α_4-Integrins Mediate Antigen-induced Late Bronchial Responses and Prolonged Airway Hyperresponsiveness in Sheep

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Abstract

Eosinophils and T lymphocytes are thought to be involved in allergic airway inflammation. Both cells express the $\alpha_4\beta_1$-integrin, very late antigen-4 (VLA-4, CD49d/CD29); α_4-integrins can promote cellular adhesion and activation. Therefore, we examined the in vivo effects of a blocking anti-α_4 monoclonal antibody, HP 1/2, on antigen-induced early and late bronchial responses, airway hyperresponsiveness, inflammatory cell influx, and peripheral leukocyte counts in allergic sheep. Sheep blood lymphocytes, monocytes, and eosinophils expressed α_4 and bound HP 1/2. In control sheep, *Ascaris* antigen challenge produced early and late increases in specific lung resistance of 380±42% and 175±16% over baseline immediately and 7 h after challenge, respectively, as well as airway hyperresponsiveness continuing for 14 d after antigen challenge. Treatment with HP 1/2 (1 mg/kg, i.v.) 30 min before antigen challenge did not affect the early increase in specific lung resistance but inhibited the late-phase increase at 5–8 h by 75% ($P < 0.05$) and inhibited the post-antigen-induced airway hyperresponsiveness at 1, 2, 7, and 14 d ($P < 0.05$, for each time). Intravenous HP 1/2 given 2 h after antigen challenge likewise blocked late-phase airway changes and postchallenge airway hyperresponsiveness. Airway administration of HP 1/2 (16-mg dose) was also effective in blocking these antigen-induced changes. Response to HP 1/2 was specific since an isotypic monoclonal antibody, 1E6, was ineffective by intravenous and aerosol administration. Inhibition of leukocyte recruitment did not totally account for the activity of anti-α_4 antibody since HP 1/2 neither diminished the eosinopenia or lymphopenia that followed antigen challenge nor consistently altered the composition of leukocytes recovered by bronchoalveolar lavage. Because airway administration of HP 1/2 was also active, HP 1/2 may have inhibited cell activation. Reduction of platelet-activating factor–induced eosinophil peroxidase release from HP 1/2–treated eosinophils supports such a mechanism. These findings indicate a role for α_4-integrins in processes that lead to airway late phase responses and persisting airway hyperresponsiveness after antigen challenge. (*J. Clin. Invest.* 1994. 93:776–787.) Key words: adhesion molecules • asthma • bronchoprovocation • eosinophils • inflammation

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Introduction

The development of late bronchial responses after airway challenge may be the initial physiologic sign of a chronic inflammatory process that leads to a prolonged increase in airway responsiveness. A number of recent studies of asthmatics have characterized the inflammatory patterns associated with the development of late responses and airway hyperresponsiveness after airway antigen challenge (1–4). Like bronchoalveolar lavage fluids (5), biopsied airway tissues (6–10) from patients with asthma contain increased numbers of eosinophils and T lymphocytes. Moreover, eosinophils and lymphocytes recruited into airway tissues after antigen challenge exhibit characteristics compatible with their activation.

One recognized adherence pathway that eosinophils and T lymphocytes might utilize in common is binding to vascular cell adhesion molecule-1 (VCAM-1)[1] that can be induced to be expressed on endothelial cells (11). Cell binding to VCAM-1 is mediated by very late antigen-4 (VLA-4), the $\alpha_4\beta_1$ heterodimeric integrin (CD49d/CD29) expressed on both T lymphocytes and eosinophils, but not neutrophils (12–16). VLA-4 also mediates binding of these cells to the CS-1 region of fibronectin (17) and potentially to an additional endothelial cell ligand (18). Blockade of the α_4 chain, CD49d, with specific monoclonal antibodies can inhibit adherence of eosinophils, lymphocytes, and monocytes to VCAM-1 and fibronectin (12, 13, 16, 19–21). Another α_4-containing heterodimeric integrin, $\alpha_4\beta_7$, mediates adherence of T lymphocytes to Peyer's patches (22), and anti-CD49d antibodies can block cellular adhesion mediated by the $\alpha_4\beta_7$-integrin (23). In addition to participation of α_4-integrins in cellular adhesion, engagement of α_4-integrins may be involved in cellular activation pathways (24–28). Thus, α_4-integrins are potentially involved by varied mechanisms in pathways of recruitment and activation of lymphocytes and eosinophils.

To formally evaluate whether inhibition of α_4-integrin functions might have a beneficial effect in allergic diseases in vivo, we have utilized an α_4-specific monoclonal antibody, HP 1/2, that blocks and does not promote α_4-integrin–dependent cellular adhesion and activation (19, 28). If the development of asthmatic late-phase airway responses and airway hyperresponsiveness is dependent on recruitment or activation of α_4-integrin expressing leukocytes, including eosinophils and T lymphocytes, then HP 1/2 might protect against these antigen-induced effects. Our results with allergic sheep demonstrate that HP 1/2 is effective in inhibiting *Ascaris* antigen-induced late phase airway responses and in blocking the airway hyperre-

1. *Abbreviations used in this paper:* BAL, bronchoalveolar lavage; BU, breath unit; EPO, eosinophil peroxidase; PAF, platelet-activating factor; VCAM-1, vascular cell adhesion molecule-1; VLA-4, very late antigen-4.

sponsiveness that normally persists for 2 wk after antigen challenge.

Methods

In vitro studies

Detection of α_4 expression on sheep leukocytes. The ability of mAb HP 1/2 against human α_4 (19, 28) to detect and inhibit sheep α_4-integrins was evaluated by flow cytometry, radioimmunoprecipitation, and leukocyte adhesion assays. Sheep venous blood anticoagulated with EDTA was diluted 1:1 with Dulbecco's PBS without calcium and magnesium (Sigma Chemical Co., St. Louis, MO) and fractionated by sedimentation on Ficoll-Hypaque (Pharmacia, Inc., Piscataway, NJ) gradients. Mononuclear leukocyte layers and granulocyte pellets (eosinophils and neutrophils) were collected. Eosinophils were separated from neutrophils by Percoll sedimentation at a density of 1.085. Leukocytes were stained with HP 1/2 (29) or an IgG_1 isotype control myeloma protein (MOPC 21, Organon-Technika, West Chester, PA) and after incubation with phycoerythrin-labeled goat $F(ab')_2$ anti-mouse IgG were analyzed by flow cytometry as previously described (12). Flow cytometry was performed on a FACStar Plus and analyzed by the program Consort 30 (Becton Dickinson & Co., Fairleigh, NJ). Because eosinophils exhibit greater green autofluorescence than do neutrophils or mononuclear leukocytes, dual-parameter flow cytometry was utilized with cellular autofluorescence analyzed with a 530-nm filter in the green detector channel and phycoerythrin staining evaluated with a 575-nm filter in the second channel. Leukocytes, in various preparations depleted of neutrophils, eosinophils or mononuclear cells, were gated by forward and orthogonal light scatter and 10,000 gated cells were analyzed.

For cell surface labeling and immunoprecipitation of the α_4-integrin subunit, sheep and control human blood mononuclear leukocytes were labeled for 30 min at 4°C with 1 mCi of $Na^{125}I$ (Du Pont-New England Nuclear, Boston, MA) using Enzymobead solid phase radioiodination reagent (Bio-Rad Laboratories, Richmond, CA) and lysed in the presence of 1% Triton X-100, 1 mM $MgCl_2$, 8 mM iodoacetamide, 1 mM PMSF in PBS for 1 h at 4°C. Lysates were clarified by centrifugation at 10,000 rpm for 10 min, precleared with 4 μl of normal rabbit serum, and 10 μg/10^6 cells of purified mouse myeloma MOPC 21 IgG_1 protein overnight at 4°C, followed by 200 μl of recombinant protein G agarose (Gibco BRL, Gaithersburg, MD) and centrifuged to remove the insoluble complexes. Precleared lysate containing 10^7 cell equivalents were then immunoprecipitated with 2 μl of rabbit α_4 COOH terminus-specific antisera: anti-α_4, N11, gift of Dr. Martin E. Hemler (Dana Farber Cancer Institute, Boston, MA) (30), 10 μg/10^6 cell equivalents of HP 1/2, 2 μl of normal rabbit serum, or 10 μg/10^6 cell equivalents of MOPC 21, followed by 100 μl of protein G agarose. The immunoprecipitates were washed five times with 1 ml of 0.1% Triton X-100 PBS, eluted with sample buffer (5% glycerol, 1.5% SDS, 3 M Tris, pH 6.8), and analyzed without reduction by SDS/PAGE utilizing 7% polyacrylamide gels. The gels were stained with Coomassie brilliant blue and autoradiographed utilizing Kodak XAR-5 film.

