Brief Definitive Report

A Monoclonal Antibody Recognizing Very Late Activation Antigen-4 Inhibits Eosinophil Accumulation In Vivo

By V. B. Weg, T. J. Williams, R. R. Lobb,* and S. Nourshargh

*From the Department of Applied Pharmacology, National Heart and Lung Institute, London SW3 6LY, UK; and *Biogen Inc., Cambridge, Massachusetts 02142*

Summary

Using an in vivo test system, the role of the β_1 integrin very late activation antigen-4 (VLA-4) in eosinophil accumulation in allergic and nonallergic inflammatory reactions was investigated. Eosinophil infiltration and edema formation were measured as the local accumulation of intravenously injected ^{111}In-labeled eosinophils and ^{125}I-human serum albumin. The inflammatory reactions investigated were a passive cutaneous anaphylaxis (PCA) reaction and responses elicited by intradermal soluble inflammatory mediators (platelet-activating factor, leukotriene B_4, C5a des Arg), arachidonic acid, and zymosan particles. The in vitro pretreatment of ^{111}In-eosinophils with the anti-VLA-4 monoclonal antibody (mAb) HP1/2, which crossreacts with guinea pig eosinophils, suppressed eosinophil accumulation in all the inflammatory reactions investigated. Eosinophil accumulation was inhibited to the same extent when mAb HP1/2 was administered intravenously. It is interesting that HP1/2 had no effect on stimulated edema formation. These results suggest a role for VLA-4 in eosinophil accumulation in vivo and indicate a dissociation between the inflammatory events of eosinophil accumulation and edema formation.

Eosinophil accumulation is a prominent feature of allergic inflammatory disorders. Allergic patients have elevated levels of circulating eosinophils and these cells accumulate after antigenic challenge in the skin, lungs, and nasal airways. Despite much research investigating the interaction of eosinophils with cultured endothelial cells, the mechanisms that mediate and control the accumulation of eosinophils in vivo remain unclear.

Adhesion of leukocytes to microvascular endothelium is essential for their migration into inflamed tissues. This response is mediated by the interaction of adhesion molecules expressed on the cell surface of leukocytes and venular endothelial cells (1). The adherence of eosinophils to cultured endothelial cells has many functional and molecular characteristics similar to neutrophil–endothelial cell interaction. However, the recent demonstration that the $\alpha_4\beta_1$ integrin VLA-4 (CD49d/CD29) is expressed on eosinophils and other leukocytes, but not the neutrophil, has led to suggestions that the VLA-4/VCAM-1 (vascular cell adhesion molecule 1) adhesion pathway may be involved in specific eosinophil, as opposed to neutrophil, migration in vivo (2–4). This proposal has not, however, been directly addressed, and was the aim of the present investigation.

Using an in vivo test system we have previously demonstrated the accumulation of eosinophils in guinea pig skin induced by preformed mediators C5a, leukotriene B_4 (LTB_4), and platelet-activating factor (PAF), and in a passive cutaneous anaphylaxis (PCA) reaction (5, 6). In the present study, using this in vivo system we have investigated the effect of an anti-VLA-4 mAb on eosinophil accumulation. The results suggest that VLA-4 plays an important role in the accumulation of eosinophils in both allergic and nonallergic inflammatory reactions.

Materials and Methods

Animals. Dunkin-Hartley guinea pigs (300–500 g) were purchased from Harlan Olac (Bicester, Oxon, UK).

Reagents. PAF and LTB_4 were obtained from Bachem (Bubendorf, Switzerland) and Cascade Biochemical Ltd. (Berkshire, UK) respectively. Arachidonic acid (AA), zymosan, and bovine gamma globulin (BGG) were from Sigma Chemical Co. (Dorset, UK). ^{125}I-human serum albumin (^{125}I-HSA) and ^{111}Indium chloride ($^{111}InCl_3$) were from Amersham International (Buckinghamshire, UK). Guinea pig zymosan-activated plasma (ZAP) was prepared as previously described and used as a source of C5a des Arg (5).

mAbs. HP1/2 is a mouse IgG1 mAb directed to the α4 chain (CD49d) of VLA-4 (7). The cell line was grown as an ascites, and mAb purified by protein A and gel filtration chromatography under endotoxin-free conditions. Endotoxin level of stock solution was assayed to be 0.03 U/ml resulting in <0.005 U being injected into each animal. Isotype-matched mAb 1E6, generated to human LFA3, was used as a control antibody. mAb 1E6, purified as above, was a gift from Dr. W. Meier (Biogen Inc., Cambridge, MA).

