Prevention of Pulmonary Injury in Isolated Perfused Rat Lungs by Activated Human Neutrophils Preincubated With Anti-Mo1 Monoclonal Antibody

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Neutrophil activation results in neutrophil adherence and may subsequently cause lung injury through the generation of oxidants, release of granule proteases, and generation of a variety of mediator substances. We hypothesized that inhibition of neutrophil adherence and subsequent lung sequestration would attenuate the lung injury caused by activated neutrophils. Using isolated perfused rat lungs, we determined if anti-Mo1 monoclonal antibody (binds to the α subunit of a neutrophil glycoprotein [gp 155,94] that facilitates adherence) would attenuate lung neutrophil sequestration and lung injury caused by human neutrophils stimulated by phorbol myristate acetate (PMA). PMA-stimulated neutrophils but not PMA or neutrophils alone caused lung injury as assessed by accumulation of 125I-bovine serum albumin into lung parenchyma and alveolar lavage fluid. Incubation of neutrophils with anti-Mo1 antibody prior to stimulation with PMA attenuated lung injury and neutrophil sequestration. Furthermore, a histological survey revealed that anti-Mo1 antibody inhibited neutrophils present in the lung from spreading following exposure to PMA. Anti-Mo1 antibody did not inhibit PMA-stimulated neutrophil release of granule constituents or toxic O2 metabolites as evidenced by lysozyme and lactoferrin release or the reduction of ferricytochrome c in the lung perfusate. The inhibition of lung injury caused by the anti-Mo1 antibody was not likely due to a nonspecific effect of the antibody, since another murine monoclonal antibody of the same class (anti-Mo5) did not inhibit lung neutrophil sequestration or lung injury. Thus, in this experimental model, interference with the close approximation of the neutrophil to its target site inhibited the ability of the activated human neutrophil to cause injury.

CONSIDERABLE EVIDENCE has accumulated over recent years suggesting that oxygen metabolites and proteases released by activated neutrophils may play an important role in the development of pulmonary inflammatory injury. Both oxidants and proteases have been detected in vivo in human disease and in models of pulmonary inflammation in experimental animals and isolated perfused lungs.12 In experimental models of lung injury, acute lung damage can occur following a single dose of phorbol myristate acetate (PMA). This PMA-induced injury of rabbit lung vascular endothelial cells is dependent on the presence of neutrophils but the precise mechanism by which the neutrophil inflicts injury is not clearly understood.10

Following activation of neutrophils, not only do the cells release both proteases and oxidants, but they adhere more avidly to each other (neutrophil aggregation) and to artificial (plastic, glass) and physiological (endothelium, epithelium) surfaces.11 Neutrophil adherence which results in intimate contact between activated neutrophils and endothelial cells may be critical to the neutrophil’s ability to inflict damage. These adherence properties are necessary in order for activated cells to migrate on and through vascular endothelium in response to inflammatory stimuli. At the molecular level, progress has been made toward the identification of neutrophil surface glycoproteins that are involved in cell adhesion. Among these adhesion-promoting molecules is a heterodimeric glycoprotein, Mo1 (gp 155,95)12 (equivalent to OKM-113 or Mac-114) that also mediates the binding of C3bi-opsonized particles to the phagocyte membrane.15 Monoclonal antibodies specific for the 155-kD alpha or 95-kD beta polypeptides of Mo1 have been shown to inhibit human neutrophil aggregation,16,17 adhesion to plastic, glass, or endothelial cell surfaces,18,19 and endothelial cell injury.20

With the availability of anti-Mo1 antibody the inhibition of neutrophil contact with its target can better be studied. We investigated in isolated salt-perfused rat lungs whether incubating human neutrophils with anti-Mo1 antibody prior to stimulation with PMA would inhibit lung neutrophil sequestration and subsequent lung injury. We also determined if anti-Mo1 antibody altered activated human neutrophil release of toxic oxygen metabolites or granule constituents. The isolated perfused rat lung was used in part since in this system we can concomitantly assess hemodynamic changes and the consequences of lung neutrophil activation (eg, sequestration, granule content release) over time. In this report, we demonstrate in isolated perfused rat lungs that PMA-activated human neutrophils induce acute lung injury which can be markedly attenuated by incubation of the neutrophils with anti-Mo1 antibody. The attenuation of the lung injury is not due to inhibition of neutrophil release of either superoxide or granule constituents. An identification of the mechanism by which neutrophils cause injury may lead to an understanding of the pathogenesis of some forms of the adult respiratory distress syndrome.

