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The role of eosinophils in airway tissue remodelling in asthma

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There is an increasing evidence that airway structural change (termed remodelling) is associated with the severity and chronicity of asthma. Recent studies support an important role for eosinophils in the remodelling process. In particular eosinophil depletion studies have demonstrated that several aspects of remodelling are attenuated. Eosinophils have been confirmed as an important source of TGF- β_1 as well as other important cytokines that can lead to the direct activation of epithelium and mesenchymal cells that are considered to drive airway remodelling. The current studies that support a role for eosinophils in airway remodelling are reviewed in article.

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Introduction

Airway structural change termed remodelling is a characteristic feature of asthma. The clinical effects of airway remodelling are at present uncertain, but the accelerated lung function decline over time in asthmatics compared to non-asthmatics [1]. The development of fixed airflow obstruction in some asthmatics [2] and the association of AHR with remodelling [3] all suggest that remodelling must be an important area of therapeutic focus. It has been appreciated for a while that tissue eosinophilia and eosinophil degranulation is commonly associated with fibrotic disease [4]. Recent studies using eosinophil depletion strategies have highlighted an important role for eosinophils as important inflammatory cells in the process of airway remodelling in asthma.

Eosinophils

Eosinophils comprise less than 5% of the circulating white cell population in healthy individuals and are easily distinguished by their bi-lobed appearance and the characteristic granules that occupy up to one fifth of the cytoplasmic content. The primary role of the eosinophil has been considered as host defence against parasitic infection through the release of cytotoxic cellular contents. Major basic protein (MBP) accounts for 50% of the secondary granule crystalloid core and the non-crystalloid core provides storage for other basic proteins such as eosinophilic cationic protein (ECP), eosinophil-derived neurotoxin (EDN) and eosinophil-derived peroxidase (EPO). The latter can generate hypohalous acids and reactive oxygen species. Eosinophils can also generate membrane phospholipids-derived eicosanoids, in particular leukotriene C4 which may act in airways smooth muscle constriction and mucus hyper-secretion. Eosinophils are also a significant source of cytokines and growth factors. Whilst eosinophil degranulation may lead to effective combat in the context of host defence, the release of such cellular contents will lead to bystander tissue damage and modulation of the accompanying inflammatory tissue response [5].

Eosinophils and asthma

The precise role of the eosinophil in asthma pathogenesis remains uncertain. Eosinophils traffic from the bone marrow into the lung in response to allergen challenge in atopic asthma. The products released by eosinophils have been demonstrated to promote some of the pathophysiological hallmarks of asthma such as airway hyper-responsiveness (AHR). For example direct installation of MBP and EPO induce both bronchoconstriction and AHR in the primate airway [6]. Post mortem analysis of asthma death patients consistently shows eosinophils and extracellular MBP associated with nerve fibres in the airway smooth muscle suggesting role for MBP in neurogenic inflammation that may modulate smooth muscle responses. MBP binds to the M₂ inhibitory muscarinic receptor on parasympathetic nerves leading to increased acetylcholine release with subsequent M₃ receptor activation and hence bronchoconstriction [7]. The cytostimulatory properties of MBP on basophils, mast cells and neutrophils [8,9] result in the propagation of the inflammatory response. Primed eosinophils have been identified as a predominant cellular source of LTC₄ in bronchial biopsies from asthmatic airways [10]. LTC₄ undergoes extracellular sequential enzymatic cleavage of glutamic acid and glycine from its sulphide chains to form the metabolites LTD₄ and LTE₄, respectively. These products can elicit paracrine effects that result

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in mucus hypersecretion [11], AHR, oedema [12] and potent bronchoconstriction [13]. Collectively these findings suggest that eosinophils may be the major pro-inflammatory effector cells in the pathogenesis of asthma and has lead to the eosinophil hypothesis in asthma.

