

EDITORIAL

Comparative, complementary and relevant: the immunological basis of ovine lung allergic responses

The UK has one of the highest prevalence rates for asthma in the world with an estimated 5.1 million people – 1 in 13 adults and 1 in 8 children – currently being treated. The high prevalence of this condition is reflected in the 74 000 emergency hospital admissions for asthma each year, and its life-threatening nature in the 1500 deaths/year that occur as a consequence of this condition [1]. The incidence appears to be increasing with the number of new cases of asthma each year being three to four times higher in adults and six times higher in children than it was 25 years ago [1].

Characterized by variable and reversible airway obstruction, eosinophilic airway inflammation and bronchial hyper-reactivity [2], allergic asthma is also strongly associated with atopy, characterized by increased levels of total IgE and IgE specific for common environmental allergens.

Our current level of understanding of the immunologic basis of allergy has benefited from studies using animal model systems, particularly those based on mice. In essence, from the original discovery by Mosmann et al. that mouse CD4⁺ T cells could be characterized into two populations on the basis of their cytokine profiles [3, 4], and the discovery that these profiles importantly influence disease outcome following protozoal infection [5, 6] we are now able to recognize that many of the features of atopic asthma are driven by Th2 cytokines. Such model systems have thus played a fundamental role in defining our current level of understanding of allergic asthma and will continue to do so.

Over the last 8 years or so there have been a number of studies that have focused on the attendant functional and pathologic features of experimentally-induced allergic airway disease in mice. These studies have quite naturally focused on similarities, where they exist, with the human phenotype and in conjunction with the ability to dissect the fine aspects of the immune and inflammatory response have offered an important insight into possible mechanisms underlying human disease pathogenesis.

However, as with any animal model system, it is important to be aware of the limits of the system as these limits define the validity of any extrapolations made beyond the species under study.

This caveat is particularly relevant to studies directed at understanding the mechanisms underlying airway hyper-responsiveness. Of the wide variety of agents that induce bronchospasm in asthmatics, only cholinergic agonists, serotonin and endothelins evoke bronchospasm in mice. Similarly, mast cells, recognized as key effector cells in human asthma [7] are present in only scant numbers in mouse lung tissue [8] and indeed the *in vitro* response of murine mast cells to a variety of stimuli often fails to mimic the response of human mast cells to the same stimuli [9, 10].

In contrast to human airways, mouse airways lack a bronchial circulation, are only sparsely furnished with afferent nerves and have no relaxant innervation, which in effect means that mice

will fail to cough in response to stimuli and may fail to mimic other clinically relevant features of asthma such as the bronchoconstrictor effect of deep inspiration.

Whilst it is important to appreciate that no single animal model system will faithfully replicate all the characteristic features of asthma, it is equally important that due regard is paid to the comparative species in their airway response to allergic inflammation as it is worth speculating that further understanding of such diversity will hold clues to unravelling species-specific mechanisms.

In this issue of *Clinical and Experimental Allergy*, Bischoff et al. demonstrate, using a local lung challenge model in sensitized sheep, an allergic response to house dust mite (HDM) [11] the characteristics of which hold 'similarities to human asthmatic disease' and indicate the potential of the model 'as a useful tool for studies of the immunological and physiological basis of allergic asthma'. Whilst undoubtedly indicating an underlying confidence in the relevance of this model, it is important to consider these claims in relation to both the current study and the extensive history of using sheep to model allergic asthma.

In a proportion of sheep having demonstrable skin test reactivity to *A. suum* extract, immediate bronchospasm and pulmonary hyperinflation is elicited in the conscious animal on exposure to aerosolized antigen [12]. A further proportion of these 'early responders', referred to as 'dual-responders' go on to develop a late bronchospastic response at 7–8 h [13] and non-specific airway responsiveness at 24 h [14], the latter persisting for 14 days [15] post-antigen exposure.

The model has been extensively characterized with the early response linked to degranulation of mast cells and release of histamine [16] with attendant immediate bronchospastic effects on airway calibre and increased bronchial blood flow [17]. Indeed, pre-emptive corticosteroid treatment [18], mast cell stabilizers [19, 20], calcium antagonists [21], and heparin [22–25] significantly attenuate the early response. In the latter instance the effect is confined to sheep that only have an early response [26]. The blocking effect is dependant on the molecular weight (MW) of the heparin [27–29] and may be mediated through inhibition of the inositol triphosphate pathway [26] the predominant second messenger in early responders.