MATERIALS AND METHODS

Ex vivo lung preparation. An ex vivo model was used to assess lung injury and pulmonary vascular constriction following infusion of human neutrophils stimulated by PMA. Lungs were isolated from male Long-Evans rats (body weights 200 to 300g) which were anesthetized with pentobarbital (40 mg/kg intraperitoneally).

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Lungs were isolated and perfused at constant flow at 0.03 mL/g rat body weight/minute as previously described. Mean pulmonary artery inflow pressure (PA) was measured with a Statham (Oxnard, CA) transducer and recorded on a Grass (Quincy, MA) recorder. Perfusion pressure was proportional to pulmonary vascular resistance, since the flow for a given lung was held constant. Left arterial pressure (pulmonary outflow) was set by adjusting the height of the outflow reservoir and was kept at 0 cm H2O. The lungs were ventilated via a tracheal cannula with an air/gas mixture containing 21% O2 and 5% CO2 at a rate of 60 strokes/minute using a Harvard animal respirator. The lungs and perfusate reservoir were kept at a temperature of 37° to 40°C.

**Lung perfusion.** Lungs were perfused from the outflow of the experiment from a reservoir containing physiologic salt solution (70 mL) osmotically stabilized with Ficoll (4 g/100 mL). The first 30 mL of lung effluent was discarded to eliminate circulating rat blood elements from the vascular space of the lungs. This resulted in no neutrophils being detected in a 1:4 dilution of perfusate in 3% acetic acid. The lungs were then perfused for 30 minutes to reach a stable perfusion pressure and temperature prior to adding ferricytochrome c (50 μmol/L) to the circulating perfusate. Ferricytochrome c was added to the perfusate so that its reduction could be used as an index of superoxide generation. Ten minutes after the addition of ferricytochrome c to the perfusate, human neutrophils were added to the lung perfusate. In some experiments catalase (100 units) was added to the lung perfusate. In other experiments, human neutrophils were incubated with either anti-Mo1 or anti-Mo5 antibody.

**Isolation of neutrophils.** Human neutrophils (98% neutrophils with >1% platelets/1,000 cells) were isolated according to the method of Curnutte and Babior. This procedure resulted in 95% viable neutrophils by the trypan blue exclusion test. The cells were washed and resuspended in a final concentration in the lung perfusate of 3 x 106 cells/mL.

**Incubation of neutrophils with monoclonal antibodies.** The generation of marine monoclonal antibodies anti-Mo1 (IgG2a) and anti-Mo5 (IgG2a) has been previously described. IgG was purified from ascites fluid by Staphylococcus protein A–sepharose chromatography. Saturating concentrations of anti-Mo1 and anti-Mo5, as determined by using indirect immunofluorescence flow cytometry, were 1 μg/106 human neutrophils. In experiments where cells were pretreated with antibodies prior to administration into the lungs, 1.2 x 106 cells in 1 mL phosphate-buffered saline (PBS) were incubated for 15 minutes at 37°C with antibody concentrations far exceeding saturation (600 μg/mL). Following incubation, neutrophils were delivered into the lung perfusate at a final concentration of 3 x 106 cells/mL without prior washing (monoclonal antibody present in the perfusate). To confirm antibody excess sufficient to saturate Mo1 sites expressed by neutrophils after PMA activation, ferricytochrome c reduction, and granule constituent release as described below. The number of circulating neutrophils was determined by using indirect immunofluorescence flow cytometry, as determined by using direct immunofluorescence flow cytometry.