Immunofluorescence Flow Cytometry. Immunofluorescence flow cytometry was carried out based on a procedure previously described

 J. Exp. Med. © The Rockefeller University Press • 0022-1007/93/02/0561/06 $2.00
Volume 177 February 1993 561–566

(8). Briefly, horse serum-induced guinea pig peritoneal eosinophils or glycogen-induced peritoneal neutrophils (>98% pure) were suspended in Ca^{2+}/Mg^{2+} free HBSS (10^6/ml). Cell aliquots were treated with 10–500 $\mu g/10^6$ cells of mAb HP1/2 for 30 min at 4°C. The cells were then washed twice in fresh HBSS and resuspended in buffer containing a saturating concentration of FITC rabbit anti-mouse antibody (Dako Ltd., Buckinghamshire, UK) for 30 min at 4°C. Finally, the cells were washed twice and analyzed using a FACS® analyzer (Becton Dickinson & Co., Mountain View, CA) as previously reported (9).

Measurement of ^{111}In-eosinophil Accumulation and Edema Formation in Guinea Pig Skin. ^{111}In-eosinophil accumulation and edema formation in guinea pig skin were simultaneously measured as previously reported (5). Briefly, peritoneal eosinophils were induced by multiple intraperitoneal injections of horse serum. The eosinophils were purified over a discontinuous Percoll gradient yielding eosinophil preparations of 95–100% purity. The cells were then radiolabeled with ^{111}In and finally injected intravenously in a volume of 1 ml of HBSS containing 5 × 10^6–10^7 eosinophils mixed with ^{125}I-HSA (5 μCi/kg).

In experiments investigating the effects of mAb HP1/2 or the control antibody, mAb 1E6, the ^{111}In-labeled eosinophils were divided into two aliquots, one of which was treated with the mAb (50 $\mu g/10^6$ cells) for 15 min at room temperature before the final wash. Alternatively, the antibodies were administered intravenously (3 mg/kg) with the radioisotopes.

For the induction of a PCA reaction, animals were passively sensitized by intradermal injection of an IgG1-rich guinea pig anti-BGG antiserum (50 μl, 1:50 dilution) 20–24 h before the intravenous administration of radioisotopes as previously described (10). 10 min after the intravenous injection of ^{111}In-eosinophils and ^{125}I-HSA, BGG was injected intradermally into sensitized sites and other stimuli (PAF, LTB_4, ZAP, AA, and zymosan particles) were injected into naive sites. Each test was performed in duplicate sites according to a balanced site injection plan with an injection volume of 100 μl/site. After a 2-h in vivo period, a cardiac blood sample was collected and animals killed by an overdose of anaesthetic. The dorsal skin was then removed and skin sites punched out. The ^{111}In counts per eosinophil was determined and used to express eosinophil accumulation in each skin site in terms of the number of labeled leukocytes, corrected for 10^7 cells injected. Exudate volumes were expressed in terms of microliters of plasma by dividing skin sample ^{125}I counts by ^{125}I counts in 1 μl of plasma.

Statistical Analysis. Results are expressed as mean ± SEM for n pairs of animals and have been analyzed for statistical significance using a paired two-way Student's t test. A p value of <0.05 was considered statistically significant.

Results

Immunofluorescence Flow Cytometry. Guinea pig eosinophils bound HP1/2 with a saturating concentration of 50 $\mu g/10^6$ cells. At this concentration, mAb HP1/2 did not bind to guinea pig neutrophils (Fig. 1). Based on these results, HP1/2 was used at the concentration of 50 $\mu g/10^6$ cells to pretreat ^{111}In-eosinophils in vitro for the in vivo experiments described below.

Effect of mAb HP1/2 on ^{111}In-Eosinophil Accumulation and Edema Formation Induced by Exogenous Inflammatory Mediators, AA, and Zymosan Particles. Intradermal injections of PAF, LTB_4, and guinea pig ZAP induced ^{111}In-eosinophil accumulation over the 2-h test period in guinea pig skin (Figs. 2 and 3). LTB_4 was found to be more potent than PAF in inducing eosinophil accumulation with little edema, whilst PAF was very effective in inducing edema.