Dual responders are distinguished from early responders in developing a more pronounced inflammatory response over the next 24 h. Although in numerical terms the inflammation is dominated by neutrophils in both groups [30] there is an associated significant increase in the number of eosinophils in the airway wall and bronchoalveolar space of the dual responders only [30, 31]. Reactive oxygen species are also increased in the airway epithelium [31]. The inflammation, which can be abrogated by corticosteroids [13], specific targeting of adhesion molecules expressed by leucocytes [15, 32–35], and anti-proteases [36–39] is accompanied by a visible increase in the

quantity of tracheal mucus and a significant and prolonged decrease in tracheal mucus velocity [40, 41]. Amongst the mediators released as a consequence of initial exposure to antigen, leukotrienes and prostaglandins [16, 42–53], platelet activating factor [54, 55], tissue kallikrein [56] and bradykinin [57, 58] are believed to contribute towards the physiologic expression of the late response.

The question arises as to whether such pronounced functional effects and attendant evidence of inflammation are reflected in histopathological evidence of abnormality. Studies by Chen et al. demonstrated that challenge with *A. suum* antigen was not associated with profound effects on the cellular composition of the lower respiratory tract as assessed 14 days after challenge [59]. No significant difference between responders and non-responders was observed with respect to the numerical density of mast cells and eosinophils, or the observed degree of degranulation of mast cells although responders had a significant increase in the numerical density of mast cell secretory granules [59]. Similarly, although hypersensitive sheep had a thinner epithelium in medium bronchi and bronchioles, fewer goblet cells in bronchioles, and greater gland area at most airway levels, changes were generally mild [60].

Given the acute and intense nature of the allergen challenge it is perhaps not surprising that the chronic and pronounced changes that typify asthma, namely thickening of the lamina reticularis, mucous gland hypertrophy, goblet and epithelial cell hypertrophy and hyperplasia, smooth muscle hypertrophy and pronounced eosinophil infiltration are not present to any significant degree. A chronic antigenic challenge protocol, involving intratracheal instillation every 2 weeks over a period of 9 months was employed by Bosse et al. [61]. These researchers were able to document an increase in lung resistance and functional residual capacity, and, in the bronchoalveolar space, increased eosinophils and histamine, and depressed cAMP [61]. Whether such effects reflected underlying structural change in the airways was not discussed.

So where does the experimental model presented by Bischoff et al. lie in relation to the considerable volume of published literature that surrounds existing ovine models of human allergic asthma?

In the first instance, these authors have sought to define the immunological features of the lung allergic response. In this regard the use of house dust mite represents a welcome and shrewd initiative in that, in addition to the well-recognized relevance of this allergen to human asthma, sensitization and challenge with an allergen to which sheep would be unlikely to be naturally exposed facilitates interpretation of immunological and cellular responses in relation to a defined exposure history. This contrasts with the situation relating to *A. suum* where allergic skin reactivity can develop in sheep unlikely to have had prior contact with this antigen [62]. Whether this latter reactivity represents cross-reaction with similar mite or nematode antigens to which the sheep have been exposed is unknown.

In the second instance these authors adopt a local lung challenge protocol to evaluate pulmonary responses to allergen. Segmental approaches have hitherto proved valuable in the context of defining, at functional, cellular and immune levels, the local lung response to antigen challenge in both experimental animals and in humans [63–71].

Systemic immunization with 50 µg HDM in Alum resulted in the development of allergic reactivity, on the basis of HDM-

specific IgE response, in 6 out of 10 sheep. Forty-eight hours after local lung challenge with HDM antigen, a significant increase in the level of eosinophils in blood, bronchoalveolar lavage and lung tissue occurred [11]. Similar trends, which attained significance in the case of bronchoalveolar eosinophil influx, were apparent for non-allergic sheep following challenge [11]. The increase in peribronchial eosinophils and activated CD4⁺ T cells is notable both when viewed in the context of human asthma and in relation to the aforementioned observations by Chen et al. that no such infiltrates were apparent 14 days after challenge with *A. suum* antigen [59].

