Selectins Mediate Eosinophil Recruitment In Vivo: A Comparison With Their Role in Neutrophil Influx

By Graca M.O. Henriques, Jadwiga M. Miotla, Renato S.B. Cordeiro, Barry A. Wolitzky, S. Terry Woolley, and Paul G. Hellewell

The role of selectins in mediating eosinophil recruitment in vivo was assessed in a model of lipopolysaccharide (LPS)-induced mouse pleurisy. LPS administration resulted in significant eosinophil influx at 24 hours, whereas neutrophil recruitment to the cavity peaked at 4 hours and persisted for 24 hours. The anti-L-selectin monoclonal antibody (MoAb) MEL-14 effectively inhibited (by 97%) eosinophil influx at 24 hours and also inhibited neutrophil recruitment at both times (75% to 95%). Eosinophil recruitment was partially reduced (54%) by the anti-P-selectin MoAb 5H1 but, in contrast, was unaffected by the anti-E-selectin MoAb 10E6. Neutrophil influx at 4 or 24 hours was not affected by the anti-P- or anti-E-selectin MoAbs. However, coadministration of anti-P-selectin and anti-E-selectin was very effective at inhibiting eosinophil influx at 24 hours (86%) and neutrophil influx at 4 (93%) and 24 hours (92%). These results show that all three selectins play a role in LPS-induced eosinophil and neutrophil recruitment in vivo, although P- and E-selectin show a degree of functional redundancy. The demonstration that P-selectin mediates eosinophil but not neutrophil influx suggests that suppressing the function of this adhesion molecule may be beneficial in blocking eosinophil accumulation in pleural inflammation.

© 1996 by The American Society of Hematology.

MATERIALS AND METHODS

Animals. Male BALB/c mice (18 to 20 g) were purchased from Harlan (Bicester, UK).

Reagents. LPS (from Escherichia coli serotype 0127:B8), goat antirat IgG fluorescein isothiocyanate (FITC), goat antirat IgG (affinity-purified) and goat antirat IgG (peroxidase-linked) were from Sigma (Poole, UK); Rat myeloma IgG1, IgG2a, and IgG2b were from Zymed (Cambridge Bioscience, Cambridge, UK). Heparin was from Baxter (London, UK; the Departamento de Fisiologia e Farmacodontica, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, Brazil; and the Department of Inflammation/Autoimmune Diseases, Hoffman-La Roche Inc, Nutley, NJ).

Submitted August 22, 1995; accepted February 16, 1996.

Supported by the National Asthma Campaign (UK), the Clinical Research Committee of the Royal Brompton National Heart & Lung Hospital, the Wellcome Trust (UK), the British Council, and CAPES (Brazil).

Address reprint requests to Paul G. Hellewell, PhD, Applied Pharmacology, National Heart & Lung Institute, London, UK; the Departamento de Fisiologia e Farmacodontica, Instituto Oswaldo Cruz/FIOCRUZ, Rio de Janeiro, Brazil; and the Department of Inflammation/Autoimmune Diseases, Hoffman-La Roche Inc, Nutley, NJ.

The publication costs of this article were defrayed in part by page charge payment. This article must therefore be hereby marked "advertisement" in accordance with 18 U.S.C. section 1734 solely to indicate this fact.

© 1996 by The American Society of Hematology.
from Leo Laboratories (Princes Risborough, UK) and RPMI was from Life Technologies (Paisley, UK).