Airway remodelling and asthma

Inflammation can be viewed as the response of tissue to injury. The aim of this inflammatory response is to repair the injury. Rapid restoration of tissue structure following injury is essential for the maintenance of organ integrity and function and any dysregulated or excessive repair will lead to tissue fibrosis and contribute to disease states. In asthma the epithelium displays structural damage with marked goblet cell, smooth muscle and fibroblast hyperplasia together with the recruitment and activation of myofibroblasts. Increased deposition of collagens and other extracellular matrix (ECM) proteins occur both in the lamina reticularis of the basement membrane (RBM) and throughout the bronchial mucosa together with prominent airway angiogenesis.

The molecular mechanisms that drive remodelling remain undefined but aberrant signalling by the transforming growth factor (TGF) superfamily, of which TGF- β_1 is the prototypic member, is increasingly recognised as playing an important role. The eosinophil is an important source of growth factors such as TGF- β_1 [14]. TGF- β_1 is an important fibroblast chemotactic factor [15] and fibroblast numbers have been shown to correlate with TGF- β_1 expression [16]. TGF- β_1 induces the differentiation of fibroblasts to myofibroblasts [17]. Collagen I and III transcription is rapidly upregulated [18] whilst at the same time collagen degradation is demonstrated in foetal lung fibroblast *in vitro* experiments [19].

Eosinophils and airway remodelling

Whilst the role of the eosinophil in asthma pathogenesis currently remains an area of vigorous debate, there has been growing recognition that the eosinophil may have an important role in airway remodelling. Most fibrotic diseases share a common paradigm of a persistent inflammatory stimulus with lymphocyte-monocyte interactions that generate fibrogenic cytokines that can initiate and even propagate fibrotic processes. It is, therefore, not unexpected that some aspects of airway structural cell activation and remodelling will be related to inflammatory consequences.

It was in an eosinophil depletion study in human asthma using a monoclonal antibody (mAb) mepolizumab (SB-240563), a humanised IgG1/ κ mAb, against interleukin (IL)-5 that an important causal role for eosinophils in airway remodelling was highlighted [20]. IL-5 acts specifically on eosinophils to promote terminal differentiation of eosinophil-lineage committed precursors and together with the chemokine eotaxin leads to eosinophil

recruitment to sites of inflammation. In this study bronchial biopsies were obtained at baseline and after three infusions of anti-IL-5 (mepolizumab) in 24 individuals with atopic asthma. Only a 55% reduction in bronchial tissue eosinophilia was achieved with no effect on AHR. There was no reduction in free airway deposition of MBP. The study was not powered to detect changes in AHR and the persistence of AHR may be explained by the residual eosinophil population. The novel finding in this study was that the decrease in eosinophilia was associated with significant reduction of markers of airway remodelling. RBM procollagen III, tenascin and lumican were all significantly decreased. This was in association with the reduction in proportion of eosinophils expressing TGF- β_1 and bronchoalveolar lavage (BAL) concentrations of TGF- β_1 . It is of note that other aspect of remodelling such as type IV collagen did not change with anti-IL-5 treatment. We were not able to assess smooth muscle changes in this study but it would be of great interest to examine effects of eosinophil depletion on myofibroblast and airway smooth muscle. It may be that some elements of remodelling are more or less eosinophil dependent and that there is a spectrum of reversibility of airway remodelling changes in asthma which may affect AHR and other aspects of lung function differentially. Certainly it has been suggested that airway muscle remodelling in asthma is associated with epigenetic changes and may thus be irreversible [56]. It is of note that tissue remodelling is also seen in other eosinophilic diseases such as eosinophilic oesophagitis [57], where eosinophil-derived TGF β is also detected.

Animal models of eosinophil depletion have supported the findings in human studies. Transgenic overexpression of IL-5 in the murine lung was associated with peribronchial collagen deposition [21]. Interestingly this group has recently gone on to develop a double airway transgenic mouse expressing IL-5 systemically in T cells and eotaxin-1 in the airway epithelium. This animal has features of spontaneous degranulation and AHR as well as remodelling changes; all of which strongly supports the potential for eosinophils in airway changes of asthma including remodelling [58]. Furthermore, airway changes did not occur in the absence of eosinophils when the double IL-5/eotaxin transgenic was crossed to the PHIL mouse. In an alternative approach, Cho *et al.* demonstrated in a repetitive ova challenge IL-5 gene deleted murine model of airway injury that the eosinophil depleted C57BL mice demonstrate significantly less total lung collagen deposition less peribronchial collagen Type III and IV deposition as well as markedly reduced evidence of fibroblast activation as detected by α -smooth muscle actin expression compared to wild type mice [22]. Eosinophils were again the predominant inflammatory cell source of TGF- β_1 and in this model epithelial expression of the integrin $\alpha_v\beta_6$, a potent activator of latent TGF- β_1 , was reduced.