Adhesion assays on recombinant soluble (rs) VCAM-1 coated plates were performed as follows: rsVCAM-1 purified from CHO cell-conditioned medium by immunoaffinity chromatography exactly as previously described (31) was added from a 1 mg/ml stock solution to 100 μl binding buffer (15 mM sodium bicarbonate/35 mM sodium carbonate, pH 9.2) in individual wells of a 96-well bacteriologic plastic plate (Linbro/Titertek, Hamden, CT), and incubated overnight at 4°C. Plates were blocked with 1% BSA in PBS for 1 h at room temperature, washed once with RPMI 1640 containing 10% FBS (RPMI/10), and adhesion assays with sheep peripheral blood mononuclear leukocytes were performed with inverted centrifugation as described (11, 32). Blood leukocyte adhesion was quantified by fluorescence as described (33). Adherence of sheep leukocytes and control VLA-4 expressing RAMOS T cells was evaluated in the presence of control IgG, the anti-α_4 mAb HP 1/2 (IgG_1), the anti-CD18 mAb, 60.3 (IgG_{2a}), and an anti-β_1 mAb TS 2/16 (IgG_1), which enhances VLA-4 mediated adhesion (34). Each was added at 10 μg/ml for 30 min at 23°C before adherence.

Eosinophil peroxidase (EPO) determination. Sheep eosinophils were isolated by sedimentation on Ficoll-Hypaque and Percoll as described above. The cells (81±7% eosinophils, in four preparations) were suspended in HBSS containing 25 mM Hepes, 1 mg/ml gelatin, 2 mg/ml BSA, and 5.5 mM glucose. Aliquots of 0.5 ml containing 0.5–1.0 × 10^6 eosinophils were then assigned to one of four groups: untreated, unstimulated (controls); HP 1/2 treated (20 μg/ml), unstimulated; untreated, stimulated; and HP 1/2 treated (20 μg/ml), stimulated. Cells were rotated at room temperature for 20 min after which cytochalasin B (5 μg/ml, Sigma) was added to all preparations. The cells were rotated for an additional 5 min at 38°C, after which platelet-activating factor (PAF) (10^{-5} M, Sigma) was added to stimulate degranulation. The cells were rotated for 30 min at 38°C. In some experiments 100 nM staurosporine (Sigma) was added to the cells 4 min before adding PAF, to increase degranulation. The cells were then centrifuged at 450 *g* for 5 min at 4°C and the supernatants collected for EPO determination using the *O*-phenylenediamine dihydrochloride (Sigma) colorimetric assay. O-phenylenediamine dihydrochloride (0.4 mg/ml) and 1.2% (vol/vol) H_2O_2 were prepared fresh in 0.5 M phosphate-citrate buffer, pH 5, and 100 μl this solution was added to 100 μl of eosinophil supernatant in a 96-well flat bottom cell culture plate (Costar, Cambridge, MA). The reaction was allowed to proceed for 30 min in a humidified CO_2 incubator at 38°C. The reaction was stopped by adding 50 μl of 2.5 M H_2SO_4 to each well. The OD of the mixture was read at 490 nm in a microplate reader (Dynatech Labs, Chantilly, VA). All reactions were run in duplicate and a blank was run in parallel in all experiments. Data were expressed as a ratio of stimulated to unstimulated optical densities.

In vivo studies

Animal preparation. A total of 12 allergic sheep weighing between 27 and 36 kg (mean 31 kg) were used in the various protocols. All sheep had previously been shown to develop both early and late bronchial responses to inhaled *Ascaris suum* antigen (35). The sheep were conscious and were restrained in a modified shopping cart in the prone position with their heads immobilized. After topical anesthesia of the nasal passages with 2% lidocaine, a balloon catheter was advanced through one nostril into the lower esophagus. The animals were intubated with a cuffed endotracheal tube through the other nostril with a flexible fiberoptic bronchoscope as a guide. All protocols used in this study were approved by the Mount Sinai Medical Center Animal Research Committee, which is responsible for assuring the humane care and use of experimental animals.

Airway mechanics. These techniques have been described previously (36–38). Pleural pressure was estimated with the esophageal balloon catheter (filled with 1 ml of air), which was positioned 5–10 cm from the gastroesophageal junction. In this position the end expiratory pleural pressure ranged between −2 and −5 cmH_2O. Once the balloon was placed, it was secured so that it remained in the same position for the duration of the experiment. Lateral pressure in the trachea was measured with a sidehole catheter (inner diameter, 2.5 mm) advanced through and positioned distal to the tip of the endotracheal tube. The tracheal and pleural pressure catheters were connected to a differential pressure transducer (MP45, Validyne, Northridge, CA) for the measurement of transpulmonary pressure which was defined as the difference between tracheal and pleural pressure. Airflow was measured by connecting the proximal end of the endotracheal tube to a pneumotachograph (Fleisch, Dyna Sciences, Inc., Blue Bell, PA). The pressure transducer-catheter system was dynamically balanced, and no phase shift was detectable between pressure and flow up to a frequency of 9 Hz. The signals of transpulmonary pressure and flow were recorded on a multichannel physiological recorder which was linked to a model 80-386 DOS personal computer (CCI Inc., Miami, FL) for on-line calculation of mean pulmonary flow resistance (R_L) by dividing the change in transpulmonary pressure by the change in flow at mid-tidal volume (obtained by digital integration). The mean of at least five

breaths, free of swallowing artifact, were used to obtain R_L in cmH_2O/liters per second. Immediately after the measurement of R_L, thoracic gas volume (V_{tg}) was measured in a constant-volume body plethysmograph to obtain specific lung resistance ($SR_L = R_L \times V_{tg}$) in liter × cmH_2O/liters per second.

Aerosol delivery systems. All aerosols were generated using a disposable medical nebulizer (Raindrop, Puritan Bennett, Lenexa, KS) that provided an aerosol with a mass median aerodynamic diameter of 3.2 μm as determined by a cascade impactor (Andersen Instruments, Inc., Atlanta, GA). The nebulizer was connected to a dosimeter system, consisting of a solenoid valve and a source of compressed air (20 psi). The output of the nebulizer was directed into a plastic T-piece, one end of which was connected to the inspiratory port of a respirator (Harvard Apparatus, S. Natick, MA). The solenoid valve was activated for one second at the beginning of the inspiratory cycle of the respirator. Aerosols were delivered at a tidal volume of 500 ml and a rate of 20 breaths per minute as previously described (36, 38).

Concentration response curves to carbachol aerosol. To assess bronchial responsiveness, we performed cumulative concentration response curves to carbachol by measuring SR_L immediately after inhalation of buffer and after each consecutive administration of 10 breaths of increasing concentrations of carbachol (0.25, 0.5, 1.0, 2.0, and 4.0% wt/vol buffered saline). The provocation test was discontinued when SR_L increased over 400% from the post-saline value or after the highest carbachol concentration had been administered. Bronchial responsiveness was assessed as previously described (36, 37) by determining the cumulative carbachol concentration (in breath units [BU]) that increased SR_L by 400% over the post-saline value (PC_{400}) by interpolation from the dose response curve. One BU was defined as one breath of a 1% wt/vol carbachol aerosol solution.

Bronchoalveolar lavage (BAL). The distal tip of a specially designed 80-cm fiberoptic bronchoscope was wedged into a randomly selected subsegmental bronchus. Lung lavage was performed by slow infusion and gentle aspiration of 3 × 30-ml aliquots of PBS (pH 7.4) at 39°C, using 30-ml syringes attached to the working channel of the bronchoscope (38). The effluent collected was strained through gauze to remove mucus and then centrifuged at 420 *g* for 15 min. The cell pellet was resuspended in buffered saline, and an aliquot of this resuspension was transferred to a hemocytometer chamber to determine total cells. Total viable cells were assessed by trypan blue exclusion. A second aliquot of the cell suspension was spun in a cytospin (600 rpm for 10 min) and stained by Wright-Giemsa to identify cell populations. 500 cells per slide were enumerated to establish the differential cell count (×100; oil objective). Cell categories included epithelial cells, macrophages, lymphocytes, neutrophils, basophils, eosinophils, and monocytes; unidentifiable cells were grouped into a category termed "others."

Agents

Ascaris suum extract (Greer Diagnostics, Lenoir, NC) was diluted with PBS to a concentration of 82,000 protein nitrogen units/ml and delivered as an aerosol (20 breaths/min × 20 min).

Carbamylcholine (Carbachol, Sigma Chemical Co.) was dissolved in buffered saline at concentrations of 0.25, 0.50, 1.0, 2.0, and 4.0% wt/vol and delivered as an aerosol.

The mAbs, HP 1/2 and IE6, were purified from ascites by protein A chromatography followed by gel filtration chromatography in endotoxin-free physiologic saline. HP 1/2 is an IgG_1 mAb directed against the α4 chain of the human $\alpha_4\beta_1$ heterodimeric intensin (39, 40). 1E6 is a nonspecific isotypic (IgG_1) antibody to leukocyte function antigen-3. The mAbs contained < 1 endotoxin unit per mg. For in vivo studies, HP 1/2 and 1E6 were dissolved in buffered saline and were given either intravenously (1 mg/kg) or as aerosols (16 mg, total dose). Levels of mouse Ig were determined by ELISA assay for evaluation of serum levels of HP 1/2.

