

## EDITORIAL

### What can be learned about the fibrosis of asthma from rodent models?

There have been many attempts to model asthma in animals and simple models of inflammation are well documented and validated showing inflammatory cell infiltration in response to repeated allergen challenge. Eosinophilic infiltration has been considered to be a good indication that the models might parallel the human disease, although recent studies suggest that even in humans the causal link to eosinophilia is not as central as we had thought [1]. One of the clearest structural changes in human asthmatic airways, but one of the most difficult aspects to convincingly model, has been the increased subepithelial fibrosis reflecting collagen deposition [2].

Two papers published in this issue of *Clinical and Experimental Allergy* [3, 4] have used rodent models to examine cellular mechanisms which might regulate fibrogenesis around the allergen-challenged airway. Corbel and colleagues [3] use a mouse model to examine the collagen expression in mice sensitized to ovalbumin and then repeatedly challenged intranasally with the allergen. In this system they see fibrotic changes by histology and increased total collagen in the lung after 16 weeks challenge. There is a peak in eosinophil numbers in bronchiolar lavage at 8 weeks and as might be expected, higher ongoing levels of infiltrating cells persist throughout the challenge period. If fibrotic disease simply involved increased deposition and reduced degradation of matrix proteins, we might expect to see reductions in matrix proteinases in fibrosis disease. However, in common with similar studies in other systems, Corbel et al. [3] see an overall increase in matrix metalloproteinases (MMPs) in their model at 8 weeks of treatment at the same time as increased numbers of infiltrating macrophages, eosinophils, lymphocytes and neutrophils. Looking in more detail at the gelatin zymography used to analyse the proteinase activity in the lavage samples immediately provides another perspective on these results. While total MMP levels go up, activated MMP-2 and MMP-9 are only detectable in 2/5 and 3/5 samples, respectively. Because of the destructive nature of proteinases, expression of their activity is tightly controlled not only by the normal transcriptional and translational controls of protein expression, but also by the requirement for zymogen activation and the coexpression of endogenous inhibitors, particularly the tissue inhibitors of metalloproteinases (TIMPs). While proteinases are essential to provide for protein breakdown, this activity is dangerous if expressed inappropriately. Outside the cell, matrix elements are well organized into complex structures showing that matrix synthesis and breakdown are tightly controlled. From developmental processes involving matrix remodelling it is clear that in order to achieve appropriate tissue architecture, control is very local. Experimental studies by Harty [5] have shown that while altered levels of cell-surface proteinases (MT-MMPs) on epithelial cells can modify a hepatocyte growth factor-driven morphogenic program of tubule formation, similarly changing levels of secreted MMPs has no effect. Another potential route of regulatory activity is the influence of MT1-MMP (MMP-14) activation on fibroblast

proliferation; the additional fibroblasts could in turn contribute to the matrix proteins [6].

The precise localization of proteinase activity in tissues is technically difficult to achieve but remains a key question in the proteinase field. This is further highlighted by the increasing recognition that proteolytic processes are involved in regulating many systems [7]. These include the activity of mediators and receptors by enzymes in the ADAM (a disintegrin and metalloproteinase) group of proteases such as TACE which cause membrane bound products to be shed from the cell [8]. The recent identification of ADAM 33 as an asthma-linked gene [9] only serves to further highlight the potential importance of proteases.

The overall amount of a protease present in a particular tissue, by zymography for example, indicates the existence or otherwise, of processes which potentially need proteases for any of these diverse functions. Overall proteinase levels in tissues therefore do not necessarily reflect activity at the site of matrix remodelling, whether this is breakdown or synthesis. Modest increases in proteinase expression in fibrotic disease if accompanied by increased levels of endogenous inhibitors such as TIMP-1, can still result in fibrosis as seen by Corbel et al. [3], although an increased sophistication in the analysis of the local functional changes underlying matrix deposition at any particular site is required.

