

A role for eosinophils in airway remodelling in asthma

A. Barry Kay¹, Simon Phipps^{1,2} and Douglas S. Robinson^{1,3}

¹Department of Allergy and Clinical Immunology, Imperial College London, National Heart & Lung Institute, Guy Scadding Building, Dovehouse Street, London, UK, SW3 6LY

²Allergy and Inflammation Group, John Curtin School of Medical Research, Australian National University, Canberra, ACT 0200, Australia

³Leukocyte Biology, Biomedical Sciences Division, Faculty of Medicine, Imperial College London, Level 3, Sir Alexander Fleming Building, South Kensington, London, UK, SW6 7AZ

Over the years, the role of the eosinophil in asthma and allergic processes has been disputed. Recent human experiments using a humanised monoclonal antibody to interleukin-5 (IL-5), and animal studies involving specific IL-5 gene deletion, indicates that eosinophils might control downstream repair and remodelling processes. Eosinophils are a rich source of fibrogenic factors, particularly transforming growth factor- β (TGF- β), the latent form of which is activated by epithelial-cell expression of the intergrin $\alpha_v\beta_6$. The emerging role for the eosinophil in airway remodelling might be important in future anti-asthma strategies. However, more effective eosinophil-depleting agents than anti-IL-5 are required before the definitive role of this cell type in asthma airway pathophysiology can be established.

Asthma is a chronic inflammatory condition of the airways characterised by a prominent eosinophilic inflammatory infiltrate in the bronchial mucosa. For many years it has been recognised that eosinophils have the potential to cause damage to the airway mucosa and associated nerves

through release of granule-associated basic proteins (which damage nerves and epithelial cells), lipid mediators (which cause bronchoconstriction, vasodilatation and mucus hypersecretion) and reactive oxygen species (which generally injure mucosal cells). These observations, as well as the broad correlation between activated eosinophils and severity of asthma, have led to the hypothesis that the eosinophil is the central effector cell responsible for ongoing airway inflammation.

Less attention has been given to a possible role for eosinophils in repair and remodelling processes, despite the well-documented association of tissue eosinophilia and eosinophil degranulation with several fibrotic syndromes [1]. In fact, eosinophils are a source of several molecules implicated in tissue remodelling processes [2]. These include transforming growth factor- α (TGF- α) [3], TGF- β 1 [4], fibroblast growth factor-2 (FGF-2) [5], vascular endothelial growth factor (VEGF) [6], matrix metalloproteinase-9 (MMP-9) [7], tissue inhibitor of metalloproteinase-1 (TIMP-1) [8], interleukin-13 (IL-13) [9] and IL-17 [10] (Table 1).

Table 1. Eosinophil-derived proteins that might be involved in airway remodelling

Protein	Properties relevant to remodelling	Refs
Angiogenin	Angiogenesis	[5]
FGF-2	Fibroblast proliferation; endothelial-cell proliferation and migration; angiogenesis	[5]
HB-EGF	Fibroblast and smooth muscle-cell proliferation	[63]
IL-4	Fibroblast activation	[64]
IL-11	<i>In vivo</i> fibroblast activation	[65]
IL-13	Increased fibroblast activity mediated through upregulation of epithelial and fibroblast TGF- β	[9,66,67]
IL-17	Inducer of fibroblast cytokine and chemokine production	[10,68]
MBP	Act synergistically with IL-1 and TGF- β to increase IL-6 production by fibroblasts	[69]
MMP-9	Matrix protein degradation	[7]
NGF	Fibroblast ECM production; endothelial-cell proliferation and migration	[70]
PDGF	Myofibroblast development	[71,72]
TGF- α	Epithelial proliferation	[3,67]
TGF- β	Myofibroblast formation; ECM production and collagen contraction	[4]
TIMP-1 and TIMP-2	Inhibitor of MMPs	[5,8]
TNF- α	Inducer of angiogenic and fibrogenic growth factors	[73]
VEGF	Angiogenic factor; induces vascular hyperpermeability	[5,6]

^aAbbreviations: ECM, extracellular matrix; FGF, fibroblast growth factor; HB-EGF, heparin-binding epidermal growth factor-like growth factor; IL-4, interleukin-4; MBP, major basic protein; MMP-9, matrix metalloproteinase-9; NGF, nerve growth factor; PDGF, platelet-derived growth factor; TGF, transforming growth factor- β ; TIMP, tissue inhibitor of metalloproteinase; TNF, tumour necrosis factor; VEGF, vascular endothelial growth factor.

Corresponding author: A. Barry Kay (a.b.kay@imperial.ac.uk).