Intravenous treatment studies. Baseline airway responsiveness was determined and BAL performed within 5 d of antigen challenge. On the antigen challenge day, SR_L was measured and then the sheep were given an intravenous infusion of 1 mg/kg HP 1/2 or an equivalent volume of saline. 30 min after treatment, SR_L was remeasured and then the animals were challenged with antigen. SR_L was remeasured immediately after, hourly from 1 to 6 h after and half-hourly from 6.5 to 8 h after antigen challenge as previously described (35, 38). Postchallenge determinations of airway responsiveness (PC_{400}) were made at 24 and 48 h, and 1 and 2 wk after antigen challenge. For these studies peripheral blood was drawn for differential analysis and determination of serum levels of HP 1/2 at baseline, 30 min post drug, 1, 2, 3, 4, 6, 8, 24, and 48 h, and 1 and 2 wk after antigen challenge. Postchallenge BAL was performed 4, 8, 24, and 48 h, and 1 and 2 wk after challenge. These studies were carried out in eight sheep in a randomized crossover fashion. Control and drug trials were separated by at least 3 wk. Our previous studies had shown that this time interval was sufficient for the sheep to recover from prior challenges (41). Consistent with these previous studies, there were no differences in baseline pulmonary function (see Results) or baseline BAL (see Table I).

A similar protocol was used for a group of five sheep challenged with the nonspecific isotypic mAb 1E6, except that the post challenge measurements of PC_{400} and peripheral blood differentials were stopped at 48 h after challenge. BAL was not performed in this series.

In a third protocol, the effect of giving HP 1/2 after antigen challenge was assessed. In five sheep, PC_{400} was measured within 5 d of antigen challenge. On the challenge day, SR_L was measured before, immediately after, and 1 and 2 h after antigen challenge. Then the sheep were given HP 1/2 (1 mg/kg, i.v.) or saline. For these posttreatment studies, the saline trials were done before the drug trials. Measurements of SR_L were continued from 3 through 8 h as previously described. PC_{400} was measured 24 and 48 h after antigen challenge.

Aerosol treatment studies. Baseline airway responsiveness was determined as described above. On the challenge day, baseline SR_L was measured and then the sheep were given 16 mg HP 1/2 ($n = 5$) or 1E6 ($n = 4$) by aerosol. Thirty minutes after drug treatment, SR_L was measured and then the sheep were challenged with antigen. Post challenge determinations of PC_{400} and peripheral bloods were obtained through 48 h as described above.

Comparison of intravenous and aerosol doses. Based on calculations by Kim et al. (42), ~ 15% of all aerosol generated is lost in the endotracheal tube. Of the remaining aerosol, ~ 40% is deposited in the lung, using this study's coordinated nebulizer-delivery system and breathing pattern. Based on these predictions, the calculated drug dose actually reaching the lung for the aerosol trial would be 5.4 mg, which is approximately one-sixth of the intravenous dose administered based on the mean weight of 31 kg for the animals used.

Statistical analysis. For the in vivo studies, within group differences overtime (i.e., drug or placebo) were analyzed by a repeated measures ANOVA for data sets of equal sample size (e.g., blood data for HP 1/2–treated sheep) or a one way ANOVA for sets of unequal sample size (e.g., blood data for control sheep). If the null hypothesis was rejected, a post hoc comparison was performed using Duncan's multiple range test. Differences between drug and placebo at any one time point for a specific variable were determined by paired or unpaired t-test where appropriate. Before analyzing the BAL data, a square root transformation with Bartlett's correction was performed. BAL was also analyzed for total accumulation of eosinophils after antigen challenge ($T_1 = 4 + 8$ h after challenge), 24 h after antigen challenge ($T_{24} = 4 + 8 + 24$ h) and 48 h after challenge ($T_{48} = 4 + 8 + 24 + 48$ h). Results were compared to baseline using Kruskall Wallis ANOVA followed by Duncan's multiple range test. In addition, the frequency of animals demonstrating BAL eosinophils at each time was compared, between controls and treated groups using Fisher's exact test. Significance was accepted when $P < 0.05$ using a two-tailed test. Values in the text, tables, and figures are reported as mean±SE.

Results

In-vitro binding and flow cytometric studies

To evaluate the binding of anti-α_4 mAb HP 1/2 to sheep leukocytes, preparations of blood leukocytes enriched for granulo-

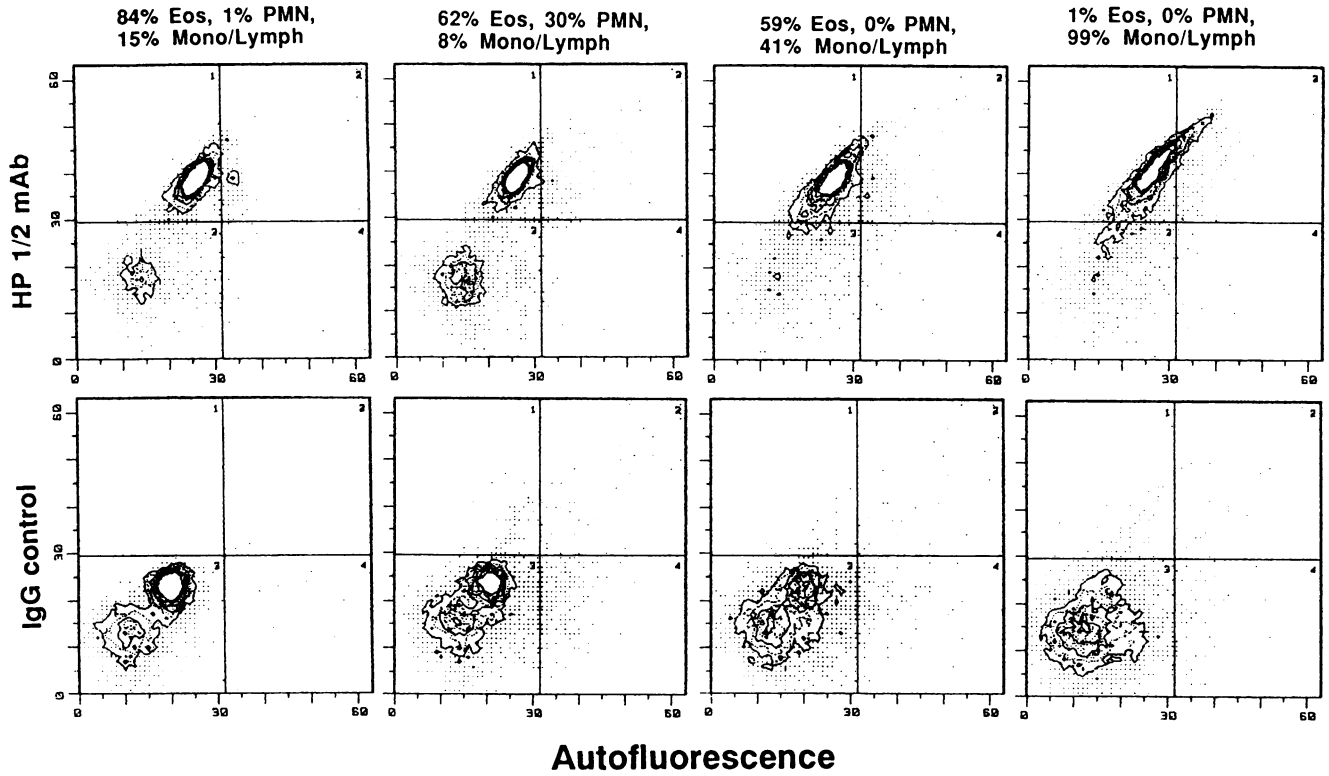


Figure 1. Two-color flow cytometric demonstration of the binding of anti-α_4 mAb, HP 1/2, to sheep blood eosinophils and mononuclear leukocytes but not neutrophils. Cell autofluorescence in the green channel is expressed on the abscissa in arbitrary units to enable the populations of eosinophils, which exhibit enhanced autofluorescence (mean channel of ~ 20) to be separated from less autofluorescent neutrophils. Intensity of phycoerythrin-labeled anti-mouse IgG binding to HP 1/2 (*top panels*) or control murine IgG (*bottom panels*) is displayed on the ordinate for four preparations of sheep blood leukocytes variously enriched in eosinophils or mononuclear leukocytes.

cytes and mononuclear leukocytes were analyzed by dual-channel immunofluorescent flow cytometry. As shown in Fig. 1, all eosinophils and almost all mononuclear leukocytes, but not neutrophils (which were distinguished by their lesser autofluorescence than eosinophils), expressed epitopes binding HP 1/2. Both HP 1/2 and a rabbit COOH-terminus specific anti-α_4 antisera immunoprecipitated bands from surface radioiodinated sheep mononuclear leukocytes identical in ~ 150 kD M_r to α_4 similarly immunoprecipitated from control human mononuclear leukocytes (data not shown). To establish that HP 1/2 inhibited sheep α_4-integrin-mediated cellular adhesion, the capacity of this antibody, in comparison with a subclass control IgG and an anti-CD18 mAb (60.3), to inhibit adhesion to rsVCAM-1 of either sheep mononuclear leukocytes or control VLA-4 expressing RAMOS T cells (34) was evaluated. HP 1/2 totally suppressed adhesion of both sheep leukocytes and RAMOS cells to VCAM-1 (Fig. 2). Moreover, an anti-β mAb, TS 2/16, that enhances VLA-4-mediated adherence (34), enhanced sheep leukocyte to VCAM-1, as it did control RAMOS cells (Fig. 2). Thus, HP 1/2 binds to an adhesion blocking epitope on sheep α_4-integrins and VLA-4, $\alpha_4\beta_1$, participates in sheep leukocyte adherence to VCAM-1.

