

Induction of allergic inflammation in the lungs of sensitized sheep after local challenge with house dust mite

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Summary

Background Previous sheep models of asthma are based on sheep sensitized to nematode (*Ascaris*) allergens and these have been used to evaluate the physiological and pharmacological effects of potential anti-asthma agents. The immunological mechanisms associated with the allergic response in sheep lungs has not been examined in detail.

Objective To develop an experimental sheep model of allergic lung inflammation based on a relevant major human allergen, house dust mite, and to define the immunological features of the allergic response in this model.

Methods Sheep immunized subcutaneously with solubilized house dust mite extract were given a single bronchial challenge with house dust mite. Bronchoalveolar lavage (BAL) and peripheral blood leucocytes were collected before and after challenge for flow cytometry, and tissue samples were taken post-mortem (48 h post-challenge) for histology and immunohistochemical analyses.

Results Immunizations with 50 µg house dust mite induced an allergen-specific IgE response in 50 to 60% of sheep (allergic sheep), with higher antigen doses increasing specific IgG₁ but not IgE. Lung challenge of allergic sheep with house dust mite led to the initial recruitment of neutrophils (at 6 h post-challenge) followed by eosinophils and activated lymphocytes into the lung tissue and BAL, similar to the late-phase allergic response seen in human asthma. Eosinophil recruitment peaked at 48 h post-challenge, representing 10 to 33% of BAL leucocytes in allergen-challenged allergic sheep compared to 0 to 3% in allergen-challenged control (naïve) sheep. Lymphocytes recovered from the lung after allergen challenge were enriched for CD4⁺ T cells and were more activated than lymphocytes in blood. There was significant down-regulation of CD62L (L-selectin) and CD49d (VLA-4) expression after allergen challenge on BAL eosinophils and lymphocytes compared to blood. In addition, VCAM-1 (ligand for VLA-4) was up-regulated on blood vessels of allergen-challenged lungs. Eosinophils, CD4⁺ T cells and CD45R⁺ B cells were the most prominent leucocytes found in lung tissue 48 h after allergen challenge.

Conclusion This study demonstrates, for the first time, the ability of house dust mite to induce allergic responses in sheep lungs. This novel sheep model of allergic lung inflammation using relevant human allergens, exhibits similarities to human asthmatic disease and will be a useful tool for studies of the immunological and physiological mechanisms of allergic asthma.

Keywords allergic lung inflammation, animal model, asthma, eosinophils, house dust mite, sheep
Submitted 15 January 2002; revised 16 May 2002; accepted 16 July 2002

Introduction

Inflammation of the airways plays a central role in the pathogenesis of asthma and involves the recruitment and activation of eosinophils, mast cells, neutrophils and lymphocytes into lung tissue and bronchoalveolar spaces [1]. House dust mite (HDM) represents the most common allergen causing allergic asthma in humans, with sensitized individuals developing

specific IgE and IgG humoral responses [2]. The ability of HDM allergens to contribute to the pathogenesis of airway inflammation by immunological [3–5] and non-immunological mechanisms [6, 7] is well established.

Appropriate animal models of disease allow defined and controlled investigations to be carried out and relate findings to human disease situations. It has been shown that the allergic response in sheep lungs closely reproduces the development of respiratory symptoms in human asthma, including a characteristic early- and late-phase asthmatic response and bronchial hyper-responsiveness [8–10]. Furthermore, the structural, histological and functional features of the sheep lung closely resemble those of the human lung [11–13]. Sheep have also been

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successfully used in many immunological studies to analyse immune responses in large animals and present therefore as an appropriate model for human respiratory disease.

While sheep have been used for evaluation of potential therapeutic agents for asthma [9, 10, 14–16], most studies have been limited to the examination of physiological (changes in airway reactivity) or pharmacological effects rather than the immunological mechanisms associated with the allergic response. In addition, all previous studies were performed using sheep sensitized to nematode allergens [10, 16, 17].

The aim of this study was to develop an experimental model of allergic lung inflammation based on a relevant human allergen, house dust mite, and to define the immunological features of the allergic response in this model.

Materials and methods

Experimental sheep

Female Merino-cross lambs aged 4 to 5 months were treated with anthelmintic to eliminate existing parasites prior to the experiment and were housed in indoor pens and fed *ad libitum*. All sheep were judged to be free of significant pulmonary disease on the basis of gross and histological examination at post-mortem.

All experimental animal procedures and the collection of tissues and cells were approved by the Animal Experimentation Ethics Committee of the University of Melbourne, following guidelines set by the National Health and Medical Research Council (NH & MRC) of Australia.

Immunizations with house dust mite

A soluble preparation of house dust mite (HDM, *Dermatophagoides pteronyssinus*; CSL Limited, Parkville, VIC, Australia) was prepared in sterile pyrogen-free saline (PFS; Baxter Healthcare Pty Ltd, Old Toongabbie, NSW, Australia), sterile-filtered and stored at -70°C prior to use. The same batch solution was used throughout the experiments.

Sheep were immunized subcutaneously with solubilized HDM, $0\text{ }\mu\text{g}$ ($n=10$), $5\text{ }\mu\text{g}$ ($n=5$), $50\text{ }\mu\text{g}$ ($n=10$) or $500\text{ }\mu\text{g}$ ($n=5$), in PFS, in aluminium hydroxide as adjuvant (1:1). As shown in Fig. 1, the immunization protocol involved three subcutaneous (s.c.) injections of 1 mL solubilized HDM in Alum at 2-week intervals. Sheep were then rested for 2 weeks prior to a single lung challenge with HDM. In a separate group of five sheep, immunizations (three at 2-week intervals) with solubilized HDM (1 mg in 5 mL PFS) were performed via the bronchial route using a fibre-optic bronchoscope as outlined below.