#### The need for animal models

The disease, or collection of diseases, which we call asthma is clearly complex and chronic elements such as fibrosis could arise in many ways. It is clear however, that multiple cell types are involved and corresponding multi-cell models are needed. Only in animals do we find the necessary functional juxtaposition of cells and the complementary acquired immune system. While the mouse is an essential animal model system, we must also recognize the 50 million years of evolutionary adaptation to different environments which separates the two species [10]. Even at the simplest level, mice are small, short-lived laboratory rodents, adapted to ground living and are obligate nasal breathers. Humans are long-lived, with an erect posture and have a much larger respiratory system. Animal models also use a deliberately inbred population which cannot reflect the underlying heterogeneity of human disease processes – the models are more equivalent to non-atopic humans in a highly provocative environment not – as in real disease – allergic individuals in an environment where normal individuals show no response.

#### Allergens are important in allergic disease

It is easy to forget that allergens are heterogeneous and their properties are important. The profile of allergen sensitivity in

a population clearly shows that not all allergens are equally potent. Indeed some allergens such as the protease containing mite-derived allergens show such a preponderance of activity as sensitizing agents that there must be pre-disposing functional properties of the allergen and these need to be modelled. While the attractions of using ovalbumin as an allergen might be clear from an immunological view, naturally occurring respiratory disease provoked by ovalbumin is not a major concern: even in mice. Equally, the route of allergen challenge is important and it is unlikely to be a coincidence that the better models use challenge direct into the airway: as in the publication by Corbel et al. [3].

### **Mast cells are a direct target for allergens and might contribute to fibrosis**

The study by Masuda et al. in this issue [4] examines the contribution of mast cells in the lung fibrotic response to chronic allergen challenge. Mast cells are traditionally associated with allergy and inflammation but there is increasing evidence that these cells may have diverse biological functions such as the control of microvascular blood flow [11], smooth-muscle contractility and proliferation [12], angiogenesis [13, 14], as well as regulation of fibrogenesis. A number of histochemical studies have shown that mast cells accumulate in tissues undergoing fibrosis. Chronic increases in tissue mast cell numbers and secretory activity are a common feature of fibrosis in several organs, with the degree of mast cell hyperplasia being correlated with the extent of collagen deposition in scleroderma [15, 16], pulmonary fibrosis [17], cardiac fibrosis [18, 19], and liver fibrosis [20, 21]. In one of the few studies of mast cells in progressive fibrosis, Seibold et al. [15] found increased numbers of mast cells in involved skin in both early and late cutaneous systemic sclerosis and observed increased mast cell degranulation in early, but not in late disease, suggesting that increases in mast cell activity precede clinically apparent dermal fibrosis. However, studies showing a causal link between mast cell activity and fibrosis in humans are notable by their absence, although individuals with systemic mastocytosis sometimes develop fibrosis in various organs and especially in the liver [22].

### **Studies with mast cell-deficient rodents do not agree on the role of mast cells in fibrosis**

The availability of mast cell-deficient mice and rats has allowed a clearer understanding of the role of mast cells in various disease models in rodents – although the well described differences between rodent and human mast cells with regard to mediator content and response to stimuli complicates the extrapolating of these models to human physiology and pathology. Masuda et al. in this issue [4] use mast cell-deficient mice to examine the role of these cells in the development of inflammation and fibrosis in a murine model of allergic asthma using animals sensitized with albumin and subsequently challenged with the protein by daily inhalation over three weeks. In both the WB6F1-W/W<sup>v</sup> (c-kit mutant) and SI/SI<sup>d</sup> (c-kit ligand mutant) mice, this regime of repeated allergen provocation induced a similar degree of bronchial hyper-responsiveness to

