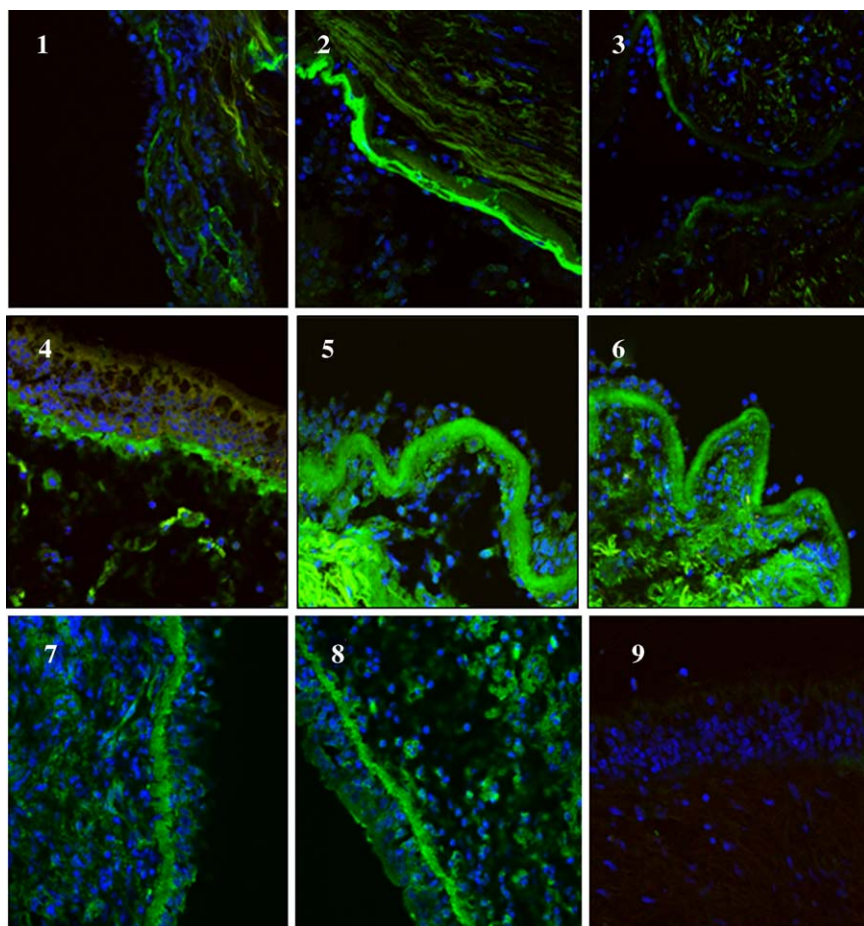


Fig. 5. Tenascin immunoreactivity in the RBM from a normal subject (Plate 1) and from an asthmatic subject before treatment (Plate 2) and after treatment (Plate 3) with anti-IL-5. Lumican immunoreactivity from a normal subject (Plate 4), before treatment (Plate 5), and after treatment (Plate 6) with anti-IL-5 is shown. Examples of procollagen III immunoreactivity before treatment (Plate 7) and after treatment (Plate 8) with anti-IL-5 are presented, although the decrease was not typical of all subjects. Plate 9 shows an example of isotype control immunoreactivity. Immunoreactivity of ECM proteins is indicated by green coloring. Sections were counterstained with 4'-6-diamidino-2-phenylindole 2HCl (DAPI) (blue). (From Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL5 treatment reduces deposition of extracellular matrix proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029–36; with permission.)

RBM, when compared with placebo treatment (Figs. 4 and 5). Anti-IL-5 treatment was associated with a significant reduction in the numbers and percentage of airway eosinophils expressing mRNA for TGF- β 1 and in the concentration of TGF- β 1 in BALF.

The data supported a novel role for the eosinophil and suggested a link between eosinophilic infiltration and airway remodeling in asthma. The findings

were consistent with those previously reported in animal studies. In an IL-5 gene knockout mouse model of airway remodeling, abrogation of eosinophil infiltration after repeated allergen challenge was associated with an absence of structural changes, including fibronectin production and mucus gland hyperplasia, which was observed in the wild-type controls [67]. In a separate animal model of atopic asthma, prophylactic treatment of ovalbumin-sensitized BALB/c mice with anti-IL-5 significantly reduced the subepithelial fibrosis that was produced by repeated allergen challenge [68]. These investigators did not report the effects of anti-IL-5 treatment on established fibrosis. In a chronic repetitive allergen challenge model, Cho et al [69] showed that IL-5 gene deletion suppresses lung eosinophilia and tissue remodeling in parallel. Specifically, IL-5-deficient mice exhibited reduced eosinophil numbers in BALF and peribronchial tissue. This finding was associated with decreased TGF- β 1 content of lung tissue and evidence that eosinophils were the major source. Epithelial cell expression of $\alpha_v\beta_6$, the integrin that activates latent TGF- β 1 [62,70], was suppressed. In IL-5-deficient animals, there was a reduction in the total lung collagen content, peribronchial fibrosis, collagen III, and collagen V. Thickness of peribronchial smooth muscle and expression of epithelial mucus also were reduced.