GATA-1 is an essential transcription factor for the development of the eosinophil lineage [23]. Δ dbl mice with eosinophil depletion as a result of deletion of a high-affinity GATA-1 promoter binding site that selectively abolishes eosinophil lineage development without effecting other GATA-1-dependent lineages were protected from peribronchial collagen deposition and increased airway smooth muscle mass in response to chronic allergen exposure [24]. AHR and mucus production did not decrease in this model. This maybe a reflection of the fact that IL-13, a key cytokine that is produced by Th2 cells, mast cells as well as epithelial and structural cells that is strongly implicated in the pathogenesis of AHR and mucus production, was not effected. In this model, there is no difference in TGF- β_1 expression between the Δ dbl and wild-type controls. This finding is in contrast to the earlier study by Cho *et al.* Airway epithelium and other inflammatory cells such as macrophages are important sources of TGF- β_1 as well other potent growth factors such as activin-A that have potent effects on airway remodelling also. However, the suggestion from this study is that eosinophils may contribute to airway remodelling by the secretion of other fibrogenic factors. In a separate study, eosinophil depletion through the lineage specific expression of the cytotoxic diphtheria toxin A under the control of the eosinophil peroxidase promoter in a transgenic line of mice (*PHIL*) demonstrated reductions in epithelial cell hypertrophy and mucus cell metaplasia [25]. Whether IL-13 production was suppressed in the *PHIL* mice is not known and therefore it is not possible to conclude that it was eosinophil depletion that leads to mucus cell changes and production. It is also not reported whether there was any reduction in other markers of airway remodelling. This study demonstrated reduction in AHR.

The contrasting findings of these studies highlight the importance of strain heterogeneity of animal models. The Δ dbl GATA mice were originally bred on a Balb/c background and it has been previously demonstrated that Balb/c IL-5 deficient mice can still develop AHR upon allergen sensitization and airway challenge [26]. By contrast the B6 background IL-5 deficient strain is protected from AHR development [27]. It is important to consider the effect of strain of animal on pathological outcomes in mouse models. For example the manifestation of airway AHR is strain dependent [28] with A/J strain mice displaying the greatest degree of AHR and re-modelling changes [29]. Strain heterogeneity effects cellular localization, in particular of eosinophils [28], and the propensity for angiogenesis [30]. Thus it would be important to perform these experiments in similar strains that are prone to develop AHR with standardised allergen challenge protocols before the exact contribution of eosinophils to remodelling and AHR can be evaluated.

In a recent study we confirmed that allergen provocation in allergen-induced asthma was associated with the rapid

and simultaneous activation of cellular inflammation and airway remodelling, but demonstrated dissociation of selected aspects of remodelling from inflammation occur at a later time point [3]. The suggestion from this study was that inflammatory cells may initiate remodelling by the release of cytokines and fibrogenic factors which will lead to activation of the remodelling pathways through activation of structural cells such as epithelium and fibroblasts. At a later time point, remodelling may proceed in the absence of further inflammatory cell recruitment through the sustained activation of structural cells which now propagate the remodelling process. Thus eosinophils that are initially recruited into the airway after allergen challenge may initiate remodelling through the release of their contents that lead to epithelial and fibroblast activation. Airway remodelling then may be propagated by sustained structural cell activation in the absence of any further eosinophil recruitment.

Mechanisms of eosinophil-induced remodelling

Eosinophils are implicated in both inflammation and remodelling by the release of cytokines and fibrogenic factors. Eosinophils have been identified as a significant source of TGF- β_1 in asthma. TGF- β_1 is one of the most fibrogenic factors known. Inflammatory cells probably account for the increased amounts of TGF- β_1 found in the asthmatic airway at baseline [16,31,32] with further increases in response to allergen challenge [32]. Eosinophils are of an activated phenotype following allergen challenge [31,33] and will, therefore, more readily release their stores of TGF- β_1 on tissue recruitment.