Molesb1. MEL-14 (purchased from ATCC, Rockville, MD) is a rat IgG2a that recognizes mouse L-selectin.24 The hybridoma was grown in a hollow fiber bioreactor and MoAb was purified by ammonium sulfate precipitation. The final MEL-14 concentration was 5.8 mg/mL and the endotoxin concentration was less than 0.01 EU/mg (QCL1000; BioWhittaker, Inc., Walkersville, MD). The control for MEL-14 was rat IgG (Sigma) or rat IgG2b (YW62.37) directed against mouse CD45 (Serotec, Oxford, UK); the latter was obtained as purified ascites at a concentration of 0.5 mg/mL. A similar MoAb has been used as a binding control for MEL-14 in previous studies.26 Rat IgG1 to murine P-selectin (both the blocker 5H1 and the non-blocker 10A10) were prepared as described27; the stock concentrations were 17.8 and 6.9 mg/mL with endotoxin concentrations of 0.05 and 0.51 EU/mg, respectively. Rat IgG2b to murine E-selectin (10E6, a blocker) was prepared as described28; the stock concentration was 18.3 mg/mL, with an endotoxin concentration of 0.03 EU/mg. A nonblocking antimurine E-selectin MoAb (14E4, rat IgG2b) was also used; the stock concentration was 7.3 mg/mL, with an endotoxin concentration of 0.66 EU/mg. Isotype controls were MoAb 8B9 (rat IgG1) and MoAb 2-4A1 (rat IgG2b). Antibodies were administered intravenously (IV) at a dose of 200 μg per mouse (approximately 10 mg/kg) 15 minutes before intrapleural injection of LPS. These doses of MoAbs have been shown previously to suppress neutrophil accumulation in vivo in the mouse.14,26-29-36 Preliminary dose-response relationship studies of MEL-14 in 6 animals confirmed that maximal inhibition (93%) of LPS-induced neutrophil recruitment at 4 hours was achieved with a dose of 200 μg MEL-14 per mouse, with an IC50 value of approximately 100 μg per mouse.

Mouse pleurisy model. Pleurisy was induced as described previously.21 Briefly, an adapted needle (13 × 5 gauge) was inserted carefully 2 mm through the parietal pleura into the right side of the thoracic cavity of mice to enable injection of LPS (250 ng/cavity in 50 μL sterile saline). Control animals received an equal volume (50 μL) of sterile saline only. After different times (4 to 48 hours), the animals were killed with an overdose of pentobarbitone (Exipral) and their chest cavities were lavaged with RPMI containing heparin (10 IU/mL). Aliquots of lavage fluid were taken for determination of total leukocytes in a hemocytometer, and differential counts on stained (Hema-Gum; BDH, Poole, UK) cytospin preparations. Tail vein blood samples were taken at 15 minutes and at 2, 4, 8, and 24 hours to assess serum MoAb concentrations; at 15 or 5 minutes and at 2, 4, and 24 hours to measure granulocyte L-selectin expression; and at 1, 4, 12, and 24 hours to monitor circulating leukocyte numbers. Total leukocytes were determined in a Coulter Counter and differentials were performed on stained (Hema-Gurr) blood smears.

Serum IgG levels. The concentration of rat MoAbs MEL-14, 5H1, and 10E6 in mouse serum were measured using an indirect enzyme-linked immunosorbent assay (ELISA). All 96 wells of a maxisorp immunoplate Nunc (Life Technologies, Paisley, UK) were coated overnight at 4°C with goat anti-rat IgG in carbonate buffer, pH 9.6. The plates were washed and 100 μL of myeloma IgG standards (1.56 to 100 ng/mL) and dilutions of mouse serum in conjugate buffer (0.05 mol/L phosphate buffer, pH 7.0, containing 1.2% NaCl, 0.5% Tween 80, 5 mg/mL bovine serum albumin [BSA]) were added, and the plates were incubated for 3 hours at room temperature. After extensive washing, bound rat IgG was detected with antirat peroxidase conjugate, followed by ABTS [2,2-azino-bis(3-ethylbenzthiazoline-6-sulfonic acid)] substrate. Optical density was measured at 405 nm on a microplate reader and concentrations were obtained by interpolation on standard curves generated using IgG myeloma protein.

Flow cytometry. Aliquots (50 μL) of whole blood were incubated at 4°C for 30 minutes with a saturating concentration of MEL-14 in phosphate-buffered saline (PBS) containing 5% heat-inactivated rabbit serum. After three washes, FITC-conjugated goat antirat IgG was added and incubated for 30 minutes at 4°C. The binding of MEL-14 on circulating leukocytes was assessed by adding FITC-conjugated goat antirat IgG directly to whole blood. Erythrocytes were lysed using Coulter Clone Immuno-Lysé (Coulter Electronics, Luton, UK) and FITC fluorescence was determined on a FACScan flow cytometer (Becton Dickinson, Oxford, UK) and analyzed using CELLQuest software.