Anti-interleukin-5, extracellular matrix proteins, and transforming growth factor β

Compared with lumican or procollagen III, there was a particularly close correlation between baseline eosinophil levels and tenascin expression and a more marked decrease in tenascin expression in response to anti-IL-5 therapy. Mepolizumab did not significantly decrease the expression of the ECM protein procollagen III, although the changes observed were significantly different from those observed with placebo. This finding suggests a strong association between eosinophils and tenascin deposition, as previously shown in allergen-induced formation of tenascin in atopic skin [65]. Longer periods of treatment may be required to reverse procollagen and lumican expression to the same degree as that observed with tenascin. Evidence from studies investigating the effect of inhaled corticosteroids on RBM thickness indicated that although short-term treatment is associated with a significant decrease in tenascin thickness in the RBM [46], longer-term studies are required to show a reduction in collagen III thickness [71,72]. Over the study period, the placebo group had nonsignificant increases in levels of eosinophils, tenascin, and procollagen, raising the possibility that these changes reflect the natural history of untreated mild atopic asthma and that anti-IL-5 may have prevented such an increase.

Tenascin expression before placebo treatment was lower than expression before mepolizumab treatment, albeit not significantly lower. Because the treatment groups were otherwise well matched in terms of clinical and histopathologic features and were selected randomly, this difference is most likely the consequence of the relatively small numbers and the large standard deviation of the baseline values for tenascin.

TGF- β 1 is a potent differentiation factor for the formation of myofibroblasts in vivo and in vitro [73,74] and has been shown to up-regulate the expression by fibroblasts of an array of ECM proteins, including tenascin [75]. Consistent with the findings of previous studies [76,77] most of the TGF- β 1 mRNA+ cells in the submucosa in these mild atopic asthmatics were eosinophils. Selective depletion of eosinophils with anti-IL-5 use significantly reduced the number of TGF- β 1 mRNA+ eosinophils in the bronchial mucosa and decreased TGF- β 1 concentrations in BALF. The proportion of cells expressing TGF- β 1 mRNA decreased significantly in the mepolizumab recipients. This finding suggests that anti-IL-5 therapy decreased the number of eosinophils producing TGF- β 1 and decreased the degree of activation of these cells for TGF- β 1 expression. The fact that most TGF- β 1 mRNA+ cells were eosinophils and that levels of TGF- β 1 in BALF were reduced supports the authors' hypothesis that eosinophils are an important source of TGF- β 1 in the bronchial mucosa of asthmatics. Eosinophil-derived TGF- β 1 may influence the expression of tenascin and other ECM proteins by way of the activation of myofibroblasts.

The observed effect of anti-IL-5 on matrix protein expression may have been mediated by the effect of the drug on IL-5 than through the reduction in eosinophil numbers. Although IL-5 has been shown to influence contraction of airway smooth muscle [78], IL-5 is not known to directly release growth factors from smooth muscle or other cells that are appropriate to the remodeling changes studied. The consistent correlation between eosinophil numbers and tenascin thickness before and after mepolizumab therapy makes a direct action of IL-5 unlikely—at least for tenascin. It also is unlikely that the changes in ECM protein expression can be explained by the action of anti-IL-5 on other cell types in the bronchial mucosa. There were no significant changes in the numbers of other cells in response to anti-IL-5. Although there was a trend toward a reduction in basophil numbers, this finding did not correlate with changes in ECM protein expression.

Relevance to asthma pathogenesis

The relevance of the authors' findings in terms of asthma pathophysiology is unclear, because the effect of airways remodeling on lung function in asthma is uncertain. Deposition of ECM proteins in the airway wall may have a mechanical effect, amplifying the degree of narrowing associated with a given degree of smooth muscle contraction [79]. A number of studies have reported a correlation between the thickness of the RBM and AHR, FEV₁, and other markers of asthma severity [51,77,80,81]. The authors found no significant correlation between baseline PC₂₀; FEV₁; and the thickness, density, and expression of tenascin, lumican, and procollagen III. Subjects had mild asthma, and the study was not powered to detect significant correlations in clinical variables such as FEV₁ or AHR. Although a significant reduction in the expression of tenascin, lumican, and procollagen III was observed in the RBM, overall RBM thickness was not measured in these biopsies, and it is possible that eosinophil depletion was not

associated with so marked a reduction in the expression of other matrix proteins, such as fibronectin and types I and V collagen.

Although anti-IL-5 administration significantly reduced the numbers of tissue eosinophils in the bronchial submucosa, it did not deplete them to the same extent as that reported previously in sputum and blood [27]. GM-CSF, IL-3, and eotaxin are involved in eosinophil homeostasis, and there is evidence that inhibition of these factors, in conjunction with IL-5 blockade, may be associated with more complete eosinophil depletion from the bronchial mucosa [39,40]. Studies showing the most marked effects of corticosteroids on RBM thickness have tended to be of longer duration, so sustained eosinophil depletion may be required to achieve a maximal effect.

Selective reduction of eosinophils by anti-IL-5 treatment was associated with a significant decrease in expression of ECM proteins in the airway RBM of patients with mild asthma. This reduction was accompanied by a decrease in TGF- β 1 expression by airway eosinophils. These data provide strong evidence of a causal association between eosinophils and markers of airway remodeling in mild atopic asthma. Nevertheless, there is no evidence to date that anti-IL-5 affects clinically relevant outcomes in asthma. As shown in Fig. 6, the eosinophil can damage the epithelium by way of IL-13 and basic granule proteins, contraction of airway smooth muscle (cysteinyl leukotrienes), AHR (IL-13), and TGF- β -mediated remodeling events.