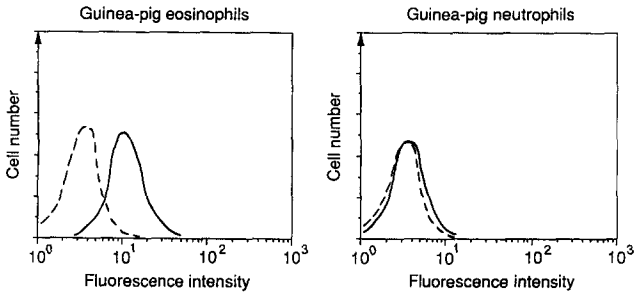

Figure 1. Flow cytometry of guinea pig eosinophils and neutrophils stained with mAb HP1/2. Cells were incubated with mAb HP1/2 at 50 $\mu g/10^6$ cells followed by staining with a FITC anti-mouse antibody as described in Materials and Methods. (*Broken lines*) Nonspecific binding; (*solid lines*) specific binding.

The in vitro pretreatment of ^{111}In-eosinophils with mAb

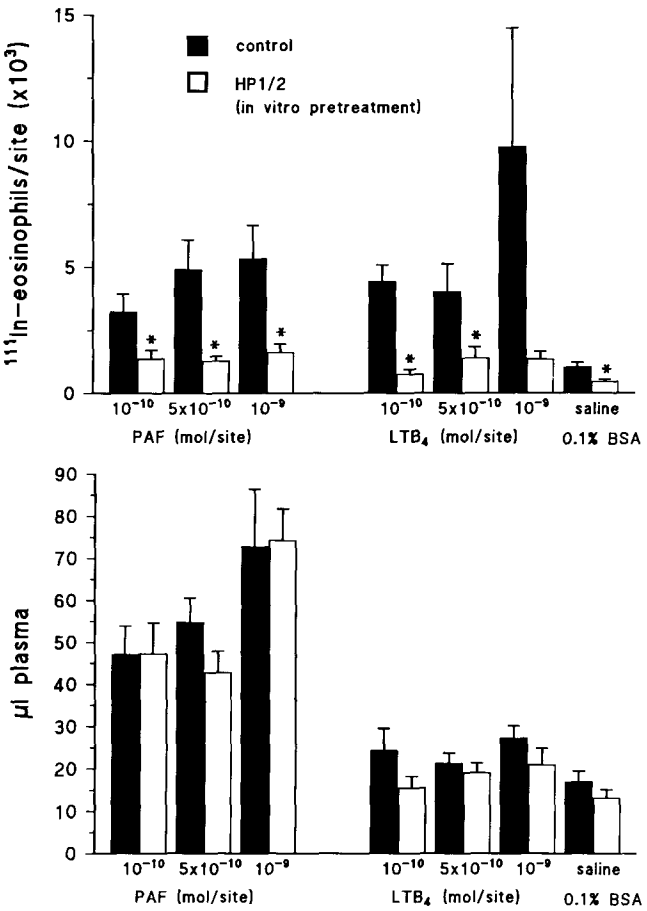

Figure 2. Effect of mAb HP1/2 on ^{111}In-eosinophil accumulation and edema formation induced by intradermal injections of PAF and LTB_4. Radiolabeled eosinophils were untreated (*control*) or pretreated with mAb HP1/2 at a concentration of 50 $\mu g/10^6$ cells before their final wash and intravenous injection into recipient guinea pigs. Results are the mean ± SEM for n = 3–8 pairs of guinea pigs. (*) Significant difference from control, p <0.05.