Statistical analysis. All results are presented as the mean ± SEM. Normalized data were analyzed by one-way analysis of variance (ANOVA) and differences between groups assessed using either the Student-Newman-Keuls post-test (pleural leukocyte recruitment and blood leukocyte data) or Dunnett’s multiple comparison test (serum IgG concentrations). A P value less than 0.05 was considered significant.

RESULTS

Time course of LPS-induced leukocyte recruitment. The kinetics of LPS-induced leukocyte recruitment to the mouse pleural cavity were similar to those observed in the rat.23 Thus, there was a marked neutrophil influx that peaked after 4 to 6 hours (approximately 106 neutrophils per cavity) and with similar numbers found up to 24 hours. There were few eosinophils in the pleural cavity up to 12 hours, but by 24 hours their number increased to reach a maximum (approximately 0.5 to 0.8 × 106 per cavity) that persisted for 48 hours. In contrast, intrapleural administration of saline resulted in little neutrophil or eosinophil accumulation throughout the time course. Mononuclear cells constituted greater than 98% of cells in the naive cavity, and their number was significantly increased 12 hours after LPS reaching an approximately twofold maximum after 24 to 48 hours. In the experiments described in the present study, the effect of antiselecin MoAbs on LPS-induced eosinophil accumulation in the pleural cavity was assessed after 24 hours. For comparison, neutrophil accumulation was assessed after 4 and 24 hours.

Effect of anti-L-selectin MoAb. Compared with animals that received intrapleural saline, administration of LPS into the pleural cavity of mice treated with a control IgG resulted in a substantial eosinophil accumulation at 24 hours (Fig 1). This represented 11.3% ± 0.7% of the pleural leukocytes at this time point. The L-selectin MoAb MEL-14 reduced this to 1.7% ± 0.7% eosinophils and blocked the total number of eosinophils in the pleural cavity by 97% (P < .001; Fig 1). In a separate experiment, LPS-induced pleural eosinophil recruitment at 24 hours was not blocked by anti-CD45 MoAb; there were 0.28 ± 0.04 × 106 eosinophils in LPS controls, 0.24 ± 0.02 × 106 eosinophils after LPS in CD45-treated mice, and no eosinophils detected after intrapleural saline (n = 6).

LPS also induced the accumulation of neutrophils in the pleural cavity at 4 and 24 hours compared with intrapleural saline (Fig 2). Total neutrophil accumulation at 4 hours was inhibited 95% by MEL-14 (P < .001; Fig 2A) but was not affected by anti-CD45 (data not shown).
Granulocyte L-selectin expression, as assessed by flow cytometry, remained constant up to 24 hours after intrapleural saline or intrapleural LPS, suggesting that LPS treatment did not cause L-selectin shedding (data not shown). Intravenous administration of 200 μg MEL-14 resulted in saturation of granulocyte L-selectin at 2 and 4 hours, although, at the latter time, there was an approximately 50% reduction in total granulocyte surface L-selectin, suggesting that MEL-14 administration induced partial L-selectin loss. However, at 24 hours, total granulocyte L-selectin was not significantly different to values at -15 minutes, although the degree of saturation by MEL-14 was reduced to 33%; this finding is consistent with clearance of MEL-14 from the circulation. Thus, whereas neutrophil and eosinophil recruitment are L-selectin-dependent, part of the inhibitory effect of MEL-14 may be due to L-selectin loss from the granulocyte surface.