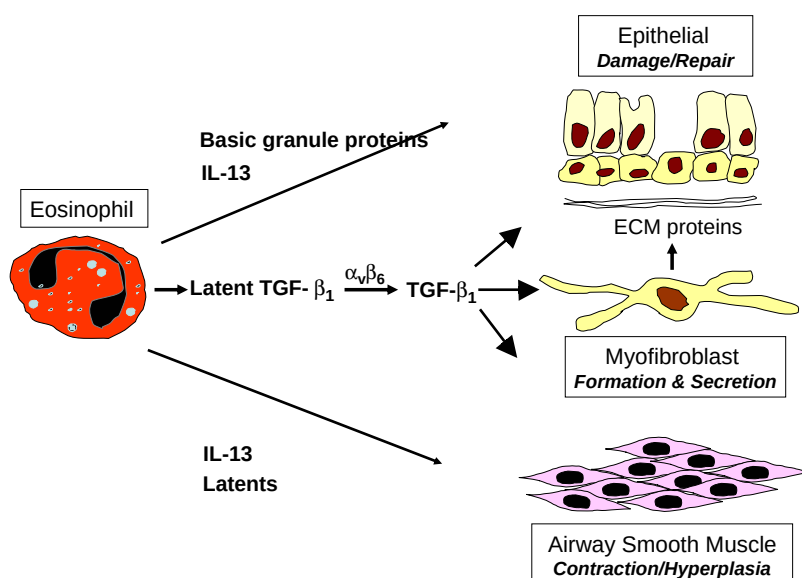


Fig. 6. Pro-inflammatory and repair and remodeling potential of eosinophils. (Modified from Williams TJ. The eosinophil enigma. J Clin Invest 2004;113:508; with permission.)

Future studies

From this information, it is reasonable to conclude that anti-IL-5 monoclonal antibodies are ineffective at depleting tissue eosinophils and that this strategy may be fundamentally flawed. Even animal models of asthma showed residual tissue eosinophilia in the airways after anti-IL-5 administration [18]. Foster et al [82] reported that deletion of IL-5 and eotaxin is required to abolish tissue eosinophilia and AHR in BALBc mice, suggesting that IL-5 blockade alone is insufficient to abrogate the tissue eosinophilia associated with allergic inflammation. Combination therapy (eg, anti-IL-5, CCR3 antagonist) may be more useful than IL-5 blockade alone, because this treatment would have the theoretic advantage of inhibiting bone marrow maturation (mainly an IL-5 effect) and tissue accumulation (predominantly a CCR3-dependent effect). Another approach would be to inhibit GATA-1, an essential transcription factor in the eosinophil lineage [83].

Effective depletion of eosinophils from the airways of patients with chronic severe asthma may prove to have useful clinical benefit, although long-term treatment probably would be required. In this context, it is relevant that a treatment strategy directed at normalization of the induced sputum eosinophil count reduced asthma exacerbations and hospital admissions without the need for additional anti-inflammatory treatment [84].

Anti-IL-5 is not a magic bullet treating for asthma. The eosinophil's potential in the asthma process cannot be overlooked until there are effective and selective methods of depleting the cell from the tissues.

Hypereosinophilic syndrome

Hypereosinophilic syndrome (HES) is a heterogeneous group of rare disorders that is characterized by levels of peripheral eosinophilia greater than 1500/mm³ of unknown cause and evidence of eosinophil-mediated end-organ damage [85–87]. Although a subset of patients with HES has an underlying clonal abnormality of the myeloid [88] or lymphocyte [89] lineages, the cause of HES remains unknown in as many as 50% of patients (A.D. Klion, unpublished data, 2004).

The spectrum of end-organ involvement in HES is broad, ranging from urticaria to life-threatening endomyocardial fibrosis, and it can be difficult to distinguish between HES and other clinical syndromes associated with marked peripheral blood and tissue eosinophilia, including eosinophilic pneumonia and eosinophilic gastroenteritis. Regardless of the underlying cause of the eosinophilia, however, eosinophil activation in the tissues is believed to be responsible for the pathology in HES, and increased levels of eosinophil granule proteins have been demonstrated in the serum of patients with HES [90] and in biopsies of affected tissues [91–93]. Successful therapy for HES, whether with steroids, hydroxyurea, interferon α , or imatinib mesylate [85,94–96], has been directed at

decreasing peripheral blood eosinophilia and preventing eosinophil infiltration into the tissues and end-organ damage. Long-term treatment with these agents is problematic, as many patients become progressively refractory to treatment or develop significant drug-related side effects.

Serum levels of IL-5 are elevated in some, but not all, patients with HES (A.D. Klion, unpublished results). Increased levels of IL-5 mRNA production by stimulated peripheral blood mononuclear cells [97] and clonal populations of IL-5-secreting lymphocytes [98] have been demonstrated in patients with HES and without detectable serum IL-5. These data suggest that IL-5 may be important in mediating the eosinophilia seen in HES, irrespective of serum IL-5 levels, and provide a compelling rationale for the treatment of patients with HES with anti-IL-5 antibody.

The potential usefulness of anti-IL-5 therapy for HES and related eosinophil-mediated syndromes has been confirmed in four small case series (Table 2) [99–102]. Six patients with HES and one patient with eosinophilic esophagitis received mepolizumab in two separate studies [99,100]. The infusion was well tolerated in all cases, and a rapid and sustained reduction in peripheral eosinophilia was seen after intravenous administration of a single 750-mg dose. Clinical manifestations, which ranged from severe steroid-resistant eosinophilic dermatitis to multisystem involvement of the lungs, gastrointestinal tract, and skin, improved dramatically. Biopsies obtained before and after treatment in two patients revealed a marked decrease in eosinophil and eosinophil cationic protein (ECP) levels in the skin [100] and reduced eosinophil numbers in the gastrointestinal tract [99]. Serum levels of IL-5, ECP, eotaxin, and thymus- and activation-regulated chemokine were reduced after mepolizumab treatment [100], a finding that is consistent with an effect of mepolizumab on eosinophil and T-cell activation.