Intravenous treatment studies (pretreatment)

Bioavailability of HP 1/2. After the intravenous administration of 1 mg/kg of HP 1/2, plasma concentrations (Fig. 3) reached steady-state levels with 30 min after injection (22.1±3.1 μg/ml) and remained constant for up to 48 h. The HP 1/2 concentrations decreased to 2.6±1.3 μg/ml by 1 wk and only two of the eight sheep showed detectable HP 1/2 by 2 wk. In two sheep treated with 0.2 mg/kg HP 1/2, plasma concentrations of HP 1/2 were 12.3±1.8, 2.5±2.5, and < 1 μg/ml at 2 min, 30 min, and 48 h after injection.

Airway responses. Fig. 4 shows the time course of the airway responses before and after antigen challenge when the animals were given 1 mg/kg HP 1/2 intravenously. In the control trial, SR_L increased 380±42% ($P < 0.05$) from a baseline value of 0.99±0.03 liter × cm_2O/liters per s immediately after challenge. SR_L returned to baseline values by 4 h postchallenge, but then began to increase again by 5 h postchallenge. The maximum increase during this late response (i.e., 5–8 h) was 175±16% ($P < 0.05$). Treatment with HP 1/2 had no effect on baseline tone. HP 1/2 provided no protection against the immediate bronchoconstrictor response to inhaled antigen, with SR_L increasing 345±33% ($P < 0.05$) from a baseline value of 0.99±0.04 liter × cm H_2O/liters per s. SR_L remained significantly elevated above baseline 1 h after challenge, but then, returned to prechallenge values and did not increase significantly above baseline throughout the remainder of the 8 h. The maximum increase in SR_L during the late response was 44±11% ($P < 0.05$ vs. control). Thus, HP 1/2 at 1.0 mg/kg provided a mean 75% protection against the late-phase response in these animals. The effect of HP 1/2 was dose dependent; reducing the dose to 0.2 mg/kg was not sufficient to protect against the late response. In two sheep treated with 0.2 mg/kg HP 1/2, the peak late response was 209±59% compared to 134±3% in the control trial.

To ensure that the protective effect of HP 1/2 did not result

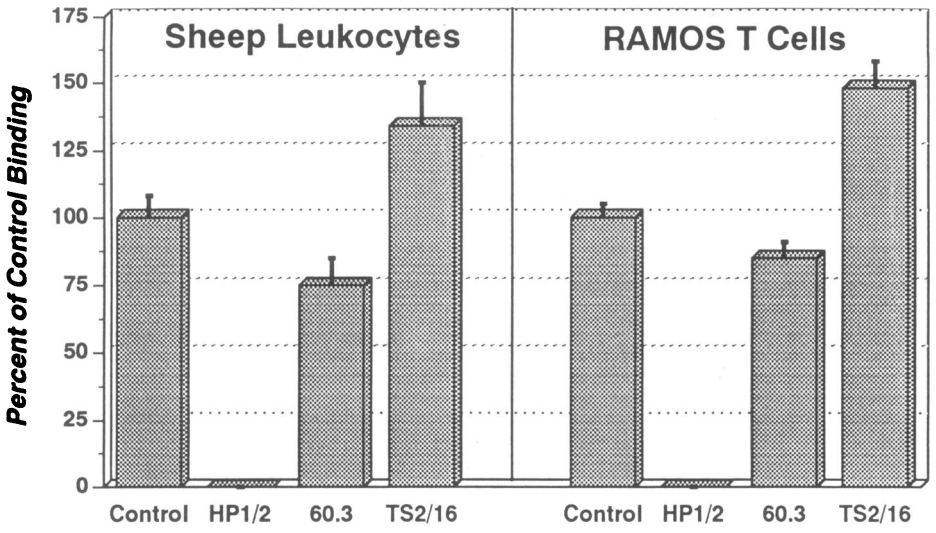


Figure 2. Adherence of sheep blood mononuclear leukocytes or control human RAMOS T cells to rsVCAM-1. Relative to control antibody, anti-α_4 HP 1/2 totally suppressed adherence, whereas an anti-CD18 mAb, 60.3, did not suppress adherence. TS 2/16, an anti-β_1 mAb, which enhances VLA-4-mediated adherence, enhanced adherence of both sheep leukocytes and RAMOS T cells. Data are mean±SEM of 3–12 experiments.

from the intravenous administration of IgG antibody, we repeated the antigen challenge studies in five sheep, but substituted a mAb to leukocyte function antigen-3 (1E6) for HP 1/2. As seen in Fig. 5, treatment with this antibody had no effect on the early or the late response. In both the control and 1E6 trials, the time courses of the early and late bronchial responses were similar to that observed in the saline trial in the HP 1/2 protocol. In the 1E6 control trial, early and late increases in SR_L were 404±68% and 206±31%, compared to 352±43% and 249±65% for the treatment trial. These responses were not different from each other.

Airway responsiveness. SR_L returned to baseline values 24 h after challenge in both the saline control and HP 1/2 trials. In the control trial, assessment of airway responsiveness at this time showed the sheep to be hyperresponsive to inhaled carbachol. PC_{400} decreased significantly to 10.2±1.5 BU from a prechallenge value of 20.3±1.7 BU (Fig. 4). Furthermore, the increased airway responsiveness persisted for 1 wk after challenge with mean PC_{400} measuring 11.6±1.4, and 11.3±1.7 BU at 48 h and 1 wk, respectively. By 2 wk, PC_{400} was 16.7±2.1 BU and returning toward prechallenge values. The prolonged hyperresponsiveness in the control sheep contrasted sharply with the HP 1/2–treated sheep in which no significant change in airway responsiveness was observed over the 2-wk period. In sheep treated with HP 1/2, PC_{400} was 18.7±1.7, 17.8±1.1, 21.5±2.0, and 20.6±2.2 BU at 24 h, 48 h, 1 wk, and 2 wk, respectively. The results with HP 1/2 also contrast sharply with those obtained with 1E6 in which airway hyperresponsiveness was present 24 and 48 h after challenge in both the control and 1E6 trial. In the control trial, PC_{400} was 21.3±2.1 BU before challenge and fell significantly to 7.43±0.9 and 10.5±2.1 BU 24 and 48 h after challenge. Likewise in the 1E6 trial, PC_{400} fell significantly from 20.2±3.3 BU to 11.8±1.4 and 11.0±1.9 BU (Fig. 5).

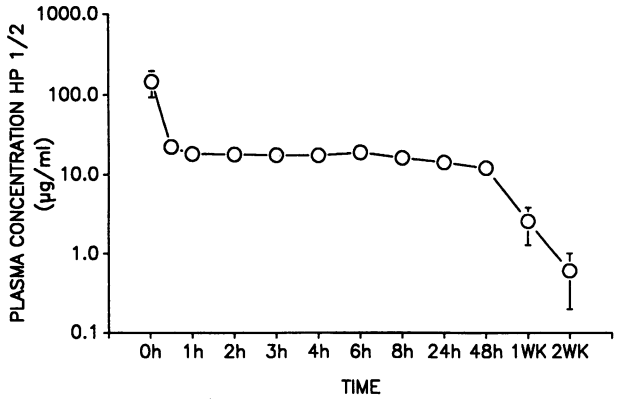
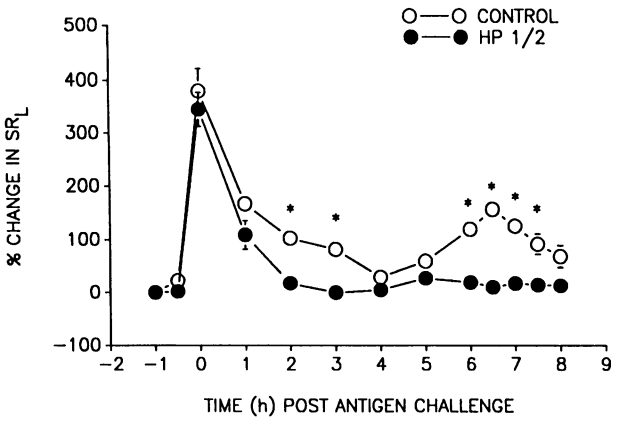


Figure 3. Plasma concentrations of HP 1/2. Levels of Ig were determined by ELISA. Values are mean±SE for eight sheep.

