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The effect of R 15.7/HO, an anti-CD18 antibody, on the late airway response and airway hyperresponsiveness in an allergic rabbit model

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1 The effects of a mouse (IgG_1 fraction) anti-CD 18 neutralizing antibody (R15.7) on allergen-induced late airway response (LAR), airway hyperresponsiveness (AHR) and cellular recruitment were investigated in an allergic rabbit model.

2 Litter-matched NZW rabbits immunized within 24 h of birth with *Alternaria tenuis* (i.p.) and subsequently exposed to the allergen (i.p.) for the first 3 months of life were challenged with inhaled allergen as adult rabbits. Lung function in terms of dynamic compliance (C_{dyn}; ml cmH_2O^{-1}) and total lung resistance (R_L; $cmH_2O^{-1}\ s^{-1}$) was monitored for 6 h following the allergen challenge. On day 16, separate groups of rabbits were pretreated with either control antibody (a non-binding mouse IgG_1, 1 mg kg^{-1}, i.v.) or R15.7 (1 mg kg^{-1}, i.v.) and 1 h later all were challenged with *Alternaria tenuis* and lung function monitored thereafter. Airway responsiveness to inhaled histamine was assessed by measuring R_L and C_{dyn} 24 h before and after allergen challenge and bronchoalveolar lavage (BAL) was also performed 24 h before and after allergen challenge.

3 Pretreatment of rabbits with the control antibody had no effect on the LAR as measured by AUC (C_{dyn}, 0–6 h). However, the magnitude of the LAR following treatment with R15.7 was significantly reduced when compared to LAR demonstrated on 1st challenge ($P<0.001$) or to that of the control group on both challenges ($P<0.01$).

4 In control antibody pretreated rabbits allergen induced a significant 3.4 fold reduction in the PC_{50} response to inhaled histamine in terms of R_L changes ($P<0.05$) and a significant 2.1 fold reduction in PC_{35} response to inhaled histamine in terms of C_{dyn} changes ($P<0.05$). However, in anti-CD 18 antibody pretreated rabbits there was no significant change in responsiveness to histamine 24 h following allergen, as assessed by either R_L PC_{50} or C_{dyn} PC_{35}.

5 Allergen challenge induced a significant increase in eosinophil and neutrophil numbers ($P<0.05$) in rabbits pre-treated with control antibody, whereas treatment with R15.7 significantly inhibited this increase in the numbers of both cell types.

6 This study demonstrates that the neutralization of CD-18 molecules reduces allergen-induced infiltration of both eosinophils and neutrophils into the airways and abolishes the accompanying LAR and AHR. These results provide evidence to support a role for CD-18 adhesion molecules in the transmigration of inflammatory cells into airways.

Keywords: Anti-CD18, antibody; inflammation; airway hyperresponsiveness

Introduction

Bronchial asthma is a complex disease which is characterized by episodes of spontaneous air flow limitation and persistent airway hyperresponsiveness (National Asthma Eduction Program, 1991). Histological assessment of asthmatic airways reveals increased presence of pro-inflammatory cells e.g. T-lymphocytes and eosinophils as well as chronic remodelling of the airway wall (Dunnill, 1960; Laitinen *et al*., 1985; Jeffery *et al*., 1989).

Vascular endothelial cells form the interface between blood and tissues and have the ability to interact with both the soluble and cellular elements of blood. It is the endothelium that is responsible for the control of transmigration of leukocytes (pro-inflammatory cells) into tissues such as the airways (Wegner *et al*., 1991). Furthermore, it has been shown that there is basal expression of adhesion molecules in normal bronchial mucosa (Montefort *et al*., 1992; 1994). However, the process of leukocyte infiltration, is likely to be related to the induction or up-regulation, over a matter of hours, of cytokine inducible endothelial and also leukocyte adhesion molecules by a process involving *de novo* protein synthesis (Hansel *et al*., 1992; Tosi *et al*., 1992). Several of the adhesion molecules on the vascular endothelium have been identified. These include members of the immunoglobulin superfamily including the intercellular adhesion molecules-1 and 2 (ICAM-1 and ICAM-2) and vascular cell adhesion molecule-1 (VCAM-1) (Springer, 1990).

The adhesion molecules of the integrin family on leukocytes act as counter ligands for the endothelial cell (receptors) adhesion molecules. The important ones in this regard being the members of the β_2 and β_1 sub-families. The β_2 integrin subfamily is found exclusively on leukocytes and is composed of three distinct, but related, α-chain polypeptides: CD11a, C11b and CD11c which are expressed on the cell surface in a non-covalent association with a common β_2 subunit, CD18 (Hellewell, 1993). These three α/β heterodimers are also referred to as, LFA-1, Mac-1 and p150/95 respectively. The CD11a/CD18 complex has been shown to mediate transient adhesion to the endothelium (Lo *et al*., 1989) and participates in acute and delayed inflammatory responses in the rabbit (Lindbom *et al*., 1990).

Interest in the role of adhesion molecules in asthma was heightened following the observations of Wegner and Gundell who characterized the relationship between eosinophilic infiltration and airway responsiveness in a primate model of

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asthma (Gundel *et al*., 1992). The administration of an anti-ICAM-1 antibody *in vivo* reduced eosinophil infiltration and airway hyperresponsiveness which was evident following inhalation of antigen (Gundel *et al*., 1992).