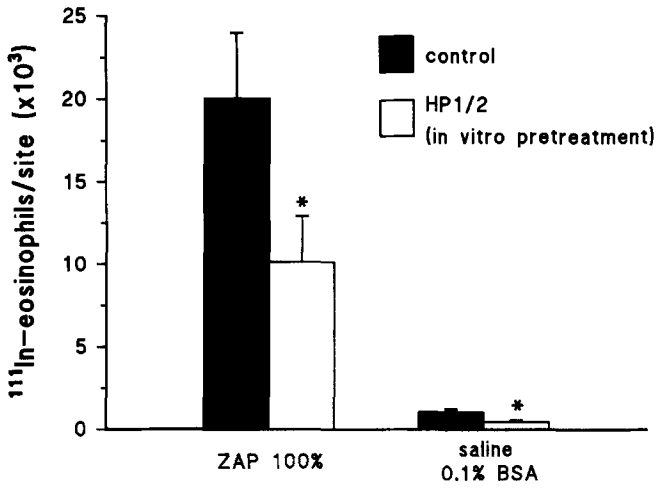

Figure 3. Effect of pretreatment of cells with mAb HP1/2 on ^{111}In-eosinophil accumulation induced by intradermal guinea pig ZAP. See legend to Fig. 2 for details. Results are the mean ± SEM for n = 9 pairs of guinea pigs. (*) Significant difference from control, p <0.05.

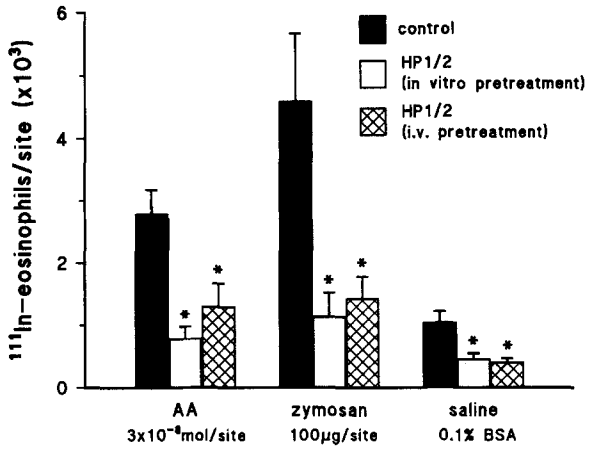

Figure 4. Effect of mAb HP1/2 on ^{111}In-eosinophil accumulation induced by intradermal AA and zymosan particles. Guinea pigs received control untreated ^{111}In-eosinophils, HP1/2 in vitro pretreated ^{111}In-eosinophils (50 µg/10^6 cells) or ^{111}In-eosinophils coinjected with HP1/2 (3 mg/kg i.v.). Arachidonic acid (*AA*) and zymosan particles were injected intradermally 10 min after the intravenous injection of radiolabeled cells and ^{125}I-HSA. Results are the mean ± SEM for n = 4–8 pairs of guinea pigs. (*) Significant difference from control, p <0.05.

HP1/2 almost completely inhibited their accumulation in response to PAF and LTB_4 (Fig. 2), whilst partially inhibiting the response to ZAP (Fig. 3). This procedure also inhibited ^{111}In-eosinophil accumulation induced by intradermal AA and zymosan particles (Fig. 4). It is interesting that the small level of eosinophil accumulation detected in sites injected with saline/BSA was also significantly inhibited. Edema formation in animals receiving treated cells was not significantly different from the responses in guinea pigs injected with control ^{111}In-eosinophils (Fig. 2 for PAF and LTB_4; data not shown for other stimuli).

mAb HP1/2 had similar inhibitory effects on ^{111}In-eosinophil accumulation, but not edema formation, when it was administered intravenously at 3 mg/kg (Table 1 and Fig. 4). Preliminary experiments demonstrated that increasing the dose to 10 mg/kg did not enhance the inhibitory effect of HP1/2, whilst 1 mg/kg gave a greatly reduced inhibition of cell accumulation.

The inhibitory effect of mAb HP1/2 was not due to a reduction in the number of circulating radiolabeled leukocytes in the guinea pigs, e.g., percentage of control labeled eosinophils and mAb HP1/2-treated ^{111}In-eosinophils circulating at the end of the 2-h in vivo test period were 10.3 ± 3.1% and 7.0 ± 2.7% (percent injected cells; mean ± SEM, n = eight pairs of animals), respectively. Pretreatment of labeled eosinophils with a control mouse IgG (mAb 1E6) or the intravenous administration of mAb 1E6, did not significantly affect ^{111}In-eosinophil accumulation, e.g., with intravenous 1E6, percent changes in ^{111}In-eosinophil accumulation over un-

Table 1. *Effect of Intravenous mAb HP1/2 on ^{111}In-eosinophil Accumulation and Edema Formation Induced by Inflammatory Mediators*