BAL. Because of the similarities of the antigen-induced airway responses between the saline treated controls in the HP 1/2 trial and the sheep treated with 1E6, the BAL results from these two experiments were combined and compared to the results obtained when the sheep were treated with HP 1/2. The findings are summarized in Table I and show that, except for the percentage of macrophages at 4 h, there were no statistical differences between the groups. In both the control and HP 1/2 trials, cell number per milliliter BAL increased over 1 wk. By 2 wk postchallenge, cell number per milliliter was returning toward baseline. The differential analyses of the cells recovered over the 2-wk period were also similar between the groups. In general, during the week after antigen challenge, there was a decrease in the percentage of macrophages, and an increase in the percentage of neutrophils and eosinophils. The percentage of lymphocytes remained relatively constant. There appeared to be a trend for a more rapid appearance of eosinophils in the control group (mean fivefold increase at 4 h compared to no change in the HP 1/2 group) but this difference did not achieve statistical significance. Analyzing the absolute number of eosinophils did not change the result. Before challenge the number of eosinophils in BAL were 2.8±2.4 and 0.6±0.6 × 10^2/ml in the control and HP 1/2 groups, respectively, and 4 h after challenge the corresponding values were 12.6±4.0 and 1.2±1.2 × 10^2/ml. There were no other apparent differences between the groups over the remainder of the trial.

Although statistical differences were not achieved at specified time points, trends were apparent in the data. Specifically, it appeared that eosinophil accumulation increased with time and was more frequent in the control sheep as compared to the treated group, whereas neutrophil influx was somewhat delayed in the treated group. To address these points, the eosino-



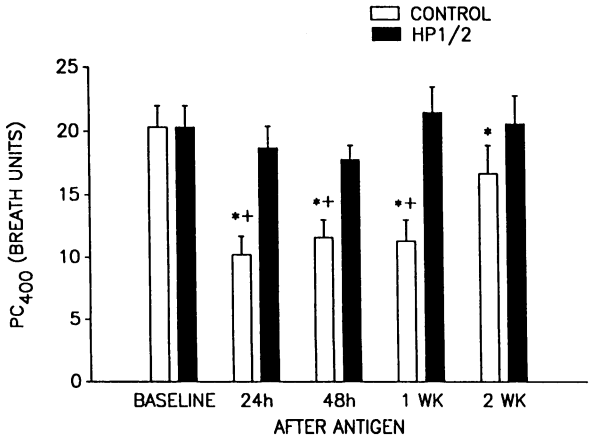
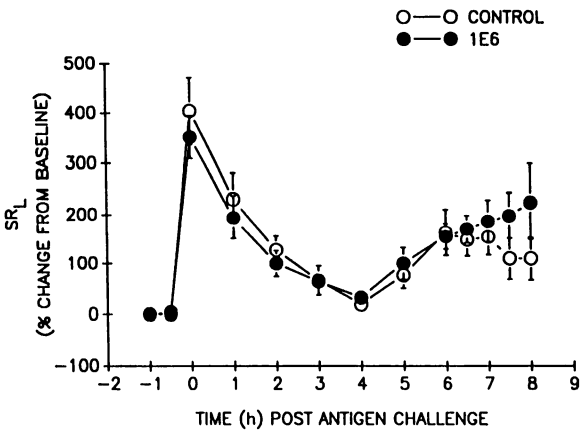


Figure 4. Top: Effect of HP 1/2 on antigen-induced early and late bronchial responses. HP 1/2 (1 mg/kg, i.v.) was given 30 min before antigen challenge. HP 1/2 did not affect the acute increase in SR_L but blocked the late response compared to control. $^*P < 0.05$ vs. HP 1/2. *Bottom:* Effect of HP 1/2 on postchallenge airway responsiveness. In the control trial, PC_{400} decreased after antigen challenge (i.e., the sheep became hyperresponsive). Treatment with HP 1/2 prevented this effect. $^*P < 0.05$ vs. baseline; $^+P < 0.05$ vs. HP 1/2. All values are mean±SE for eight sheep.

phil and neutrophil recoveries (expressed as percentages) were summed over the first 48 h and the data reanalyzed. The results are reported in Table II. Between group comparisons using these calculated values still did not achieve significance at any time point ($P = 0.1042$ at T_1 and $P = 0.1008$ at T_{24}). Within group analysis, however, showed that in the control animals, but not in the HP 1/2–treated animals, the percentage of eosinophils increased at all times with respect to baseline. Furthermore, the frequency of animals demonstrating eosinophils in BAL was significantly greater in the control group as compared to the treated group. Despite the differences in the eosinophil response between the treated and control group, no significant correlation between the eosinophil recovery and the severity of the late response could be found. Using this same analysis did not uncover any differences in the neutrophil response between or within the two groups. Both control and treated groups showed significant increases in neutrophil recoveries over time.

Peripheral leukocytes. These data were analyzed as were the BAL results and are summarized in Fig. 6. In general, the changes in total WBCs and leukocyte differentials were similar for both groups, with very few differences between control and the HP 1/2 group. In the control trial, total WBCs increased significantly after antigen challenge from a baseline value of $3.9\pm0.3 \times 10^3/mm^3$ to a maximum value of $5.5\pm0.5 \times 10^3/mm^3$, 8 h after antigen challenge. After this, total WBCs began to return to prechallenge values. The starting (baseline) total WBC count for the HP 1/2 trial was ($4.8\pm0.2 \times 10^3/mm^3$, $P < 0.05$) greater than that seen in the control trial. 30 min after injection of HP 1/2, WBC number fell to a value of $2.9\pm0.5 \times 10^3/mm^3$ ($P < 0.05$ vs baseline) which was equivalent to that seen in the control trial. Total WBCs rose by 2 h to $4.4\pm0.6 \times 10^3/mm^3$ and then reached a peak value of $5.6\pm0.6 \times 10^3/mm^3$ at 48 h postchallenge. The differential leukocyte responses were also similar between the groups. The percentage of neutrophils increased steadily from 1 to 8 h after antigen challenge ($P < 0.05$ vs baseline for both groups), where as eosinophils and lymphocytes decreased from 1 to 8 h postchallenge, except for the sheep receiving HP 1/2 in which the fall in eosinophils occurred after 2 h after challenge. Eosinophils and lymphocytes began to increase 24–48 h after challenge in both groups.



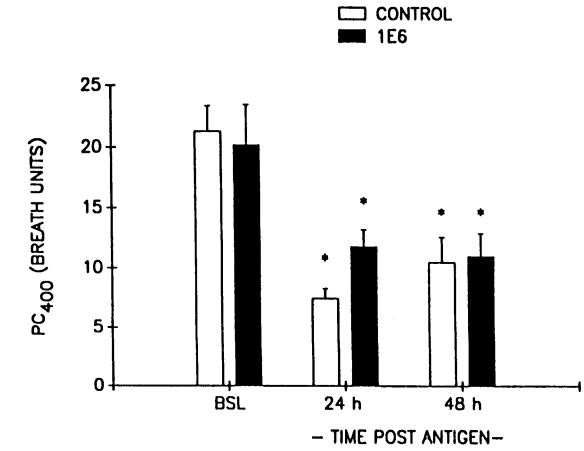


Figure 5. Top: Effect of control 1E6 monoclonal antibody on antigen-induced early and late bronchial responses. 1E6 (1 mg/kg, i.v.) was given 30 min before antigen challenge. 1E6 had no effect. *Bottom:* Effect of 1E6 on postchallenge airway responsiveness. 1E6 failed to protect against the fall in PC_{400}. $^*P < 0.05$ vs. baseline. BSL indicates prechallenge baseline.

