Soluble ICAM-1 Activates Lung Macrophages and Enhances Lung Injury

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Soluble ICAM-1 Activates Lung Macrophages and Enhances Lung Injury

Hagen Schmal,* Boris J. Czemak,* Alex B. Lentsch,† Nicolas M. Bless,* Beatrice Beck-Schimmer,† Hans P. Friedl,* and Peter A. Ward‡

Because of the important role of rat ICAM-1 in the development of lung inflammatory injury, soluble recombinant rat ICAM-1 (sICAM-1) was expressed in bacteria, and its biologic activities were evaluated. Purified sICAM-1 did bind to rat alveolar macrophages in a dose-dependent manner and induced production of TNF-α and the CXC chemokine, macrophage inflammatory protein-2 (MIP-2). Alveolar macrophages exhibited cytokine responses to both sICAM-1 and immobilized sICAM-1, while rat PBMCs failed to demonstrate similar responses. Exposure of alveolar macrophages to sICAM-1 resulted in NFκB activation (which was blocked by the presence of the aldehyde peptide inhibitor of 28S proteosome and by genistein, a tyrosine kinase inhibitor). As expected, cross-linking of CD18 on macrophages with Ab resulted in generation of TNF-α and MIP-2. This response was also inhibited in the presence of the proteosome inhibitor and by genistein. Alveolar macrophages showed adherence to immobilized sICAM-1 in a CD18-dependent manner. Finally, airway instillation of sICAM-1 intensified lung injury produced by intrapulmonary deposition of IgG immune complexes in a manner associated with enhanced lung production of TNF-α and MIP-2 and increased neutrophil recruitment. Therefore, through engagement of β2 integrins, sICAM-1 enhances alveolar macrophage production of MIP-2 and TNF-α, the result of which is intensified lung injury after intrapulmonary disposition of immune complexes. The Journal of Immunology, 1998, 161: 3685–3693.
Protein expression of ICAM-1

A soluble fragment of ICAM-1 was expressed by means of pet15b (Novagen, Madison, WI)-transformed Escherichia coli that were stimulated with isopropyl b-D-thiogalactoside (IPTG) (14). The ICAM-1 derivative consisted of the three N-terminal domains responsible for its interactions with b2 integrins (15, 16). Nickel affinity chromatography facilitated rapid purification of the recombinant ICAM-1 using imidazole. A 200 µg/ml stock solution of sICAM-1 containing 0.01% thimerosal was maintained at 4°C. Abs to sICAM-1 were obtained in rabbits as described previously (14).

Determination of endotoxin content

A Limulus amebocyte lysate assay QCL-1000 was used according to manufacturer’s instructions (BioWhittaker, Walkersville, MD). The LPS concentration of the 200 µg/ml stock solution of sICAM-1 was 120 ng/ml. Since most sICAM-1 applications entailed sICAM-1 concentrations from 0.125 to 2 µg/ml, the working concentrations of LPS did not exceed 1 ng/ml. Using this concentration of LPS, only modest amounts of MIP-2 were measured with isopropyl b-D-thiogalactoside (IPTG) (14). The incubation time with this Ab was 1 h. This was followed by an incubation period of 45 min with an anti-rabbit horseradish peroxidase-conjugated Ab (Amersham, Arlington Heights, IL) in a dilution of 1/5000. The assay was developed by addition of o-phenylenediamine dihydrochloride substrate. The developing reaction was stopped by adding 50 ml of 3 M H2SO4, and the OD at 490 nm was analyzed by a MicroELISA Autoreader (Bio-Tek Instruments, Winooski, VT).