	^{111}In-eosinophils/site (× 10^3)		µl Plasma	
	Control	Intravenous HP1/2	Control	Intravenous HP1/2
Saline/0.1% BSA	1.02 ± 0.29	0.4 ± 0.07*	16.23 ± 3.66	16.65 ± 6.52
LTB_4 (5 × 10^{-10} mol/site)	3.07 ± 0.84	1.61 ± 0.48*	20.38 ± 2.51	19.04 ± 2.27
PAF (10^{-9} mol/site)	6.45 ± 2.27	1.86 ± 0.35*	94.70 ± 21.64	90.53 ± 17.0
ZAP (100%)	19.79 ± 3.25	8.75 ± 2.0*	44.35 ± 8.64	52.65 ± 7.32

mAb HP1/2 was administered intravenously (3 mg/kg) 10 min before intradermal administration of LTB_4, PAF, and ZAP. ^{111}In-eosinophil accumulation and edema formation in guinea pig skin were measured over a 2-h in vivo test period as described in Materials and Methods. Results are mean ± SEM for n = 4–5 pairs of animals.
* p <0.05, a significant difference from control.

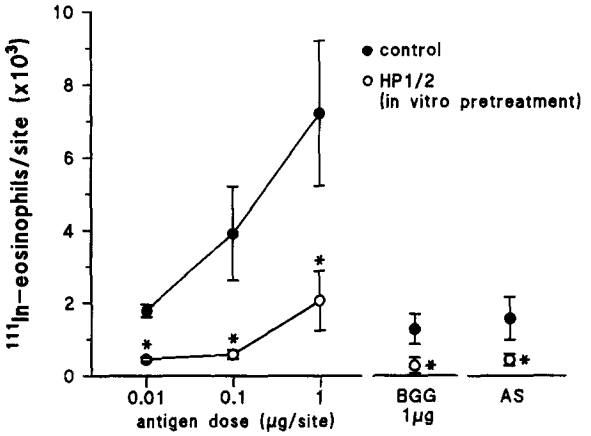

Figure 5. Effect of mAb HP1/2 pretreatment on ^{111}In-eosinophil accumulation in the PCA reaction. To elicit the PCA reaction, antigen (BGG) was administered intradermally into previously sensitized sites. Control skin sites injected with BGG or antisera (*AS*) alone were also included. Radiolabeled eosinophils were untreated (*control*) or pretreated with mAb HP1/2 at a concentration of 50 $\mu g/10^6$ cells before their final wash and intravenous injection into recipient guinea pigs. Results are the mean ± SEM for n = 4–6 pairs of animals. (*) Significant difference from control, $p < 0.05$.

treated cells were 22.3 ± 19.3% and 10.6 ± 26.4% (n = four pairs) for PAF and LTB_4, respectively.

Effect of mAb HP1/2 on ^{111}In-Eosinophil Accumulation in the PCA Reaction. Injection of antigen (BGG) into sensitized sites led to a marked and dose-dependent ^{111}In-eosinophil accumulation in the guinea pig skin, whilst BGG and antisera injected alone induced very small responses (Fig. 5).

As observed with the exogenous mediators, AA and zymosan particles, the in vitro pretreatment of ^{111}In-eosinophils with mAb HP1/2 significantly inhibited their accumulation in the PCA reaction. HP1/2 also inhibited the small cell accumulation induced by BGG and antisera where small inflammatory responses were possibly induced.

Discussion

Eosinophils have been implicated in the pathogenesis of a wide variety of inflammatory disease states including allergic disorders. The mechanisms that mediate and regulate the selective accumulation of eosinophils in sites of allergic inflammation remain unclear. Both neutrophils and eosinophils respond to chemoattractants such as C5a, LTB_4, and PAF and exhibit enhanced adhesion to cytokine-activated endothelial cells in a CD18-dependent manner (11, 12). Intercellular adhesion molecule 1 (ICAM-1), an important ligand for CD11a/CD18 and CD11b/CD18 (13–15) has been shown to be involved in neutrophil and eosinophil adhesion in vitro (15–17) and accumulation in vivo (18, 19). E-selectin has also been implicated in neutrophil and eosinophil adhesion to cytokine-activated endothelial cells in vitro (2, 16, 17), however, of the two leukocyte types, only eosinophils express VLA-4 that binds to VCAM-1 on activated endothelial cells (2–4). The characterization of the VLA-4/VCAM-1 adhesion pathway has led to suggestions that this interaction may mediate the accumulation of eosinophils, as opposed to neutrophils, into sites of eosinophilic inflammation. In the present study using a neutralizing anti-VLA-4 mAb, which blocks both VLA-4/VCAM-1 and VLA-4/fibronectin interactions (7), we have demonstrated a role for VLA-4 in eosinophil accumulation in vivo.