acetylcholine and airway inflammation. This suggests that mast cell deficiency had no effect on inflammatory response to allergen which is a key issue as the inflammatory response is a major determinant of subsequent fibrosis. However, development of fibrosis was reduced in the mast cell-deficient animals when extracellular matrix accumulation was assessed by either hydroxyproline content of lung or by image analysis of the subepithelial area stained by Van Gieson elastica to show connective tissue. The reported reduction in connective tissue staining was ~29% in WB6F1-W/W<sup>v</sup> mice and ~32% in SI/SI<sup>d</sup> mice, with smaller differences in hydroxyproline reduction. Reconstitution of the WB6F1-W/W<sup>v</sup> mice with mast cells by injection returned the fibrotic response of the animals to normal. This provides crucial support for a role of mast cells in the fibrotic response, rather than as some consequence of the c-kit/kit ligand deficiency unrelated to mast cells. The facilitatory role of mast cells in lung fibrosis reported by Masuda and colleagues [4] is supported by previous studies [23] showing that silica-induced lung fibrosis was diminished in WB6F1-W/W<sup>v</sup> mice. However, other studies with mast cell-deficient rodents suggest that mast cells make little contribution to fibrosis, or indeed might have an anti-fibrotic function. Collagen content of scleroderma lesions induced by 4 weeks of intradermal bleomycin treatment was uninfluenced by mast cell deficiency – although the fibrosis developed more rapidly in the normal mice in the first week suggesting mast cells may accelerate fibrogenesis [24]. Okazaki et al. [25] showed that collagen accumulation in chronically injured lung and liver in mast cell-deficient Ws/Ws rats (which have a critical deletion at the tyrosine kinase domain of the c-kit gene) was no less than in normal rats – indeed the deficient rats had more collagen accumulation. Sugihara et al. [26] made similar conclusions – that mast cell deficiency did not inhibit liver fibrosis – using WB6F1-W/W<sup>v</sup> mice and Ws/Ws rats in two separate models of liver injury. Similarly, Mori et al. [27] showed that there was little histological difference between lungs of normal mice or WB6F1-W/W<sup>v</sup> mice treated for 10 days with bleomycin, and again there were indications that collagen deposition was actually greater in the latter mice. In the light of these findings, it would be interesting to delve further into the mechanisms explaining why mast cells were required for the full fibrotic response in the model of Masuda et al. [4]. Taking the various studies of fibrosis in mast cell-deficient rodents into account, it appears that the contribution of mast cells to fibrosis might be tissue and injury dependent. Furthermore, in those cases where mast cells appear to regulate fibrosis, much more information is required about the mechanisms involved which necessitates investigating the many variables regulating matrix deposition such as matrix synthesis, TIMP and MMP expression. However, given the current lack of accord on the role of mast cells in fibrosis in rodents, the jury is still very much out on whether mast cells contribute to the development of fibrosis in humans.

### **Matrix homeostasis is complex, but TGF- $\beta$ is accepted as a key player**

Extracellular matrix deposition is a complex phenomenon depending not only on synthesis of matrix proteins but also the expression of matrix-degrading pathways. A key family of

enzymes mediating matrix degradation are the MMPs. As mentioned above, these are antagonized by the TIMPs. Post-secretory modifications to matrix such as cross-linking by lysyl oxidase or tissue transglutaminase may also enhance its stability towards MMPs [28]. Studies of fibrosis in liver, kidney and lung [29, 30] suggest that TIMPs contribute to collagen accumulation by stabilizing matrix against degradation by MMPs. Therefore, mast cells might promote lung fibrosis by a combination of: augmenting matrix synthesis by resident fibroblasts or myofibroblasts; down-regulating MMP expression; and increasing TIMP expression. Although mast cells themselves apparently have limited ability to synthesize extracellular matrix, it is well established that mast cells produce a plethora of mediators which in *in vitro* culture studies either enhance fibroblast proliferation and/or promote fibroblast matrix production. These include tryptase, histamine, IL-4, heparin and basic fibroblast growth factor [31–33]. Mast cells may also promote fibrosis by producing TIMP-1 [34], although  $\alpha$ -chymase which is released abundantly by human mast cells reportedly inactivates TIMP-1 [35] which would limit its inhibitory efficacy. Human, mouse and dog mast cells also produce TGF- $\beta$  which is a potent stimulator of both extracellular matrix and TIMP-1 synthesis by fibroblasts, while simultaneously inhibiting their MMPs synthesis [36–38]. TGF- $\beta$  is a key player in organ fibrosis and induced over-expression of TGF- $\beta$  in mouse lung has been shown to cause rapid and extensive fibrosis, greatly in excess of the levels reported in these animal models [39]. TGF- $\beta$  neutralizing antibodies and receptor antagonists are also proving to be effective anti-fibrotics *in vivo* [40–42]. However, Masuda et al. [4] found no difference in total TGF- $\beta$  expression in bronchoalveolar lavage fluid (BALF) of mast cell-deficient vs. normal mice. A key step regulating TGF- $\beta$  activity is the activation of its latent form by proteolysis, and reports suggest that mast cell chymase can achieve this [43]. BALF levels of endogenous active TGF- $\beta$  were not reported in the study of Masuda et al. [4], so potential differences in activation within the normal vs. mast cell-deficient mice remain unknown. In contrast, Corbel et al. [3] report increased TGF- $\beta$  in their model although since the activation state of the TGF- $\beta$  is unclear, and no increase is seen until 36 weeks, it may reflect other ongoing processes by this stage of sensitization. TGF- $\beta$  activation might be intimately related to the expression of MMPs by infiltrating inflammatory cells and resident cells during matrix remodelling in inflamed airway as MMP-9 and -14 can activate latent TGF- $\beta$  [44, 45].