The purpose of the present study was to investigate further the role of the β_2 integrin in the development of allergen induced LAR, AHR and cellular recruitment into the lungs with a monoclonal antibody (R15.7) directed against CD18 in a well characterized allergic rabbit model.

Methods

Immunization protocol

New Zealand White (NZW) rabbits (Froxfield Farms, Petersfield, Hampshire) of either sex, were used for this study. The immunization of neonatal rabbits has been described previously (Herd *et al*., 1994). Rabbits were injected on the day of birth with 0.5 ml (i.p.) *Alternaria tenuis* extract at 40,000 protein nitrogen units (PNu) ml^{-1} in Al $(OH)_3$ moist gel and saline in the ratio of 2:1:1. The intraperitoneal administration of antigen and adjuvant was repeated weekly for the first month and then biweekly for the following 2 months. Sham immunized littermates were injected intraperitoneally with 0.5 ml of $Al(OH)_3$ and saline in the ratio of 1:3. This was repeated as described above. The methods described in this study were subject to Home Office approval and performed under the Animals (Scientific Procedures) Act, 1986.

Pulmonary function measurement

For measurement of pulmonary function all rabbits were sedated with diazepam (2.5 mg kg^{-1}, i.p.) and then anaesthetized with Hypnorm (0.4 ml kg^{-1}, i.m.; a mixture of fentanyl citrate 0.315 mg ml^{-1} and fluanisone 10 mg ml^{-1}). Animals were placed in a supine position and intubated with a 3.00 mm endotracheal tube (Mallinckrodt Laboratories, Athlone, Ireland). The cuff was inflated and the tube attached to a heated Fleisch pneumotachograph. Flow was measured with a validyne differential pressure transducer (model MP 45-14-871; Validyne Engineering Corp., Northridge, California, U.S.A.). Pleural pressure was estimated by placing an oesophageal balloon in the lower third of the oesophagus to obtain the maximum expiratory pressure. Transpulmonary pressure, the difference between atmospheric and pleural pressure, was measured with another Validyne differential pressure transducer (model MP 45-24-871) connected between the outflow of the endotracheal tube and oesophageal balloon. The flow was integrated to obtain a continuous recording of tidal volume. Measurements of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated by an on-line respiratory analyser (PMS version 5.1, Mumed Ltd., London, U.K.) as previously described (Herd *et al*., 1994) at isovolumatic and zero flow points respectively.

Antigen challenge

At the age of three months rabbits were challenged (by inhalation) with an aerosol of *Alternaria tenuis* extract in 0.9% saline (20,000 PNu ml^{-1}) by use of a DeVilbiss nebulizer (Herd *et al*., 1994). The antigen challenge consisted of a 2 min aerosol of saline, followed by two 2 min antigen aerosols, then 4 consecutive antigen aerosols of 4 min duration (total 20 min). After each challenge respiratory function was recorded as described above. Lung function was monitored for a period of 6 h after the end of the challenge. Measurements of dynamic compliance were taken every 15 min for the first 1 h and then every 30 min for the next 5 h. This period of exposure has been previously shown to induce both early (EAR) and late (LAR) airway responses in this model (Coyle *et al*., 1989; 1990). Moreover, the period of antigen exposure is so designed to allow for measurements of acute changes to allergen inhalation to be recorded during the allergen challenge. Neuroleptanalgesia was maintained throughout the course of the experiment by administration of Hypnorm i.m. approximately every 30 min (Flecknall 1987).

Airway responsiveness

After measurement of baseline lung function, rabbits were exposed to an aerosol of 0.9% saline for 2 min, and lung function parameters were recorded. Airway responsiveness was determined by exposing the rabbits to cumulative concentrations of an aerosol of histamine (1.25–160 mg ml^{-1}; 2 min per dose) administered directly to the lungs via the endotracheal tube. Pulmonary function was recorded following each 2 min exposure and the mean values of total lung resistance (R_L) and dynamic compliance (C_{dyn}) were calculated.