Available online 23 July 2004

Asthma and remodelling

The remodelled phenotype in asthma, which might be the consequence of excessive repair processes following repeated airway injury, includes increased deposition of several extracellular matrix (ECM) proteins in the reticular basement membrane (RBM) and bronchial mucosa, as well as increases in airway smooth muscle mass, goblet-cell hyperplasia and new blood vessel formation [11]. These ECM proteins include pro-collagen III (the mature precursor for collagen III) and the proteoglycans, tenascin and lumican. Apart from the effects on airway structure, these ECM proteins might also influence cellular function, including adhesion, differentiation and survival [12]. The origin of fibroblasts below the RBM (the attenuated fibroblast sheath) is of particular interest. Current evidence suggests that following allergen challenge of atopic asthmatics, or appropriately sensitised mice, CD34⁺-collagen1- α -smooth muscle actin⁺ fibrocytes are recruited, together with eosinophils, to the airway wall from the circulation, that is, circulating fibrocytes act as myofibroblast precursors [13]. The physiological consequences of this deposition of ECMs within the sub-epithelial basement membrane are debated, however, in severe asthmatics there is a clear association with increased numbers of bronchial mucosal eosinophils [14]. It has been suggested that airway remodelling might contribute to airway hyper-responsiveness, particularly through smooth muscle increases [15], and/or to fixed airway obstruction, which develops in some severe asthmatics [16] and might explain the accelerated loss of lung capacity seen in asthmatics [17]. However, it is also of note that airway remodelling changes are seen in children with asthma, or soon after diagnosis, and there is no clear evidence of progression of these changes [18].

Role of TGF- β

TGF- β , in particular, is a potent regulator of fibroblast and myofibroblast function and controls the production of several ECM proteins, including collagens, proteoglycans and tenascin [19]. Of the three closely related isoforms (TGF- β 1, β 2 and β 3), TGF- β 1 is the most widely studied. All isoforms induce fibroblast proliferation, collagen production and collagen gel contraction. TGF- β synthesis, storage and action are complex. The molecule is secreted as part of a large latent complex from which it is released by several activators, including integrins (e.g. $\alpha_v\beta_6$), proteases and thrombospondin-1. TGF- β s either act locally, by binding of the TGF- β large latent complex to the extracellular matrix through the latent TGF- β -binding protein, or distally, through proteolytic release of the latent complex from the ECM.

Although eosinophils are a rich source of TGF- β 1, other cell types involved in allergic inflammation are also potential sources of this fibrogenic factor. These include macrophages, T cells, mast cells, neutrophils, endothelial and epithelial cells, as well as smooth muscle cells and fibroblasts themselves [20]. Furthermore, there is important 'crosstalk' between mast cells, eosinophils and fibroblasts [21]. For example, TGF- β release from eosinophils is enhanced by mast cell-derived tumour necrosis factor- α (TNF- α), and mast-cell tryptase also induces

fibroblast proliferation, both through proteinase-activated receptor 2 (PAR2) and peroxisome proliferator-activated receptor γ (PPAR γ) [through newly synthesised products of cyclooxygenase 2 (COX2) activity]. Fibroblasts, in turn, secrete stem-cell factor (SCF) and granulocyte-macrophage-colony stimulating factor (GM-CSF), which support the survival of mast cells and eosinophils, respectively. In addition TGF- β 1 is chemotactic for both mast cells and macrophages.

Support for an association among IgE-mediated sensitivity, eosinophils and markers of repair in humans was obtained from an *in vivo* model of allergic inflammation in atopic skin. After allergen cutaneous challenge, eosinophil-derived TGF- β 1 was temporally associated with myofibroblast formation and the expression of tenascin and procollagen-I by dermal fibroblasts [22]. When eosinophils and fibroblasts were co-cultured *in vitro*, α -SM (smooth muscle) actin⁺ myofibroblasts were observed, together with the generation of tenascin transcripts and protein product. Both of which could be inhibited using neutralising antibodies to TGF- β 1. Tenascin is of particular interest because this matricellular protein is expressed primarily during growth and in response to injury and is believed to have a role in cell trafficking by enabling cell influx at low density and inhibiting migration at high density [23].

Anti-IL-5 and asthma

There have been several recent attempts to dissect the role of the eosinophil in asthma by the use of humanised monoclonal antibodies (mAbs) against IL-5. To date, two mAbs against human IL-5, SCH55700 (Schering-Plough Research Institute, www.sch-plough.com) and mepolizumab (GlaxoSmithKline, www.gsk.com), have been used in clinical trials. SCH55700 is a humanised mAb of the IgG4/ κ subtype derived from the rat anti-human antibody, 39D10 [24]. Mepolizumab (SB-240563) is a humanised mAb of the IgG1/ κ subtype derived in a similar fashion from the murine monoclonal anti-human IL-5 antibody, 2B6, and expressed in Chinese hamster ovary cells [25].