Certainly, TGF- $\beta$  is also involved in the interactions between epithelial and mesenchymal tissues both in development and wound healing [46] and is produced by repairing epithelial cells [47].

## Conclusions

Animal models such as that set up by Corbel et al. [3] and Masuda et al. [4] reflect the range of cells, from infiltrating and resident inflammatory cells to structural epithelial and connective tissue fibroblasts, which we expect to be involved in human airway disease. They also encompass the potential complexity of interaction between cells and the dynamic processes of matrix synthesis and degradation and the regulation of the balance

between these. If we could understand how these processes are controlled in the presence of infiltrating cells and resident mast cells – and there is still a long way to go – we would be in a significantly better position to understand fibrosis in human allergic disease. One caveat in extrapolating to human data from rodent models, apart from the known differences in mast cell phenotype discussed above, is that rodent models develop fibrosis very rapidly, implying that there is a rapid and profound dysfunction in connective tissue homeostasis. In contrast, development of fibrosis in humans is much slower and might involve a more subtle but prolonged imbalance in matrix deposition vs. degradation. However, rodent models are irreplaceable and when used carefully as analytical tools can yield exciting results which inform the necessarily rather descriptive studies of human asthma. What is equally clear however, is that they cannot yet provide a model of asthma and if we attempt to use them as such we are only likely to be misled.

## References

- 1 Leckie MJ, ten Brinke A, Khan J 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.
- 2 Roche WR, Beasley R, Williams JH, Holgate ST. Subepithelial fibrosis in the bronchi of asthmatics. *Lancet* 1989; 1:520–4.
- 3 Corbel M, Caulet-Maugendre S, Germain N, Lagente V, Biochet E. Enhancement of gelatinase activity during development of subepithelial fibrosis in a murine model of asthma. *Clin Exp Allergy* 2003; 33:695–703.
- 4 Masuda T, Tanaka H, Komai M et al. Mast cells play a partial role in allergen-induced subepithelial fibrosis in a murine model of allergic asthma. *Clin Exp Allergy* 2003; 33:704–12.
- 5 Hotary K, Allen E, Punturieri A, Yana I, Weiss SJ. Regulation of cell invasion and morphogenesis in a three-dimensional type I collagen matrix by membrane-type matrix metalloproteinases 1, 2, and 3. *J Cell Biol* 2000; 149:1309–23.
- 6 Xu J, Benyon RC, Leir SH, Zhang S, Holgate ST, Lackie PM. Matrix metalloproteinase-2 from bronchial epithelial cells induces the proliferation of subepithelial fibroblasts. *Clin Exp Allergy* 2002; 32:881–8.
- 7 Shapiro SD. Matrix metalloproteinase degradation of extracellular matrix: biological consequences. *Curr Opin Cell Biol* 1998; 10:602–8.
- 8 Black RA, White JM. ADAMs: focus on the protease domain. *Curr Opin Cell Biol* 1998; 10:654–9.
- 9 Van Eerdewegh P, Little RD, Dupuis J et al. Association of the ADAM33 gene with asthma and bronchial hyperresponsiveness. *Nature* 2002; 418:426–30.
- 10 Gordon CJ, Grafton G, Wood PM, Larche M, Armitage RJ. Modelling the human immune response: can mice be trusted? *Commentary Curr Opin Pharmacol* 2001; 1:431–5.
- 11 Hammarlund A, Olsson P, Pipkorn U. Dermal blood flow after local challenges with allergen, histamine, bradykinin and compound 48/80. *Clin Exp Allergy* 1991; 21:333–42.
- 12 Page S, Ammit AJ, Black JL, Armour CL. Human mast cell and airway smooth muscle cell interactions: implications for asthma. *Am J Physiol Lung Cell Mol Physiol* 2001; 281:L1313–23.
- 13 Levi-Schaffer F, Pe'er J. Mast cells and angiogenesis. *Clin Exp Allergy* 2001; 31:521–4.
- 14 Blair RJ, Meng H, Marchese MJ et al. Human mast cells stimulate vascular tube formation. Tryptase is a novel, potent angiogenic factor. *J Clin Invest* 1997; 99:2691–700.