Improvement in eosinophilia and clinical symptoms persisted for up to 7 weeks after treatment with a single dose of mepolizumab and was maintained at a stable level during monthly dosing. In the study by Plötz et al [100], clinical remission persisted after discontinuation of mepolizumab therapy in two of three patients.

SCH55700 has been used to treat eight patients with HES or eosinophilic gastroenteritis with eosinophilia (EGE) (Table 2) [101,102]. Two of the four patients with HES and all of the four patients with EGE responded to a single 1-mg/kg dose of SCH55700 with a rapid and sustained reduction in eosinophilia. Significant improvement in clinical symptoms was apparent only in two patients with HES, despite gastrointestinal biopsies performed before and at 1 month after SCH55700 infusion, which showed a 50% to 70% decrease in tissue eosinophilia in three of four patients with EGE. One of the two patients with HES who did not demonstrate clinical improvement in response to SCH55700 had a markedly elevated baseline level of serum IL-5 (223 pg/mL) and only a brief reduction in eosinophilia, suggesting that a higher dose may have been necessary.

A self-limited exacerbation of eosinophilia above baseline levels occurred in all of the six responding patients (with a concomitant exacerbation of symptoms in six of eight patients) as drug levels waned. This rebound was preceded by an

Table 2
Anti-interleukin-5 treatment of hypereosinophilic syndrome

Characteristic	Plötz et al [100]	Garrett et al [99]	Klion et al [101]	Prussin et al [102]
No. of patients	3	4	4	4
Gender (M/F)	0/3	2/2	2/2	2/2
Age (y)	60–82	18–48	32–52	38–53
Diagnosis	HES with eosinophilic dermatitis	HES (3 patients), EE (1 patient)	HES	EGE
Drug	Mepolizumab	Mepolizumab	SCH55700	SCH55700
Dose	750 mg $\times$ 2–8 doses	750 mg $\times$ 3 doses	1–3 mg/kg $\times$ 6 doses	1 mg/kg $\times$ 6 doses
Frequency	Every 3 days to monthly	Monthly	Monthly	Monthly
Baseline eosinophil count	1118–3811	300–1500	2620–6133	819–1883
Elevated baseline serum IL-5 (no. of patients)	3/3	1/4	1/4	1/4
Presence of FIP1L1/PDGFR α mutation (no. of patients)	0/3	0/4	2/4	0/4
Response rate (no. of patients)	3/3 ^a	4/4	2/4 ^b	4/4 ^b

Abbreviation: M/F, male/female.

^a A sustained response was seen in two patients after drug administration was stopped.

^b Attenuation of clinical response was seen over time despite monthly dosing.

increase in serum IL-5 levels. Although both patients with HES responded to the reinstitution of anti-IL5 therapy (1 mg/kg/mo for five doses), the magnitude and duration of the response decreased with each subsequent dose, despite adequate drug levels. A similar attenuation of response was seen in the four patients with EGE who received monthly dosing.

Despite the heterogeneity in study design and patient populations, several conclusions can be drawn from the four case studies published to date. Anti-IL-5 therapy is safe and effective in reducing blood and tissue eosinophilia in most patients with HES and related eosinophil-mediated diseases, even in the absence of detectable serum IL-5 levels (see Table 2). The high response rate of these patients with varied end-organ involvement to anti-IL-5 therapy confirms previous data pointing to a primary role for activated eosinophils in the pathogenesis of HES. The underlying cause of the eosinophilia does not seem to predict response, as exemplified by the disparate outcomes of treatment in two patients with the FIP-1-like 1/platelet-derived growth factor receptor α (FIP1L1/PDGFR α)-positive clonal myeloproliferative variant of HES [101]. Additional studies are needed to determine the markers of responsiveness to anti-IL-5 therapy in HES and related disorders, to define the optimal dosing regimen, and to better understand the mechanisms of the rebound eosinophilia and attenuation of response observed in the SCH55700 studies.

References

- [1] Sanderson CJ, Warren DJ, Strath M. Identification of a cytokine that stimulates eosinophil differentiation in vitro: its relationship to IL-3 and functional properties of eosinophils produced in cultures. *J Exp Med* 1985;162:60–74.
- [2] Clutterbuck EJ, Hirst EM, Sanderson CJ. Human interleukin-5 (IL-5) regulates the production of eosinophils in human bone marrow cultures: comparison and interaction with IL-1, IL-3, IL-6 and GM-CSF. *Blood* 1989;73:1504–12.
- [3] Yamaguchi Y, Suda T, Suda J, Eguchi M, Miura Y, Harada N, et al. Purified interleukin-5 supports the terminal differentiation and proliferation of murine eosinophil precursors. *J Exp Med* 1988;167:43–56.
- [4] Tavernier J, Van der Heyden J, Verhee A, Brusselle G, Van Ostade X, Vandekerckhove J, et al. Interleukin 5 regulates the isoform expression of its own receptor alpha-subunit. *Blood* 2000;95:1600–7.
- [5] Erb KJ, LeGros G. The role of Th2 type CD4 + cells and Th2 type CD8 + T cells in asthma. *Immunol Cell Biol* 1996;74:206–8.
- [6] Warren HS, Kinnear BF, Phillips JH, Lanier LL. Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10 and IL-12. *J Immunol* 1995;154:5144–52.
- [7] Okayama Y, Petit-Frere C, Kassel O, Semper A, Quint D, Tunon-de-Lara JM. IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J Immunol* 1995;155:1796–808.
- [8] Tanaka Y, Delaporte E, Dubucquoi S, Gounni AS, Porchet E, Capron A. Interleukin-5 messenger RNA and immunoreactive protein expression by activated eosinophils in lesional atopic dermatitis skin. *J Invest Dermatol* 1994;103:589–92.
- [9] Salvi S, Semper A, Blomberg A, Holloway J, Jaffar Z, Papi A. Interleukin-5 production by human airway epithelial cells. *Am J Respir Cell Mol Biol* 1999;20:984–91.
- [10] Lopez AF, Sanderson CJ, Gamble JR, Campbell HR, Young IG, Vadas MA. Recombinant