Animal model of IgG immune complex-mediated alveolitis

Male Long-Evans rats (275–300 g; specific pathogen free; Harlan Industries, Rochester, MI) were anesthetized with i.p. ketamine (175 mg/kg). Injury was induced by the intratracheal instillation of rabbit polyclonal IgG (1.25 mg) rich in Ab to BSA (anti-BSA) in the presence or the absence of added sICAM-1 in a volume of 300 µl of PBS via an intratracheal catheter during inspiration. Immediately thereafter, 10 mg of BSA with trace amounts of [125I]BSA (as a quenching agent) was injected i.v. Rats were sacrificed 4 h later, the pulmonary circulation was flushed, and lung injury was quantitated by increases in vascular permeability. For calculations of the permeability index, the amount of radioactivity (125I[BSA] remaining in the saline perfused lungs was compared with the amount of radioactivity present in 1.0 ml of blood obtained from the inferior vena cava at the time of sacrifice. Negative control animals received the same volume of PBS intratracheally instead of anti-BSA.

Bronchoalveolar lavage

For cell isolation, 10 ml of PBS was gently instilled into the lung via a tracheal catheter and then withdrawn. This process was repeated five times. The cells were analyzed by manual microscopy. Neutrophils were easily distinguished from mononuclear cells (lymphocytes and macrophages). In each preparation the cells were 100% viable and contained only minor contamination with erythrocytes (0.002%). For neutrophil cell counting, 5 ml of PBS were instilled into the lung, withdrawn, and reassayed repeatedly (three times). After centrifugation at 400 × g, the cell pellet underwent a lysis step to remove contaminating RBCs.

ELISA for MIP-2

A sandwich ELISA was used as previously described (18). Briefly, 50 µl of a 10 µg/ml MIP-2 Ab solution in carbonate coating buffer (pH 9.6) was applied to coat a 96-well Immulon 4 ELISA plate (Fisher, Pittsburgh, PA). The coating procedure was conducted overnight. A 2% BSA solution in PBS was added to block specific binding (30 min at 37°C). Following a washing step, 100 µl/sample was added per well, and incubation was performed for 1 h at 37°C. Biotinylated Ab (100 µl, diluted 1/750) was added and incubated for 1 h at 37°C. Following a 30-min incubation with an anti-rabbit horseradish peroxidase-conjugated Ab (Pierce, Rockford, IL), the assay was developed by addition of the substrate o-phenylenediamine dihydrochloride. The developing reaction was stopped by adding 50 µl of 3 M H2SO4.

TNF-α assay

Samples were diluted 1/10 to 1/1000. One hundred microliters of samples (BAL fluids or cell culture supernatant fluids) were added to 96-well microtiter plates (Costar). WHeL cells (WHeL 164, subclone 13) in a volume of 0.1 ml/well were added in a final concentration of 5 × 104 cells/ml in the presence of actinomycin D (Life Technologies; 0.5 µg/ml). In a separate set of wells, a standard consisting of serially diluted recombinant rat TNF-α (Biosource, Camarillo, CA; starting at a concentration of 100 pg/ml stock solution were added per well. This was followed by another 4-h incubation period. Then 150 µl of supernatant fluids per well were carefully removed, and 100 µl of acidified isopropanol (isopropanol in 0.04
N HCl) was added. The plate was then wrapped in foil and left on the bench top overnight. The OD at 550 nm was analyzed by a MicroELISA Auto-reader (Bio-Tek).

**Nuclear extraction and electrophoretic mobility shift assay (EMSA)**

Alveolar macrophages were harvested by BAL and were plated on 100-mm tissue culture plates (1 × 10^7 cells/plate). Cells were incubated with vehicle (DMSO) or with the aldehyde inhibitor of NF-κB (30 μg/ml) for 15 min before the addition of sICAM-1 (500 ng/ml). After 1 h, cells were harvested by scraping, and nuclear extracts were prepared as previously described (19). Protein concentrations were determined by bicinchoninic acid assay with trichloroacetic acid precipitation using BSA as a reference standard (Pierce). The double-stranded NF-κB consensus oligonucleotide (5′-AGT GAG GGG ACT TTC CCA GGC-3′; Promega) was end labeled with [32P]ATP (3000 Ci/mmol at 10 mCi/ml; Amersham, Arlington Heights, IL). Binding reactions containing equal amounts of protein (5 μg) and 35 fmol (~50,000 cpm, Cherenkov counting) of oligonucleotide were performed for 30 min in binding buffer (4% glycerol, 1 mM MgCl2, 0.5 mM EDTA (pH 8.0), 0.5 mM DTT, 50 mM NaCl, 10 mM Tris (pH 7.6), and 50 μg/ml poly(dI-dC); Pharmacia, Piscataway, NJ). Reaction volumes were held constant at 15 μl. Reaction products were separated in a 4% polyacrylamide gel and analyzed by autoradiography.