Intradermal chemoattractants PAF, LTB_4, and C5a des Arg (in ZAP) induced eosinophil accumulation in guinea pig skin which was inhibited by mAb HP1/2, whether used to pretreat the ^{111}In-eosinophils in vitro or given intravenously. These chemoattractants, which do not appear to activate endothelial cells, are believed to stimulate eosinophil adhesion in the venule lumen primarily by a CD18-dependent mechanism. It was therefore interesting to observe that an anti-VLA-4 mAb inhibited eosinophil accumulation induced by these mediators, although other workers have shown that VLA-4 is not upregulated by a chemoattractant such as PAF and the antibody does not inhibit eosinophil adherence in vitro to endothelial cells induced by PAF (3). Whilst HP1/2 almost completely inhibited the responses induced by PAF and LTB_4, it only partially suppressed eosinophil accumulation induced by C5a des Arg. These findings indicate that VLA-4 plays an important role in chemoattractant-induced eosinophil accumulation in vivo, but other VLA-4–independent adhesion pathways perhaps involving CD18 and ICAM-1 may also be involved in eosinophil accumulation induced by C5a des Arg. HP1/2 also inhibited eosinophil accumulation induced by the endogenous generation of mediators in response to AA, zymosan, and in PCA reactions. However, in no case was inhibition of edema formation seen when using HP1/2. These findings indicate a dissociation between the inflammatory events of eosinophil accumulation and plasma protein leakage. Edema formation induced by chemoattractants in this model, as found in other in vivo models (20, 21), may be mediated by the process of neutrophil accumulation.

Although in vitro studies have shown that VLA-4 can interact with VCAM-1 (22), fibronectin (23), and a ligand involved in leukocyte homotypic aggregation (7), the existence of as yet uncharacterized VLA-4 ligands is strongly suggested (7, 24). In the present in vivo study, whilst demonstrating an important role for VLA-4 in eosinophil accumulation, we have not attempted to identify the possible ligands with which VLA-4 may be interacting. A possible candidate is clearly VCAM-1, which may be basally expressed on venular endothelial cells in vivo. In addition, upregulation of VCAM-1 expression in vivo may be involved. Time course experiments with cytokine-activated cultured endothelial cells have shown that significant levels of VCAM-1 can be detected as early as 1–2 h, though expression peaks after 6–10 h of cytokine treatment (25, 26). It is possible that induction of VCAM-1 is faster on venular endothelial cells in vivo to account for the rapid appearance of eosinophils. The availability of mAbs to guinea pig VCAM-1 will allow us to investigate directly the involvement of this molecule in eosinophil accumulation in our in vivo model and to determine the time course of

VCAM-1 expression within skin sites by immunohistology. In addition, whilst numerous studies have investigated the interaction of VLA-4 with VCAM-1 with respect to leukocyte-endothelial cell adhesion, very few have addressed the involvement of this adhesion pathway in the process of leukocyte transendothelial cell migration. VLA-4 may interact with a different ligand to VCAM-1 during this process. Clearly, further in vivo studies are required to dissect the involvement of endothelial cell adhesion molecules in the process of eosinophil accumulation in vivo. The interaction of VLA-4 with fibronectin may also partly explain the present results. Fibronectin can be deposited on the lumenal surface of the endothelium at sites of skin inflammation (27), and its interaction with circulating leukocytes may contribute to the process of leukocyte migration in vivo (28).

In summary, the results presented here strongly indicate a role for VLA-4 in the process of eosinophil accumulation in both allergic and nonallergic inflammatory reactions. Although the ligands with which VLA-4 is interacting in vivo are yet to be determined, our findings suggest that VLA-4 blockers may be highly effective therapeutic tools in the treatment of inflammatory conditions where eosinophil accumulation is a prominent feature.

The authors thank Miss A. Hartnell for assistance in flow cytometry studies and Miss K. Kimball for technical help in purifying mAb HP1/2.

This work was supported by Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES) (Brasil), the National Asthma Campaign (UK), and the Wellcome Trust (UK).