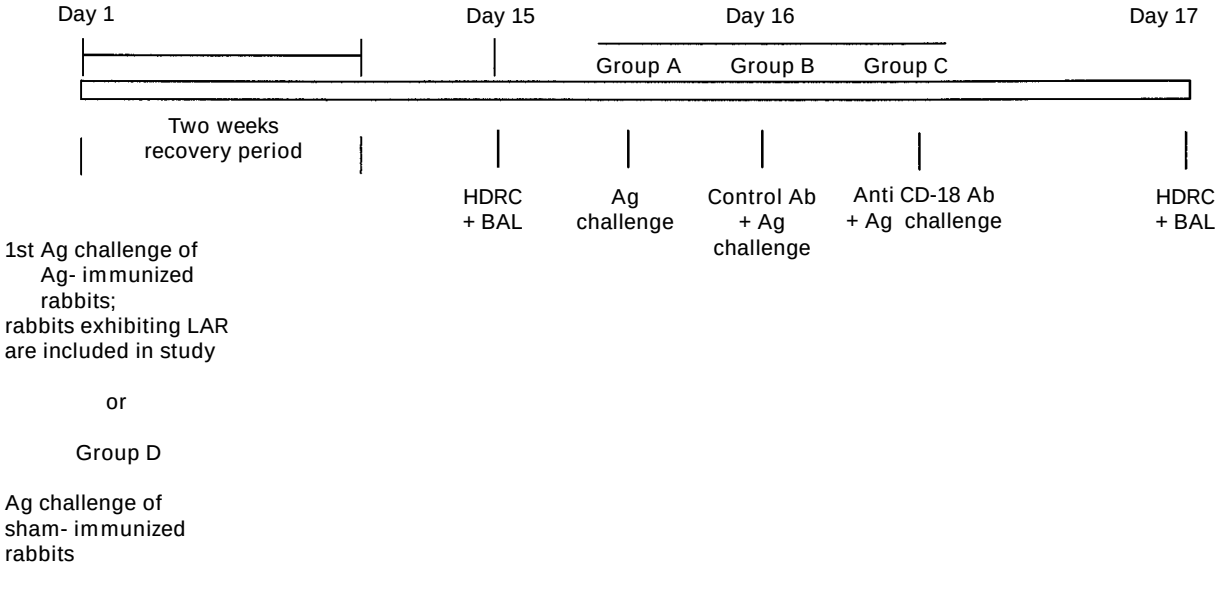

Figure 1 The experimental design. In Ag/immunized Ag challenged rabbits, only those rabbits from groups B and C had undergone bronchoalveolar lavage (BAL) procedure performed on days 15 and 17.

The provocation concentration (PC) of histamine which produced a 50% increase in R_L (PC_{50}) and a 35% decrease in C_{dyn} (PC_{35}) was determined for each animal. These were used as indices of airway responsiveness.

Aerosols were generated by an ultrasonic nebulizer (De-Vilbiss Health Care, U.K., Ltd, Hounslow, Middlesex) which had previously been shown to generate particles of which the majority were in the 0.5–5 μm diameter range.

Bronchoalveolar lavage (BAL)

BAL was performed after the determination of airway responsiveness to histamine on both day 15 and 17. A polythene catheter was inserted via the endotracheal tube into the lung, until resistance was felt. Then 5 ml of sterile saline was injected into the lungs and retrieved by gentle suction under vacuum. Cytospin preparations were made and the cells enumerated after haemotoxylin and chromotrope staining as described previously (Herd *et al*., 1994).

Experimental protocol

The experimental protocol used in this study is depicted in Figure 1.

Day 1 (screening) All Alternaria immunized rabbits were challenged by inhalation with *Alternaria tenuis* (20,000 PNu ml^{-1}) for a total of 20 min and lung function was monitored for 6 h thereafter as described earlier. Only those rabbits exhibiting a LAR were subsequently used to evaluate the anti-CD 18 antibody. Two weeks after the screening challenge, a time that baseline lung function values had returned to pre antigen challenge values, immunized rabbits were randomly assigned to one of 3 groups: allergen challenge only, $n=9$, (group A); (allergen challenge in mouse IgG_1-pretreated rabbits, $n=7$, (group B); and allergen challenge in R15.7 pretreated rabbits, $n=9$ (group C). Sham immunized rabbits ($n=4$, group D) were also challenged with *Alternaria tenuis* and lung function was monitored over 6 h to act as control rabbits. (Figure 1).

Day 15 Airway responsiveness to inhaled histamine was determined in animals from all groups. Bronchoalveolar lavage (BAL) was performed immediately after completion of the histamine dose-response curve (HDRC).

Day 16 Animals from group A were challenged with *Alternaria tenuis* aerosol and lung function was assessed as on day 1. Animals from groups B and C were administered either control (whole molecule IgG_1) or test (R15.7) antibody intravenously, 1 h before challenge, at a concentration of 1 mg kg^{-1} which, in other studies was shown to exert a significant biological effect (Welbourn *et al*., 1990; Ma *et al*., 1991). Aerosol antigen challenge and lung function measurements were performed as on day 1.

Day 17 HDRC and BAL were performed as on day 15.

Expression and analysis of results

Late airway response (LAR) Percentage changes in C_{dyn} (0–6 h) from baseline following antigen challenge were calculated for animals in all groups. The LAR was defined as a significant fall in % change in C_{dyn} (0–6 h) from baseline in allergen immunized, allergen challenged rabbits when compared to sham immunized, allergen challenged animals. The area under the curve (AUC, % fall in C_{dyn} × min) was determined by use of the Origin programme, (MicroCal Software, Inc, U.S.A.). The AUC was measured from 0–6 h as opposed to 1–6 h as there was no significant difference in the magnitude of the early bronchoconstriction between the different treatment groups. Therefore differences in the early response could not account for changes in overall AUC. Dunnett's test was used to compare the magnitude of LAR between sham immunized/allergen challenged and *Alternaria* immunized/allergen challenged rabbits. Student's paired and unpaired t tests were used to compare the magnitude of LAR before and after treatment and between different treatment groups.

We have also shown the temporal response to antigen in terms of R_L for group C before R15.7 pretreatment. However, because there was not a consistently measurable response in this parameter to allergen inhalation challenge, it was not possible to test the anti CD-18 antibody on allergen-induced changes in R_L (as an index of lung function changes to allergen).