Table I. Effect of HP 1/2 on BAL Cell Responses before and after Antigen Challenge in Allergic Sheep

	Baseline	+4 h	+8 h	+24 h	+48 h	+1 wk	+2 wk
BAL return				*Total cells* $\times 10^4$/ml			
Control	41.99±6.07	49.95±15.52	43.75±8.99	98.02±20.79*	82.89±23.0*	55.02±6.92*	36.70±5.57
HP 1/2	28.02±3.92	25.25±2.00	54.74±25.88	57.13±24.30	110.42±31.43*	65.62±7.38	54.07±3.40*
Macrophages (%)							
Control	71.0±2.97	70.0±4.58	70.3±5.33	53.8±6.21*	61.8±7.18	82.2±3.19	75.7±3.86
HP 1/2	80.3±4.52	85.8±2.05‡	65.9±7.81	65.2±9.65	60.1±10.22	78.6±5.02	78.7±3.32
Lymphocytes (%)							
Control	14.4±2.16	11.7±2.00	10.4±2.00	11.1±2.08	9.8±1.64	7.0±0.36	18.3±2.52
HP 1/2	12.0±3.43	9.9±2.02	15.4±0.53	9.2±2.51	7.7±2.19	14.2±5.13	14.0±3.08
Neutrophils (%)							
Control	3.7±1.39	7.4±3.44	13.1±5.88	31.9±7.93*	24.4±7.57*	4.3±1.66	1.9±0.94
HP 1/2	1.8±0.99	1.1±0.46	12.5±7.88	22.3±9.90*	29.5±4.10*	4.6±2.58	3.4±1.34
Eosinophils (%)							
Control	0.05±0.03	0.25±0.10	0.29±0.08	0.45±0.17	0.72±0.52	0.50±0.33	0±0
HP 1/2	0.02±0.01	0.02±0.01	0.23±0.18	0.45±0.33	0.23±0.16	0.35±0.19	0.08±0.05

Values are mean±SE for 13 control sheep and 8 HP 1/2–treated sheep. * $P < 0.05$ vs. baseline; ‡ $P < 0.05$ vs. control.

Intravenous treatment studies (postchallenge treatment)

Postchallenge treatment with intravenous HP 1/2 was also effective in blocking the late airway response and the post antigen-induced airway hyperresponsiveness (Fig. 7). The changes in SR_L were the same in the control and HP 1/2 trials before and for 4 h after antigen challenge. By 5 h, however, SR_L began to increase in the control trial, but not in the HP 1/2 trial. The peak late response in the control trial was 177±17% compared to 33±7%, when the animals were treated with HP 1/2 ($P < 0.05$). Posttreatment with HP 1/2 was also effective in blocking the post–antigen-induced airway hyperresponsiveness over 48 h ($P < 0.05$).

Aerosol treatment

Airway responses. Fig. 8 shows that treating sheep with HP 1/2 aerosol (16 mg total dose) 30 min before antigen challenge was also effective in blocking the late response and airway hyperresponsiveness. The protective effect was specific for HP 1/2 because the same dose of 1E6 had no protective effect. Fig. 9 shows that the differences in the physiological responses between aerosol HP 1/2 and 1E6 were not the result of differences in total WBC or differential counts between the groups. Total WBC and differential in both the HP 1/2 and 1E6 groups showed a pattern of responses similar to that seen in the intravenous trial. This protection afforded by aerosol HP 1/2 occurred in the absence of detectable serum levels of the mAb. None of the serum samples obtained from the five sheep at the times where mAb was most likely to be present, i.e., 30 min after drug treatment, immediately after antigen challenge and 1 h after antigen challenge contained detectable mAb.

Table II. Accumulated Eosinophils and Neutrophils in BAL in Allergic Sheep after Antigen Challenge

	Baseline	T_1	T_{24}	T_{48}
		Eosinophils		
Control	0 (0–0.4)	0.4 (0–2.0)* [[10/13]]‡	0.6 (0–2.8)* [[11/13]]‡	1.0 (0–9.6)* [[11/13]]§
HP 1/2	0 (0–0.2)	0 (0–1.4) [2/8]	0 (0–40) [2/8]	0 (0–5.2) [3/8]
		Neutrophils		
Control	1.4 (0–17)	7.9 (0.4–101)*	39.4 (4–170)*	60.4 (5.8–217)*
HP 1/2	0.7 (0–8.6)	1.5 (0–67)*	23.5 (1.2–119)*	55.8 (5.0–152)*

Median and ranges (in parentheses) for summed eosinophil and neutrophils in BAL over 48 h. (T_1 = 4 + 8 h postchallenge, T_{24} = 4 + 8 + 24 h postchallenge; and T_{48} = 4 + 24 + 48 h postchallenge.) Note that ranges can be >100% because values are summed. Values in brackets represent fraction of animals from which eosinophils were recovered in BAL. * $P < 0.05$ vs. baseline; ‡ $P < 0.05$ vs. HP 1/2; § $P < 0.06$ vs. HP 1/2.

Airway responsiveness. In the HP 1/2–treated sheep PC_{400} was not significantly changed 24 or 48 h after challenge from the prechallenge value. Prechallenge PC_{400} was 20.3±0.6 BU whereas after antigen challenge BU were 16.0±2.3 and 27.1±1.64 for 24 and 48 h, respectively (P = NS). In the 1E6 sheep, PC_{400} fell significantly ($P < 0.05$) 24 and 48 h after antigen challenge, respectively, from a baseline value of 23.7±5.6 to 11.6±2.7 and 8.4±2.9 BU (Fig. 8).

Effect of HP 1/2 on EPO activity. Although the in vivo data provide some support that HP 1/2 can slow eosinophil recruitment to the airway, it is possible that binding of HP 1/2 to the α_4-integrin can modulate cell function as well. To test this possibility, sheep eosinophils were stimulated with PAF (10^{-5} M) in the presence and absence of HP 1/2 and EPO production was measured as an index of cell activation. In the control cells, PAF caused a 3.5±0.9 (n = 6)-fold increase in EPO production, which was significantly greater than the 1.9±0.1-fold increase observed in the HP 1/2–treated cells. HP 1/2 had no effect on unstimulated eosinophils (1.1±0.1, n = 4).

Discussion

The results of this study indicate that a blocking mAb to α_4-integrins, HP 1/2, can modify antigen-induced late bronchial responses and the prolonged airway hyperresponsiveness that

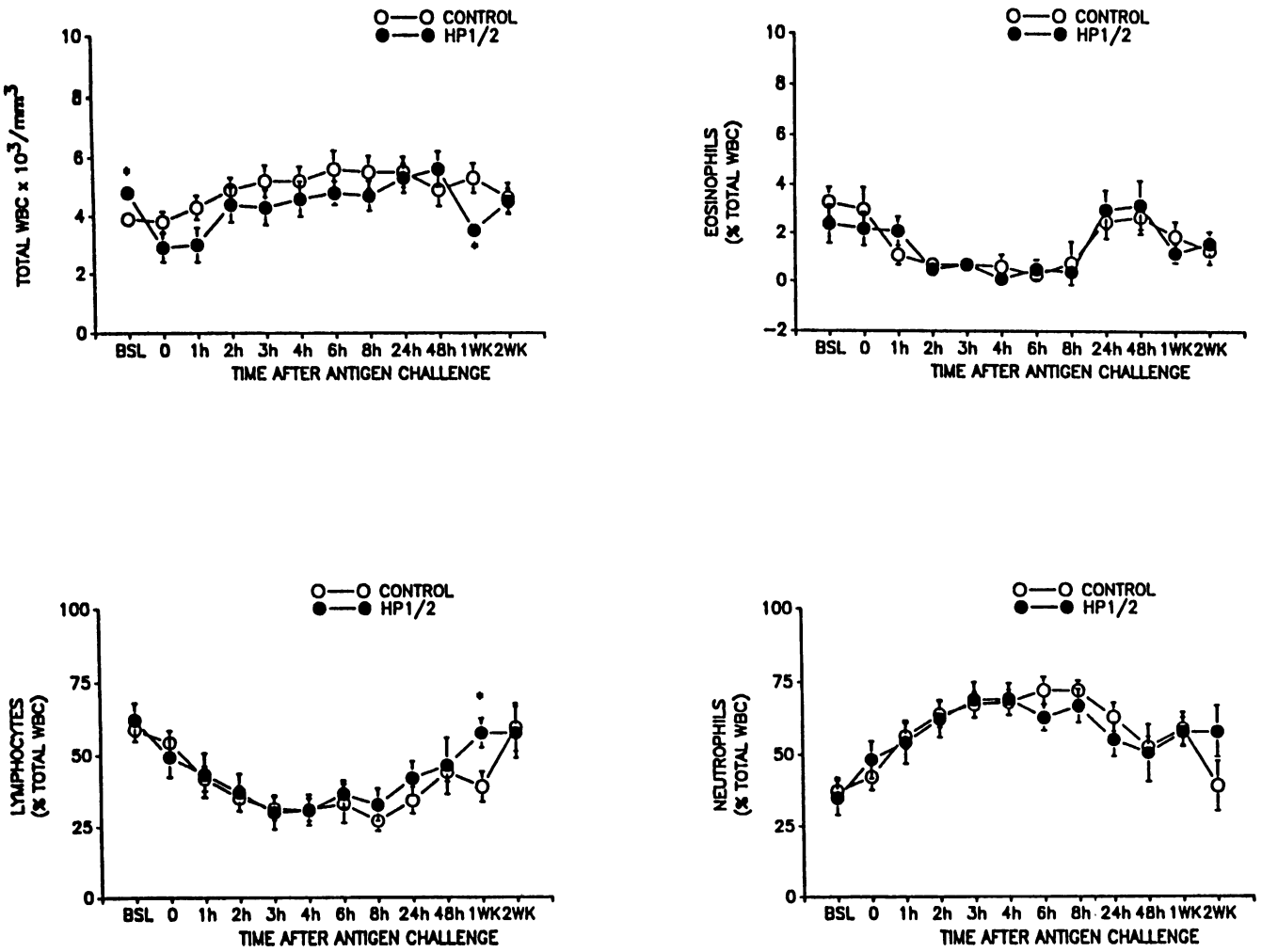


Figure 6. Effect of HP 1/2 (1 mg/kg, i.v.) on peripheral blood leukocytes after antigen challenge. WBCs and differential cell counts for lymphocytes, eosinophils, and neutrophils at baseline (*BSL*) and during 2 wk after antigen challenge. Values are mean±SE for HP 1/2 (n = 8); control (n = 13). *$P < 0.05$ vs. control.