Address correspondence to Dr. S. Nourshargh, Department of Applied Pharmacology, National Heart & Lung Institute, Dovehouse Street, London SW3 6LY, UK.

Received for publication 20 October 1992 and in revised form 16 November 1992.

References

1. Springer, T.A. 1990. Adhesion receptors of the immune system. *Nature (Lond.).* 346:425.
2. Weller, P.F., T.H. Rand, S.E. Goelz, G. Chi-Rosso, and R.R. Lobb. 1991. Human eosinophil adherence to vascular endothelium mediated by binding to vascular cell adhesion molecule 1 and endothelial leukocyte adhesion molecule 1. *Proc. Natl. Acad. Sci. USA.* 88:7430.
3. Walsh, G.M., J.-J. Mermod, A. Hartnell, A.B. Kay, and A.J. Wardlaw. 1991. Human eosinophil, but not neutrophil, adherence to IL-1–stimulated human umbilical vascular endothelial cells is $\alpha_4\beta_1$ (very late antigen-4) dependent. *J. Immunol.* 146:3419.
4. Dobrina, A., R. Menegazzi, T.M. Carlos, E. Nardon, R. Cramer, T. Zacchi, J.M. Harlan, and P. Patriarca. 1991. Mechanisms of eosinophil adherence to cultured vascular endothelial cells. Eosinophils bind to the cytokine-induced endothelial ligand vascular cell adhesion molecule-1 via the very late activation antigen-4 integrin receptor. *J. Clin. Invest.* 88:20.
5. Faccioli, L.H., S. Nourshargh, R. Moqbel, F.M. Williams, R. Sehmi, A.B. Kay, and T.J. Williams. 1991. The accumulation of ^{111}In-eosinophils induced by inflammatory mediators in vivo. *Immunology.* 73:222.
6. Weg, V.B., M.L. Watson, L.H. Faccioli, and T.J. Williams. 1992. [^{111}In]-eosinophil accumulation during passive cutaneous anaphylaxis in the guinea pig. *Br. J. Pharmacol.* 105:127P.
7. Pulido, R., M.J. Elices, M.R. Campanero, L. Osborn, S. Schiffer, A. Garcia-Pardo, R. Lobb, M.E. Hemler, and F. Sanchez-Madrid. 1991. Functional evidence for three distinct and independently inhibitable adhesion activities mediated by the human integrin VLA-4. *J. Biol. Chem.* 266:10241.
8. Nourshargh, S., M. Rampart, P.G. Hellewell, P.J. Jose, J.M. Harlan, A.J. Edwards, and T.J. Williams. 1989. Accumulation of ^{111}In-neutrophils in rabbit skin in allergic and nonallergic inflammatory reactions in vivo: inhibition by neutrophil pretreatment in vitro with a monoclonal antibody recognising the CD18 antigen. *J. Immunol.* 142:3193.
9. Hartnell, A., R. Moqbel, G.M. Walsh, B. Bradley, and A.B. Kay. 1990. Fc-gamma and CD11/CD18 receptor expression on normal density and low density human eosinophils. *Immunology.* 69:264.
10. Weg, V.B., M.L. Watson, R.S.B. Cordeiro, and T.J. Williams. 1991. Histamine, leukotriene D_4 and platelet activating factor in guinea pig passive cutaneous anaphylaxis. *Eur. J. Pharmacol.* 204:157.
11. Smith, C.W., R. Rothlein, B.J. Hughes, M.M. Mariscalco, H.E. Rudloff, F.C. Schmalstieg, and D.C. Anderson. 1988. Recognition of an endothelial determinant for CD18-dependent human neutrophil adherence and transendothelial migration. *J. Clin. Invest.* 82:1746.
12. Lamas, A.M., C.M. Mulroney, and R.P. Schleimer. 1988. Studies on the adhesive interaction between purified human eosinophils and cultured vascular endothelial cells. *J. Immunol.* 140:1500.
13. Rothlein, R., M.L. Dustin, S.D. Marlin, and T.A. Springer. 1986. An intercellular adhesion molecule (ICAM-1) distinct from LFA-1. *J. Immunol.* 137:1.
14. Marlin, S.D., and T.A. Springer. 1987. Purified intercellular adhesion molecule-1 (ICAM-1) is a ligand for lymphocyte function-associated antigen 1 (LFA-1). *Cell.* 51:813.
15. Smith, C.W., S.D. Marlin, R. Rothlein, C. Toman, and D.C. Anderson. 1989. Cooperative interactions of LFA-1 and Mac-1 with intercellular adhesion molecule-1 in facilitating adherence