Airway responsiveness Airway responsiveness to inhaled histamine is expressed as the percentage change in R_L and C_{dyn} from baseline values in response to increasing doses of inhaled histamine. The doses of histamine required to provoke a 50% increase (PC_{50}) R_L and a 35% decrease (PC_{35}) in C_{dyn} were determined. A one way ANOVA test was used to assess the histamine responsiveness between groups before antigen challenge. Student's paired t tests were used to assess the histamine responsiveness data before and after antigen challenge by use of geometric mean log 10 transformed PC_{50} and PC_{35} values.

Bronchoalveolar lavage At least 200 cells were differentiated as either neutrophils, eosinophils or mononuclear cells based on standard morphological criteria and expressed as absolute cell counts ml^{-1} lavage fluid. Cell counts were compared by a paired t test.

Drugs

Alternaria tenuis was obtained from Greer laboratories Inc. Lenoir, N.C. U.S.A. (40,000 PNu ml^{-1}) and aluminium hydroxide ($Al(OH)_3$ moist gel from FSA laboratory supplies, Loughborough, U.K.); fentanyl citrate (Hypnorm, Janssen Pharmaceutical Ltd., U.K.). The test antibody, R15.7/HO4 (a gift of Boehringer Ingelheim Inc., Ridgefield, CT, U.S.A.) directed against CD18, is a mouse IgG_1 antibody. The Ig concentration was 2.15 mg ml^{-1}. The antibody was thawed (previously having been stored at $-70^{\circ}C$), and diluted in sterile PBS immediately before use. The control antibody was a mouse whole IgG_1 (a gift from Boehringer Ingelheim Inc., Ridgefield, CT, U.S.A.) and was prepared in the same way as the test antibody.

Results

Late airway response (LAR)

Alternaria challenge of sham immunized rabbits failed to induce a significant fall in baseline C_{dyn} over the 6 h period as assessed by the AUC (Table 1). In contrast allergen challenge

Table 1 Area under curve (AUC) $_{0-6\ h}$ in sham and *Alternaria* immunised rabbits challenged with *Alternaria tenuis*

Treatment	n	*Day 1*	*Day 16*
Allergen/immunized	9	157.8±12.4*	186.3±15.1
Control antibody	7	157.5±25.0*	180.6±24.1
Test antibody	9	192.9±20.8*	53.1±17.5#
Allergen/sham	4	−16±29.2	ND

Results are expressed as mean±s.e.mean in 4–9 rabbits. Immunized (allergen/immunized) rabbits were challenged with allergen on day 1 and day 16. In other groups immunized rabbits were pretreated with control antibody (IgG) or with anti-CD 18 antibody before the 2nd allergen challenge. *Indicates $P<0.01$ compared to sham challenged value (Dunnett's test). #Indicates $P<0.001$ compared to challenge on day 1 (paired t test). ND: not determined.

of immunized rabbits on day 1 caused a significant fall in C_{dyn} that is reflected by a significantly greater AUC in groups A, B and C compared with the sham immunized group (Table 1; $P<0.01$). There was no significant response to allergen in terms of R_L during the LAR in any of the groups. Response in terms of R_L to allergen challenge on day 1 is shown for group C (Figure 2).

The ability of allergen challenge to induce a LAR was shown to be reproducible with no significant difference in AUC compared with that observed at day 1 (Figure 3, Table 1, $P>0.05$). Similarly, the allergen-induced LAR in rabbits pretreated with control antibody (group C) was not significantly different when compared with the LAR demonstrated by the same rabbits on day 1 (Figure 4a, Table 1, $P>0.05$). In contrast, the ability of R15.7 pretreated rabbits to develop a LAR following allergen challenge on day 16 was significantly inhibited compared with the LAR obtained on day 1 (Figure 4b, Table 1, $P<0.001$) or to that of the control group on both challenges ($P<0.01$).

Airway responsiveness to histamine

There was no significant difference in airway responsiveness to histamine between allergen immunized rabbits before allergen challenge between the 3 groups (Figures 5, 6 and 7, $P>0.05$). In group A, antigen-induced airway hyperresponsiveness to inhaled histamine 24 h after challenge with Ag was shown by a 3.7 fold reduction in R_L PC_{50} (Figure 5a, $P<0.05$) and a 2.3 fold reduction in C_{dyn} PC_{35} (Figure 5b, $P<0.05$). In the control antibody treated group, antigen-induced airway hyperresponsiveness was also demonstrated by a 3.4 fold reduction in R_L PC_{50} (Figure 6a, $P<0.05$) and a 2.1 fold in C_{dyn} PC_{35} (Figure 6b, $P<0.05$). However in the anti-CD 18 antibody pretreated rabbits, there were no significant changes in airway responsiveness to histamine 24 h following Ag challenge as assessed by either R_L PC_{50} or C_{dyn} PC_{35} (Figure 7a and b).