**Statistical analyses**

As appropriate, the percent protection or reduction in injury was calculated by first subtracting the background negative control values from both the positive control values and the values obtained from positive control animals given various treatments. The data were analyzed with one- or two-way analysis of variance. Additional Student’s t tests were performed to determine the significance of differences between means of individual groups. All values were expressed as the mean ± SEM unless otherwise indicated. Statistical significance was defined when p < 0.05.

**Results**

**Expression of sICAM-1**

After IPTG-induced expression of sICAM-1 in BL21(DE)pLysS E. coli, the carboxyl-terminal six-histidine tag facilitated rapid purification using a nickel column for metal ion affinity chromatography (20). Elutions were obtained with increasing concentrations of imidazole (100–500 mM), providing an enriched fraction of sICAM-1, as demonstrated by SDS-PAGE and staining with Coomassie blue (Fig. 1, elution 2). Material from this elution procedure was used for the experiments described below.

**Northern blot analysis of lung mRNA for ICAM-1**

To obtain information related to up-regulation of lung ICAM-1, RNA from lungs of animals sacrificed at different time points after intrapulmonary deposition of IgG immune complexes was extracted and analyzed by Northern blot analysis (Fig. 2, A and B). Since different isoforms of sICAM-1 have been described, we sought to determine whether differently spliced ICAM-1 mRNAs were present. Only one distinct mRNA band for ICAM-1 could be detected, suggesting that a single mRNA species is expressed. ICAM-1 mRNA demonstrated constitutive expression followed by up-regulation at 2 and 4 h. At 6 h, mRNA expression for ICAM-1 declined to background levels (Fig. 2). Approximately equal loading was confirmed by the 18S (lower) and 28S (upper) RNA bands (Fig. 2C).

**Binding of sICAM-1 to alveolar macrophages**

Binding of sICAM-1 to monolayers of rat alveolar macrophages was demonstrated by means of a cell-based ELISA. The cells were incubated with different concentrations of sICAM-1 (7.8–2000 ng/ml), and then detector anti-ICAM-1 IgG or preimmune IgG was added to the monolayers. The OD of the monolayers was directly proportional to the sICAM-1 concentrations added to the wells, indicating binding and immobilization of the Ab to ICAM-1 present on surfaces of macrophages (Fig. 3). Differences between background values (0.202 ± 0.008) and those values of sICAM-1-treated cells were observed at a concentration of 125 ng/ml (0.246 ± 0.027) or greater. In macrophages exposed to 2000 ng of sICAM-1, the OD (at 490 nm) reached a value of 0.929 ± 0.075.
Preimmune IgG was used as control Ab, in which case no increase in binding to cell monolayers was observed at any of the concentrations, indicating that the differences were not a result of binding of Ab via Fc receptors of macrophages. The data shown are representative of two separate and independent experiments that produced similar results.

**Generation of MIP-2 and TNF-α by sICAM-1**

When alveolar macrophages were incubated with increasing concentrations of sICAM-1, production of TNF-α and the CXC chemokine, MIP-2, was observed (Fig. 4). For these studies both unheated and heated (5 min at 95°C) sICAM-1 were used. Over a dose range of 125 to 2000 ng sICAM-1/ml, there were progressive increases in the amounts of MIP-2 and TNF-α produced, with peak levels of 373 ± 1.40 ng/ml and 6170 ± 1367 pg/ml, respectively (Fig. 4). At the highest dose (2.0 μg) of sICAM-1 employed, cytokine responses were equivalent to those obtained with 10 ng LPS/ml. Heated sICAM-1 was considerably less effective than unheated sICAM-1. To assess whether the production of cytokines could be due to contamination of sICAM-1 with LPS, sICAM-1 was degraded by either heat denaturation or digestion with proteinase K. The results are shown in Table I. Both forms of treatment with sICAM-1 resulted in a significant decrease in the cytokine-inducing effects of sICAM-1. In the case of MIP-2, inhibition was 60% when sICAM-1 was treated with the proteinase and 64% when sICAM-1 was heat denatured. In the case of TNF-α, reductions were 90 and 100%, respectively. This experiment was repeated four times with two different sICAM-1 preparations (n = 3–6/group) and produced similar results (data not shown).