IL-5 is a key cytokine in eosinophil differentiation [26], maturation, recruitment and activation at sites of inflammation [27]. It stimulates the expansion and differentiation of eosinophil precursors [28] and upregulates expression of its own specific receptor α chain during human eosinophil development [29]. IL-5 is produced primarily by activated T cells, although secretion by CD8⁺ T cells [30], natural killer (NK) cells [31], mast cells [32], eosinophils [33] and airway epithelial cells [34], has also been reported. In addition, it is chemotactic for eosinophils, stimulates their activation [35], prolongs their survival [36], and facilitates their degranulation in response to specific stimuli. In contrast to the broad range of biological activities of the other cytokines known to have a role in eosinophilia (i.e. IL-3 and GM-CSF), the action of IL-5 is specific for eosinophils in humans. This specificity is probably conferred by the restriction of expression of the IL-5 receptor α chain to eosinophils and basophils [37].

We recently reported that anti-IL-5 (mepolizumab) arrests maturation of the human eosinophil lineage in

the bone marrow [38] and depletes the cell from the peripheral blood [39]. However, it only partially (but selectively) reduces eosinophils in the airway wall, even after multiple dosing. Thus, there was a 55% decrease in bronchial mucosa eosinophils, compared to a 100% median reduction in eosinophils in blood and 79% reduction in bronchoalveolar lavage. An earlier study, based on a single infusion of mepolizumab, showed significant reduction in both blood and induced sputum eosinophils, but no changes in either the late asthmatic reaction or airway hyper-responsiveness [40]. This study did not include bronchial biopsies, and was criticised with respect to statistical power [41], however, the lack of significant clinical changes seen in this, and other studies with anti-IL-5 in asthma [42], might be as a result of a failure to deplete eosinophils sufficiently from the bronchial mucosa. This might be because airway eosinophils have downregulated IL-5R α expression and thus become less dependent on this cytokine [12,43].

Anti-IL-5 and remodelling

We then proceeded to test the hypothesis that reduction of bronchial mucosal eosinophils with anti-IL-5 would reduce markers of airway remodelling. Bronchial biopsies were obtained before and after three infusions of anti-IL-5 (mepolizumab) in 24 atopic asthmatics in a randomised, double-blind, placebo-controlled study [44]. The thickness and density of tenascin, lumican and procollagen III, in the RBM were measured by confocal microscopy. At baseline, there was airway eosinophil infiltration and ECM protein deposition was increased in the RBM of asthmatics compared with non-asthmatic controls. However, after anti-IL-5 treatment there was a significant reduction in tenascin, lumican and procollagen III, when compared with placebo. There was also a significant reduction in the number and percentage of tissue eosinophils expressing mRNA for TGF- β 1, as well as the concentration of TGF- β 1 in bronchoalveolar (BAL) fluid. Although there were no appreciable improvements in clinical outcomes, the study was not powered to detect changes in lung function or airway hyper-responsiveness. The study did, however, provide strong evidence that there is a causal relation between eosinophils and airway remodelling.

IL-5, eosinophils and TGF- β in animal models of airway remodelling with allergen challenge

Until recently, dissection of the mechanisms of airway remodelling was hampered by the lack of a good animal model. However, in recent years several groups have developed models involving repetitive allergen inhalation challenge in sensitised mice that can reproduce many of the features of the remodelled asthmatic airway [45–50]. These models differ from each other, particularly the timing of the last allergen challenge with respect to killing of the animal and study of airway pathology. In the model of Leigh *et al.* [45], airway hyper-responsiveness, collagen deposition and smooth muscle increases persisted for 8 weeks after the last allergen challenge, despite resolution of airway eosinophilia and BAL IL-13 concentrations. McMillan *et al.* [46] confirmed persistence of the

remodelled phenotype up to 80 days after the last challenge, although in this model there was resolution of both bronchial hyper-responsiveness (BHR) and inflammation. Blyth *et al.* [47] studied the role of IL-5 in their model by using an anti-IL-5 antibody, giving intravenous anti-IL-5 antibody 30 min before each of three intratracheal allergen challenges in sensitised animals. They noted a reduction in subepithelial reticulin in the airways with selective and almost complete depletion of airway eosinophilia. Trifilieff *et al.* [48] used IL-5 deficient mice and showed absence of bronchoalveolar eosinophilia together with reduced BAL fibronectin and epithelial-cell proliferation compared to wild-type controls in a model involving repeat allergen challenge on days 21–25. Interestingly intratracheal IL-5 alone in sensitised wild type animals was not sufficient to lead to remodelling changes.