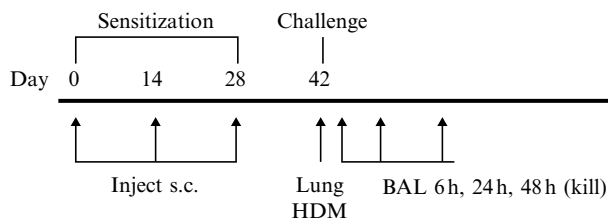


Fig. 1. Schematic representation of the immunization and challenge protocol employed for sheep immunized subcutaneously with HDM.

Lung challenges and collection of bronchoalveolar lavage cells and lung tissue

During the experimental lung challenge procedure, sheep were mildly sedated with Xylazil-20 (containing 2% w/v of Xylazine (2-(2,6-xylidino)-5,6-dihydro-4H-1,3-thiazine hydrochloride); Troy Laboratories, Smithfield, NSW, Australia) and restrained in a custom-made canvas body sheath and head harness tethered in a modified metabolism cage. Allergen challenge of the lungs was made using a flexible fibre-optic bronchoscope (Model FG-16X; Pentax Ltd, Slough, UK) for localized delivery of solubilized HDM (1 mg in 5 mL PFS at 39°C) deep into the left caudal lung lobe. The HDM preparation was slowly infused into the lung via the biopsy port of the bronchoscope using a 10-mL syringe. The right caudal lobe of the lung of the same sheep was challenged with PFS as a control. Baseline bronchoalveolar lavage (BAL) samples were collected using the bronchoscope by slow infusion and withdrawal of 10 mL PFS (39°C) 1 week before challenge. Sequential BAL samples (returning $1\text{--}20 \times 10^6$ cells) were collected after challenge from the left and right lung at 6 h, 24 h and 48 h post-challenge for the preparation of cytosots.

Sheep were sacrificed at 48 h post-challenge and lung tissue samples and BAL cells were prepared for histology and flow cytometry. BAL cells were collected from whole lung lavage of left and right lung lobes as previously described [18]. On ice, BAL cells were washed and centrifuged (400 g , 5 min) twice with 1% bovine serum albumin (BSA, Fraction V; Trace Biosciences, Melbourne, VIC, Australia) in PBS prior to immunostaining.

Peripheral blood leucocytes

Peripheral blood was drawn from the jugular vein of sheep into a plastic tube containing ethylenediaminetetra-acetic acid (EDTA; BDH Merck, Kilsyth, VIC, Australia). Red blood cells were lysed with the addition of Tris-buffered ammonium chloride (170 mM Tris, 160 mM NH_4Cl , pH 7.2) at 39°C and leucocytes washed twice with phosphate-buffered saline (PBS), resuspended in 1% BSA/PBS and stored on ice prior to immunostaining.

Determination of HDM-specific serum immunoglobulin responses

Blood samples were collected prior to the first HDM immunization as well as 7 days and 14 days following the third immunization, and sera assayed for the presence of immunoglobulins (Ig) to house dust mite by standard enzyme-linked immunosorbent assay (ELISA). Briefly, for the detection of HDM-specific IgG_1 and IgG_2 , ELISA plates were coated with $2.5\text{ }\mu\text{g}$ HDM antigen in carbonate buffer (pH 9.6), then washed, blocked and incubated with serum (diluted 1/100). After washing, plates were incubated with anti- IgG_1 or anti- IgG_2 monoclonal antibody (mAb) (gifts from K. Beh, CSIRO, Parkville, VIC, Australia) culture supernatants (undiluted), washed and incubated with horseradish peroxidase (HRP)-conjugated rabbit anti-mouse Ig (Dako, Carpinteria, CA, USA), followed by development with 3', 3', 5', 5'-tetramethyl-benzidine dihydrochloride hydrate (TMB; Sigma, Castle Hill, NSW, Australia) as substrate.

HDM-specific IgE was determined by ELISA of NH_4SO_4 -treated serum samples as described previously [19]. Briefly, equal volumes of serum and 80% NH_4SO_4 solution were mixed and the sample centrifuged after 30 min in a microcentrifuge

(17 900 g for 10 min). NH_4SO_4 -treated serum samples (diluted 1 : 1 with 0.05% Tween 20/distilled water) were added to HDM antigen-coated plates and incubated overnight, followed by washing and incubation with anti-IgE mAb (clone XB6/YD3 [19]; AgResearch, Upper Hutt, New Zealand) culture supernatant (1/10 dilution). After washing, plates were incubated with HRP-conjugated goat anti-mouse IgG (γ -chain specific; Sigma), then washed and developed with TMB substrate.

For each of the ELISAs performed, optical density (OD) was determined with a TitreTek Multiscan MCC plate reader using a dual wavelength (A_{450} - A_{690}).

Flow cytometry

BAL and peripheral blood leucocytes were enumerated using a Coulter counter[®] (Coulter Electronics, Luton, UK). MAbs against the sheep cell surface molecules CD1, CD2, CD4, CD5, CD8, CD45R, WC2 ($\gamma\delta$ -TCR), CD45, CD25, MHC class II, CD11a (LFA-1), CD11b, CD29 (β 1 integrin), CD44, CD49d (α 4 integrin/VLA-4) and CD62L (L-selectin) were used as detailed elsewhere [20]. The mAb SBU-3, non-reactive with sheep leucocytes, was used as a negative control. Leucocytes obtained from BAL and peripheral blood were stained by indirect immunofluorescence for two-colour flow cytometry as previously described [20] and analysed on a FACSCalibur[®] instrument (Becton-Dickinson, Mountain View, CA, USA) using Cellquest[®] software (Becton-Dickinson). Changes in the expression of adhesion molecules were assessed on the basis of relative mean fluorescence intensity (RMFI) of staining, calculated as the ratio of mean fluorescence intensity (MFI) of expression of the adhesion molecule to that of the negative control.