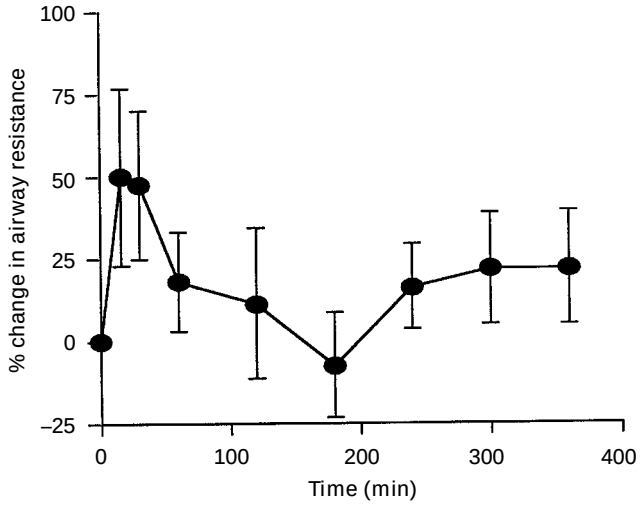

Figure 2 Effect of *Alternaria tenuis* on lung function as measured by % change in airways resistance (0–6 h). Vertical lines show s.e.mean.

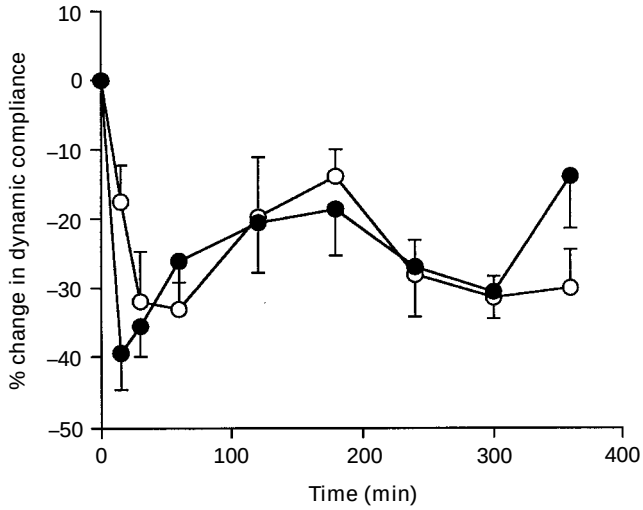

Figure 3 Effect of inhaled *Alternaria tenuis* on lung function as measured by changes in dynamic compliance in NZW rabbits ($n=9$) on two different days, day 1 (●) and day 16 (○). There was a significant fall in dynamic compliance on both days ($P<0.01$; Dunnett's test) between this group and sham immunized rabbits. However, there was no significant difference in the fall in dynamic compliance between day 1 and day 16 ($P>0.05$; paired t test). Vertical lines show s.e.mean.

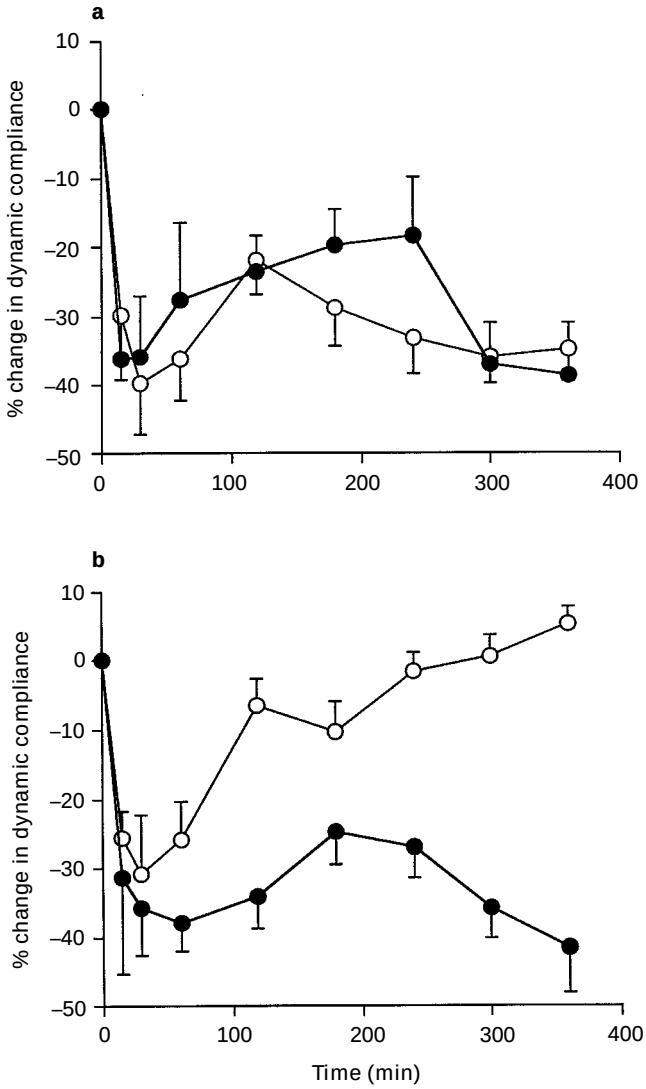

Figure 4 Effect of (a) control antibody and (b) anti-CD18 antibody on *Alternaria tenuis*-induced changes in dynamic compliance in NZW rabbits ($n=7-9$). *Alternaria tenuis* challenge resulted in a fall in dynamic compliance on day 1 from 0–6 h in both groups (●; $P<0.01$; Dunnett's test). Pretreatment of the rabbits with control antibody on day 16 did not significantly affect *Alternaria tenuis*-induced fall in dynamic compliance (○; $P>0.05$ paired t test). In contrast pretreatment of the rabbits with anti-CD18 antibody on day 16 resulted in a significant reduction in the *Alternaria tenuis*-induced fall in dynamic compliance (○; $P<0.001$; paired t test). Vertical lines show s.e.mean.