follow an acute antigen challenge in the allergic sheep model. The mAb was effective when given intravenously before and after antigen challenge or by aerosol before antigen. The mAb did not have an effect on peripheral blood leukocyte numbers but the mAb did show some ability to lessen the overall accumulation of eosinophils over the first 48 h after challenge, as well as to diminish the frequency with which eosinophils appeared in the BAL. Studies with PAF-stimulated eosinophils indicate that HP 1/2 decreases the production of EPO, thereby providing an additional mechanism for its protective effect. Taken together, these findings suggest that α_4-integrins participate in pathophysiological responses associated with the prolonged inflammatory events that follow antigen challenge, in part, by down-regulating the function of eosinophils and/or other cells that express this integrin.

Studies of the late response and post-antigen-induced airway hyperresponsiveness support a prominent effector role for the eosinophil. In the peripheral blood of patients who develop late responses and airway hyperresponsiveness, antigen challenge causes a transient blood eosinopenia at 6 h followed by an eosinophilia that continues through 24 h postchallenge (43). The eosinopenia has been interpreted as a consequence of selective recruitment of these cells and has been correlated with the magnitude of the late bronchial response, whereas the post challenge eosinophilia occurs only in those patients that develop late responses and has been correlated with the degree of airway hyperresponsiveness (44). Similar correlations have been found in the airways between the number of eosinophils in BAL and the late bronchial response (45). Conversely, the role of the neutrophil in these pathophysiologic events is less convincing. Unlike eosinophils, recovery of neutrophils in BAL and in biopsy specimens is sporadic and, in contrast to eosinophils obtained from BAL, markers of neutrophil activation have not been correlated with asthma symptoms (46). That the eosinophil may be more prominent in mediating the late response and airway hyperresponsiveness is not surprising considering the differences in the biochemical pathways of the two cell types. The neutrophil does not produce sulfidopeptide leukotrienes (47, 48), i.e., leukotrienes C_4, D_4, and E_4) major mediators of the late response (38, 49–51), whereas the eosinophil, is a potent source of these spasmogens. In addition, eosinophils release major basic protein which damages respiratory epithelium (52, 53) and can provoke airway hyperresponsiveness (54, 55).

Although these data support a role for the eosinophil in eliciting late responses and airway hyperresponsiveness, defini-

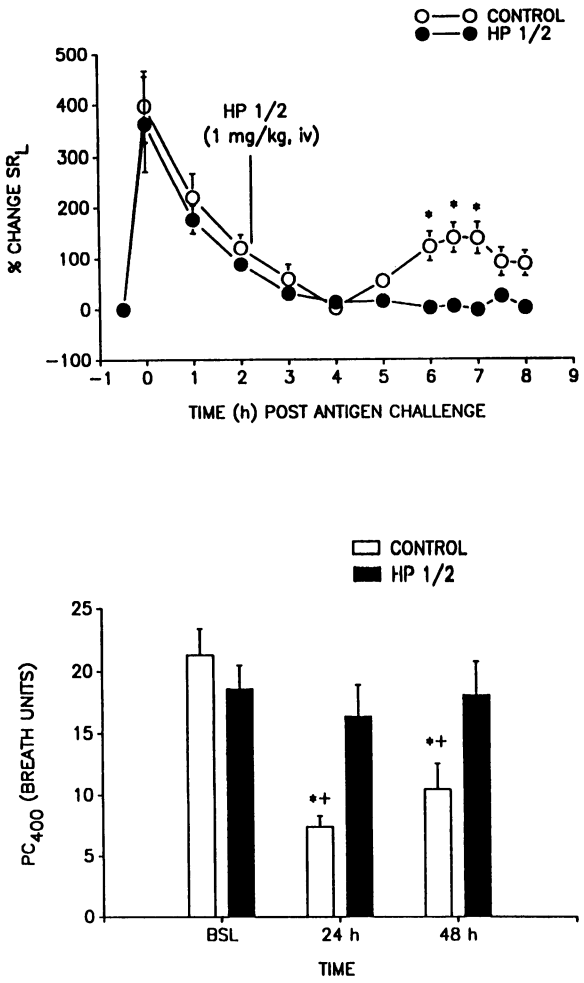


Figure 7. Top: Protective effect of HP 1/2 (1 mg/kg, i.v.) on antigen-induced late responses when given 2 h after antigen challenge. *Bottom:* Effect of posttreatment with HP 1/2 on airway hyperresponsiveness after antigen challenge. Posttreatment with HP 1/2 blocked the late response and the postchallenge airway hyperresponsiveness. *Top:* $^*P < 0.05$ vs. HP 1/2. *Bottom:* $^*P < 0.05$ vs. baseline (BSL). $^+P < 0.05$ vs. HP 1/2. Values are mean±SE for five sheep.

tive evidence is still lacking. The present study adds to our previous findings demonstrating the association between the presence of eosinophils and the development of late bronchial responses in this animal model (41). The current data, which shows gradual eosinophil recruitment and increased frequency of response over time in the control animals, but not in HP 1/2-treated animals is supportive of the eosinophil–late response interaction. Despite this evidence there are still a number of concerns in concluding that the eosinophil is the only cell involved including the inability to demonstrate a significant correlation (using either parametric or non parametric analyses) between the eosinophil percentage and the severity of the late response, the failure to show significant differences in cell recoveries at specified time points and the appearance of eosinophils in the treated animals. These findings, in conjunction with the effectiveness of the aerosol treatment, suggest that additional pathways, possibly regulation of cell activation, might be involved. The ability of HP 1/2 to reduce PAF-induced EPO formation in isolated eosinophils supports this idea.

Previous studies in *Ascaris suum*-sensitive monkeys suggest that selective endothelial adhesion proteins regulate specific pathophysiological events that follow antigen challenge. In an acute challenge model, an anti-human E-selectin mAb (CL2, 2 mg/kg, i.v.) was found to block the late bronchial response (56). This protection was associated with a reduction in leukocyte, specifically in neutrophil, infiltration into the lung. No protection against the late bronchial response or the cellular influx was observed when the animals were treated with an anti-intracellular adhesion molecule-1 mAb (R6.5, 2 mg/kg, i.v.), a mAb that when used in a chronic challenge model had been shown to prevent the eosinophilic inflammatory response and the development of airway hyperresponsiveness (57). The conclusion from these studies was that E-selectin modulates the acute airway neutrophilic inflammation that is characterized by a late bronchial obstruction, and that intracellular adhesion molecule-1 mediates the chronic eosinophilic inflammatory response which contributes to airway hyperresponsiveness.

Which cell(s) is responsible for the late response and the persistent airway hyperresponsiveness is still uncertain. In the

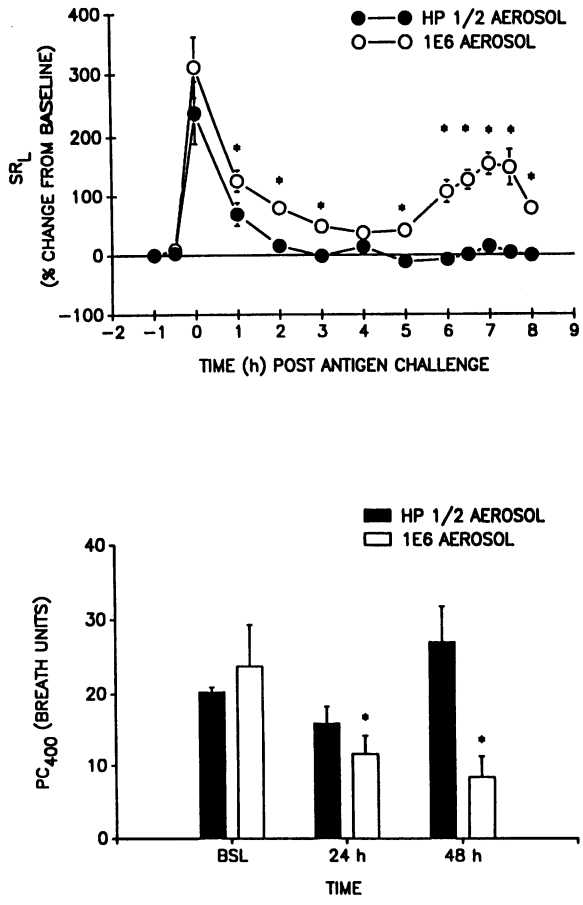


Figure 8. Top: Effect of aerosol administration of monoclonal antibodies HP 1/2 (16 mg) and 1E6 (16 mg) on antigen-induced early and late bronchial responses. HP 1/2 had no effect on the early increase in SR$_L$ but blocked the late response, whereas 1E6 was without effect early and late. $^*P < 0.05$ vs. HP 1/2. *Bottom:* Effect of HP 1/2 and 1E6 on airway responsiveness. 1E6-treated animals showed a significant fall in PC$_{400}$, whereas HP 1/2 blocked the effect. $^*P < 0.05$ vs. baseline (*BSL*). Values are mean±SE for HP 1/2 ($n = 5$); 1E6 ($n = 4$).