**Measurement of lung injury.** 125I-bovine serum albumin (125I-BSA) was prepared by the standard chloramine T method. Unbound iodine was removed by gel filtration using a G-75 column. 125I-BSA was added to the perfusion reservoir 30 minutes after the addition of PMA. This was done in part so that pulmonary venous effluence could be sampled and assays performed on venous effluent that did not contain 125I-BSA. Thirty minutes after the addition of the 125I-BSA to the perfusate, 1 mL of pulmonary venous effluent was obtained, weighed, and counted for two minutes in a gamma scintillation counter. This allowed the determination of the number of 125I-BSA counts in 1 g of circulating perfusate.

The reservoir supplying perfusate to the lung was then changed to contain only physiologic salt solution osmotically stabilized with Ficoll (4 g/100 mL). After the lung was perfused with 15 mL of this perfusate, 1.0 mL of pulmonary venous effluent was obtained and assessed for radioactivity in a gamma scintillation counter. This was done to confirm that minimal 125I-BSA counts (<1% of the counts present in 1.0 gram of the circulating perfusate) remained within the intravascular space of the lung. The lungs and attached structures were then weighed prior to performing whole lung lavage, which was done by instilling 3.0 mL of normal saline into the tracheal cannula. This was instilled and withdrawn three times and 1 to 1.5 mL of total fluid was recovered. One mL of this lavage fluid was then counted for two minutes in a gamma scintillation counter to assess the amount of 125I-BSA that accumulated in alveolar lavage fluid. The structures attached to the lung tissue were then dissected away from the lung and weighed. This weight was subtracted from the previously determined weight of the lung with attached structures to determine lung wet weight. The lung tissue was then counted for two minutes in a gamma scintillation counter to assess the amount of 125I-BSA that had accumulated in lung parenchyma. 125I-BSA counts present in alveolar lavage fluid, lung tissue, and pulmonary venous effluent were normalized by dividing the number of counts measured by the number of 125I-BSA counts present in 1.0 g of circulating perfusate. The ratio of lung wet weight to the rat body weight was calculated as an index of lung water.

To assess neutrophil–pulmonary endothelial interactions in situ, a light and transmission electron microscopic survey of lung sections was performed. Lungs perfused with PMA or PMA and neutrophils plus PMA, and neutrophils incubated with anti-Mo1 antibody plus PMA. Thirty minutes following the addition of the neutrophils to the perfusion reservoir the lung was fixed with 4% glutaraldehyde via tracheal instillation (at 15 cm H2O). Sections (1 μ) were examined following staining of plastic-embedded tissue with toluidine blue.

**Analysis of lung perfusate.** The number of circulating neutrophils in the perfusate were counted on a hemocytometer. Perfusion samples that contained an undetectable number of cells at a 1:4 dilution in 3% acetic acid were considered devoid of circulating neutrophils. Ferricytochrome c reduction was used as an index of superoxide generation and was determined by optical density measurement of the perfusate at 551 nm. In separate experiments, it was demonstrated that the reduction of ferricytochrome c was abolished by the addition of superoxide dismutase (50 μmol/L) to the perfusate. Lysosome activity (units/min/mL) and lactoferrin concentration (μg/mL) were measured as described previously.

**Assay for hydrogen peroxide generation.** Hydrogen peroxide generation by resting and stimulated neutrophils was determined by the method of Root and Metcalfe. Neutrophils (2.5 x 106/mL) in Krebs-ringer-phosphate, pH 7.4, were incubated with 5 ng/mL PMA or 10-7 mol/L 1-Methyl-Leu-Phe (FMLP) for 15 minutes. In some instances, 50 μmol/L ferricytochrome c was added to the buffer in order to evaluate the effect of this scavenger of superoxide on hydrogen peroxide formation.

**Statistics.** Values were expressed as mean ± SEM. Groups were
NEUTROPHIL-MEDIATED LUNG INJURY

Fig 1. Typical tracing showing the experimental protocol and change in perfusion pressure following the addition of ferricytochrome c, human neutrophils (PMN), PMA, and 125I-BSA (downward arrows) to a lung perfuse consisting of physiologic salt solution containing Ficoll.

jcompared using one-way analysis of variance and the Neuman-Keuls multiple comparison test.