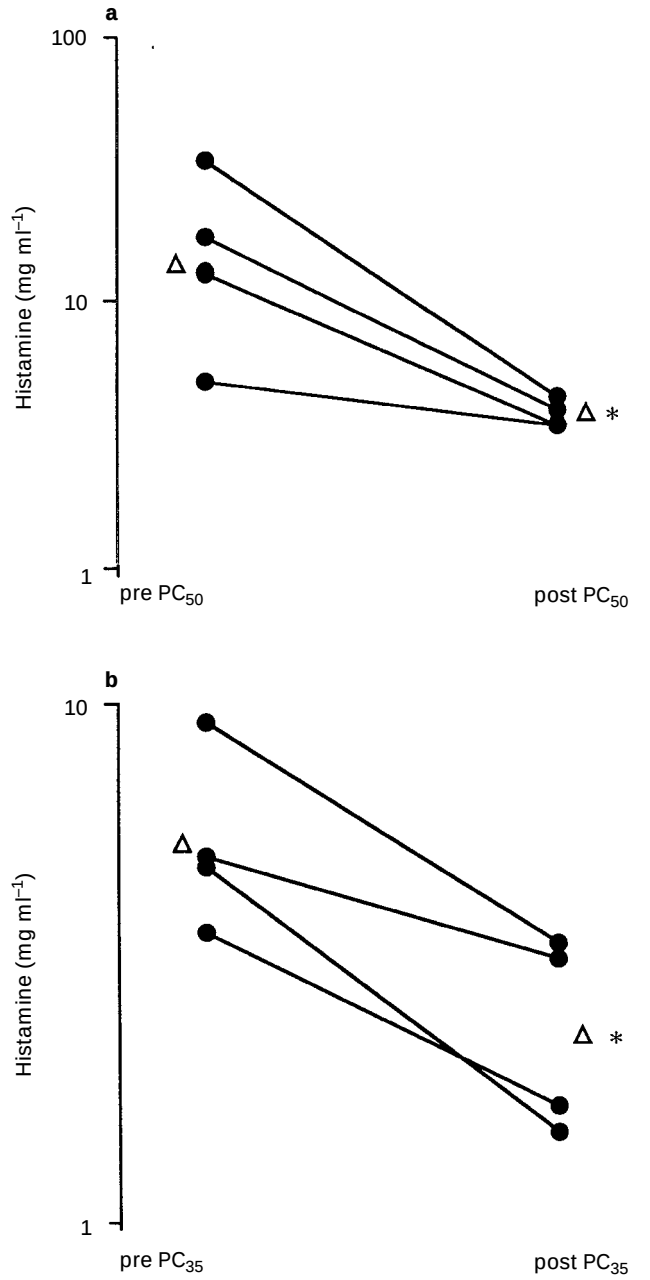

Figure 5 The dose of inhaled histamine required to provoke a 50% increase in (a) R_L and 35% decrease in (b) C_{dyn} in the reproducibility group is shown as individual values and the means before and after allergen challenge. *Indicates $P<0.05$ vs pre allergen (paired t test).