More recently, Cho *et al.* [49] confirmed, in a chronic repetitive allergen challenge model, that IL-5 gene deletion suppresses lung eosinophilia and tissue remodelling in parallel. Specifically, IL-5-deficient mice exhibited reduced eosinophil numbers in BAL fluid and in peribronchial tissue. This was associated with decreased TGF- β 1 content of the lung tissue with evidence that eosinophils are the major source of this cytokine. Interestingly, epithelial-cell expression of $\alpha_v\beta_6$, the integrin that activates latent TGF- β 1 [51], was also suppressed. Allergen-induced increases in peribronchial fibrosis, collagen III, collagen IV and total lung collagen content, was reduced in IL-5-deficient animals. Peribronchial smooth muscle thickness and epithelial mucus expression were also reduced. Tanaka *et al.* also observed reduced airway eosinophilia, TGF- β 1 production and remodelling in IL-5 knockout animals, and reported increased airway fibrosis in IL-5 transgenic animals after repeated allergen inhalation [52]. Taken together, these animal studies strongly support the hypothesis that eosinophils contribute, to a greater or lesser extent, to the remodelled phenotypes, and that eosinophil-derived TGF- β 1 is central in facilitating matrix deposition in the airway wall.

Other factors that might link eosinophils to airway remodelling

As mentioned earlier, and illustrated in Figure 1, numerous cytokines and fibrogenic factors are implicated in airway remodelling in asthma in human studies and in animal models. Transgenic expression of IL-5, IL-11 and IL-13 [53–55] in the airway of mice leads to tissue remodelling, which in the case of IL-13 were partially TGF- β 1-dependent [56]. Whether these remodelling events are eosinophil-dependent remains to be established. Many of the factors implicated in remodelling in animal models are also expressed by eosinophils and are increased in the asthmatic airway, including FGF-2, IL-11, IL-4, IL-13, IL-17, nerve growth factor (NGF) and VEGF (Table 1).

Eosinophil infiltration of the airway is also seen in other lung conditions. Particular interest has been focused recently on eosinophilic bronchitis (EB), a condition characterised by sputum eosinophilia and cough but without the airway hyper-responsiveness or variable