- human interleukin-5 is a selective activator of human eosinophil function. *J Exp Med* 1988; 167:219–24.
- [11] Yamaguchi Y, Suda T, Ohta S, Tominaga K, Miura Y, Kasahara T. Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 1991;78:2542–7.
- [12] Lopez AF, Elliott MJ, Vadas MA. GM-CSF, IL-3 and IL-5: cross-competition on human hematopoietic cells. *Immunol Today* 1992;13:495–500.
- [13] Egan RW, Athwal D, Bodmer MW, Carter JM, Chapman RW, Chou C, et al. Effect of Sch 55700, a humanized monoclonal antibody to human interleukin-5, on eosinophilic responses and bronchial hyperreactivity. *Arzneimittelforschung* 1999;49:779–90.
- [14] Zhang J, Kuvelkar R, Murgolo NJ, Taremi SS, Chou C, Wang P, et al. Mapping and characterization of the epitope(s) of Sch 55700, a humanized mAb that inhibits human IL-5. *Int Immunol* 1999;11:1935–43.
- [15] Zia-Amirhosseini P, Minthorn E, Benincosa LJ, Hart TK, Hottenstein CS, Tobia LAP, et al. Pharmacokinetics and pharmacodynamics of SB-240563, a humanized monoclonal antibody directed to human interleukin-5, in monkeys. *J Pharm Exp Therapeut* 1999;291:1060–7.
- [16] Wardlaw AJ, Moqbel R, Kay AB. Eosinophils and the allergic inflammatory response. In: Kay AB, editor. *Allergy and allergic diseases*. Oxford: Blackwell Science; 1997. p. 171–97.
- [17] Dent LA, Strath M, Mellor AL, Sanderson CJ. Eosinophilia in transgenic mice expressing interleukin 5. *J Exp Med* 1990;172:1425–31.
- [18] Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG. Interleukin-5 deficiency abolishes eosinophilia, airway hyperreactivity, and lung damage in a mouse model. *J Exp Med* 1996; 183:195–201.
- [19] Denburg JA, Silver JE, Abrams JS. Interleukin-5 is a human basophilopoietin: induction of histamine content and basophilic differentiation of HL-60 cells and peripheral blood basophil-eosinophil progenitors. *Blood* 1991;77:1462–8.
- [20] Macfarlane AJ, Kon OM, Smith SJ, Zeibecoglou K, Khan LN, Barata LT, et al. Basophils, eosinophils and mast cells in atopic and nonatopic asthma and in late-phase allergic reactions in the lung and skin. *J Allergy Clin Immunol* 2000;105:99–107.
- [21] Wedemeyer J, Tsai M, Galli SJ. Roles of mast cells and basophils in innate and acquired immunity. *Curr Opin Immunol* 2000;12:624–31.
- [22] Hamid Q, Azzawi M, Ying S, Moqbel R, Wardlaw AJ, Corrigan CJ, et al. Expression of mRNA for IL-5 in mucosal bronchial biopsies from asthma. *J Clin Invest* 1991;87:1541–6.
- [23] Humbert M, Corrigan CJ, Durham SR, Kimmit P, Till SJ, Kay AB. Relationship between bronchial mucosal IL-4 and IL-5 mRNA expression and disease severity in atopic asthma. *Am J Respir Crit Care Med* 1997;156:704–8.
- [24] Robinson DS, Hamid Q, Bentley A, Ying S, Kay AB, Durham SR. Activation of CD4+ T cells, increased Th2-type cytokine mRNA expression, and eosinophil recruitment in bronchoalveolar lavage after allergen inhalation challenge in atopic asthmatics. *J Allergy Clin Immunol* 1993; 92:313–24.
- [25] Robinson DS, Hamid Q, Ying S, Bentley AM, Assoufi B, North J, et al. Prednisolone treatment in asthma is associated with modulation of bronchoalveolar lavage cell IL-4, IL-5 and interferon-gamma cytokine gene expression. *Am Rev Respir Dis* 1993;148:401–6.
- [26] Mauser PJ, Pitman A, Fernandez X, Foran SK, Adams III GK, Kreutner W, et al. Effects of an antibody to interleukin-5 in a monkey model of asthma. *Am J Respir Crit Care Med* 1995; 152:467–72.
- [27] Leckie MJ, ten Brinke A, Khan J, Diamant Z, O'Connor BJ, Walls CM, et al. Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 2000;356:2144–8.
- [28] Flood-Page PT, Menzies-Gow AN, Kay AB, Robinson DS. Eosinophil's role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am J Respir Crit Care Med* 2003;167:199–204.
- [29] Büttner C, Lun A, Spletstoesser T, Kunkel G, Renz H. Monoclonal anti-interleukin-5 treatment suppresses eosinophil but not T-cell functions. *Eur Respir J* 2003;21:799–803.