Mononuclear cell numbers in the pleural cavity at 4 hours were not significantly altered by MEL-14 treatment with 0.92 ± 0.18 × 10⁶ (n = 6) cells in the saline-treated cavity and 1.06 ± 0.19 × 10⁶ and 0.73 ± 0.14 × 10⁶ cells in the LPS-treated cavities in control IgG- and MEL-14-treated mice, respectively. At 24 hours, mononuclears increased from 2.08 ± 0.18 × 10⁶ (n = 6) cells in the saline-treated cavity to 3.65 ± 0.12 × 10⁶ (P < .01, n = 6) cells in the LPS-treated cavity and this was significantly (P < .05) reduced by MEL-14 to 2.60 ± 0.46 × 10⁶ (n = 6) cells. A similar effect of MEL-14 on thioglycollate-induced mononuclear cell influx into the mouse pleural cavity has been reported and emphasizes the important role of L-selectin in leukocyte recruitment in vivo.

**Effect of anti-P- and E-selectin MoAbs.** The effect of the anti-P-selectin MoAb 5H1 on LPS-induced eosinophil recruitment at 24 hours is shown in Fig 3. Accumulation in

---

**Fig 1. Effect of blocking L-selectin on LPS-induced eosinophil recruitment.** Mice received intrapleural saline (saline group) or were treated with IV control IgG (200 μg) or anti-L-selectin MoAb MEL-14 (200 μg) followed by intrapleural LPS (250 ng). Eosinophils in pleural lavage were quantified after 24 hours. Values are the mean ± SEM of 6 mice per group.

---

**Fig 2. Effect of blocking L-selectin on LPS-induced neutrophil recruitment.** Mice received intrapleural saline (saline group) or were treated IV with control IgG (200 μg) or anti-L-selectin MoAb MEL-14 (200 μg) followed by intrapleural LPS (250 ng). Neutrophils in pleural lavage were quantified after 4 or 24 hours. Values are the mean ± SEM of 6 mice per group.

---
the pleural cavity was significantly ($P < .01$) inhibited 54% by anti-P-selectin, which represented a 56% reduction in the percentage of total pleural leukocytes that were eosinophils from 9.8% ± 1.0% to 4.3% ± 0.9% ($P < .005$). These data provide the first evidence for a role of P-selectin in mediating eosinophil accumulation in vivo. In contrast to the effect of anti-P-selectin, there was no effect of the anti-E-selectin MoAb 10E6 on LPS-induced eosinophil recruitment at 24 hours (Fig 3); 10.2% ± 1.6% of pleural leukocytes were eosinophils in control IgG-treated mice, and in anti-E-selectin-treated mice, this number was 11.2% ± 1.4%. However, the combination of anti-P- and anti-E-selectin was very effective at blocking eosinophil recruitment, with an 86% inhibition ($P < .001$; Fig 3). This represented a similar reduction in the percentage of eosinophils from 11.7% ± 1.4% in mice treated with control MoAbs to 2.2% ± 0.2% in animals that received anti-P- and anti-E-selectin (200 μg of each MoAb). In a separate study, there

![Graph](https://via.placeholder.com/150)

**Fig 3.** Effect of blocking P-selectin and E-selectin on LPS-induced eosinophil recruitment. Mice received intrapleural saline (saline group) or were treated IV with control MoAbs (IgG1, IgG2b, or both) or anti-P-selectin (SH1), anti-E-selectin (10E6), or both. All MoAbs were administered at a dose of 200 μg per mouse. This was followed by intrapleural LPS (250 μg) and eosinophils in pleural lavage were quantified after 24 hours. Values are the mean ± SEM of 4 to 6 mice per group.
was no significant effect of a combination of the nonblocking P-selectin MoAb 10A10 and E-selectin MoAb 14E4 on LPS-induced eosinophil recruitment; there were 0.28 ± 0.04 × 10^6 eosinophils in controls and 0.35 ± 0.08 × 10^6 eosinophils in mice treated with nonblocking MoAbs (n = 6) and 0 after intrapleural saline.