An *in vivo* model of allergen-induced inflammation in atopic skin has provided convincing support for an association of eosinophils with expression of markers of remodelling [34]. In this model eosinophils expressing TGF- β_1 were temporally associated with fibroblast transformation to myofibroblasts and ECM expression. Direct eosinophil activation of mesenchymal cells leading to ECM production was demonstrated *in vitro* using eosinophil-dermal fibroblast co-culture experiments. Neutralising antibodies against TGF- β_1 inhibited fibroblast-myofibroblast transformation as well as tenascin and procollagen I production. It is, therefore, conceivable that eosinophil-derived products will directly interact with airway fibroblasts and other structural cells in the asthmatic airway leading to activation and ECM production.

Airway epithelium is a significant source of the TGF- β superfamily of ligands. Eosinophil cytotoxicity can lead to direct activation of epithelium. For example the *in vitro* effects of MBP include epithelial detachment and ciliary dysfunction which may contribute to airway injury. It is possible that such MBP-induced epithelial cytotoxicity leads to epithelial cell activation and release of growth factors such as TGF- β_1 that can now act upon the

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underlying fibroblast layers leading to induction of collagen and ECM production [35]. It is also probable that eosinophil contents will modulate mesenchymal function directly. Fibroblasts in inflamed tissue will be exposed to granule contents. ECP has been shown to inhibit the degradation of proteoglycans by fibroblasts [36]. MBP has also been recently identified as having potent heparanase inhibitor function. Heparanases serve to cleave the heparin-sulphate intrachain, with subsequent release of heparin-bound growth and differentiation factors present in tissue ECM reservoirs. Newly released growth factors can now undergo activation and signal to activate downstream remodelling processes. Thus MBP may have a functional role in the repair response to injury by regulating ECM growth factor release that leads to limitation of tissue injury and promotion of healing.

Eosinophils are implicated as a source of several other fibrogenic cytokines and modulators of remodelling such as fibroblast and smooth muscle cell mitogen heparin-binding epidermal growth factor-like growth factor (HB-EGF) [37], nerve growth factor (NGF) [38] and TGF- α [39]. IL-4 and IL-13, both predominantly Th₂ cytokines, are also produced by eosinophils [40,41]. IL-13 can induce fibroblast differentiation into myofibroblasts with activation of collagen production. IL-13 leads to sub-epithelial fibrosis [42] and goblet cell hyperplasia and secretion. The ability of IL-13 to induce the production of TGF- β_1 and also activate stored latent TGF- β_1 [43] may explain the potent fibrogenic properties of IL-13.

A balance between production and degradation achieves the regulation of ECM turnover. ECM degradation is dependent on the family of proteinases termed matrix metalloproteinases (MMPs). The tissue inhibitors of metalloproteinases (TIMPs) serve to inhibit MMP activity by binding to MMPs in a 1:1 manner and this exact balance is critical in determining the normal matrix turnover. An imbalance can lead to either excess degradation or accumulation of ECM. Eosinophils express both MMP-9 and TIMPs [44,45]. IL-5 is an inducer of MMP-9 expression in eosinophils.

There is growing evidence of a role for CysLTs such as LTC₄ in airway remodelling. *In vitro* studies confirm increased structural cell activation with marked proliferation of airway epithelium as well as fibroblasts in response to LTC₄ [46] whilst LTD₄ participates with growth factors in induction of ASM proliferation [47].

Eosinophils infiltration in particular is associated with increased vascularity [48]. Vascularity in turns correlates with asthma severity [49] whilst inversely correlating with the degree of airway obstruction and AHR [50]. Vascularity is driven by the overproduction of multiple growth factors that include vascular endothelial growth factor (VEGF), fibroblast growth factor (FGF) and angiogenin. Using

double immunohistochemical staining with MBP, eosinophils have been identified as a significant cellular source of VEGF and also FGF and angiogenin in asthmatic airways [51]. VEGF in particular is a potent multifunctional cytokine which can drive not only inflammation and vascular and tissue remodelling but also enhance antigen presentation and Th2 inflammation in asthma [52].