- [30] Bentley A, Hamid Q, Robinson D, Schotman E, Meng Q, Assoufi B, et al. Prednisolone treatment in asthma. Reduction in the numbers of eosinophils, T cells, tryptase-only positive mast cells, and modulation of IL-4, IL-5 and interferon-gamma cytokine gene expression within the bronchial mucosa. *Am J Respir Crit Care Med* 1996;153:551–6.
- [31] Compton C. ATS symposium. 2000.
- [32] Kips JC, O'Connor BJ, Langley SJ, Woodcock A, Kerstjens HAM, Postma DS, et al. Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am J Respir Crit Care Med* 2003;167:1655–9.
- [33] Sehmi R, Wardlaw AJ, Cromwell O, Kurihara K, Waltmann P, Kay AB. Interleukin-5 selectively enhances the chemotactic response of eosinophils obtained from normal but not eosinophilic subjects. *Blood* 1992;79:2952–9.
- [34] Rizzo CA, Yang R, Greenfeder S, Egan RW, Pauwels RA, Hey JA. The IL-5 receptor on human bronchus selectively primes for hyperresponsiveness. *J Allergy Clin Immunol* 2002;109:404–9.
- [35] Hart TK, Cook RM, Zia-Amirhosseini P, Minthorn E, Sellers TS, Meleeff BE, et al. Preclinical efficacy and safety of mepolizumab (SB-240563), a humanized monoclonal antibody to IL-5, in cynomolgous monkeys. *J Allergy Clin Immunol* 2001;108:250–7.
- [36] Robinson DS, Hamid Q, Ying S, Tsicopoulos A, Barkans J, Bentley AM, et al. Predominant TH2-like bronchoalveolar T-lymphocyte population in atopic asthma. *N Engl J Med* 1992;326:298–304.
- [37] Liu LY, Sedgwick JB, Bates ME, Vrtis RF, Gern JE, Kita H, et al. Decreased expression of membrane IL-5 receptor alpha on human eosinophils: I. Loss of membrane IL-5 receptor alpha on airway eosinophils and increased soluble IL-5 receptor alpha in the airway after allergen challenge. *J Immunol* 2002;169:6452–8.
- [38] Gregory B, Kirchem A, Phipps S, Gevaert P, Pridgeon C, Rankin SM, et al. Differential regulation of human eosinophil IL-3, IL-5 and GM-CSF down regulate IL-5 α expression with loss of IL-5 responsiveness, but up-regulate IL-3R α expression. *J Immunol* 2003;170:5359–66.
- [39] Nishinakamura R, Miyajima A, Mee PJ, Tybulewicz VL, Murray R. Hematopoiesis in mice lacking the entire granulocyte-macrophage colony-stimulating factor/interleukin-3/interleukin-5 functions. *Blood* 1996;88:2458–64.
- [40] Foster PS, Mould AW, Yang M, Mackenzie J, Mattes J, Hogan SP, et al. Elemental signals regulating eosinophil accumulation in the lung. *Immunol Rev* 2001;179:173–81.
- [41] Ying S, Robinson DS, Meng Q, Rottman J, Kennedy R, Ringler D, et al. Enhanced expression of eotaxin and CCR3 mRNA and protein in atopic asthma: association with airway hyperresponsiveness and predominant co-localization of eotaxin mRNA to bronchial epithelial and endothelial cells. *Eur J Immunol* 1997;27:3507–16.
- [42] Hogan SP, Matthaei KI, Young JM, Koskinen A, Young IG, Foster PS. A novel T cell-regulated mechanism modulating allergen-induced airways hyperreactivity. *J Immunol* 1998;161:1501–9.
- [43] Todd R, Donoff BR, Chiang T, Chou MY, Elovic A, Gallagher GT, et al. The eosinophil as a cellular source of transforming growth factor alpha in healing cutaneous wounds. *Am J Pathol* 1991;138:1307–13.
- [44] Wong DT, Donoff BR, Yang J, Song BZ, Matossian K, Nagura N, et al. Sequential expression of transforming growth factors alpha and beta 1 by eosinophils during cutaneous wound healing in the hamster. *Am J Pathol* 1993;143:130–42.
- [45] Noguchi H, Kephart GM, Colby TV, Gleich GJ. Tissue eosinophilia and eosinophil degranulation in syndromes associated with fibrosis. *Am J Pathol* 1992;40:521–8.
- [46] Wong DT, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, et al. Human eosinophils express transforming growth factor alpha. *J Exp Med* 1990;172:673–81.
- [47] Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989;1:520–4.
- [48] Wilson JW, Li X. The measurement of reticular basement membrane and submucosal collagen in the asthmatic airway. *Clin Exp Allergy* 1997;27:363–71.