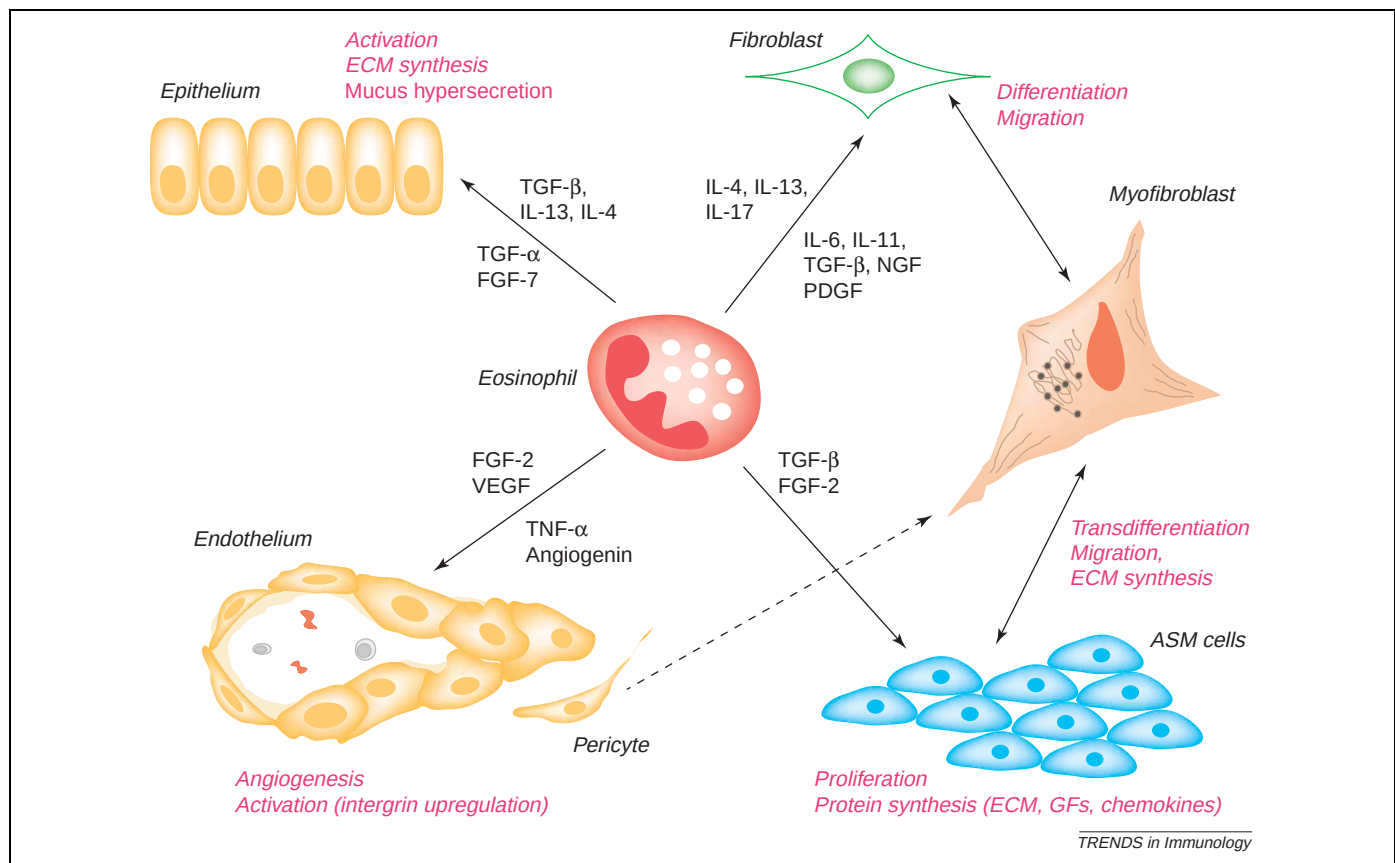


Figure 1. Potential involvement of eosinophils in airway remodelling. Eosinophils produce a wide range of proteins involved in fibrogenesis and angiogenesis, as well as cytokines, which activate various mesenchymal cells and induce the synthesis of ECM proteins. Activation of fibroblasts is a property of IL-4, IL-6, IL-11, IL-13, IL-17, TGF-β, NGF and PDGF. This, in turn, can lead to fibroblast migration and differentiation to the myofibroblast phenotype, trans-differentiation of myofibroblasts to ASM cells, as well as the migration of various mesenchymal cells and ECM synthesis. Thus, there is considerable plasticity between fibroblasts, myofibroblasts and ASM cells. The pericyte is also involved in these trans-differentiation processes. TGF-β and FGF-2 have direct effects on ASM, such as proliferation and protein synthesis. Eosinophils also produce angiogenic factors, including VEGF and angiogenin. Endothelial cells can also be activated by FGF-2 and TNF-α. Epithelial-cell activation, ECM synthesis and mucus hypersecretion might be the result of the release of eosinophil-derived TGF-β, IL-4, IL-13 and TGF-α. Abbreviations: ASM, airway smooth muscle; ECM, extracellular matrix; FGF, fibroblast growth factor; GFs, growth factors; IL-4, interleukin-4; NGF, nerve growth factor; PDGF, platelet-derived growth factor; TGF-β, transforming growth factor-β; TNF-α, tumour necrosis factor-α; VEGF, vascular endothelial growth factor.

airflow obstruction that characterise asthma [57]. Brightling *et al.* [58] showed subepithelial thickening in EB similar to that seen in asthma, supporting the link between eosinophils and, at least this element of, remodelling. The major reported difference between EB and asthma is the infiltration of airway smooth muscle by mast cells in asthma, which is not seen in EB. It will therefore be important to see if airway smooth muscle remodelling occurs in EB and asthma: one possibility is that eosinophils contribute to some elements of fibrosis and ECM deposition whereas mast cells and other cells are related to smooth muscle and epithelial changes.

Future work

To provide definitive evidence that eosinophils are key cytokines in airway remodelling more effective strategies are required to deplete tissue eosinophils. Even in animal models of asthma, there is residual tissue eosinophilia in the airways after anti-IL-5 administration [59]. In fact, deletion of both IL-5 and eotaxin are required to abolish tissue eosinophils and airway hyper-responsiveness in mice, suggesting that IL-5 blockade alone is insufficient. Combination therapy with, for example, anti-IL-5 and a

CCR3 antagonist, might be more useful than IL-5 blockade alone because this would have the theoretical advantage of inhibiting both 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 [60]. TGF-β polymorphisms have been linked to asthma severity, and it will be of interest to see if there is differential regulation of the *TGF-β* gene between asthmatics and non-asthmatics and whether this genetic effect is by changes in remodelling [61].

Whether the eosinophil is involved in late asthmatic reactions and airway hyper-responsiveness remains uncertain. Its participation in remodelling events now seems persuasive. Effective depletion of eosinophils from the airways of chronic severe asthmatics could have useful clinical benefit, although long-term treatment would probably be required. In this context, it is relevant that a treatment strategy directed at normalisation of the induced sputum eosinophil count reduced asthma exacerbations and hospital admissions without the need for additional anti-inflammatory treatment [62]. Interest in the eosinophil as an important cell in asthma has waned over the past few years because of the first negative

mepolizumab (mAb to IL-5) study [40], which showed no change in the late asthmatic reaction or airway hyper-reactivity. However, recent data show that this cell has still to be reckoned with, and furthermore, could even turn out to have more, unexpected, tricks up its sleeve.

Acknowledgements

The work is supported by grants from the Wellcome Trust and GlaxoSmithKline. DSR is supported in part by a Wellcome Trust Research Leave Award for Clinical Academics. SP is supported by an International Fellowship from The Royal Society.