It is important to consider how eosinophils and other inflammatory cells interactions may propagate airway remodelling. Eosinophils secrete mast cell stem cell factor (SCF) essential for mast cell growth, activation, chemotaxis and degranulation. This may explain why tissue eosinophilia is often in association with mast cell recruitment. In turn mast cell-derived TNF- α is a potent stimulus for eosinophil TGF- β_1 release.

Mast cells have significant potential to contribute to remodelling as they are important sources of several fibrogenic factors. Of particular interest is tryptase which signals through the G-protein coupled protease activated receptor type-2 (PAR-2) present on inflammatory cells as well as epithelium, fibroblasts and ASM [53]. PAR-2 activation leads to structural cell activation and proliferation [54,55]. In turn fibroblasts secrete GM-CSF and SCF leading to sustenance of eosinophil and mast cell-derived inflammation and repair in the tissue.

In conclusion the emerging evidence suggests that the eosinophil may have an important role in airway remodelling in asthma. The development of studies that more effectively deplete eosinophils are eagerly awaited as only then can it become more certain that eosinophils are a major contributory source of growth factors and cytokines that drive the remodelling pathways.

References

1. Lange P, Parner J, Vestbo J, Schnohr P, Jensen G: **A 15-year follow-up study of ventilatory function in adults with asthma.** *N Engl J Med* 1998, **339**:1194-1200.
2. Bumbacea D, Campbell D, Nguyen L, Carr D, Barnes PJ, Robinson D, Chung KF: **Parameters associated with persistent airflow obstruction in chronic severe asthma.** *Eur Respir J* 2004, **24**:122-128.
3. Kariyawasam HH, Aizen M, Barkans J, Robinson DS, Kay AB: **Remodeling and airway hyperresponsiveness but not cellular inflammation persist after allergen challenge in asthma.** *Am J Respir Crit Care Med* 2007, **175**:896-904.
4. Noguchi H, Kephart GM, Colby TV, Gleich GJ: **Tissue eosinophilia and eosinophil degranulation in syndromes associated with fibrosis.** *Am J Pathol* 1992, **140**:521-528.
5. Kariyawasam HH, Robinson DS: **The eosinophil: the cell and its weapons, the cytokines, its locations.** *Semin Respir Crit Care Med* 2006, **27**:117-127.
6. Gundel RH, Letts LG, Gleich GJ: **Human eosinophil major basic protein induces airway constriction and airway hyper-responsiveness in primates.** *J Clin Invest* 1991, **87**:1470-1473.
7. Fryer AD, Costello RW, Yost BL, Lobb RR, Tedder TF, Steeber DA, Bochner BS: **Antibody to VLA-4, but not to L-selectin, protects neuronal M2 muscarinic receptors in antigen-challenged guinea pig airways.** *J Clin Invest* 1997, **99**:2036-2044.