Histology and immunohistochemistry

Cytospots of BAL cells and blood smears were prepared and stained with Wright's stain (Sigma) for differential leucocyte cell counts. Tissue samples were collected at 48 h post-challenge from left and right lungs, fixed in 10% neutral-buffered formalin and processed in paraffin for histology, or frozen in OCT embedding medium (Tissue Tek, Miles Scientific, Naperville, IL, USA) for immunohistochemistry. Paraffin-embedded tissue sections (5 μm) were stained with haematoxylin and eosin Y (Sigma) for general histology. For immunohistochemistry, frozen tissue sections (5 μm) were stained by the indirect immunoperoxidase technique as described previously [21] using mAbs against sheep cell surface molecules (see above). The anti-ovine VCAM-1 mAb QE4G9 [22] was kindly provided by R. Krishnan (The University of Adelaide, Adelaide, SA, Australia).

Statistical analyses

Statistical analysis was carried out using the Kruskal-Wallis non-parametric test to assess if there was any significant difference ($P < 0.05$) between means of the sample groups.

Results

Serum immunoglobulin responses to HDM immunizations

Sheep were immunized with HDM by the bronchial or s.c. routes and serum was collected for analysis of HDM-specific antibody responses. Immunization via the bronchial route showed no

detectable induction of specific Ig (data not shown). Significant allergen-specific serum Ig responses were induced by s.c. HDM immunizations as presented in Fig. 2. HDM-specific IgE responses were strongest in the group immunized s.c. with 50 μg HDM. In contrast, HDM-specific IgG₁ responses were maximal in sheep immunized at 500 μg . There was no particular correlation between IgE and IgG₁ responses. No differences were detected in IgG₂ levels. A total of 6/10 sheep in the group immunized at 50 μg HDM responded with high levels of HDM-specific IgE in serum and are referred to as allergic sheep. Other immunized sheep that failed to show an IgE response (i.e. non-responders) are referred to as non-allergic sheep.

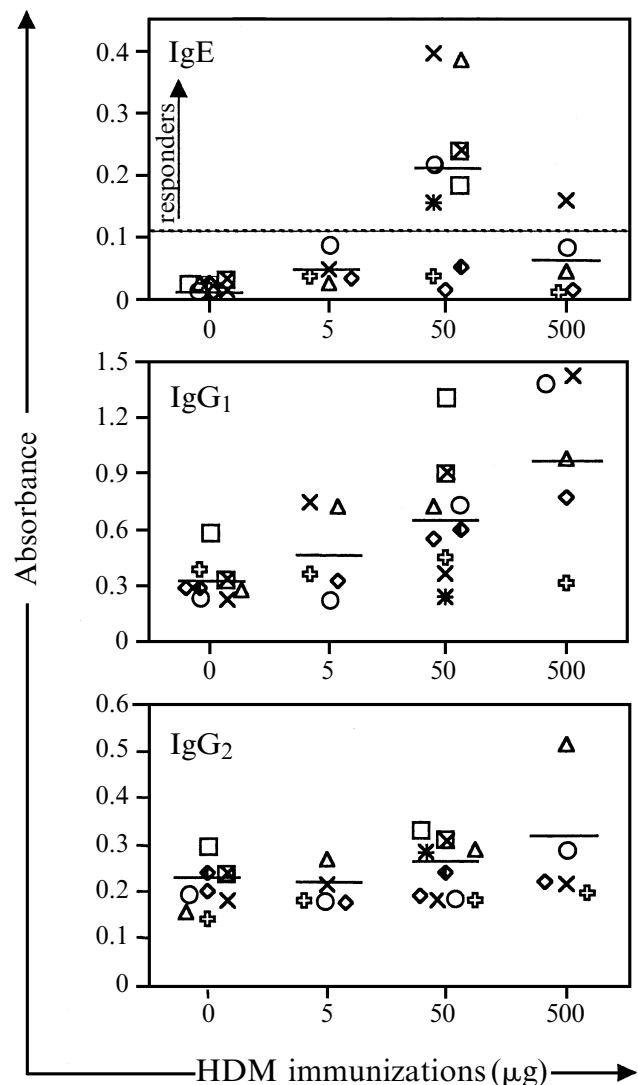


Fig. 2. HDM-specific IgE, IgG₁ and IgG₂ levels in final bleeds (7 days post third immunization) obtained from sheep immunized s.c. with HDM at 0, 5, 50 or 500 μg per immunization. Within each group, the different markers correspond to values obtained for individual sheep. Responder (allergic) sheep, as indicated above the dashed line, were identified as those sheep with serum HDM-specific IgE levels greater than the mean + 5 $\times$ SD of the control (0 μg HDM) group. Horizontal lines depict the mean values.