One aspect of this and other studies is the tendency to pre-select groups on the basis of given functional responses, whether these relate to immunological, physiological or clinical endpoints. Whilst such groupings undoubtedly facilitate robust statistical scrutiny and thus help define the mechanisms underlying observed differences, they do tend to feed the notion that dichotomous responses are a feature of the wider population rather than there being a continuum of response. Subjective evaluation of the data presented by Bischoff et al. [11] suggests that HDM sensitization and challenge generates a continuum rather than dichotomous cellular and immunological response in sheep.

It is well recognized that, in common with other species, ovine mast cell populations are heterogenous with respect to morphology, histochemical characteristics and granule proteinase content [72, 73]. As the predominant cell type involved in the immediate airway response to allergen and the putative architect of subsequent eosinophilic inflammation, dynamic flux in the relative proportions of phenotypically distinct mast cell populations may have important bearing on lung allergic responses. It is therefore important to consider what factors are likely to play a role in influencing the makeup of mast cell populations in the lung particularly in relation to existing and proposed ovine models of asthma.

Prior exposure to nematode antigen will have a profound bearing on pulmonary mast cell populations. Indeed, the pathology of patent lungworm (*Dictyocaulus* spp.) infection in ruminants is characterized by chronic catarrhal bronchitis and bronchiolitis, bronchial epithelial and mucous cell hyperplasia, increased peribronchiolar fibrous tissue and smooth muscle, and prominent eosinophil and mast cell infiltrates [74], and the local lung response to recombinant lungworm antigen in sensitized sheep is characterized by increased ratios of sheep mast cell proteinase-1-expressing cells and tryptase-expressing cells, to toluidine blue positive cells in airways [71].

Wherein nematode infections are ubiquitous within small ruminants, it is tempting to suggest that such infections could play a predominant role in shaping pulmonary mast cell populations, particularly at the mucosal interface.

Similarly, the potential role of central neural networks in mediating dynamic flux in mucosal mast cell populations should be borne in mind given the close morphological association that exists between mast cells and neuropeptide-containing nerves [75] and the evidence that suggests that epithelium, nerves and mast cells do indeed interact in a functional manner in the lung [76, 77]. Certainly, the observation that neuropeptides are capable of lowering the threshold parasite antigen concentration for mast cell degranulation in the sheep [78] adds credence to the notion that mechanisms exist to tailor effector responses to the nature and level of parasitic

insult and that such prior history is pertinent to interpreting 'allergic' mechanisms that have their evolutionary roots intertwined in parasite : host defence issues.

Notwithstanding the potential influence of these and other factors in the response of sheep to sensitization and challenge with allergen it is encouraging that steps are finally being taken to characterize the immunological aspects of the ovine lung allergic response. Indeed, such progress will presumably look to take advantage of the sweeping progress in genomic and proteomic technology that will undoubtedly impinge on our ability to more comprehensively dissect comparative disease mechanisms over the next several years. Whether such steps will fully validate this particular model at the functional and pathological level remains to be seen; however, it is with some anticipation that we can look forward to this story unfold.