and transendothelial migration of human neutrophils in vitro. *J. Clin. Invest.* 83:2008.
16. Kyan-Aung, U., D.O. Haskard, R.N. Poston, M.H. Thornhill, and T.H. Lee. 1991. Endothelial leukocyte adhesion molecule-1 and intercellular adhesion molecule-1 mediate the adhesion of eosinophils to endothelial cells in vitro and are expressed by endothelium in allergic cutaneous inflammation in vivo. *J. Immunol.* 146:521.
17. Bochner, B.S., F.W. Luscinskas, M.A. Gimbrone, Jr., W. Newman, S.A. Sterbinsky, C.P. Derse-Anthony, D. Klunk, and R.P. Schleimer. 1991. Adhesion of human basophils, eosinophils, and neutrophils to interleukin 1–activated human vascular endothelial cells: contributions of endothelial cell adhesion molecules. *J. Exp. Med.* 173:1553.
18. Barton, R.W., R. Rothlein, J. Ksiazer, and C. Kennedy. 1989. The effect of anti-intercellular adhesion molecule-1 on phorbol-ester-induced rabbit lung inflammation. *J. Immunol.* 143:1278.
19. Wegner, C.D., R.H. Gundel, P. Reilly, N. Haynes, L.G. Letts, and R. Rothlein. 1990. Intercellular adhesion molecule-1 (ICAM-1) in the pathogenesis of asthma. *Science (Wash. DC).* 247:456.
20. Wedmore, C.V., and T.J. Williams. 1981. Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature (Lond.).* 289:646.
21. Nourshargh, S., and T.J. Williams. 1991. Mechanisms of granulocyte-dependent oedema. *In* Research Monographs on Cell and Tissue Physiology. No: 17 Vascular Endothelium: Interactions with Circulating Cells. J.L. Gordon, editor. Elsevier Science Publishers B.V., Amersterdam. 161–174.
22. Elices, M.J., L. Osborn, Y. Takada, C. Crouse, S. Luhowskyj, M.E. Hemler, and R.R. Lobb. 1990. VCAM-1 on activated endothelium interacts with the leukocyte integrin VLA-4 at a site distinct from the VLA-4/fibronectin binding site. *Cell.* 60:577.
23. Wayner, E.A., A. Garcia-Pardo, M.J. Humphries, J.A. McDonald, and W.G. Carter. 1989. Identification and characterisation of the lymphocyte adhesion receptor for an alternative cell attachment domain in plasma fibronectin. *J. Cell Biol.* 109:1321.
24. Vonderheide, R.H., and T.A. Springer. 1992. Lymphocyte adhesion through very late antigen 4: evidence for a novel binding site in the alternatively spliced domain of vascular cell adhesion molecule 1 and an additional $\alpha 4$ integrin counter-receptor on stimulated endothelium. *J. Exp. Med.* 175:1433.
25. Osborn, L., C. Hession, R. Tizard, C. Vassallo, S. Luhowskyi, G. Chi-Rosso, and R. Lobb. 1989. Direct expression cloning of vascular cell adhesion molecule 1, a cytokine-induced endothelial protein that binds to lymphocytes. *Cell.* 59:1203.
26. Rice, G.E., J.M. Munro, and M.P. Bevilacqua. 1990. Inducible cell adhesion molecule 110 (INCAM-110) is an endothelial receptor for lymphocytes: a CD11/CD18-independent adhesion mechanism. *J. Exp. Med.* 171:1369.
27. Clark, R.A.F., C.R. Horsburgh, A.A. Hoffman, H.F. Dvorak, M.W. Mosesson, and R.B. Colvin. 1984. Fibronectin deposition in delayed-type hypersensitivity reactions of normals and a patient with afibrinogenemia. *J. Clin. Invest.* 74:1011.
28. Doherty, D.E., P.M. Henson, and R.A.F. Clark. 1990. Fibronectin fragments containing the RGDS cell-binding domain mediate monocyte migration into the rabbit lung. A potential mechanism for C5 fragment-induced monocyte lung accumulation. *J. Clin. Invest.* 86:1065.