References

- Noguchi, H. *et al.* (1992) Tissue eosinophilia and eosinophil degranulation in syndromes associated with fibrosis. *Am. J. Pathol.* 140, 521–528
- Robinson, D.S. *et al.* (2002) Eosinophils. *Clin. Allergy Immunol.* 16, 43–75
- Wong, D.T. *et al.* (1990) Human eosinophils express transforming growth factor α . *J. Exp. Med.* 172, 673–681
- Wong, D.T. *et al.* (1991) Eosinophils from patients with blood eosinophilia express transforming growth factor β 1. *Blood* 78, 2702–2707
- Hoshino, M. *et al.* (2001) 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.* 107, 295–301
- Horiuchi, T. and Weller, P.F. (1997) Expression of vascular endothelial growth factor by human eosinophils: upregulation by granulocyte-macrophage colony-stimulating factor and interleukin-5. *Am. J. Respir. Cell Mol. Biol.* 17, 70–77
- Okada, S. *et al.* (1997) Migration of eosinophils through basement membrane components *in vitro*: role of matrix metalloproteinase-9. *Am. J. Respir. Cell Mol. Biol.* 17, 519–528
- Levi-Schaffer, F. *et al.* (1999) Human eosinophils regulate human lung- and skin-derived fibroblast properties *in vitro*: a role for transforming growth factor β (TGF- β). *Proc. Natl. Acad. Sci. U. S. A.* 96, 9660–9665
- Schmid-Grendelmeier, P. *et al.* (2002) Eosinophils express functional IL-13 in eosinophilic inflammatory diseases. *J. Immunol.* 169, 1021–1027
- Molet, S. *et al.* (2001) IL-17 is increased in asthmatic airways and induces human bronchial fibroblasts to produce cytokines. *J. Allergy Clin. Immunol.* 108, 430–438
- Bousquet, J. *et al.* (2000) Asthma. From bronchoconstriction to airways inflammation and remodeling. *Am. J. Respir. Crit. Care Med.* 161, 1720–1745
- Liu, L.Y. *et al.* (2002) Decreased expression of membrane IL-5 receptor α on human eosinophils: IL-5 down-modulates its receptor via a proteinase-mediated process. *J. Immunol.* 169, 6459–6466
- Schmidt, M. *et al.* (2003) Identification of circulating fibrocytes as precursors of bronchial myofibroblasts in asthma. *J. Immunol.* 171, 380–389
- Wenzel, S.E. *et al.* (1999) Evidence that severe asthma can be divided pathologically into two inflammatory subtypes with distinct physiologic and clinical characteristics. *Am. J. Respir. Crit. Care Med.* 160, 1001–1008
- Macklem, P.T. (1996) A theoretical analysis of the effect of airway smooth muscle load on airway narrowing. *Am. J. Respir. Crit. Care Med.* 153, 83–89
- Robinson, D.S. *et al.* (2003) Systematic assessment of difficult-to-treat asthma. *Eur. Respir. J.* 22, 478–483
- Lange, P. *et al.* (1998) A 15-year follow-up study of ventilatory function in adults with asthma. *N. Engl. J. Med.* 339, 1194–1200
- Laitinen, L.A. *et al.* (1993) Airway mucosal inflammation even in patients with newly diagnosed asthma. *Am. Rev. Respir. Dis.* 147, 697–704
- Annes, J.P. *et al.* (2003) Making sense of latent TGF β activation. *J. Cell Sci.* 116, 217–224
- Duvernelle, C. *et al.* (2003) Transforming growth factor- β and its role in asthma. *Pulm. Pharmacol. Ther.* 16, 181–196
- Munitz, A. and Levi-Schaffer, F. (2004) Eosinophils: ‘new’ roles for ‘old’ cells. *Allergy* 59, 268–275
- Phipps, S. *et al.* (2002) The relationship between allergen-induced tissue eosinophilia and markers of repair and remodeling in human atopic skin. *J. Immunol.* 169, 4604–4612
- Treasurywala, S. and Berens, M.E. (1998) Migration arrest in glioma cells is dependent on the α v integrin subunit. *Glia* 24, 236–243
- Zhang, J. *et al.* (1999) Mapping and characterization of the epitope(s) of Sch 55700, a humanized mAb, that inhibits human IL-5. *Int. Immunol.* 11, 1935–1944