8. O'Donnell MC, Ackerman SJ, Gleich GJ, Thomas LL: **Activation of basophil and mast cell histamine release by eosinophil granule major basic protein.** *J Exp Med* 1983, **157**:1981-1991.
9. Moy JN, Gleich GJ, Thomas LL: **Noncytotoxic activation of neutrophils by eosinophil granule major basic protein. Effect on superoxide anion generation and lysosomal enzyme release.** *J Immunol* 1990, **145**:2626-2632.
10. Cowburn AS, Sladek K, Soja J, Adamek L, Nizankowska E, Szczeklik A, Lam BK, Penrose JF, Austen FK, Holgate ST, Sampson AP: **Overexpression of leukotriene C4 synthase in bronchial biopsies from patients with aspirin-intolerant asthma.** *J Clin Invest* 1998, **101**:834-846.
11. Marom Z, Shelhamer JH, Bach MK, Morton DR, Kaliner M: **Slow-reacting substances, leukotrienes C4 and D4, increase the release of mucus from human airways in vitro.** *Am Rev Respir Dis* 1982, **126**:449-451.
12. Joris I, Majno G, Corey EJ, Lewis RA: **The mechanism of vascular leakage induced by leukotriene E4. Endothelial contraction.** *Am J Pathol* 1987, **126**:19-24.
13. Adelroth E, Morris MM, Hargreave FE, O'Byrne PM: **Airway responsiveness to leukotrienes C4 and D4 and to methacholine in patients with asthma and normal controls.** *N Engl J Med* 1986, **315**:480-484.
14. Wong DT, Elovic A, Matossian K, Nagura N, McBride J, Chou MY, Gordon JR, Rand TH, Galli SJ, Weller PF: **Eosinophils from patients with blood eosinophilia express transforming growth factor beta 1.** *Blood* 1991, **78**:2702-2707.
15. Postlethwaite AE, Seyer JM: **Identification of a chemotactic epitope in human transforming growth factor-beta 1 spanning amino acid residues 368-374.** *J Cell Physiol* 1995, **164**:587-592.
16. Vignola AM, Chanez P, Chiappara G, Merendino A, Pace E, Rizzo A, la Rocca AM, Bellia V, Bonsignore G, Bousquet J: **Transforming growth factor-beta expression in mucosal biopsies in asthma and chronic bronchitis.** *Am J Respir Crit Care Med* 1997, **156**:591-599.
17. Thannickal VJ, Lee DY, White ES, Cui Z, Larios JM, Chacon R, Horowitz JC, Day RM, Thomas PE: **Myofibroblast differentiation by transforming growth factor-beta1 is dependent on cell adhesion and integrin signaling via focal adhesion kinase.** *J Biol Chem* 2003, **278**:12384-12389.
18. Igotz RA, Endo T, Massague J: **Regulation of fibronectin and type I collagen mRNA levels by transforming growth factor-beta.** *J Biol Chem* 1987, **262**:6443-6446.
19. McAnulty RJ, Chambers RC, Laurent GJ: **Regulation of fibroblast procollagen production. Transforming growth factor-beta 1 induces prostaglandin E2 but not procollagen synthesis via a pertussis toxin-sensitive G-protein.** *Biochem J* 1995, **307**(Pt 1):63-68.
20. 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.
21. Lee JJ, McGarry MP, Farmer SC, Denzler KL, Larson KA, Carrigan PE, Brenneise IE, Horton MA, Haczku A, Gelfand EW *et al.*: **Interleukin-5 expression in the lung epithelium of transgenic mice leads to pulmonary changes pathognomonic of asthma.** *J Exp Med* 1997, **185**:2143-2156.
22. Cho JY, Miller M, Baek KJ, Han JW, Nayar J, Lee SY, McElwain K, McElwain S, Friedman S, Broide DH: **Inhibition of airway remodeling in IL-5-deficient mice.** *J Clin Invest* 2004, **113**:551-560.
23. Yu C, Cantor AB, Yang H, Browne C, Wells RA, Fujiwara Y, Orkin SH: **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, **195**:1387-1395.
24. Humbles AA, Lloyd CM, McMillan SJ, Friend DS, Xanthou G, McKenna EE, Ghiran S, Gerard NP, Yu C, Orkin SH *et al.*: **A critical role for eosinophils in allergic airways remodeling.** *Science* 2004, **305**:1776-1779.