References

- National Asthma Campaign. Asthma Audit 2001. *Asthma J* 2001; 6:1–14.
- Mygind N, Dahl R, Thestrup-Pedersen K, eds. *Essential Allergy*. Oxford: Blackwell Science, 2002.
- Mosmann TR, Cherwinski H, Bond MW, Giedlin MA, Coffman RL. Two types of murine helper T cell clone. I. Definition according to profiles of lymphokine activities and secreted proteins. *J Immunol* 1986; 136:2348–57.
- Mosmann TR, Coffman RL. TH1 and TH2 cells: different patterns of lymphokine secretion lead to different functional properties. *Annu Rev Immunol* 1989; 7:145–73.
- Heinzel FP, Sadick MD, Holaday BJ, Coffman RL, Locksley RM. Reciprocal expression of interferon gamma or interleukin 4 during the resolution or progression of murine leishmaniasis. Evidence for expansion of distinct helper T cell subsets. *J Exp Med* 1989; 169:59–72.
- Scott P, Natovitz P, Coffman RL, Pearce E, Sher A. Immunoregulation of cutaneous leishmaniasis. T cell lines that transfer protective immunity or exacerbation belong to different T helper subsets and respond to distinct parasite antigens. *J Exp Med* 1988; 168:1675–84.
- Rossi GL, Olivieri D. Does the mast cell still have a key role in asthma? *Chest* 1997; 112:523–9.
- Gersch C, Dewald O, Zoerlein M, Michael LH, Entman ML, Frangogiannis NG. Mast cells and macrophages in normal C57/BL/6 mice. *Histochem Cell Biol* 2002; 118:41–9.
- Ali H, Leung KB, Pearce FL, Hayes NA, Foreman JC. Comparison of the histamine-releasing action of substance P on mast cells and basophils from different species and tissues. *Int Arch Allergy Appl Immunol* 1986; 79:413–8.
- Pearce FL, Al Laith M, Bosman L et al. Effects of sodium cromoglycate and nedocromil sodium on histamine secretion from mast cells from various locations. *Drugs* 1989; 37 (Suppl. 1):37–43.
- Bischoff R, Snibson K, Shaw R, Meeusen ENT. Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite. *Clin Exp Allergy* 2003; 33:367–75.
- Wanner A, Mezey RJ, Reinhart ME, Eyre P. Antigen-induced bronchospasm in conscious sheep. *J Appl Physiol* 1979; 47:917–22.
- Abraham WM, Delehunt JC, Yerger L, Marchette B. Characterization of a late phase pulmonary response after antigen challenge in allergic sheep. *Am Rev Respir Dis* 1983; 128:839–44.
- Abraham WM, Lanes S, Stevenson JS, Yerger LD. Effect of an inhaled glucocorticosteroid (budesonide) on post-antigen induced increases in airway responsiveness. *B Eur Physiopath Res* 1986; 22:387–92.
- Abraham WM, Sielczak MW, Ahmed A et al. Alpha 4-integrins mediate antigen-induced late bronchial responses and prolonged airway hyperresponsiveness in sheep. *J Clin Invest* 1994; 93:776–87.
- Okayama H, Aikawa T, Ohtsu H, Sasaki H, Takishima T. Leukotriene C4 and B4 in bronchoalveolar lavage fluid during biphasic allergic bronchoconstriction in sheep. *Am Rev Respir Dis* 1989; 139:725–31.
- Long WM, Yerger LD, Martinez H et al. Modification of bronchial blood flow during allergic airway responses. *J Appl Physiol* 1988; 65:272–82.
- Delehunt JC, Yerger L, Ahmed T, Abraham WM. Inhibition of antigen-induced bronchoconstriction by methylprednisolone succinate. *J Allergy Clin Immunol* 1984; 73:479–83.
- Abraham WM, Stevenson JS, Chapman GA, Tallent MW, Jackowski J. The effect of nedocromil sodium and cromolyn sodium on antigen-induced responses in allergic sheep in vivo and in vitro. *Chest* 1987; 92:913–7.
- Abraham WM, Stevenson JS, Eldridge M, Garrido R, Nieves L. Nedocromil sodium in allergen-induced bronchial responses and airway hyperresponsiveness in allergic sheep. *J Appl Physiol* 1988; 65:1062–8.
- D'Brot J, Abraham WM, Ahmed T. Effect of calcium antagonist gallopamil on antigen-induced early and late bronchoconstrictor responses in allergic sheep. *Am Rev Respir Dis* 1989; 139:915–20.
- Ahmed T, Syriste T, Mendelssohn R et al. Heparin prevents antigen-induced airway hyperresponsiveness: interference with IP3-mediated mast cell degranulation? *J Appl Physiol* 1994; 76:893–901.
- Ahmed T, Syriste T, Lucio J, Abraham W, Robinson M, D'Brot J. Inhibition of antigen-induced airway and cutaneous responses by heparin: a pharmacodynamic study. *J Appl Physiol* 1993; 74:1492–8.
- Lucio J, D'Brot J, Guo CB et al. Immunologic mast cell-mediated responses and histamine release are attenuated by heparin. *J Appl Physiol* 1992; 73:1093–101.
- Ahmed T, Abraham WM, D'Brot J. Effects of inhaled heparin on immunologic and nonimmunologic bronchoconstrictor responses in sheep. *Am Rev Respir Dis* 1992; 145:566–70.
- Ahmed T, D'Brot J, Abraham WM et al. Heterogeneity of allergic airway responses in sheep: differences in signal transduction? *Am J Respir Crit Care Med* 1996; 154:843–9.
- Martinez-Salas J, Mendelssohn R, Abraham WM, Hsiao B, Ahmed T. Inhibition of allergic airway responses by inhaled low-molecular-weight heparins: molecular-weight dependence. *J Appl Physiol* 1998; 84:222–8.
- Molinari JF, Campo C, Shakir S, Ahmed T. Inhibition of antigen-induced airway hyperresponsiveness by ultralow molecular-weight heparin. *Am J Respir Crit Care Med* 1998; 157:887–93.
- Ahmed T, Ungo J, Zhou M, Campo C. Inhibition of allergic late airway responses by inhaled heparin-derived oligosaccharides. *J Appl Physiol* 2000; 88:1721–9.
- Abraham WM, Sielczak MW, Wanner A et al. Cellular markers of inflammation in the airways of allergic sheep with and without allergen-induced late responses. *Am Rev Respir Dis* 1988; 138:1565–71.
- Lieberman H, Mariassy AT, Sorace D, Suster S, Abraham WM. Morphometric estimation of superoxide generation in allergen-induced airway hyperresponsiveness. *Laboratory Invest* 1995; 72:348–54.
- Abraham WM, Ahmed A, Sielczak MW, Narita M, Arrhenius T, Elices MJ. Blockade of late-phase airway responses and airway hyperresponsiveness in allergic sheep with a small-molecule peptide inhibitor of VLA-4. *Am J Respir Crit Care Med* 1997; 156:696–703.
- Lin K, Ateeq HS, Hsiung SH et al. Selective, tight-binding inhibitors of integrin alpha 4 beta 1 that inhibit allergic airway responses. *J Med Chem* 1999; 42:920–34.
- Abraham WM, Gill A, Ahmed A et al. A small-molecule, tight-binding inhibitor of the integrin alpha (4) beta (1) blocks