RESULTS

Effect of anti-Mo1 antibody on lung neutrophil sequestration and lung injury. Addition of human neutrophils to rat lung perfuse containing physiologic salt solution in the absence of PMA caused PA pressure to increase 3 ± 0.4 mm Hg (n = 9, Fig 1). More than 95% of the neutrophils entering the lung were sequestered, since they were not recovered in the pulmonary venous effluent (Table 1, experiment A). Despite lung neutrophil sequestration, in the absence of PMA there was no increase in rat lung wet weight to body weight ratio or increase in accumulation of 125I-BSA into lung parenchyma or alveolar lavage fluid (Fig 2, column two). Similarly, as seen in Fig 2, column one, PMA alone in the absence of neutrophils failed to cause increased rat lung weight to body weight ratio or increase in accumulation of 125I-BSA into lung parenchyma or alveolar lavage fluid.

Neutrophils could not longer be detected in the lung effluent following the addition of PMA to lung perfuse containing neutrophils (Table 1, experiment A). Correspondingly, in these lungs the administration of PMA resulted in lung injury as assessed by increased rat lung weight to body weight ratio and increased accumulation of 125I-BSA into lung parenchyma and alveolar lavage fluid (Fig 2, column three). This increased albumin accumulation in lung parenchyma and alveolar lavage fluid was not likely due to hydrostatic changes, since the PA pressure measured during the period of albumin accumulation (12 ± 2 mm Hg) in these lungs (PMN and PMN/PMA) was not different (P = NS) from that in lungs in which nontreated neutrophils were stimulated by PMA (12 ± 2 mm Hg). Lung albumin accumulation was also compared in a subset of lungs from each of these two groups. Lungs were paired such that the PA pressures during the period of albumin accumulation were identical (±.5 mm Hg difference). Comparing these subgroups (paired t test, n = 3) addition of PMA to lung perfuses containing neutrophils incubated with anti-Mo1 antibody, as opposed to nonincubated neutrophils, still resulted in less accumulation of 125I-BSA into lung parenchyma (.43 ± .09 v 1.1 ± .1, P < .05) and alveolar lavage fluid (.03 ± .01 v .36 ± .3, P < .05). Treatment of neutrophils with anti-Mo1 antibody not only reduced the total number of cells sequestered in the lung, but anti-Mo1-treated neutrophils failed to spread on pulmonary endothelium after exposure to PMA as assessed microscopically (Fig 3, column C). In addition, the vascular endothelium appeared intact following exposure to PMA-stimulated neutrophils incubated with anti-Mo1 antibody. However, the anti-Mo1 antibody did not impair the development of vacuolization, a finding consistent with degranulation. In contrast, as seen in Fig 3, column B, PMA-activated neutrophils not exposed to anti-Mo1 antibody underwent spreading on endothelium, caused marked endothelial cell damage, and demonstrated vacuolization consistent with injury to occur since addition of catalase (n = 5) to the lung perfuse prior to the addition of PMA inhibited the increased accumulation of 125I-BSA in lung parenchyma (.36 ± .06, P < .05).