Eosinophil levels in peripheral blood and bronchoalveolar lavage of allergic sheep after lung challenge with HDM

Groups of three to four allergic, non-allergic and control (naïve) sheep were challenged with solubilized HDM in PFS deep into the left caudal lung lobe and PFS alone into the right lobe using a fibre-optic bronchoscope. Peripheral blood eosinophil levels

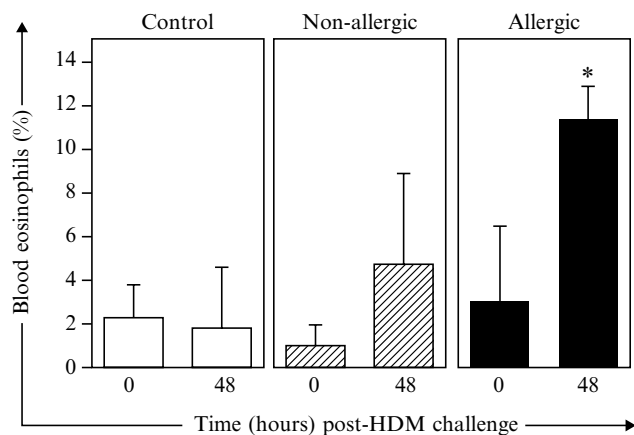


Fig. 3. Peripheral blood eosinophil numbers before (0 h) and after (48 h) lung challenge with HDM in groups of three to four control, non-allergic and allergic sheep. Data shown are the mean $\pm$ SD; *denotes significant difference ($P < 0.05$) compared with the 0 h time-point, and both 0 h and 48 h in the control and non-allergic sheep.

in the three groups of sheep before and after lung HDM challenge are shown in Fig. 3. The percentage of eosinophils in blood prior to lung challenge (0 h) was similar between groups and in control and non-allergic sheep their levels did not change significantly after challenge. However, in allergic sheep the percentage of blood eosinophils increased four- to six-fold after HDM challenge of the lungs and these levels were significantly higher when compared with the corresponding time-point in non-allergic and control sheep (Fig. 3).

BAL collections were performed with the infusion of 10 mL PFS into the lungs using a bronchoscope. The total volume of BAL fluid recovered ranged from 3.0 to 6.8 mL with no discernible difference between groups throughout the experimental period. There was also considerable variation in total cells recovered (0.3 to 43×10^5 cells/mL), with no obvious differences between groups (Fig. 4). Large variations in BAL fluid and total BAL cell recoveries have been reported in other studies [17] and may be partly due to inter-animal differences in tissue architecture. A comparison of the changes in BAL eosinophil number and eosinophil percentage for individual sheep over time suggested that the percentages may be a more reliable indicator of BAL cell responses when comparing the experimental groups in this study (Fig. 4). The kinetics of the cell response in the BAL after lung challenge was therefore analysed with respect to the percentage of the different cell populations recovered in BAL, as depicted in Fig. 5.

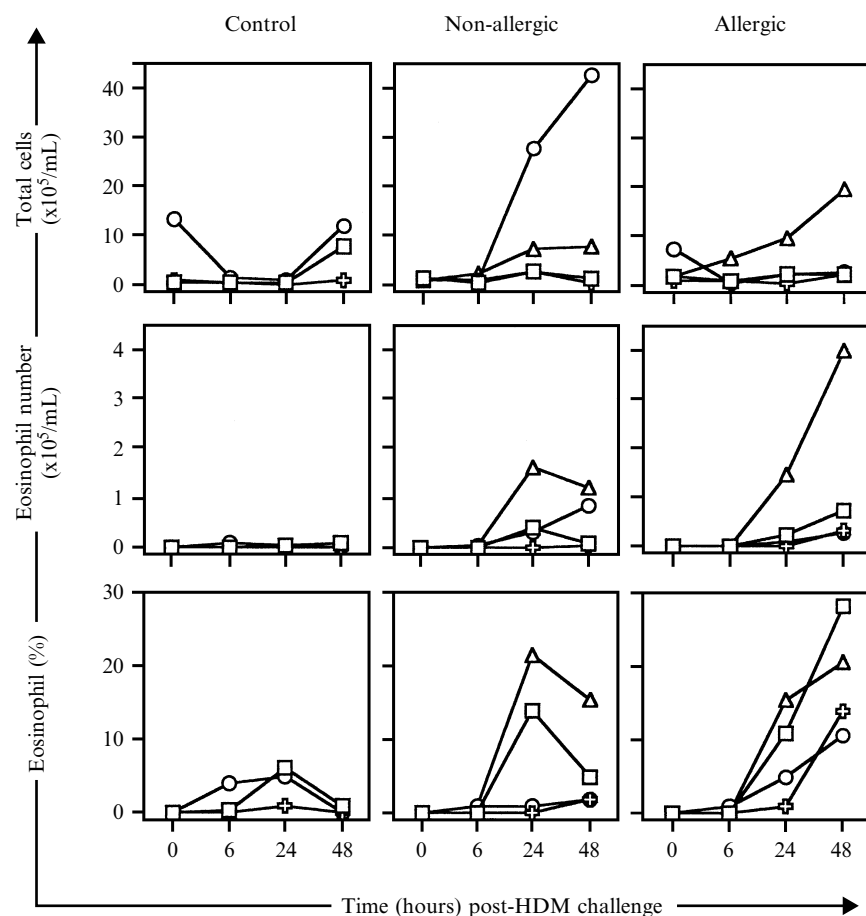


Fig. 4. Changes in total BAL leucocyte cell numbers, eosinophil cell numbers and BAL eosinophil percentages for individual control ($n = 3$), non-allergic ($n = 4$) and allergic ($n = 4$) sheep 0 to 48 h following HDM lung challenge.