25. Lee JJ, Dimina D, Macias MP, Ochkur SI, McGarry MP, O'Neill KR, Protheroe C, Pero R, Nguyen T, Cormier SA *et al.*: **Defining a link with asthma in mice congenitally deficient in eosinophils.** *Science* 2004, **305**:1773-1776.
26. Foster PS, Hogan SP, Ramsay AJ, Matthaei KI, Young IG: **Interleukin 5 deficiency abolishes eosinophilia, airways hyperactivity, and lung damage in a mouse asthma model.** *J Exp Med* 1996, **183**:195-201.
27. Hogan SP, Matthaei KI, Young JM, Koskinen A, Young IG, Foster PS: **A novel T cell-regulated mechanism modulating allergen-induced airways hyperactivity in BALB/c mice independently of IL-4 and IL-5.** *J Immunol* 1998, **161**:1501-1509.
28. Takeda K, Haczku A, Lee JJ, Irvin CG, Gelfand EW: **Strain dependence of airway hyperresponsiveness reflects differences in eosinophil localization in the lung.** *Am J Physiol Lung Cell Mol Physiol* 2001, **281**:L394-L402.
29. Shinagawa K, Kojima M: **Mouse model of airway remodeling: strain differences.** *Am J Respir Crit Care Med* 2003, **168**:959-967.
30. Rohan RM, Fernandez A, Udagawa T, Yuan J, D'Amato RJ: **Genetic heterogeneity of angiogenesis in mice.** *FASEB J* 2000, **14**:871-876.
31. Minshall EM, Leung DY, Martin RJ, Song YL, Cameron L, Ernst P, Hamid Q: **Eosinophil-associated TGF-beta1 mRNA expression and airways fibrosis in bronchial asthma.** *Am J Respir Cell Mol Biol* 1997, **17**:326-333.
32. Redington AE, Madden J, Frew AJ, Djukanovic R, Roche WR, Holgate ST, Howarth PH: **Transforming growth factor-beta 1 in asthma. Measurement in bronchoalveolar lavage fluid.** *Am J Respir Crit Care Med* 1997, **156**:642-647.
33. Chanez P, Bousquet J, Couret I, Cornillac L, Barneon G, Vic P, Michel FB, Godard P: **Increased numbers of hypodense alveolar macrophages in patients with bronchial asthma.** *Am Rev Respir Dis* 1991, **144**:923-930.
34. 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**:4604-4612.
35. Zhang S, Smartt H, Holgate ST, Roche WR: **Growth factors secreted by bronchial epithelial cells control myofibroblast proliferation: an in vitro co-culture model of airway remodeling in asthma.** *Lab Invest* 1999, **79**:395-405.
36. Hernnas J, Sarnstrand B, Lindroth P, Peterson CG, Venge P, Malmstrom A: **Eosinophil cationic protein alters proteoglycan metabolism in human lung fibroblast cultures.** *Eur J Cell Biol* 1992, **59**:352-363.
37. Powell PP, Klagsbrun M, Abraham JA, Jones RC: **Eosinophils expressing heparin-binding EGF-like growth factor mRNA localize around lung microvessels in pulmonary hypertension.** *Am J Pathol* 1993, **143**:784-793.
38. Solomon A, Aloe L, Pe'er J, Frucht-Pery J, Bonini S, Bonini S, Levi-Schaffer F: **Nerve growth factor is preformed in and activates human peripheral blood eosinophils.** *J Allergy Clin Immunol* 1998, **102**:454-460.
39. Wong DT, Weller PF, Galli SJ, Elovic A, Rand TH, Gallagher GT, Chiang T, Chou MY, Matossian K, McBride J: **Human eosinophils express transforming growth factor alpha.** *J Exp Med* 1990, **172**:673-681.
40. Moqbel R, Ying S, Barkans J, Newman TM, Kimmitt P, Wakelin M, Taborda-Barata L, Meng Q, Corrigan CJ, Durham SR *et al.*: **Identification of messenger RNA for IL-4 in human eosinophils with granule localization and release of the translated product.** *J Immunol* 1995, **155**:4939-4947.
41. Schmid-Grendelmeier P, Altnauer F, Fischer B, Bizer C, Straumann A, Menz G, Blaser K, Wuthrich B, Simon HU: **Eosinophils express functional IL-13 in eosinophilic inflammatory diseases.** *J Immunol* 2002, **169**:1021-1027.
42. Zhu Z, Homer RJ, Wang Z, Chen Q, Geba GP, Wang J, Zhang Y, Elias JA: **Pulmonary expression of interleukin-13 causes inflammation, mucus hypersecretion, subepithelial fibrosis,**