Total circulating leukocytes at 4 and 24 hours were not significantly altered by anti–P-selectin or anti–E-selectin MoAbs (data not shown). The only significant change in circulating eosinophils was seen at 24 hours, when the blood counts were as follows: intrapleural saline, 0.11 ± 0.03 × 10^6/mL; intrapleural LPS, intravenous IgG1, 0.12 ± 0.05 × 10^6/mL; and intrapleural LPS, intravenous anti–P-selectin, plus anti–E-selectin, 0.46 ± 0.13 × 10^6/mL (P < .05). Serum rat IgG concentrations measured by ELISA were determined in 4 mice after the administration of anti–P-selectin and anti–E-selectin MoAbs. The concentrations (in micrograms per milliliter) at 15 minutes and at 2, 4, 8, and 24 hours were as follows: 247 ± 52, 240 ± 60, 127 ± 45, 177 ± 36, and 93 ± 27, respectively (significant compared with 15 minutes value at 4 and 24 hours, P < .01, one-way ANOVA and Dunnert’s test).

In contrast to the effect of MEL-14, the anti–P-selectin MoAb 5H1 had no effect on neutrophil accumulation at 4 hours, suggesting that P-selectin was not involved in this response (Table 2). Similar data were found with the anti–E-selectin MoAb 10E6 (Table 2). However, simultaneous blockade of P- and E-selectin was very effective; neutrophil accumulation was inhibited by 93% (P < .01), which represented a decrease from 34.2% ± 4.2% to 5.6% ± 2.1% neutrophils in the pleural cavity. Combined treatment with nonblocking anti–P- and E-selectin MoAbs did not alter neutrophil accumulation (LPS, 0.62 ± 0.10 × 10^6; LPS + MoAbs, 0.65 ± 0.07 × 10^6 neutrophils). Qualitatively similar data were found for LPS-induced neutrophil accumulation at 24 hours, such that neutrophil recruitment was inhibited by 92% only when both P- and E-selectin were blocked (Table 2) and there was no effect of the nonblocking MoAbs (data not shown). Whereas circulating neutrophils were significantly (P < .01) increased at 4 hours from 1.08 ± 0.20 × 10^6/mL in animals that received intrapleural saline to 4.06 ± 0.71 × 10^6/mL, in animals that received intrapleural LPS, this increase was not altered by IV administration of anti–P- and anti–E-selectin blocking MoAbs. At 24 hours, blood neutrophils in animals that received intrapleural saline (1.31 ± 0.23 × 10^6/mL) were not increased after intrapleural LPS, but there was an increase when P- and E-selectin were blocked (2.69 ± 0.32 × 10^6/mL, P < .01).

Mononuclear cell numbers in the pleural cavity are shown in Table 3. At 4 hours, they were not significantly altered by LPS treatment with anti–P-selectin or anti–E-selectin MoAbs. At 24 hours, total mononuclears increased approximately twofold in the LPS-treated cavity and this increase was not significantly affected by control, anti–P-selectin, or a combination of anti–E- and P-selectin MoAbs. In contrast, LPS-induced a significantly (P < .05) greater mononuclear cell recruitment in animals treated with anti–E-selectin MoAb compared with the control IgG2b. In a separate study, there was no significant effect of nonblocking P- and E-selectin MoAbs on cavity mononuclear cells at both 4 and 24 hours (data not shown).

**DISCUSSION**

The aim of this study was to investigate the possible role of selectins in mediating eosinophil recruitment to an inflammatory site. We used the pleural cavity of the mouse because this is a straightforward and well-established model that allows the sampling of endogenous cells to be monitored at different times. In addition, the mouse is the species of choice because of the availability of MoAbs specific for mouse selectins.

Using doses of antiselctin MoAbs that have been shown previously to cause maximal inhibition of neutrophil accumulation, our results suggest that L-selectin plays an obligatory role in LPS-induced eosinophil recruitment. We also confirmed that L-selectin is important for neutrophil accumulation. Other studies using MEL-14 or a soluble L-selectin/IgG chimera have shown the important role of L-selectin in thioglycollate-induced early neutrophil influx in the mouse peritoneum. The data presented here indicate that L-selectin also plays a requisite role in LPS-induced neutrophil influx; in addition, preliminary studies show that neutrophil influx in the mouse pleural cavity induced by carrageenan is abrogated by MEL-14 (data not shown). The lesser inhibition of neutrophil recruitment at 24 hours may be because there was less MEL-14 in the circulation, although, in the same animals, eosinophil influx at the same time was reduced by 95%. A plausible explanation for this paradox is that, be-