In all sheep, BAL samples taken before HDM lung challenge comprised mainly macrophages (79 to 99%) and small numbers of lymphocytes (1 to 21%) (Figs 5a, b). The cellular response to lung challenge with HDM was characterized by an early influx of neutrophils (Fig. 5c) into the BAL, 6 h post-challenge. In immunized (non-allergic and allergic) sheep the neutrophil response was more sustained, with a higher percentage of neutrophils in the BAL at 24 h and 48 h post-challenge compared with

the HDM-challenged controls. Eosinophils were detected in the BAL at 6 h post-HDM challenge in all groups. In the controls, eosinophilia reached a maximum ($4.0 \pm 2.6\%$) at 24 h and declined to minimal levels by 48 h post-challenge (Fig. 5d). In both immunized (non-allergic and allergic) groups of sheep, the percentage of BAL eosinophils remained high at 48 h compared with control sheep. The peak was reached at 24 h in non-allergic sheep but eosinophils in all allergic sheep peaked at 48 h

Fig. 5. Changes in the percentages of macrophages (a), lymphocytes (b), neutrophils (c) and eosinophils (d) present in the BAL of control, non-allergic and allergic sheep 0 to 48 h after challenge with HDM. Data shown are the mean $\pm$ SD from three to four sheep per group; *denotes significant difference ($P < 0.05$) compared with the controls.

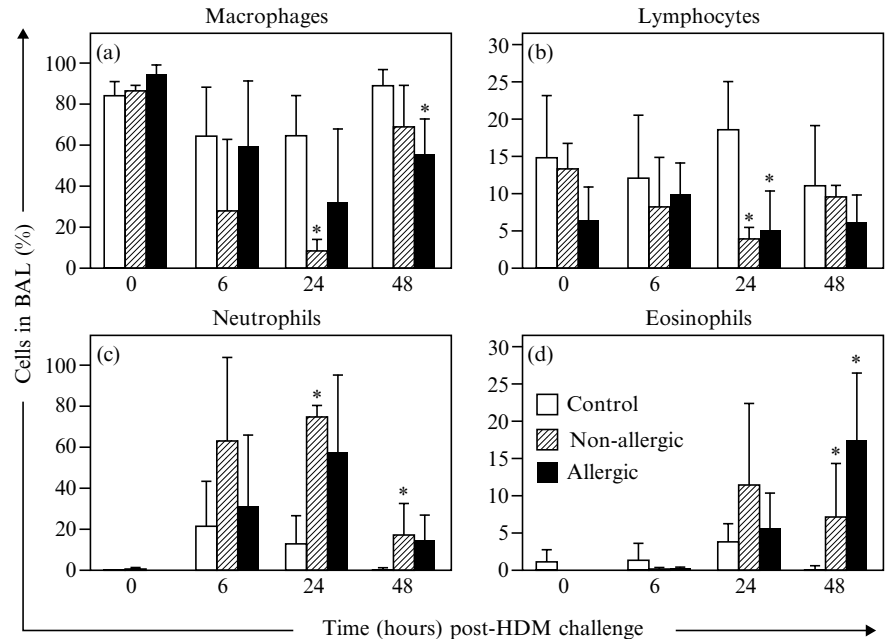
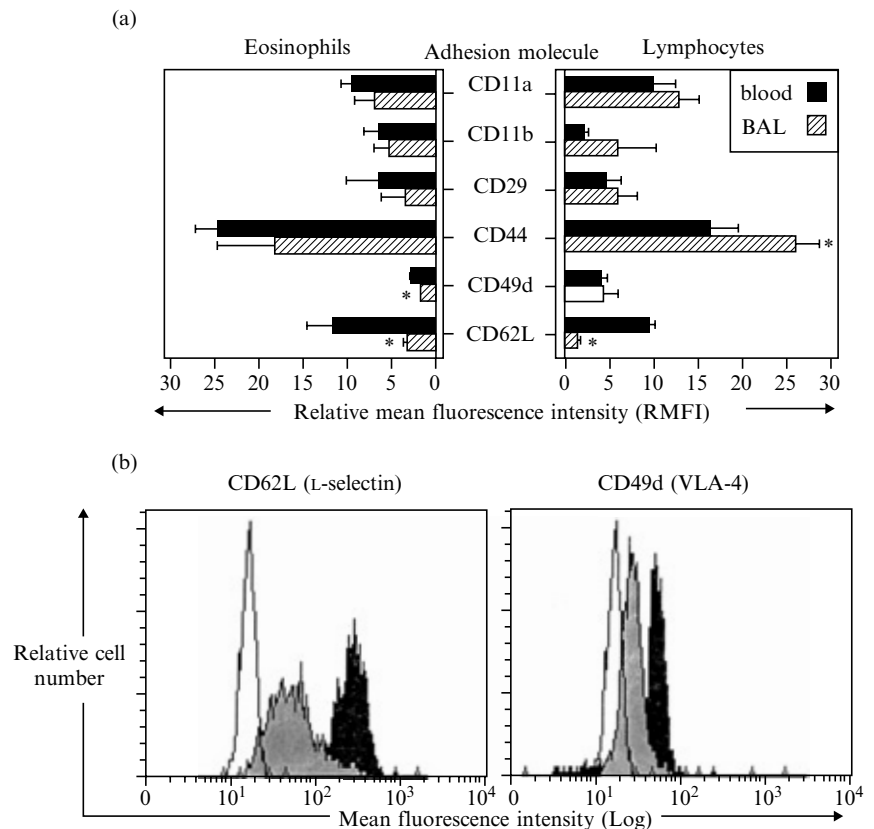