- Zia-Amirhosseini, P. *et al.* (1999) Pharmacokinetics and pharmacodynamics of SB-240563, a humanized monoclonal antibody directed to human interleukin-5, in monkeys. *J. Pharmacol. Exp. Ther.* 291, 1060–1067
- Sanderson, C.J. (1992) Interleukin-5, eosinophils, and disease. *Blood* 79, 3101–3109
- Clutterbuck, E.J. *et al.* (1989) 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* 73, 1504–1512
- Yamaguchi, Y. *et al.* (1988) Highly purified murine interleukin 5 (IL-5) stimulates eosinophil function and prolongs *in vitro* survival. IL-5 as an eosinophil chemotactic factor. *J. Exp. Med.* 167, 1737–1742
- Tavernier, J. *et al.* (2000) Interleukin 5 regulates the isoform expression of its own receptor α -subunit. *Blood* 95, 1600–1607
- Erb, K.J. and Le Gros, G. (1996) The role of Th2 type CD4⁺ T cells and Th2 type CD8⁺ T cells in asthma. *Immunol. Cell Biol.* 74, 206–208
- Warren, H.S. *et al.* (1995) Production of IL-5 by human NK cells and regulation of IL-5 secretion by IL-4, IL-10, and IL-12. *J. Immunol.* 154, 5144–5152
- Okayama, Y. *et al.* (1995) IgE-dependent expression of mRNA for IL-4 and IL-5 in human lung mast cells. *J. Immunol.* 155, 1796–1808
- Tanaka, Y. *et al.* (1994) Interleukin-5 messenger RNA and immunoreactive protein expression by activated eosinophils in lesional atopic dermatitis skin. *J. Invest. Dermatol.* 103, 589–592
- Salvi, S. *et al.* (1999) Interleukin-5 production by human airway epithelial cells. *Am. J. Respir. Cell Mol. Biol.* 20, 984–991
- Lopez, A.F. *et al.* (1988) Recombinant human interleukin 5 is a selective activator of human eosinophil function. *J. Exp. Med.* 167, 219–224
- Yamaguchi, Y. *et al.* (1991) Analysis of the survival of mature human eosinophils: interleukin-5 prevents apoptosis in mature human eosinophils. *Blood* 78, 2542–2547
- Yamada, T. *et al.* (1998) IL-3, IL-5, granulocyte-macrophage colony-stimulating factor receptor α -subunit, and common β -subunit expression by peripheral leukocytes and blood dendritic cells. *J. Allergy Clin. Immunol.* 101, 677–682
- Menzies-Gow, A. *et al.* (2003) Anti-IL-5 (mepolizumab) therapy induces bone marrow eosinophil maturational arrest and decreases eosinophil progenitors in the bronchial mucosa of atopic asthmatics. *J. Allergy Clin. Immunol.* 111, 714–719
- Flood-Page, P.T. *et al.* (2003) Eosinophil’s role remains uncertain as anti-interleukin-5 only partially depletes numbers in asthmatic airway. *Am. J. Respir. Crit. Care Med.* 167, 199–204
- Leckie, M.J. *et al.* (2000) Effects of an interleukin-5 blocking monoclonal antibody on eosinophils, airway hyper-responsiveness, and the late asthmatic response. *Lancet* 356, 2144–2148
- O’Byrne, P.M. *et al.* (2001) The trials and tribulations of IL-5, eosinophils, and allergic asthma. *J. Allergy Clin. Immunol.* 108, 503–508
- Kips, J.C. *et al.* (2003) Effect of SCH55700, a humanized anti-human interleukin-5 antibody, in severe persistent asthma: a pilot study. *Am. J. Respir. Crit. Care Med.* 167, 1655–1659
- Gregory, B. *et al.* (2003) Differential regulation of human eosinophil IL-3, IL-5, and GM-CSF receptor α -chain expression by cytokines: IL-3, IL-5, and GM-CSF down-regulate IL-5 receptor α expression with loss of IL-5 responsiveness, but up-regulate IL-3 receptor α expression. *J. Immunol.* 170, 5359–5366
- Flood-Page, P. *et al.* (2003) Anti-IL-5 treatment reduces deposition of ECM proteins in the bronchial subepithelial basement membrane of mild atopic asthmatics. *J. Clin. Invest.* 112, 1029–1036