Incubation of neutrophils with anti-Mo1 antibody inhibited lung neutrophil sequestration both prior to and following the addition of PMA to the lung perfuse (Table 1, experiment B). Incubation of neutrophils with anti-Mo1 antibody had no effect on the PA pressure increase following addition of these neutrophils to the lung perfuse (3 ± .4 mm Hg, n = 6, P = NS). However, addition of PMA to lung perfusates containing neutrophils incubated with anti-Mo1 antibody resulted in less lung injury since the increase in lung wet weight to body weight ratio and accumulation of 125I-BSA into lung parenchyma and alveolar lavage fluid were inhibited (Fig 2, column four). This inhibition of the lung parenchyma and alveolar lavage albumin accumulation was not likely due to hydrostatic changes, since the PA measured during the period of albumin accumulation (10 ± .6 mm Hg) in these lungs (PMN incubated with Mo1 antibody and PMA) was not different (P = NS) from that in lungs in which nontreated neutrophils were stimulated by PMA (12 ± 2 mm Hg). Lung albumin accumulation was also compared in a subset of lungs from each of these two groups. Lungs were paired such that the PA pressures during the period of albumin accumulation were identical (±.5 mm Hg difference). Comparing these subgroups (paired t test, n = 3) addition of PMA to lung perfuses containing neutrophils incubated with anti-Mo1 antibody, as opposed to nonincubated neutrophils, still resulted in less accumulation of 125I-BSA into lung parenchyma (.43 ± .09 v 1.1 ± .1, P < .05) and alveolar lavage fluid (.03 ± .01 v .36 ± .3, P < .05). Treatment of neutrophils with anti-Mo1 antibody not only reduced the total number of cells sequestered in the lung, but anti-Mo1-treated neutrophils failed to spread on pulmonary endothelium after exposure to PMA as assessed microscopically (Fig 3, column C). In addition, the vascular endothelium appeared intact following exposure to PMA-stimulated neutrophils incubated with anti-Mo1 antibody. However, the anti-Mo1 antibody did not impair the development of vacuolization, a finding consistent with degranulation. In contrast, as seen in Fig 3, column B, PMA-activated neutrophils not exposed to anti-Mo1 antibody underwent spreading on endothelium, caused marked endothelial cell damage, and demonstrated vacuolization consistent with

Table 1. Circulating Neutrophils in Perfusate Following Infusion of Untreated or Antibody-Treated Human Neutrophils

<table>
<thead>
<tr>
<th>Experimental Conditions*</th>
<th>Before PMA Injection</th>
<th>After PMA Injection</th>
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<tr>
<td></td>
<td>Neutrophils (x 10⁶ Cells/mL)</td>
<td>Percentage Recovery†</td>
</tr>
<tr>
<td>A. PMN alone</td>
<td>1.58 ± 0.63</td>
<td>4.9 ± 1.95</td>
</tr>
<tr>
<td>B. PMN-AbMo</td>
<td>7.03 ± 1.07</td>
<td>23.3 ± 3.54</td>
</tr>
<tr>
<td>C. PMN-AbMo5</td>
<td>2.50 ± 0.82</td>
<td>8.2 ± 2.68</td>
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*Neutrophils were added to the reservoir in a concentration of 3 x 10⁶ cells/mL.
†Percentage recovery was calculated by dividing the number of neutrophils measured in 1 mL of pulmonary venous effluent by the number of neutrophils in the lung perfuse entering the lung.
‡P values represent comparisons between PMN alone and each of the other two conditions.
§ND indicates no neutrophils detected in a 1:4 dilution of perfusate in 3% acetic acid.
perforation resulted in the secretion of the specific granule components lysozyme and lactoferrin into the lung perfusate (Fig 4). The addition of PMA in the absence of neutrophils resulted in no detectable increase in granule components. Incubation of human neutrophils with anti-Mol antibody had no significant effect on release of granule constituents into the lung perfusate following the addition of PMA. On the other hand, mere exposure of nonstimulated human neutrophils to rat lung endothelium led to the release of a small but significant (p < .001) amount of lactoferrin.

**Effect of anti-Mol antibody on ferricytochrome c reduction.** Stimulation of neutrophils with PMA caused a time-dependent reduction of ferricytochrome c in the lung perfusate (Fig 5). The average rate of ferricytochrome c reduction by untreated, anti-Mol−, and anti-Mo5−treated neutrophils occurred at 30 nmol/min/10⁷ cells during the first ten minutes following stimulation with PMA. There was no significant reduction of ferricytochrome c in perfusates containing either PMA or neutrophils alone. PMA-stimulated human neutrophils preincubated with either anti-Mol or anti-Mo5 antibody produced rates (P = NS) of ferricytochrome c reduction comparable to those observed with nontreated neutrophils.