- antigen-induced airway responses and inflammation in experimental asthma in sheep. *Am J Respir Crit Care Med* 2000; 162:603–11.
- 35 Abraham WM, Ahmed A, Sabater JR et al. Selectin blockade prevents antigen-induced late bronchial responses and airway hyperresponsiveness in allergic sheep. *Am J Respir Crit Care Med* 1999; 159:1205–14.
 - 36 Wright CD, Havill AM, Middleton SC et al. Secretory leukocyte protease inhibitor prevents allergen-induced pulmonary responses in animal models of asthma. *J Pharm Exp Therap* 1999; 289:1007–14.
 - 37 Clark JM, Abraham WM, Fishman CE et al. Tryptase inhibitors block allergen-induced airway and inflammatory responses in allergic sheep. *Am J Respir Crit Care Med* 1995; 152:2076–83.
 - 38 Elrod KC, Moore WR, Abraham WM, Tanaka RD. Lactoferrin, a potent tryptase inhibitor, abolishes late-phase airway responses in allergic sheep. *Am J Respir Crit Care Med* 1997; 156:375–81.
 - 39 Wright CD, Havill AM, Middleton SC et al. Inhibition of allergen-induced pulmonary responses by the selective tryptase inhibitor 1, 5-bis-[4-[(3-carbamimidoyl-benzenesulfonylamino)-methyl]-phenoxy]-pen tane (AMG-126737). *Biochem Pharmacol* 1999; 58:1989–96.
 - 40 Weissberger D, Oliver W, Abraham WM, Wanner A. Impaired tracheal mucus transport in allergic bronchoconstriction: effect of terbutaline pretreatment. *J Allergy Clin Immunol* 1981; 67:357–62.
 - 41 Allegra L, Abraham WM, Chapman GA, Wanner A. Duration of mucociliary dysfunction following antigen challenge. *J Appl Physiol* 1983; 55:726–30.
 - 42 Abraham WM, Russi E, Wanner A, Delehunt JC, Yerger LD, Chapman GA. Production of early and late pulmonary responses with inhaled leukotriene D₄ in allergic sheep. *Prostaglandins* 1985; 29:715–26.
 - 43 Delehunt JC, Perruchoud AP, Yerger L, Marchette B, Stevenson JS, Abraham WM. The role of slow-reacting substance of anaphylaxis in the late bronchial response after antigen challenge in allergic sheep. *Am Rev Respir Dis* 1984; 130:748–54.
 - 44 Abraham WM, Ahmed A, Cortes A, Sielczak M, Hallmon J. The effects of a cysteinyl leukotriene antagonist (ONO-1078) on antigen-induced responses in allergic sheep. *Prostag Leukotr Ess* 1993; 48:233–9.
 - 45 Abraham WM, Stevenson JS, Garrido R. A leukotriene and thromboxane inhibitor (Sch 37224) blocks antigen-induced immediate and late responses and airway hyperresponsiveness in allergic sheep. *J Pharm Exp Therap* 1988; 247:1004–11.
 - 46 Abraham WM, Stevenson JS, Garrido R. The effect of an orally active leukotriene (LT) D₄ antagonist WY-48, 252 on LTD₄ and antigen-induced bronchoconstrictions in allergic sheep. *Prostaglandins* 1988; 35:733–45.
 - 47 Tomioka K, Garrido R, Stevenson JS, Abraham WM. The effect of an orally active leukotriene (LT) antagonist YM-16638 on antigen-induced early and late airway responses in allergic sheep. *Prostag Leukotr Ess* 1989; 36:43–7.
 - 48 Tagari P, Abraham WM, McGolrick J et al. Increased leukotriene E₄ excretion during antigen-induced bronchoconstriction in allergic sheep. *J Appl Physiol* 1990; 68:1321–7.
 - 49 Soler M, Sielczak M, Abraham WM. Separation of late bronchial responses from airway hyperresponsiveness in allergic sheep. *J Appl Physiol* 1991; 70:617–23.
 - 50 Abraham WM, Ahmed A, Cortes A et al. The 5-lipoxygenase inhibitor zileuton blocks antigen-induced late airway responses, inflammation and airway hyperresponsiveness in allergic sheep. *Eur J Pharmacol* 1992; 217:119–26.
 - 51 Abraham WM, Laufer S, Tries S. The effects of ML 3000 on antigen-induced responses in sheep. *Pulm Pharmacol Ther* 1997; 10:167–73.
 - 52 Scuri M, Allegra L, Abraham WM. The effects of multiple dosing with zileuton on antigen-induced responses in sheep. *Pulm Pharmacol Ther* 1998; 11:277–80.