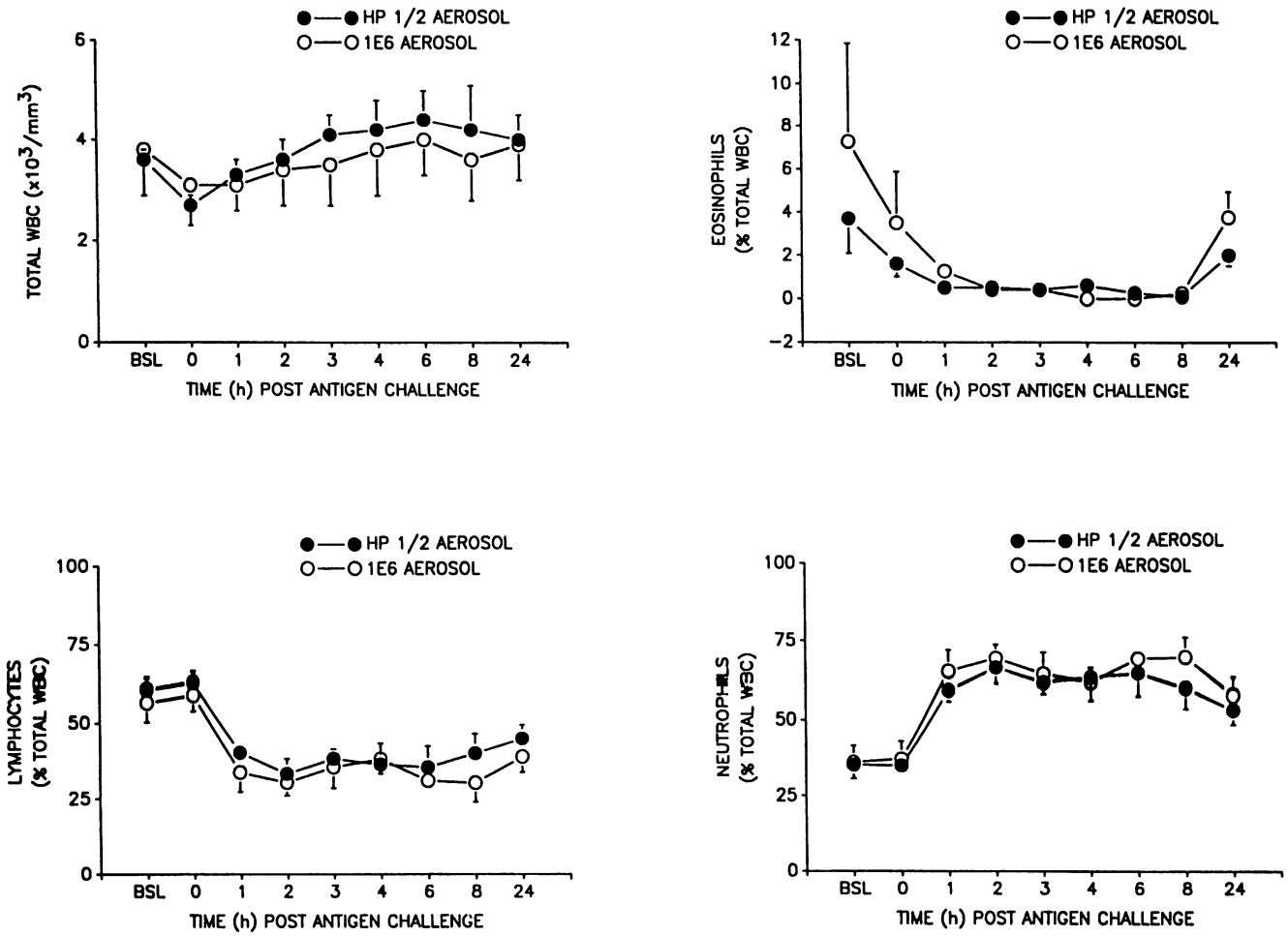


Figure 9. Effect of HP 1/2 and 1E6 aerosols on peripheral blood leukocytes after antigen challenge. WBC and differential lymphocyte, eosinophil, and neutrophil counts at baseline (*BSL*) and during 2 wk after antigen challenge given at time 0. Values are mean±SE for HP 1/2 (n = 5) and 1E6 (n = 4).

present study, the eosinophil response was somewhat overshadowed by the large numbers of neutrophils present in the BAL. This is a common feature in protocols where multiple lavages are performed (such as this one). Studies both in our own laboratory as well as in humans (58) have suggested that this neutrophilic response, although at times quite striking, is in part nonspecific. Nevertheless there was a reduced (although) non significant slowing of the neutrophil response in the HP 1/2-treated sheep. Based on the flow cytometric data, which demonstrates that sheep neutrophils do not bind HP 1/2, it is unlikely that the reduction results from a direct effect of the mAb on the neutrophil itself. If, however, binding the α_4-integrin can reduce cell activity, then it is possible that HP 1/2 could act on cells that control neutrophil recruitment. The binding of HP 1/2 to eosinophils and/or macrophages which also express the α_4-integrin, could reduce the respective release of leukotriene C_4 which can enhance the binding of neutrophils to vascular endothelium (59), and leukotriene B_4 a potent neutrophil chemotaxin, from these cells. Both of these mechanisms could explain the slower neutrophil recruitment in the treated group.

There is other evidence to suggest that although administration of an α_4 blocking mAb inhibit VLA-4 dependent pathways of cellular adhesion and mobilization, these effects might not totally account for the beneficial airway effects of α_4-integrin blockade in antigen challenged sheep. First, the differences in lymphocytes, eosinophils and other cells recovered from bronchoalveolar lavages after antigen challenge were not consistently altered by HP 1/2 administration. Second, the post challenge eosinopenia and lymphopenia that presumably reflects mobilization of these leukocytes from the bloodstream into the lungs was not inhibited by HP 1/2 administration. Third, the efficacy of HP 1/2 administered into the airway suggests that inhibition of α_4-integrins could also occur in tissues and, therefore, may not only be acting by blocking leukocyte mobilization from the vasculature. Fourth, the inability to demonstrate increased expression of VCAM-1 in sheep airways after antigen challenge (data not shown).

Despite the appearance of inflammatory cells in the airways of challenged sheep, the HP 1/2-treated animals did not develop late phase airway responses or airway hyperresponsiveness. The efficacy of HP 1/2, whether administered systemically or by inhalation into the airway, suggests that blockage of α_4-integrin function may be explained by inhibition of pathways involved in activation of resident or recruited airway cells. Inhibition of lymphocyte activation can be achieved by anti-α_4 antibodies (25–29). Such inhibition of lymphocyte activation might also serve to diminish eosinophil activation if

lymphocyte-derived cytokines, such as interleukin 5, are involved in eosinophil activation in the airways (11). Preventing eosinophil activation could inhibit the release of sulfidopeptide leukotrienes, modulators of late phase airway responses (38, 50), and eosinophil granular proteins that can contribute to airway hyperresponsiveness (54, 55). Recent observations by Wardlaw and colleagues showed that VLA-4 dependent adhesion of eosinophils to fibronectin resulted in short-term priming of eosinophils for calcium dependent leukotriene release (60). Likewise, Neeley et al. (61) found that VLA-4 mediated interaction with fibronectin results in increased FMLP-induced degranulation of peripheral blood eosinophils. These findings are consistent with our own studies showing inhibition of EPO activity in PAF-stimulated eosinophils treated with HP 1/2 which indicate that α_4 antibodies can down-regulate eosinophil function directly. Such a mechanism might explain why biopsies taken from individuals with the most responsive airways had greater numbers of VLA-4–positive staining cells (62).

In conclusion, these results support a role for α_4-integrins in the production of asthmatic late phase airway obstruction and hyperresponsiveness. Inhibition of airway hyperresponsiveness was persistent for over 2 wk after systemic administration of the anti-α_4 blocking antibody. Moreover, administration of the anti-α_4 antibody into the airway was effective indicating the potential utility of inhalational approaches to mediating airway cell activation that contributes to asthma pathophysiology.

Acknowledgments

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