**Effect of extracellular ferricytochrome c on PMA-stimulated H₂O₂ generation.** As shown in Table 2, ferricytochrome c totally inhibited H₂O₂ release from FMLP-stimulated cells. In contrast, the amount of H₂O₂ release detected from cells stimulated by PMA was only partially attenuated by the presence of ferricytochrome c.

**DISCUSSION**

Following stimulation, activated neutrophils are able to damage neighboring target tissues such as endothelial cells. This model has been broadly adopted to explain inflammatory vascular damage such as lung injury in the adult respiratory distress syndrome. Because neutrophils are capable of releasing a broad spectrum of effector substances, neutrophil contact with vascular endothelium may be relevant to the pathogenesis of vascular injury and subsequent pulmonary edema. Previous studies have directed considerable attention toward the mechanisms by which neutrophils adhere to endothelium in vivo and in vitro. It has been observed that monoclonal antibodies directed against the Mol surface glycoprotein can inhibit neutrophil aggregation and their adhesion to substrates. Employing cells from a patient with a congenital deficiency of adhesive glycoproteins, Harlan et al found that activated neutrophils from the patient failed to induce disruption of an endothelial monolayer or increase [¹²⁵I]-albumin passage through the monolayers. Activated control neutrophils caused a nonlytic disruption of the endothelial monolayers which was mediated by an oxygen radical–dependent mechanism. In contrast, Shasby et al demonstrated a neutrophil-mediated increased albumin passage across endothelial monolayers by an oxygen-dependent mechanism. Since the cultured monolayers may not mimic the response of endothelium in vivo, we chose to study the effect of neutrophil activation on changes in permeability using an intact vasculature. We found that treatment of human neutrophils with anti-Mol monoclonal...
Fig 3. Photomicrographs of rat lungs exposed to human neutrophils. Line 1 original magnification 400x, current magnification; line 2 original magnification 2,600x, current magnification; line 3 original magnification 5600x, current magnification. Column A shows nonstimulated human neutrophils. Column B shows PMA-stimulated human neutrophils. Column C shows PMA-stimulated human neutrophils incubated with anti-Mo1 antibody. As seen in Column B, stimulation of PMNs by PMA resulted in the close approximation of the neutrophil to the endothelium and vacuolization consistent with degranulation. Gross endothelial cell injury is evident. Anti-Mo1–treated PMA-stimulated PMNs (Column C) or unstimulated PMNs (Column A) remained round and endothelial damage was not evident. Anti-Mo1 antibody did not prevent the development of PMN vacuolization. A contaminating red blood cell is seen in B2.
antibody attenuated lung injury following exposure of neutrophils to PMA. The attenuation of the lung injury likely resulted from the inability of the neutrophils to undergo spreading and firmly attach to vascular endothelium. Simon et al.31,32 have demonstrated in epithelial cell cultures that spreading and firmly attach to the target cells.

We demonstrated that treatment with anti-Mo1 monoclonal antibody did not prevent the PMA neutrophil–induced lung injury by inhibiting the production of potentially noxious substances such as oxidants and granule proteases. This is consistent with in vitro studies that have shown that anti-Mo1 antibody does not inhibit degranulation or superoxide production.10 Finally, inhibition of lung injury did not arise from a nonspecific effect of a murine IgG2a antibody, since anti-Mo5, an IgG2a antibody that recognizes another neutrophil membrane constituent,34 failed to attenuate lung injury.