Fig. 6. Comparison of adhesion molecule expression on eosinophils and lymphocytes in peripheral blood and BAL of allergic sheep 48 h after HDM lung challenge. Eosinophils and lymphocytes were gated on FSC and SSC characteristics and 10 000 events analysed. (a) Relative mean fluorescence intensity (RMFI) expression of adhesion molecules on blood (solid bars) and BAL (hatched bars). Data presented are the means $\pm$ SD from three sheep; *denotes significant difference ($P < 0.05$) in levels of expression between blood and BAL. (b) Representative FACS profiles from an individual sheep showing a shift in cell surface expression of CD62L and CD49d on eosinophils in blood (black histograms) vs. BAL (grey histogram). Staining with an isotype-matched control Ab (open histogram) is included.



post-challenge (Figs 4 and 5d). Segmental bronchi challenge of all sheep with PFS alone in the right caudal lung lobe resulted in a cellular response clearly separate to the HDM-challenged region, characterized by a transient neutrophilic response (5 to 10% of BAL leucocytes) resolving by 24 to 48 h post-challenge and devoid of the appearance of eosinophils (data not shown).

Phenotype of eosinophils and lymphocytes in blood and BAL after lung challenge with HDM

In allergic sheep, blood and BAL leucocytes were collected at 48 h post-HDM lung challenge, stained with mAbs to leucocyte cell surface molecules and analysed by flow cytometry. Eosinophils and lymphocytes were gated on the basis of forward- and side-scatter profiles [20]. Compared with blood, there was a significant decline in the level of expression of CD62L (L-selectin) on BAL eosinophils and lymphocytes, and CD49d (VLA-4) on BAL eosinophils at 48 h post-challenge (Figs 6a, b). In contrast, CD44 was significantly up-regulated on BAL compared with blood lymphocytes (Fig. 6a).

The majority of lymphocytes in the BAL at 48 h post-challenge were CD4⁺ T cells, with smaller numbers of CD8⁺ T cells, $\gamma\delta$ ⁺ T cells and CD45R⁺ B cells (Fig. 7a). A large proportion of the BAL lymphocytes expressed high levels of MHC Class II (59 to 81%) and the IL-2 receptor (20 to 43%), indicative of their activation status. MHC class II expression on both CD4⁺ and CD4⁻ lymphocytes was elevated on BAL compared with blood (Fig. 7b).

Histological and immunohistological features of the allergic lung after HDM challenge

Tissues were collected from left and right lungs at 48 h post-challenge for histology and immunohistology. In allergic sheep, HDM challenge of the left lung lobe (Figs 8a, c) resulted in a striking increase in tissue cellularity compared with the saline challenged right lung (Figs 8b, d). This was in contrast to non-immunized control sheep, where there was only a modest recruitment of inflammatory cells after HDM challenge of the lungs at 48 h (data not shown). The tissue response to HDM challenge in allergic sheep was characterized by marked infiltration of eosinophils and lymphocytes into the peribronchial regions and the surrounding alveoli. Eosinophils were also associated with aggregations of mononuclear cells beneath the bronchiolar epithelium and around blood vessels (Fig. 8c). Eosinophils and lymphocytes were also recruited into the HDM-challenged lung tissue of non-allergic sheep but to a lesser degree than in allergic sheep (not shown).

Immunohistochemistry performed on lung tissues of allergic sheep at 48 h post-challenge revealed that CD4⁺ T cells (Fig. 8e) and CD45R⁺ B cells (Fig. 8f) were the main lymphocyte subpopulations, while smaller numbers of CD8⁺ and $\gamma\delta$ ⁺ T cells were also observed. An increase in the expression of the activation markers MHC class II and CD25, associated particularly with the mononuclear cell infiltrates, was noted in allergen-challenged (left) compared with saline-challenged (right) lung lobes (data not shown). Staining for VCAM-1, the ligand for CD49d (VLA-4) expressed on leucocytes such as eosinophils and lymphocytes, was strong on endothelial cells of the majority of blood vessels in inflamed allergen-challenged left lung lobes (Fig. 8g) but was absent in saline-challenged right lobes (Fig. 8h).