- 15 Seibold JR, Giorno RC, Claman HN. Dermal mast cell degranulation in systemic sclerosis. *Arthritis Rheum* 1990; 33:1702–9.
- 16 Claman HN. On scleroderma. Mast cells, endothelial cells, and fibroblasts. *JAMA* 1989; 262:1206–9.
- 17 Kawanami O, Ferrans VJ, Fulmer JD, Crystal RG. Ultrastructure of pulmonary mast cells in patients with fibrotic lung disorders. *Laboratory Invest* 1979; 40:717–34.
- 18 Panizo A, Mindan FJ, Galindo MF, Cenarruzabeitia E, Hernandez M, Diez J. Are mast cells involved in hypertensive heart disease? *J Hypertens* 1995; 13:1201–8.
- 19 Li QY, Raza Ahmad A, MacAulay MA et al. The relationship of mast cells and their secreted products to the volume of fibrosis in posttransplant hearts. *Transplantation* 1992; 53:1047–51.
- 20 Rioux KP, Sharkey KA, Wallace JL, Swain MG. Hepatic mucosal mast cell hyperplasia in rats with secondary biliary cirrhosis. *Hepatology* 1996; 23:888–95.
- 21 Farrell DJ, Hines JE, Walls AF, Kelly PJ, Bennett MK, Burt AD. Intrahepatic mast cells in chronic liver diseases. *Hepatology* 1995; 22:1175–81.
- 22 Mican JM, Di Bisceglie AM, Fong TL et al. Hepatic involvement in mastocytosis: clinicopathologic correlations in 41 cases. *Hepatology* 1995; 22:1163–70.
- 23 Suzuki N, Horiuchi T, Ohta K et al. Mast cells are essential for the full development of silica-induced pulmonary inflammation: a study with mast cell-deficient mice. *Am J Respir Cell Mol Biol* 1993; 9:475–83.
- 24 Yamamoto T, Takahashi Y, Takagawa S, Katayama I, Nishioka K. Animal model of sclerotic skin. II. Bleomycin induced scleroderma in genetically mast cell deficient WBB6F1-W/W(V) mice. *J Rheumatol* 1999; 26:2628–34.
- 25 Okazaki T, Hirota S, Xu ZD et al. Increase of mast cells in the liver and lung may be associated with but not a cause of fibrosis: demonstration using mast cell-deficient Ws/Ws rats. *Laboratory Invest* 1998; 78:1431–8.
- 26 Sugihara A, Tsujimura T, Fujita Y, Nakata Y, Terada N. Evaluation of role of mast cells in the development of liver fibrosis using mast cell-deficient rats and mice. *J Hepatol* 1999; 30:859–67.
- 27 Mori H, Kawada K, Zhang P, Uesugi Y, Sakamoto O, Koda A. Bleomycin-induced pulmonary fibrosis in genetically mast cell-deficient WBB6F1-W/Wv mice and mechanism of the suppressive effect of tranilast, an antiallergic drug inhibiting mediator release from mast cells, on fibrosis. *Int Arch Allergy Appl Immunol* 1991; 95:195–201.
- 28 Vater CA, Harris EDJ, Siegel RC. Native cross-links in collagen fibrils induce resistance to human synovial collagenase. *Biochem J* 1979; 181:639–45.
- 29 Selman M, Ruiz V, Cabrera S et al. TIMP-1, -2, -3, and -4 in idiopathic pulmonary fibrosis. A prevailing nondegradative lung microenvironment? *Am J Physiol Lung Cell Mol Physiol* 2000; 279: L562–74.
- 30 Benyon RC, Arthur MJ. Mechanisms of hepatic fibrosis. *J Pediatr Gastroenterol Nutr* 1998; 27:75–85.
- 31 Garbuzenko E, Nagler A, Pickholtz D et al. Human mast cells stimulate fibroblast proliferation, collagen synthesis and lattice contraction: a direct role for mast cells in skin fibrosis. *Clin Exp Allergy* 2002; 32:237–46.