- 45 Leigh, R. *et al.* (2002) Dysfunction and remodeling of the mouse airway persist after resolution of acute allergen-induced airway inflammation. *Am. J. Respir. Cell Mol. Biol.* 27, 526–535
- 46 McMillan, S.J. and Lloyd, C.M. (2004) Prolonged allergen challenge in mice leads to persistent airway remodelling. *Clin. Exp. Allergy* 34, 497–507
- 47 Blyth, D.I. *et al.* (2000) Airway subepithelial fibrosis in a murine model of atopic asthma: suppression by dexamethasone or anti-interleukin-5 antibody. *Am. J. Respir. Cell Mol. Biol.* 23, 241–246
- 48 Trifilieff, A. *et al.* (2001) IL-5 deficiency abolishes aspects of airway remodelling in a murine model of lung inflammation. *Clin. Exp. Allergy* 31, 934–942
- 49 Cho, J.Y. *et al.* (2004) Inhibition of airway remodeling in IL-5-deficient mice. *J. Clin. Invest.* 113, 551–560
- 50 Johnson, J.R. *et al.* (2004) Continuous exposure to house dust mite elicits chronic airway inflammation and structural remodeling. *Am. J. Respir. Crit. Care Med.* 169, 378–385
- 51 Munger, J.S. *et al.* (1999) The integrin α v β 6 binds and activates latent TGF β 1: a mechanism for regulating pulmonary inflammation and fibrosis. *Cell* 96, 319–328
- 52 Tanaka, H. *et al.* (2004) Role of IL-5 and eosinophils in allergen-induced airway remodeling in mice. *Am. J. Respir. Cell Mol. Biol.* 31, 62–68
- 53 Lee, J.J. *et al.* (1997) Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma. *J. Exp. Med.* 185, 2143–2156
- 54 Tang, W. *et al.* (1996) Targeted expression of IL-11 in the murine airway causes lymphocytic inflammation, bronchial remodeling, and airways obstruction. *J. Clin. Invest.* 98, 2845–2853
- 55 Zhu, Z. *et al.* (1999) Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis, physiologic abnormalities, and eotaxin production. *J. Clin. Invest.* 103, 779–788
- 56 Lee, C.G. *et al.* (2001) Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor β 1. *J. Exp. Med.* 194, 809–821
- 57 Gibson, P.G. *et al.* (1989) Chronic cough: eosinophilic bronchitis without asthma. *Lancet* 1, 1346–1348
- 58 Brightling, C.E. *et al.* (2002) Mast-cell infiltration of airway smooth muscle in asthma. *N. Engl. J. Med.* 346, 1699–1705
- 59 Foster, P.S. *et al.* (2001) Elemental signals regulating eosinophil accumulation in the lung. *Immunol. Rev.* 179, 173–181
- 60 Yu, C. *et al.* (2002) 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.* 195, 1387–1395
- 61 Pulleyn, L.J. *et al.* (2001) TGF β 1 allele association with asthma severity. *Hum. Genet.* 109, 623–627
- 62 Green, R.H. *et al.* (2002) Asthma exacerbations and sputum eosinophil counts: a randomised controlled trial. *Lancet* 360, 1715–1721
- 63 Powell, P.P. *et al.* (1993) Eosinophils expressing heparin-binding EGF-like growth factor mRNA localize around lung microvessels in pulmonary hypertension. *Am. J. Pathol.* 143, 784–793
- 64 Moqbel, R. *et al.* (1995) Identification of mRNA for interleukin-4 in human eosinophils with granule localization and release of the translated product. *J. Immunol.* 155, 4939–4947
- 65 Minshall, E. *et al.* (2000) IL-11 expression is increased in severe asthma: association with epithelial cells and eosinophils. *J. Allergy Clin. Immunol.* 105, 232–238
- 66 Zheng, T. *et al.* (2001) IL-11: insights in asthma from overexpression transgenic modeling. *J. Allergy Clin. Immunol.* 108, 489–496
- 67 Booth, B.W. *et al.* (2001) Interleukin-13 induces proliferation of human airway epithelial cells *in vitro* via a mechanism mediated by transforming growth factor- α . *Am. J. Respir. Cell Mol. Biol.* 25, 739–743
- 68 Maertzdorf, J. *et al.* (2002) IL-17 expression in human herpetic stromal keratitis: modulatory effects on chemokine production by corneal fibroblasts. *J. Immunol.* 169, 5897–5903
- 69 Rochester, C.L. *et al.* (1996) Eosinophil-fibroblast interactions. Granule major basic protein interacts with IL-1 and transforming growth factor- β in the stimulation of lung fibroblast IL-6-type cytokine production. *J. Immunol.* 156, 4449–4456
- 70 Solomon, A. *et al.* (1998) Nerve growth factor is preformed in and activates human peripheral blood eosinophils. *J. Allergy Clin. Immunol.* 102, 454–460
- 71 Ohno, I. *et al.* (1995) Eosinophils as a potential source of platelet-derived growth factor B-chain (PDGF-B) in nasal polyposis and bronchial asthma. *Am. J. Respir. Cell Mol. Biol.* 13, 639–647
- 72 Bostrom, H. *et al.* (1996) PDGF-A signaling is a critical event in lung alveolar myofibroblast development and alveogenesis. *Cell* 85, 863–873
- 73 Costa, J.J. *et al.* (1993) Human eosinophils can express the cytokines tumor necrosis factor- α and macrophage inflammatory protein-1 α . *J. Clin. Invest.* 91, 2673–2684

Free journals for developing countries

The WHO and six medical journal publishers have launched the Access to Research Initiative, which enables nearly 70 of the world's poorest countries to gain free access to biomedical literature through the Internet.

The science publishers, Blackwell, Elsevier, the Harcourt Worldwide STM group, Wolters Kluwer International Health and Science, Springer-Verlag and John Wiley, were approached by the WHO and the *British Medical Journal* in 2001. Initially, more than 1000 journals will be available for free or at significantly reduced prices to universities, medical schools, research and public institutions in developing countries. The second stage involves extending this initiative to institutions in other countries.

Gro Harlem Brundtland, director-general for the WHO, said that this initiative was 'perhaps the biggest step ever taken towards reducing the health information gap between rich and poor countries'.

See <http://www.healthinternetwork.net> for more information.