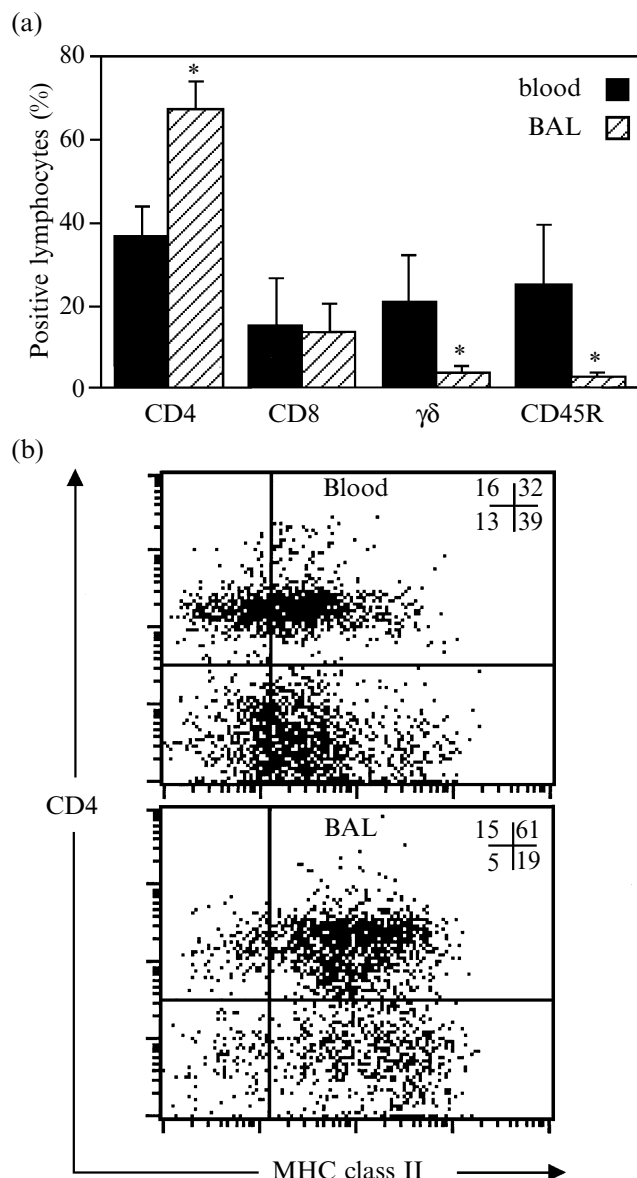


Fig. 7. Lymphocyte subpopulations and their activation status in blood and BAL of allergic sheep collected 48 h after HDM lung challenge. (a) Percentage of lymphocyte subsets in blood and BAL; *denotes significant difference ($P < 0.05$) between blood and BAL; means $\pm$ SD from three sheep. (b) Representative FACS profiles from an individual sheep showing two colour staining for CD4 and MHC class II on blood and BAL lymphocytes. 10 000 events were analysed and the percentage of cells in each quadrant is noted.

Discussion

Sensitization to HDM is one of the most common underlying causes of human respiratory allergy [2] and was therefore chosen as a relevant antigen for the development of a sheep model of experimental asthma. Previously, natural or experimental sensitization of sheep to *Ascaris suum* has been used as a physiological model of asthma to study the pharmacological effect of new anti-allergic compounds [9, 15, 16, 23]. However, little attention has been directed to studying the immunological basis of the response to allergen challenge in sheep lungs. The