- [49] Jeffery PK, Godfrey RW, Adelroth E, Nelson F, Rogers A, Johansson SA. Effects of treatment on airway inflammation and thickening of basement membrane reticular collagen in asthma: a quantitative light and electron microscopic study. *Am Rev Respir Dis* 1992;145:890–9.
- [50] Laitinen A, Altraja A, Kampe M, Linden M, Virtanen I, Laitinen LA. Tenascin is increased in airway basement membrane of asthmatics and decreased by an inhaled steroid. *Am J Respir Crit Care Med* 1997;156:951–8.
- [51] Huang J, Olivenstein R, Taha R, Hamid Q, Ludwig M. Enhanced proteoglycan deposition in the airway wall of atopic asthmatics. *Am J Respir Crit Care Med* 1999;160:725–9.
- [52] Murphy-Ullrich JE. The de-adhesive activity of matricellular proteins: is intermediate cell adhesion an adaptive state? *J Clin Invest* 2001;107:785–90.
- [53] Anwar AR, Moqbel R, Walsh GM, Kay AB, Wardlaw AJ. Adhesion to fibronectin prolongs eosinophil survival. *J Exp Med* 1993;177:839–43.
- [54] Ebina M, Takahashi T, Chiba T, Motomiya M. Cellular hypertrophy and hyperplasia of airway smooth muscles underlying bronchial asthma: a 3-D morphometric study. *Am Rev Resp Dis* 1993;148:720–6.
- [55] Ordonez CL, Khashayar R, Wong HH, Ferrando R, Wu R, Hyde DM, et al. Mild and moderate asthma is associated with airway goblet cell hyperplasia and abnormalities in mucin gene expression. *Am J Respir Crit Care Med* 2001;163:517–23.
- [56] Li X, Wilson JW. Increased vascularity of the bronchial mucosa in mild asthma. *Am J Respir Crit Care Med* 1997;156:229–33.
- [57] Wenzel SE, Schwartz LB, Langmack EL, Halliday JL, Trudeau JB, Gibbs RL, et al. Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am J Respir Crit Care Med* 1999;160:1001–8.
- [58] Wong DT, Elovic A, Matossian K, Nagura N, McBride J, Chou MY, et al. Eosinophils from patients with blood eosinophilia express transforming growth factor beta 1. *Blood* 1991; 78:2702–7.
- [59] Hoshino M, Takahashi M, Aoike N. Expression of vascular endothelial growth factor, basic fibroblast growth factor, and angiogenin immunoreactivity in asthmatic airways and its relationship to angiogenesis. *J Allergy Clin Immunol* 2001;107:295–301.
- [60] Okada S, Kita H, George TJ, Gleich GJ, Leiferman KM. Migration of eosinophils through basement membrane components in vitro: role of matrix metalloproteinase-9. *Am J Respir Cell Mol Biol* 1997;17:519–28.
- [61] Levi-Schaffer F, Garbuzenko E, Rubin A, Reich R, Pickholz D, Gillery P, et al. Human eosinophils regulate human lung- and skin-derived fibroblast properties in vitro: a role for transforming growth factor. *Proc Natl Acad Sci USA* 1999;96:9660–5.
- [62] Schmid-Grendelmeier P, Altnauer F, Fischer B, Bizer C, Straumann A, Menz G, et al. Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J Immunol* 2002; 169:1021–7.
- [63] Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. Myofibroblasts: I. Paracrine cells important in health and disease. *Am J Physiol* 1999;277:C1–9.
- [64] Paakko P, Kaarteenaho-Wiik R, Pollanen R, Soini Y. Tenascin mRNA expression at the foci of recent injury in usual interstitial pneumonia. *Am J Respir Crit Care Med* 2000;161:967–72.
- [65] Phipps S, Ying S, Wangoo A, Ong YE, Levi-Schaffer F, Kay AB. The relationship between allergen-induced tissue eosinophilia and markers of repair and remodeling in human atopic skin. *J Immunol* 2002;169:4604–12.
- [66] Flood-Page P, Menzies-Gow A, Phipps S, Ying S, Wangoo A, Ludwig MS, et al. Anti-IL5 treatment reduces deposition of extracellular matrix proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J Clin Invest* 2003;112:1029–36.
- [67] Trifileff A, Fujitani Y, Coyle AJ, Kopf M, Bertrand C. IL-5 deficiency abolishes aspects of airway remodelling in a murine model of lung inflammation. *Clin Exp Allergy* 2001;31: 934–42.
- [68] Blyth DI, Wharton TF, Pedrick MS, Savage TJ, Sanjar S. Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. *Am J Respir Cell Mol Biol* 2000;23:241–6.