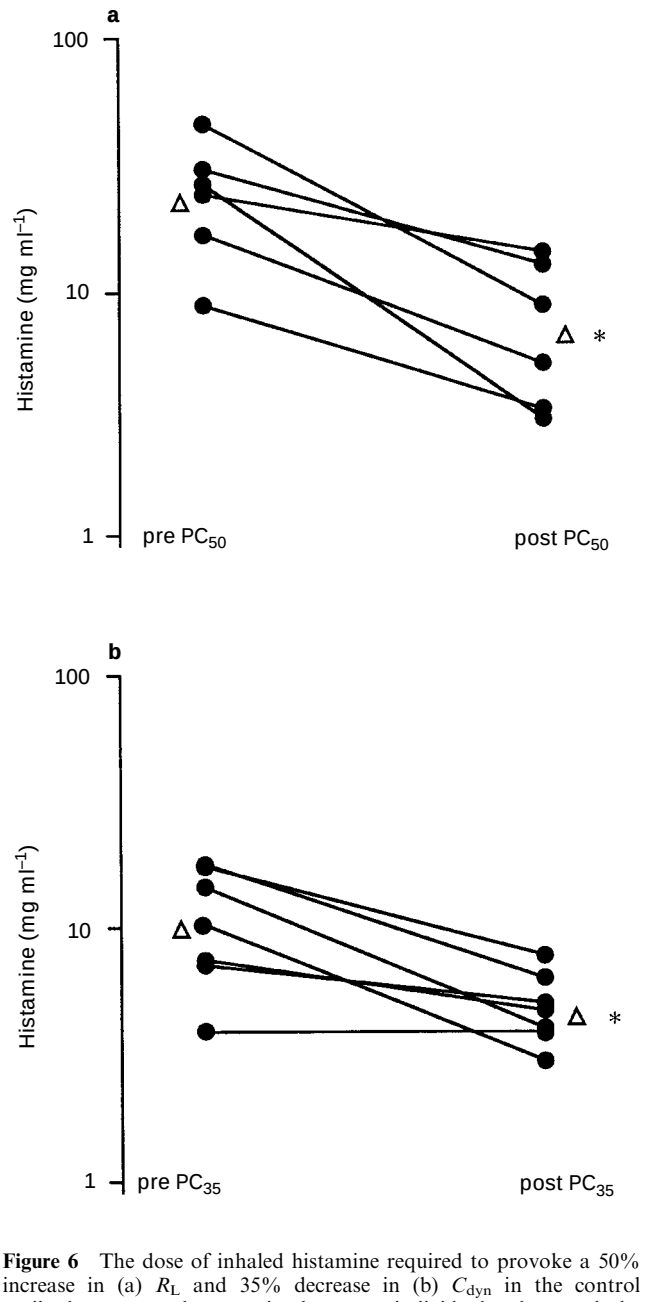

Figure 6 The dose of inhaled histamine required to provoke a 50% increase in (a) R_L and 35% decrease in (b) C_{dyn} in the control antibody pretreated group is shown as individual values and the means before and after allergen challenge. *Indicates $P<0.05$ vs pre allergen (paired t test).

Bronchoalveolar lavage (BAL)

The volume of fluid recovered from BAL was not significantly different between the control and anti-CD 18 antibody pretreated groups before or after allergen challenge (2.0–3.5 ml, 40–70% recovery, data not shown). The total leukocytes recovered in BAL fluid 24 h after allergen challenge were increased by 30% in the control antibody pretreated rabbits (although this just failed to reach statistical significance; Table 2). However, in R15.7 pretreated rabbits there was only a 5.5% increase in total leukocyte numbers 24 h after allergen inhalation (Table 2, $P>0.05$). Analysis of the differential cell counts revealed that the neutrophil numbers were significantly elevated by 10.2 fold ($P<0.05$) following allergen challenge in control antibody-treated rabbits with a significantly lower, 2.6 fold ($P<0.05$), increase in R15.7 pretreated rabbits (Table 2). Eosinophils were not detectable before allergen challenge but the number of eosinophils recovered in BAL was significantly increased in control antibody pretreated rabbits ($P<0.05$). However, by comparison R15.7 pretreatment significantly reduced the eosinophilia ($P<0.05$, Table 2) compared with control antibody pretreated rabbits.

Discussion

Our results provide evidence that neutralizing CD18 with the antibody R15.7 significantly reduced the magnitude of the LAR, the AHR and the influx of inflammatory cells into the airways that are induced following allergen inhalation in an established allergic rabbit model (Herd & Page, 1996). These results provide further evidence that expression of the β_2 integrins on the surface of leukocytes plays an important role in the manifestation of several of the features of allergic asthma.

The LAR following allergen inhalation has been used extensively as a model of asthma and is considered to be a consequence of the release of both preformed and newly syn-

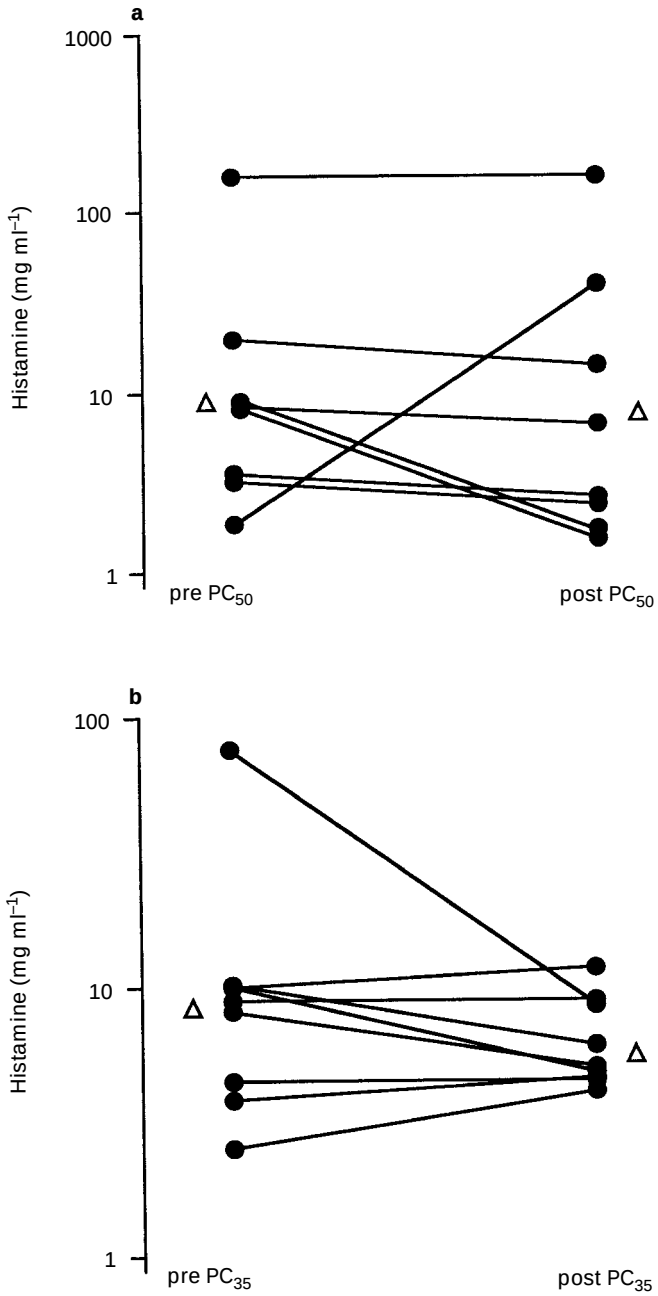

Figure 7 The dose of inhaled histamine required to provoke a 50% increase in (a) R_L and 35% decrease in (b) C_{dyn} in the anti CD-18 antibody pretreated group is shown as individual values and the means before and after allergen challenge. There was no significant difference between pre and post allergen PC_{50} and PC_{35} values.