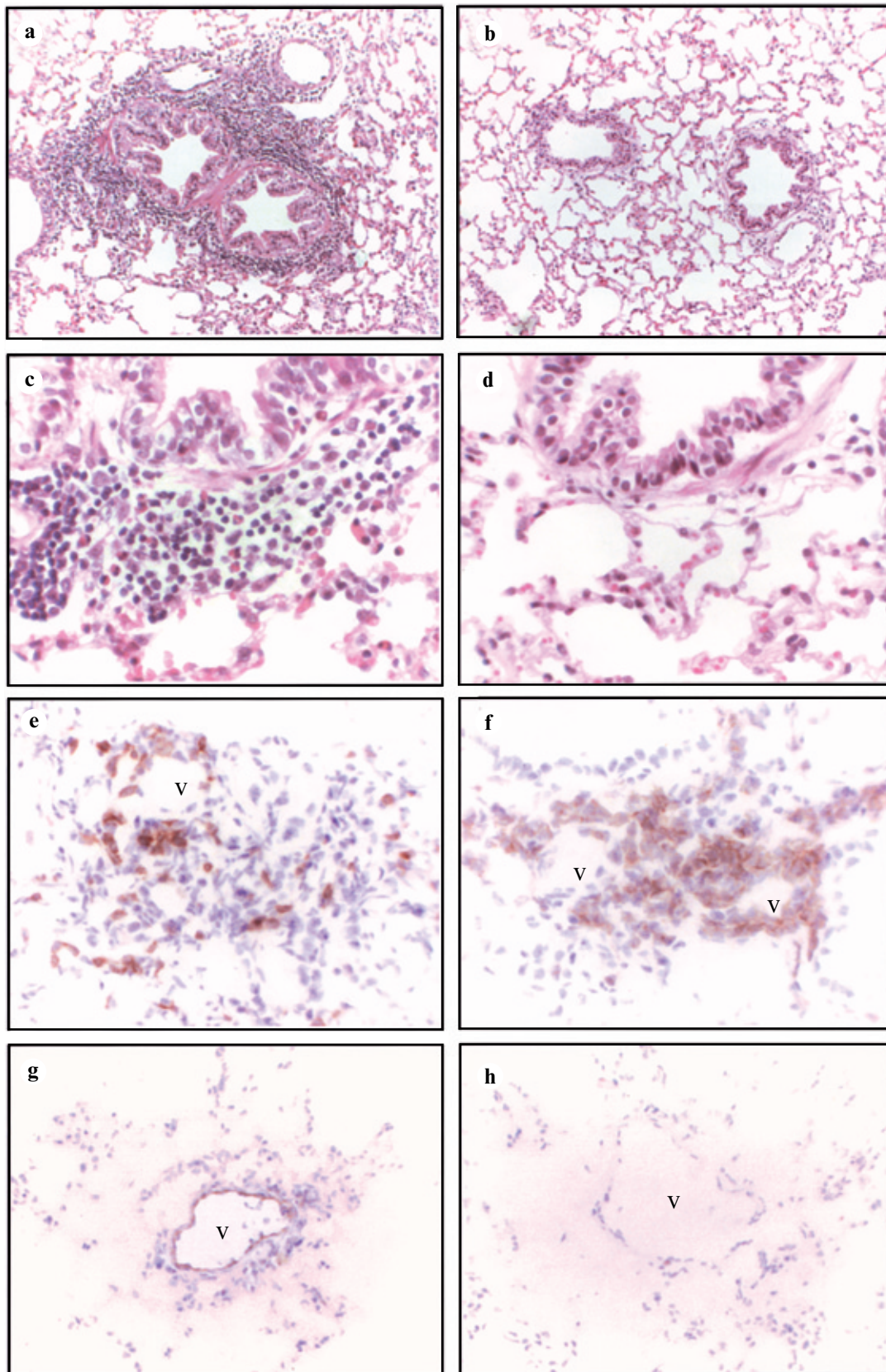


Fig. 8. Histology and immunohistology of the lung 48 h after HDM challenge in allergic sheep. Numerous aggregations of eosinophils and lymphocytes were seen around bronchioles and blood vessels in HDM-challenged (a, c) but not saline-challenged lungs (b, d); H&E stain. The majority of infiltrating lymphocytes in HDM-challenged lungs were CD4⁺ T cells (e) and CD45R⁺ B cells (f). VCAM-1 staining of bronchial endothelial cells was clearly up-regulated in allergen-challenged (g) vs. saline-challenged (h) lungs; indirect immunoperoxidase staining, lightly counterstained with haematoxylin. v = blood vessel. Original magnification $\times 100$ (a, b), $\times 400$ (c–h).

present studies characterized in detail the inflammatory response in a sheep model of experimental asthma, using an allergen relevant to human asthma.

Subcutaneous immunizations with 50 µg HDM resulted in approximately 60% of sheep developing high levels of HDM-specific serum IgE. Lower (5 µg) or higher (500 µg) doses of HDM were less effective in inducing a specific IgE response, although higher doses further increased the IgG₁ response. These findings are in agreement with previous studies showing that antigen dose can influence the development of allergen-specific antibody responses [24, 25].

A novel feature of the present study is that it provided the opportunity to examine the response to allergen challenge of the lung in allergic and non-allergic sheep. Indeed, this is the first study to correlate the induction of allergen-specific serum IgE responses to an allergic inflammatory response in the sheep lung. It was shown that experimental sensitized sheep segregate into high and low IgE responders to HDM, similar to humans. Sheep that display high specific IgE serum titres (allergic sheep) generated an elevated and prolonged BAL eosinophilia when challenged locally with HDM, compared with non-allergic and control sheep. Parallels can be drawn with humans in that allergic individuals with allergen-specific IgE in their sera are biased toward the production of Th2 cytokines such as interleukin (IL)-4 and IL-5 [26, 27], which are known inducers of IgE switching and eosinophilia [1, 28].

A distinct advantage of sheep over smaller animal models of asthma is that multiple samples and measurements can be taken from one or more lung compartments in the same animal. The segmental approach has proven to be a valuable tool for studying local responses in the lung following antigen challenge in humans [29, 30] and sheep [17]. We used this approach in the present study to examine the kinetics of the allergic response to HDM challenge in sheep lungs.

Lung challenge with HDM in allergic sheep leads to a transient appearance of neutrophils and the recruitment of eosinophils and activated lymphocytes into the lung tissue and bronchoalveolar fluid. This was similar to previous studies in a sheep mammary infusion model of allergic inflammation [20], although the level of eosinophilia obtained in the mammary gland (40 to 65%) was not achieved in the lung model. The cellular and tissue changes described in the present study resemble some of the pathological features associated with the late asthmatic response in human bronchial asthma [31]. The prolonged eosinophilia, as well as the representation of eosinophils in BAL (10 to 30%), is similar to that found in human asthmatics [31, 32]. In contrast, previous studies in sheep have reported only 0.5 to 1% of BAL eosinophils after local lung challenge with *Ascaris suum* [33] or a recombinant lungworm antigen [17].

Adhesion molecules play a pivotal role in the recruitment of leucocytes to the site of inflammation and we have previously shown that their expression on eosinophils and lymphocytes is modulated during the course of an allergic response [20]. In allergen-challenged lungs, CD62L (L-selectin) was down-regulated on eosinophils and lymphocytes in BAL compared with blood. In addition, the modulation of CD49d (VLA-4) on BAL eosinophils and the elevated expression of VCAM-1 (the ligand for VLA-4) on endothelial cells in inflamed allergen-challenged lungs, as reported in human lung biopsies [34], supports an important role for VLA-4 in the recruitment of inflammatory cells during the allergic response in the sheep lung.

These observations are in agreement with previous work in the sheep *Ascaris* model where *in vivo* blocking of VLA-4 [16, 33] and L-selectin [35] resulted in suppression of antigen-induced late bronchial responses and airway hyper-responsiveness.

A significant advantage of using sheep as a model for human respiratory disease lies in the structural, histological and functional features of the sheep lung, which are similar to those of the human lung. Examples include the characteristics and distribution of respiratory tract mast cells [13, 36] and histochemical attributes of the tracheobronchial epithelium [12, 37]. The induction of bronchial hyper-responsiveness, another feature of the late asthmatic response [38, 39], has been demonstrated in experimental allergic sheep [8]. These physiological and anatomical similarities indicate that appropriate experimental studies in sheep will be of direct relevance to human respiratory disease. The HDM sheep lung inflammation model will be a useful tool for studying underlying molecular interactions in allergen-challenged lungs and will allow correlations to be made between immunological events and airway responsiveness. Recent studies using this model have demonstrated up-regulation of mRNA of two ovine CC chemokines, monocyte chemoattractant protein (MCP)-1 α and MCP-2, in allergen-challenged lungs [18], and the release of a novel eosinophil-specific molecule into the BAL fluid [40].

In conclusion, we have presented a new animal model of human asthma based on sensitization to house dust mite. The immunological characteristics of the model include development of an allergen-specific IgE response and allergen-induced inflammation of the lung similar to that observed in atopic asthmatics.

Acknowledgements

The authors would like to thank Margaret McNeill (AgResearch, New Zealand) for performing the HDM-specific IgE ELISAs. This work was supported by the NH & MRC of Australia.

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