- 32 Levi Schaffer F, Rubinchik E. Mast cell role in fibrotic diseases. *Isr J Med Sci* 1995; 31:450–3.
- 33 Gruber BL. Mast cells: accessory cells which potentiate fibrosis. *Int Rev Immunol* 1995; 12:259–79.
- 34 Di Girolamo N, Wakefield D. In vitro and in vivo expression of interstitial collagenase/MMP-1 by human mast cells. *Dev Immunol* 2000; 7:131–42.
- 35 Frank BT, Rossall JC, Caughey GH, Fang KC. Mast cell tissue inhibitor of metalloproteinase-1 is cleaved and inactivated extracellularly by alpha-chymase. *J Immunol* 2001; 166:2783–92.
- 36 Kanbe N, Kurosawa M, Nagata H, Yamashita T, Kurimoto F, Miyachi Y. Production of fibrogenic cytokines by cord blood-derived cultured human mast cells. *J Allergy Clin Immunol* 2000; 106:85–90.
- 37 Gordon JR. TG Fbeta1 and TNFalpha secreted by mast cells stimulated via the FcepsilonRI activate fibroblasts for high-level production of monocyte chemoattractant protein-1 (MCP-1). *Cell Immunol* 2000; 201:42–9.
- 38 Pennington DW, Lopez AR, Thomas PS, Peck C, Gold WM. Dog mastocytoma cells produce transforming growth factor beta 1. *J Clin Invest* 1992; 90:35–41.
- 39 Gauldie J, Sime PJ, Xing Z, Marr B, Tremblay GM. Transforming growth factor-beta gene transfer to the lung induces myofibroblast presence and pulmonary fibrosis. *Curr Top Pathol* 1999; 93:35–45.
- 40 George J, Roulot D, Kotliansky VE, Bissell DM. In vivo inhibition of rat stellate cell activation by soluble transforming growth factor beta type II receptor: a potential new therapy for hepatic fibrosis. *Proc Natl Acad Sci USA* 1999; 96:12719–24.
- 41 Qi Z, Atsuchi N, Ooshima A, Takeshita A, Ueno H. Blockade of type beta transforming growth factor signaling prevents liver fibrosis and dysfunction in the rat. *Proc Natl Acad Sci USA* 1999; 96:2345–9.
- 42 Wang Q, Hyde DM, Gotwals PJ, Giri SN. Effects of delayed treatment with transforming growth factor-beta soluble receptor in a three-dose bleomycin model of lung fibrosis in hamsters. *Exp Lung Res* 2002; 28:405–17.
- 43 Lindstedt KA, Wang Y, Shiota N et al. Activation of paracrine TGF-beta1 signaling upon stimulation and degranulation of rat serosal mast cells: a novel function for chymase. *FASEB J* 2001; 15:1377–88.
- 44 Mu D, Cambier S, Fjellbirkeland L et al. The integrin alpha (v) beta8 mediates epithelial homeostasis through MT1-MMP-dependent activation of TGF-beta1. *J Cell Biol* 2002; 157:493–507.
- 45 Yu Q, Stamenkovic I. Cell surface-localized matrix metalloproteinase-9 proteolytically activates TGF-beta and promotes tumor invasion and angiogenesis. *Genes Dev* 2000; 14:163–76.
- 46 O'Kane S, Ferguson MW. Transforming growth factor beta's and wound healing. *Int J Biochem Cell Biol* 1997; 29:63–78.
- 47 Howat WJ, Holgate ST, Lackie PM. TGF-beta isoform release and activation during in vitro bronchial epithelial wound repair. *Am J Physiol Lung Cell Mol Physiol* 2002; 282:L115–23.

P. M. LACKIE and R. C. BENYON\*  
*Respiratory Cell and Molecular Biology and*  
*\*Tissue Remodelling and Repair Division of*  
*Infection Inflammation and Repair*  
*University of Southampton*  
*Southampton General Hospital*  
*Southampton SO16 6YD*  
 UK  
 E-mail: p.m.lackie@soton.ac.uk