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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 hyperreactivity [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 hyperresponsiveness. 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 preselect 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.

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