- [69] Cho JY, Miller M, Je Baek K, Han JW, Nayar JS, Lee SY, et al. Inhibition of airway remodeling in IL-5 deficient mice. *J Clin Invest* 2004;113:551–60.
- [70] Huang XZ, Wu JF, Cass D, Erle DJ, Corry D, Young SG, et al. Inactivation of the integrin beta 6 subunit gene reveals a role of epithelial integrins in regulating inflammation in the lung and skin. *J Cell Biol* 1996;133:921–8.
- [71] Trigg CJ, Manolitsas ND, Wang J, Calderon MA, McAulay A, Jordan SE, et al. Placebo-controlled immunopathologic study of four months of inhaled corticosteroids in asthma. *Am J Respir Crit Care Med* 1994;150:17–22.
- [72] Ward C, Pais M, Bish R, Reid D, Feltis B, Johns D, et al. Airway inflammation, basement membrane thickening and bronchial hyperresponsiveness in asthma. *Thorax* 2002;57:309–16.
- [73] Zhang HY, Phan SH. Inhibition of myofibroblast apoptosis by transforming growth factor beta-1. *Am J Respir Cell Mol Biol* 1999;21:658–65.
- [74] Desmouliere A. Factors influencing myofibroblast differentiation during wound healing and fibrosis. *Cell Biol Int* 1995;19:471–6.
- [75] Pearson CA, Pearson D, Shibahara S, Hofsteenge J, Chiquet-Ehrismann R. Tenascin: cDNA cloning and induction by TGF-beta. *EMBO J* 1988;7:2977–82.
- [76] Ohno I, Nitta Y, Yamauchi K, Hoshi H, Honma H, Woolley K, et al. Transforming growth factor beta 1 (TGF beta 1) gene expression by eosinophils in asthmatic airway inflammation. *Am J Respir Cell Mol Biol* 1996;15:404–9.
- [77] Minshall EM, Leung DYM, Martin RJ, Song YL, Cameron L, Ernst P, et al. Eosinophil-associated TGF- β 1 mRNA expression and airways fibrosis in bronchial asthma. *Am J Respir Cell Mol Biol* 1997;17:326–33.
- [78] Hakonarson H, Maskeri N, Carter C, Chuang S, Grunstein MM. Autocrine interaction between IL-5 and IL-1beta mediates altered responsiveness of atopic asthmatic sensitized airway smooth muscle. *J Clin Invest* 1999;104:657–67.
- [79] Wiggs BR, Bosken C, Pare PD, James A, Hogg JC. A model of airway narrowing in asthma and in chronic obstructive pulmonary disease. *Am Rev Resp Dis* 1992;145:1251–8.
- [80] Hoshino M, Nakamura Y, Sim J, Shimojo J, Isogai S. Bronchial subepithelial fibrosis and expression of matrix metalloproteinase-9 in asthmatic airway inflammation. *J Allergy Clin Immunol* 1998;102:783–8.
- [81] Chetta A, Foresi A, Del Donno M, Bertorelli G, Pesci A, Olivieri D. Airways remodeling is a distinctive feature of asthma and is related to severity of disease. *Chest* 1997;111:852–7.
- [82] Foster PS, Hogan SP, Yang M, Mattes J, Young IG, Matthaei KI, et al. Interleukin-5 and eosinophils as therapeutic targets for asthma. *Trends Mol Med* 2002;8:162–7.
- [83] Yu C, Cantor AB, Yang H, Browne C, Wells RA, Fujiwara Y, et al. Targeted deletion of a high-affinity GATA-binding site in the GATA-1 promoter leads to selective loss of the eosinophil lineage in vivo. *J Exp Med* 2002;19:1387–95.
- [84] Green RH, Brightling CE, McKenna S, Hargadon B, Parker D, Bradding P, et al. Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 2002;360:1715–21.
- [85] Chusid MJ, David DC, West BC, Wolff SM. The hypereosinophilic syndrome: analysis of fourteen cases with review of the literature. *Medicine* 1975;54:1–27.
- [86] Weller PF, Bubley GJ. The idiopathic hypereosinophilic syndrome. *Blood* 1994;83:2759–79.
- [87] Assa'ad AH, Spicer RL, Nelson DP, Zimmerman N, Rothenberg ME. Hypereosinophilic syndromes. *Chem Immunol* 2000;76:208–29.
- [88] Cools J, DeAngelo DJ, Gotlib J, Stover EH, Legare RD, Cortes J, et al. A novel tyrosine kinase created by the fusion of the *PDGFRA* and *FIP1L1* genes is a therapeutic target of imatinib in idiopathic hypereosinophilic syndrome. *N Engl J Med* 2003;348:1201–14.
- [89] Simon HU, Plotz SG, Dummer R, Blaser K. Abnormal clones of T cells producing interleukin-5 in idiopathic eosinophilia. *N Engl J Med* 1999;341:1112–20.
- [90] Fauci AS, Harley JB, Roberts WC, Ferrans VJ, Gralnick HR, Bjornson BH. The idiopathic hypereosinophilic syndrome. *Ann Intern Med* 1982;97:78–92.
- [91] Tai P, Spry CJF, Olsen EGJ, Ackerman SJ, Dunnette S, Gleich GJ. Deposits of eosinophil

- granule proteins in cardiac tissues of patients with eosinophilic endomyocardial disease. *Lancet* 1987;1:643–7.
- [92] Aractingi S, Janin A, Zini JM, Gauthier MS, Chauvenet L, Tobelem G, et al. Specific mucosal erosions in the hypereosinophilic syndrome: evidence for eosinophil protein deposition. *Arch Dermatol* 1996;132:535–41.
- [93] Tajima K, Katagiri T. Deposits of eosinophil granule proteins in eosinophilic cholecystitis and eosinophilic colitis associated with hypereosinophilic syndrome. *Dig Dis Sci* 1996;41:282–8.
- [94] Parrillo JE, Fauci AS, Wolff SM. Therapy of the hypereosinophilic syndrome. *Ann Intern Med* 1978;89:167–72.
- [95] Butterfield JH, Gleich GJ. Response of six patients with idiopathic hypereosinophilic syndrome to interferon alfa. *J Allergy Clin Immunol* 1994;94:1318–26.
- [96] Klion AD, Robyn J, Akin C, Noel P, Brown M, Law MA, et al. Molecular remission and reversal of myelofibrosis in response to imatinib mesylate treatment in patients with the myeloproliferative variant of hypereosinophilic syndrome. *Blood* 2003;103:473–8.
- [97] Satoh T, Sun L, Li MS, Spry CJF. Interleukin-5 mRNA levels in blood and bone marrow mononuclear cells from patients with the idiopathic hypereosinophilic syndrome. *Immunology* 1994;83:308–12.
- [98] Roufosse F, Schandene L, Sibille C, Willard-Gallo K, Kennes B, Efra A, et al. Clonal Th2 lymphocytes in patients with the idiopathic hypereosinophilic syndrome. *Br J Haematol* 2000;109:540–8.
- [99] Garrett JK, Jameson SC, Thomson B, Collins MH, Wagoner LE, Freese DK, et al. Anti-interleukin-5 (mepolizumab) therapy for hypereosinophilic syndromes. *J Allergy Clin Immunol* 2003;113:115–9.
- [100] Plötz S, Simon HU, Darsow U, Simon D, Vassina E, Yousefi S, et al. Use of an anti-interleukin-5 antibody in the hypereosinophilic syndrome with eosinophilic dermatitis. *N Engl J Med* 2003;349:2334–9.
- [101] Klion AD, Law MA, Noel P, Haverty TP, Nutman TB. Safety and efficacy of the monoclonal anti-interleukin 5 antibody, SCH55700, in the treatment of patients with the hypereosinophilic syndrome. *Blood* 2004;103:2939–41.
- [102] Prussin C, James SP, Huber MM, Klion AD, Metcalfe DD. Pilot study of anti-IL-5 in eosinophilic gastroenteritis [abstract]. *J Allergy Clin Immunol* 2003;111:827.