**Table 2. Effect of Blocking P-Selectin and E-Selectin on LPS-Induced Pleural Neutrophil Accumulation at 4 and 24 Hours**

<table>
<thead>
<tr>
<th>Time</th>
<th>Saline</th>
<th>IgG1 LPS</th>
<th>Anti-P LPS</th>
<th>IgG2b LPS</th>
<th>Anti-E LPS</th>
<th>IgG1 + IgG2b LPS</th>
<th>Anti-P + Anti-E LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>0.06 ± 0.03</td>
<td>0.83 ± 0.14*</td>
<td>1.06 ± 0.19*</td>
<td>0.76 ± 0.15*</td>
<td>0.61 ± 0.06i</td>
<td>0.62 ± 0.111</td>
<td>0.09 ± 0.04i</td>
</tr>
<tr>
<td>24 h</td>
<td>0.18 ± 0.07</td>
<td>1.05 ± 0.253</td>
<td>0.87 ± 0.213</td>
<td>1.24 ± 0.231</td>
<td>0.84 ± 0.205</td>
<td>1.13 ± 0.261</td>
<td>0.26 ± 0.081</td>
</tr>
</tbody>
</table>

Mice received intrapleural saline (n = 9 to 10) or were treated IV with control MoAbs (IgG1, IgG2b, or both) or anti–P-selectin (5H1), anti–E-selectin (10E6), or both (n = 5 to 6 per group). This was followed by intrapleural LPS and neutrophils in pleural lavage were quantified after 4 or 24 hours.

* P < .001, a significant difference from the response to animals treated with intrapleural saline.
† P < .01, a significant difference from the response to animals treated with intrapleural saline.
‡ P < .05, a significant difference from the response to LPS in animals treated with control MoAbs.
§ P < .05, a significant difference from the response to animals treated with intrapleural saline.
¶ P < .05, a significant difference from the response to LPS in animals treated with control MoAbs.
MEL-14 might have other actions in addition to L-selectin. If the endothelial ligand for this epitope were expressed predominantly at later time points after eosinophil recruitment on L-selectin, this could lead to a greater dependency of cyte L-selectin after 4 hours, suggesting that part of its inhibitory action could be due to differences in the sLe^C-containing surface molecules. Such differences in neutrophil and eosinophil adhesion to P-selectin were not observed, but differences in the P-selectin ligands of the two cells were reported. Whether this contributes to differences in selectin requirement in vivo is unknown, but the in vitro data are suggestive of a dominant role for P-selectin during eosinophil recruitment. Our findings in the mouse provide the in vivo evidence to support the in vitro studies. P-selectin, but not E-selectin, also plays an important role in eosinophil adhesion to nasal polyp endothelium, although the contribution to neutrophil adhesion in this system is not known.

In contrast to the effects of L- and P-selectin, there was no inhibitory effect of the anti-E-selectin MoAb on eosinophil accumulation, except when P-selectin was also blocked. Under these conditions, eosinophil accumulation was reduced greater than 85%. Similar findings were made for neutrophil accumulation at 4 and 24 hours, although the effect of the anti-P plus anti-E-selectin combination was more dramatic because neither MoAb alone was inhibitory. These data are consistent with findings in E-selectin deficient mice in which thioglycollate-induced neutrophil influx into the peritoneal cavity at 6 hours was similar to wild-type mice; anti-P-selectin was also ineffective in the wild-type, but in the E-selectin-deficient animals it reduced neutrophil influx by 85%. In another study of thioglycollate-induced neutrophil influx into the mouse peritoneal cavity, it was found that MoAbs against L-, P-, and E-selectin were all partial inhibitors at 4 hours, but a combination of anti-L and anti-P-selectin MoAbs provided maximum inhibitory effects. However, the MoAbs to P- and E-selectin were different to those used in the present study and in the study of E-selectin knockouts; therefore the reasons for the differences remains to be resolved. Our results suggest that either P-selectin or E-selectin can mediate eosinophil and neutrophil influx and that there is some redundancy of function of the two molecules, particularly with respect to the neutrophil. An alternative explanation is that P- and E-selectin share a similar function in regulating neutrophil and eosinophil recruitment.