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- physiologic abnormalities, and eotaxin production.** *J Clin Invest* 1999, **103**:779-788.
43. Lee CG, Homer RJ, Zhu Z, Lanone S, Wang X, Kotliansky V, Shipley JM, Gotwals P, Noble P, Chen Q *et al.*: **Interleukin-13 induces tissue fibrosis by selectively stimulating and activating transforming growth factor beta(1).** *J Exp Med* 2001, **194**:809-821.
 44. 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**:519-528.
 45. Levi-Schaffer F, Garbuzenko E, Rubin A, Reich R, Pickholz D, Gillery P, Emonard H, Nagler A, Maquart FA: **Human eosinophils regulate human lung- and skin-derived fibroblast properties in vitro: a role for transforming growth factor beta (TGF-beta).** *Proc Natl Acad Sci U S A* 1999, **96**:9660-9665.
 46. Leikauf GD, Claesson HE, Doupnik CA, Hybbinette S, Grafstrom RC: **Cysteinyl leukotrienes enhance growth of human airway epithelial cells.** *Am J Physiol* 1990, **259**:L255-L261.
 47. Panettieri RA, Tan EM, Ciocca V, Luttmann MA, Leonard TB, Hay DW: **Effects of LTD4 on human airway smooth muscle cell proliferation, matrix expression, and contraction in vitro: differential sensitivity to cysteinyl leukotriene receptor antagonists.** *Am J Respir Cell Mol Biol* 1998, **19**:453-461.
 48. Salvato G: **Quantitative and morphological analysis of the vascular bed in bronchial biopsy specimens from asthmatic and non-asthmatic subjects.** *Thorax* 2001, **56**:902-906.
 49. Vrugt B, Wilson S, Bron A, Holgate ST, Djukanovic R, Aalbers R: **Bronchial angiogenesis in severe glucocorticoid-dependent asthma.** *Eur Respir J* 2000, **15**:1014-1021.
 50. Hoshino M, Nakamura Y, Hamid QA: **Gene expression of vascular endothelial growth factor and its receptors and angiogenesis in bronchial asthma.** *J Allergy Clin Immunol* 2001, **107**:1034-1038.
 51. 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.
 52. Lee CG, Link H, Baluk P, Homer RJ, Chapoval S, Bhandari V, Kang MJ, Cohn L, Kim YK, McDonald DM *et al.*: **Vascular endothelial growth factor (VEGF) induces remodeling and enhances TH2-mediated sensitization and inflammation in the lung.** *Nat Med* 2004, **10**:1095-1103.
 53. Hallgren J, Pejler G: **Biology of mast cell tryptase. An inflammatory mediator.** *FEBS J* 2006, **273**:1871-1895.
 54. Akers IA, Parsons M, Hill MR, Hollenberg MD, Sanjar S, Laurent GJ, McNulty RJ: **Mast cell tryptase stimulates human lung fibroblast proliferation via protease-activated receptor-2.** *Am J Physiol Lung Cell Mol Physiol* 2000, **278**:L193-L201.
 55. Berger P, Perng DW, Thabrew H, Compton SJ, Cairns JA, McEuen AR, Marthan R, Tunon De Lara JM, Walls AF: **Tryptase and agonists of PAR-2 induce the proliferation of human airway smooth muscle cells.** *J Appl Physiol* 2001, **91**:1372-1379.
 56. Roth M, Johnson PR, Borger P, Bihl MP, Rüdiger JJ, King GG, Ge Q, Hostettler K, Burgess JK, Black JL *et al.*: **Dysfunctional interaction of C/EBPalpha and the glucocorticoid receptor in asthmatic bronchial smooth-muscle cells.** *N Engl J Med* 2004, **351**:560-574.
 57. Aceves SS, Newbury RO, Dohil R, Bastian JF, Broide DH: **Esophageal remodeling in pediatric eosinophilic esophagitis.** *J Allergy Clin Immunol* 2007, **119**:206-212.
 58. Ochkur SI, Jacobsen EA, Protheroe CA, Biechele TL, Pero RS, McGarry MP, Wang H, O'Neill KR, Colbert DC, Colby TV *et al.*: **Coexpression of IL-5 and eotaxin-2 in mice creates an eosinophil-dependent model of respiratory inflammation with characteristics of severe asthma.** *J Immunol* 2007, **178**:7879-7889.