thesized mediators from a range of inflammatory cells, which are recruited into the airways by various chemotactic agents (Weersink *et al*., 1994). The inflammatory cell infiltration into the airways that follows allergen exposure (De Monchy *et al*., 1985) has been suggested to contribute to the exacerbation of AHR that can occur (Durham & Kay, 1985). Such a conclusion is supported by observations showing that anti-inflammatory drugs, such as glucocorticosteroids, effectively abolish the LAR, cellular infiltration and AHR, both clinically (Cockcroft & Murdock, 1987; O'Byrne *et al*., 1987) and experimentally (Abraham *et al*., 1986; Gozzard *et al*., 1996). Our results further support the hypothesis that airway inflammation is related to AHR and are consistent with previous studies in ovalbumin-sensitized rats where anti-LFA-1 or anti-Mac-1 antibodies were shown to reduce significantly the LAR (Rabb *et al*., 1994) and studies in guinea-pigs where an anti-CD18 antibody reduced airways inflammation and AHR (Milne & Piper, 1994).

However, we did not completely inhibit the infiltration of neutrophils and eosinophils into the airways, despite almost complete inhibition of the LAR and the AHR. It is possible therefore that the antibody is merely slowing down the migration of these cells into the airways rather than actually inhibiting this process. Another possibility for the incomplete inhibition of the cellular infiltration is that the cells we measured were migrating in part via mechanisms independent of β_2 integrins. An anti-VLA 4 antibody has certainly been observed to inhibit the accumulation of eosinophils into guinea pig skin (Weg *et al*., 1993) and neutrophil migration out of the pulmonary microcirculation has been shown to have a CD18-independent component (Doerschuk *et al*., 1990; Hellewell *et al*., 1994). Other investigators have shown that an anti-LFA-1 antibody inhibited both allergen induced eosinophilia and AHR in ovalbumin sensitized rats, whereas an anti-VLA 4 antibody that binds VCAM-1 (Hellewell, 1993), prevented only the AHR (Laberge *et al*., 1995). These results have been taken as evidence that the presence of eosinophils in the airways may not necessarily be causally related to the exacerbation of AHR (Laberge *et al*., 1995). Moreover, in the study by Milne & Piper (1994) two different anti-CD18 antibodies were used (R15.7 and 6.5E); both were effective in blocking the allergen-induced eosinophilia but only R15.7 blocked both allergen-induced eosinophilia and AHR. Clearly these studies would suggest that the relationship between eosinophil recruitment and AHR is not simple. Interestingly, it has been shown in rabbits that mechanical manipulation of the lungs results in a cellular infiltrate, which unlike that induced by allergen is not associated with AHR (Irvin *et al*., 1986). Some investigators have gone so far as to question whether airways infiltration has any role in AHR (Smith & McFadden, 1995) and there are certainly a number of pharmacological studies that have dissociated airways infiltration from exacerbation of AHR (Spina *et al*., 1991; Milne & Piper, 1994; Herd *et al*., 1995; Laberge *et al*., 1995). Furthermore, several clinical studies have now also revealed the

Table 2 Total and differential cell numbers recovered from bronchoalveolar fluid before and 24 h following antigen challenge in immunized rabbits pretreated with IgG_1 (control antibody) or R15.7 anti-CD 18 antibody

	Cells ($\times 10^4$ ml^{-1} BAL)			
	Total	*Neutrophils*	*Eosinophils*	*Mononuclear cells*
Control antibody				
Pretreated	32.0±6.12	0.48±0.13	0.00	31.52±0.13
24 h post	41.7±1.8	4.92±0.23*	1.73±0.2*	35.53±0.52*
Anti CD-18 antibody				
Pretreated	36.0±5.0	0.59±0.20	0.00	35.3±0.18
24 h post	38.0±5.2	1.52±0.32#	0.57±0.23#	35.5±0.55

Results are expressed as mean±s.e.mean from $n=3$ rabbits. *Indicates $P<0.05$ vs pre antigen (paired t test). #Indicates $P<0.05$ vs post antigen control antibody pretreated (unpaired t test).

presence of AHR in the absence of airways infiltration (Lundgren *et al*., 1988; Gibson *et al*., 1989).

Over the last few years, the upregulation of adhesion molecules has been noted in asthmatics (Montefort *et al*., 1992; 1994; Hansel *et al*., 1992) and clearly plays a crucial role in the migration of infiltrating cells into tissues such as lungs. Our results support this crucial role as we have shown that neutralizing CD18 significantly reduces the infiltration of inflammatory cells into the airways and abolishes the LAR and AHR in an animal model. Our data also lend support to the idea that these molecules may be potential targets for future anti-inflammatory drugs used to treat inflammatory diseases of the lung.

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