 - 53 Chapman GA, Signoretti F, Lauredo IT et al. Cellular LTB₄ production differs in allergic sheep with and without late airway responses. *Am J Physiol* 1990; 259:L136–43.
 - 54 Soler M, Sielczak MW, Abraham WM. A PAF antagonist blocks antigen-induced airway hyperresponsiveness and inflammation in sheep. *J Appl Physiol* 1989; 67:406–13.
 - 55 Abraham WM, Stevenson JS, Garrido R. A possible role for PAF in allergen-induced late responses: modification by a selective antagonist. *J Appl Physiol* 1989; 66:2351–7.
 - 56 Forteza R, Botvinnikova Y, Ahmed A et al. The interaction of alpha 1-proteinase inhibitor and tissue kallikrein in controlling allergic ovine airway hyperresponsiveness. *Am J Respir Crit Care Med* 1996; 154:36–42.
 - 57 Soler M, Sielczak M, Abraham WM. A bradykinin-antagonist blocks antigen-induced airway hyperresponsiveness and inflammation in sheep. *Pulmonary Pharmacol* 1990; 3:9–15.
 - 58 Abraham WM, Burch RM, Farmer SG, Sielczak MW, Ahmed A, Cortes A. A bradykinin antagonist modifies allergen-induced mediator release and late bronchial responses in sheep. *Am Rev Respir Dis* 1991; 143:787–96.
 - 59 Chen W, Alley MR, Manktelow BW. Airway inflammation in sheep with acute airway hypersensitivity to inhaled *Ascaris suum*. *Int Arch Allergy Appl Immunol* 1991; 96:218–23.
 - 60 Chen W, Alley MR, Manktelow BW. Morphological and morphometric studies of the airways of sheep with acute airway hypersensitivity to inhaled *Ascaris suum*. *Int J Exp Pathol* 1991; 72:543–51.
 - 61 Bosse J, Boileau R, Begin R. Chronic allergic airway disease in the sheep model: functional and lung-lavage features. *J Allergy Clin Immunol* 1987; 79:339–44.
 - 62 Chen W, Pack RJ, Alley MR, Carr DH, Manktelow BW. Airway hypersensitivity induced by *Ascaris suum* extract in New Zealand Romney sheep. *NZ Vet J* 1990; 38:57–61.
 - 63 Shaver JR, O'Connor JJ, Pollice M et al. Pulmonary inflammation after segmental ragweed challenge in allergic asthmatic and nonasthmatic subjects. *Am J Respir Crit Care Med* 1995; 152:1189–97.
 - 64 Sur S, Kita H, Gleich GJ, Chenier TC, Hunt LW. Eosinophil recruitment is associated with IL-5, but not with RANTES, twenty-four hours after allergen challenge. *J Allergy Clin Immunol* 1996; 97:1272–8.
 - 65 Hohlfield JM, Ahlf K, Enhorning G et al. Dysfunction of pulmonary surfactant in asthmatics after segmental allergen challenge. *Am J Respir Crit Care Med* 1999; 159:1803–9.
 - 66 Weissman DN, Bice DE, Crowell RE, Schuyler MR. Intrapulmonary antigen deposition in the human lung: local responses. *Am J Respir Cell Mol Biol* 1994; 11:607–14.
 - 67 Shaver JR, Zangrilli JG, Cho SK et al. Kinetics of the development and recovery of the lung from IgE-mediated inflammation: dissociation of pulmonary eosinophilia, lung injury, and eosinophil-active cytokines. *Am J Respir Crit Care Med* 1997; 155:442–8.
 - 68 Gratziau C, Carroll M, Montefort S, Teran L, Howarth PH, Holgate ST. Inflammatory and T-cell profile of asthmatic airways 6 hours after local allergen provocation. *Am J Respir Crit Care Med* 1996; 153:515–20.
 - 69 Kane GC, Pollice M, Kim C-J et al. A controlled trial of the effect of the 5-lipoxygenase inhibitor, zileuton, on lung inflammation produced by segmental antigen challenge in human beings. *J Allergy Clin Immunol* 1996; 97:646–54.
 - 70 Kroegel C, Liu MC, Hubbard WC, Lichtenstein LM, Bochner BS. Blood and bronchoalveolar eosinophils in allergic subjects after segmental antigen challenge: surface phenotype, density heterogeneity, and prostanoic production. *J Allergy Clin Immunol* 1994; 93:725–34.
 - 71 Collie DD, Macalldowie CN, Pemberton AD et al. Local lung responses following local lung challenge with recombinant