One of the apparent advantages of our isolated perfused rat lung–human neutrophil system is the ability to quantify neutrophil activation in situ by monitoring both the generation of oxidants and the release of specific granule constituents into the perfusate. We found that the presence of ferricytochrome c in our lung perfusate did not quantitatively alter acute pulmonary injury of PMA-activated neutrophils as compared to those experiments carried out in the absence of ferricytochrome c (data not shown). A possible explanation for the lack of inhibition of injury by ferricytochrome c may be explained by the ability of PMA to initiate vesicles within intact neutrophils that are sequestered from the direct inhibitory effect of the superoxide scavenger. Other investigators have shown that during contact of human neutrophils with opsonized particles, the presence of ferricytochrome c failed to completely abolish H2O2 release from the cells. In contrast, there was no detectable H2O2 release from neutrophils pretreated with cytochalasin B to prevent phagosome formation in the presence of ferricytochrome c. These data suggest that the formation of “privileged sites” within the phagosome that are inaccessible to ferricytochrome c can lead to the generation of diffusible H2O2. In our model, ferricytochrome c which was used as an extracellular indicator of superoxide production likely did not scavenge superoxide formed within the neutrophil. Cytochemical studies previously have demonstrated that PMA induces formation of cytoplasmic vesicles and channels in neutrophils which exhibit H2O2 staining.13 It is likely that PMA stimulation of the human neutrophils in our lung model induced similar vesicles hidden from the scavenging effect of ferricytochrome c. Indeed, we showed that the rate of H2O2 formation by PMA-stimulated human neutrophils in vitro was not completely abolished in the presence of extracellular ferricytochrome c, indicating that a substantial
amount of H2O2 produced during PMA stimulation escaped the scavenging effects of ferricytochrome c. In contrast, neutrophils activated by FMLP, which leads to total release of superoxide to the extracellular environment, were inhibited from generating H2O2 by the presence of ferricytochrome c in the supernatant.

We found that pretreatment of neutrophils with anti-Mol antibody failed to completely inhibit sequestration of unstimulated neutrophils or cells exposed to PMA. The detection of residual soluble anti-Mol monoclonal antibody in lung perfusate even after PMA administration (which has previously been shown to induce an increase in surface Mol expression by neutrophils44) indicates that the concentration of anti-Mol antibody used in these experiments was far in excess of that required to saturate all neutrophil Mol antigenic sites. Another possible explanation for the failure to completely prevent cell adherence may relate to the existence of other adherence-promoting leukocyte surface antigens besides that recognized by our anti-Mol reagent. In support of this possibility are studies of Harlan, Beatty, Wallis, and their colleagues41,43,45 in which a monoclonal antibody (60.336) directed against the 95-kD β chain that is common to Mol and two other surface heterodimeric glycoproteins (LFA-1 and p150,95) resulted in a significant inhibition of the adhesion of neutrophils18,19 and monocytes35 to endothelial surfaces. Whether the structurally related glycoproteins LFA-1 and p150,95 are also involved in neutrophil adhesion is a subject for further study.

In our studies, we observed that the exposure of neutrophils to rat endothelium resulted in the release of a small but significant amount of the specific granule constituent lactoferrin. These observations are similar to reports by other investigators who have found that attachment of neutrophils to surfaces leads to exocytosis of specific granule constituents.28 Despite the release of granule constituents, this was insufficient to lead to detectable lung injury in our system. Similarly, PMA by itself or by stimulating cells present in the lung did not lead to the intravascular generation of superoxide or lung injury. This is in contrast to the report by Dyer39 demonstrating a neutrophil independent effect of PMA on lung injury in sheep.

In conclusion, PMA-activated human neutrophils in isolated perfused rat lungs induced acute edematous injury which could be markedly attenuated by incubation of the neutrophils with anti-Mol antibody. The extent of lung injury paralleled that observed in isolated rat lungs utilizing a homologous system employing rat neutrophils and rat plasma activated by the complement activator cobra venom factor.25 In this homologous system lung injury was also neutrophil-dependent, since the activation of complement by cobra venom factor in plasma-perfused lungs in the absence of neutrophils did not result in lung injury. Anti-Mol antibody did not inhibit the release of either superoxide or granule constituents but led to an increase in the number of circulating cells in the perfusate, suggesting that the attenuation of lung injury by the monoclonal antibody resulted from interference of neutrophil adhesion to the endothelial cell surface by the specific monoclonal antibody. These studies indicate the necessity of intimate neutrophil-endothelial cell contact for the initiation of target cell injury.

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Prevention of pulmonary injury in isolated perfused rat lungs by activated human neutrophils preincubated with anti-Mo1 monoclonal antibody

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