Table 3. Effect of Blocking P-Selectin and E-Selectin on Mononuclear Cell Numbers in the Pleural Cavity at 4 and 24 Hours

<table>
<thead>
<tr>
<th>Time</th>
<th>Saline</th>
<th>IgG1 LPS</th>
<th>Anti-P LPS</th>
<th>IgG2b LPS</th>
<th>Anti-E LPS</th>
<th>IgG1 + IgG2b LPS</th>
<th>Anti-P + Anti-E LPS</th>
</tr>
</thead>
<tbody>
<tr>
<td>4 h</td>
<td>1.48 ± 0.22</td>
<td>1.18 ± 0.15</td>
<td>1.72 ± 0.20</td>
<td>1.61 ± 0.33</td>
<td>1.82 ± 0.24</td>
<td>1.14 ± 0.14</td>
<td>2.01 ± 0.63</td>
</tr>
<tr>
<td>24 h</td>
<td>2.48 ± 0.19</td>
<td>3.56 ± 0.31</td>
<td>4.15 ± 0.29</td>
<td>4.64 ± 0.54</td>
<td>6.23 ± 0.39</td>
<td>4.92 ± 0.33</td>
<td>4.13 ± 0.49</td>
</tr>
</tbody>
</table>

Mice received intrapleural saline (n = 9 to 10) or were treated IV with control MoAbs (IgG1, IgG2b, or both) or anti-P-selectin (5H1), anti-E-selectin (10E8), or both (n = 5 to 6 per group). This was followed by intrapleural LPS and mononuclear cells in pleural lavage were quantified after 4 or 24 hours.

* P < .05, a significant difference from the response to animals treated with intrapleural saline.
† P < .01, a significant difference from the response to animals treated with intrapleural saline.
‡ P < .001, a significant difference from the response to animals treated with intrapleural saline.
§ P < .05, a significant difference from the response to the saline group.
ligand, which is consistent with the report that P-selectin glycoprotein ligand (PSGL-1) binds both P- and E-selectin. In contrast, E-selectin ligand (ESL-1) binds E-selectin only. It will therefore be of interest to examine the role of these selectin ligands when appropriate tools for the mouse become available.

Because macrophages and T lymphocytes are important intermediates in LPS-induced eosinophil recruitment to the rat pleural cavity, an effect of antisel ectin MoAbs on mononuclear cell influx may contribute to their inhibitory actions on eosinophil (and perhaps neutrophil) accumulation at 24 hours. An anti-VLA-4 MoAb has been reported to attenuate eosinophil influx in a delayed hypersensitivity reaction in mouse skin, perhaps an indirect effect as a result of inhibiting mononuclear cell recruitment. However, this hypothesis did not appear to account for the inhibition in our study of eosinophil accumulation by anti-P- and anti-E-selectin MoAbs because these MoAbs, either alone or in combination, did not significantly affect mononuclear cell numbers after intrapleural LPS. We have yet to examine responses to LPS in selectin-deficient mice but, in a contact hypersensitivity reaction, monocyte and CD4+ T lymphocyte (and neutrophil) influx was markedly less in P-selectin-deficient animals than seen in wild-type counterparts, suggesting that the involvement of P-selectin may depend on the stimulus.

The presence of eosinophils in tissues is a feature of allergic disease. These leukocytes and their secretory products have been implicated in these diseases, yet the mechanisms of their recruitment are not completely understood. The results presented here show that selectins play a crucial role in mediating eosinophil recruitment in a model of pleural space recruitment and suggest that blocking selectin function will be of benefit in allergic inflammation. Whether a carbohydrate-based therapeutic strategy that blocks all selectins offers the best approach or whether blocking P-selectin alone would be favorable, which would reduce eosinophil migration without affecting neutrophil migration, remains the subject of further study.

REFERENCES

Selectins mediate eosinophil recruitment in vivo: a comparison with their role in neutrophil influx

GM Henriques, JM Miotla, SB Cordeiro, BA Wolitzky, ST Woolley and PG Hellewell