- lungworm antigen in systemically sensitized sheep. *Clin Exp Allergy* 2001; 31:1636–47.
- 72 Sture GH, Huntley JF, MacKellar A, Miller HRP. Ovine mast cell heterogeneity is defined by the distribution of sheep mast cell proteinase. *Vet Immunol Immunopathol* 1995; 48:275–85.
- 73 Chen W, Alley MR, Manktelow BW, Davey P. Mast cells in the ovine lower respiratory tract: heterogeneity, morphology and density. *Int Arch Allergy Appl Immunol* 1990; 93:99–106.
- 74 Jubb KVF. The respiratory system. In: Jubb KVF, Kennedy PC, Palmer N, eds. *Pathology of the Domestic Animals*. Orlando: Academic Press Inc., 1985; 520–4.
- 75 Williams RM, Bienenstock J, Stead RH. Mast cells: the neuroimmune connection. *Chem Immunol* 1995; 61:208–35.
- 76 Kiernan JA. Degranulation of mast cells in the trachea and bronchi of the rat following stimulation of the vagus nerve. *Int Arch Allergy Appl Immunol* 1990; 91:398–402.
- 77 Leff AR, Stimler NP, Munoz NM, Shioya T, Tallet J, Dame C. Augmentation of respiratory mast cell secretion of histamine caused by vagus nerve stimulation during antigen challenge. *J Immunol* 1986; 136:1066–73.
- 78 Stewart MJ, Emery DL, McClure SJ, Bendixsen T. The effects of four neuropeptides on the degranulation of mucosal mast cells from sheep. *Immunol Cell Biol* 1996; 74:255–7.

D. D. S. COLLIE

*Department of Veterinary Clinical Studies
University of Edinburgh, Easter Bush Veterinary Centre
Roslin, Midlothian EH25 9RG
UK*

E-mail: david.collie@ed.ac.uk