Using a protocol similar to that described in Figure 4 and employing rat PBMC, incubation with sICAM-1 caused generation of small amounts of MIP-2 and no generation of TNF-α (Table II). In the case of MIP-2, there was no statistically significant difference between treatment with unheated or heat-inactivated sICAM-1. In the case of TNF-α, no increases occurred with any of the sICAM-1 preparations. In fact, in all such cases TNF-α levels were less than those found with untreated PBMC. As expected, LPS was an effective stimulus for PBMC generation of MIP-2 or TNF-α. Thus, PBMC, in contrast to alveolar macrophages, show poor cytokine responses to sICAM-1 under the conditions employed.

**Signaling pathways following binding of sICAM-1 to alveolar macrophages**

To investigate the ability of sICAM-1 to activate NF-κB in macrophages, cells were incubated with sICAM-1 (3.0 μg/ml) in the absence or the presence of the aldehyde proteasome inhibitor (PSI), Z-Ile-Glu(O-tBu)-Ala-Leu-H, and activation of NF-κB was assessed by EMSA. PSI has been shown to block the chymotrypsin-like activity of the multicatalytic protease complex (20S proteasome), preventing NF-κB translocation by stabilizing the complex of phosphorylated IκB-α with NF-κB (21, 22). Alveolar

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**Table I. Heat and protease lability of sICAM-1**

<table>
<thead>
<tr>
<th>Additions</th>
<th>MIP-2 (ng/ml)</th>
<th>% Inhibition</th>
<th>TNF-α (pg/ml)</th>
<th>% Inhibition</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>44.2 ± 1.40</td>
<td></td>
<td>109 ± 23.8</td>
<td></td>
</tr>
<tr>
<td>sICAM-1</td>
<td>162 ± 29.4</td>
<td></td>
<td>515 ± 81.1</td>
<td></td>
</tr>
<tr>
<td>sICAM-1 heat inactivated</td>
<td>85.0 ± 6.54</td>
<td>64 (p &lt; 0.04)</td>
<td>96.0 ± 3018</td>
<td>100 (p &lt; 0.01)</td>
</tr>
<tr>
<td>sICAM-1 digested with proteinase K</td>
<td>91.4 ± 2.48</td>
<td>60 (p &lt; 0.04)</td>
<td>149 ± 32.7</td>
<td>90 (p &lt; 0.01)</td>
</tr>
</tbody>
</table>

*All sICAM-1 concentrations were 500 ng/ml. Heat inactivation was at 95°C for 5 min. Proteinase treatment of sICAM-1 is described in the text.*
macrophages underwent a 15-min preincubation period with PSI before exposure to sICAM-1. Using EMSA, exposure of macrophages to sICAM-1 (500 ng/ml) caused NF-κB activation; the shift was diminished in the presence of PSI (Fig. 5). The amount of total protein added to each lane was 5 μg. The effects of PSI on cytokine production in sICAM-1-stimulated macrophages was studied concurrently. The control groups (with or without sICAM-1) were incubated with DMSO, since PSI required similar amounts of DMSO for PSI solubility. Under conditions identical with those described in Figure 5, pretreatment of macrophages with PSI caused an 80% reduction (p < 0.001) in levels of MIP-2 (to 52.3 ± 1.9 ng/ml) and an 81% decrease (p < 0.01) in TNF-α levels (to 737 ± 340 pg/ml; Fig. 6).

To further explore the effects of sICAM-1 (500 ng/ml) on activation of alveolar macrophages, cells were preincubated with the tyrosine kinase inhibitor, genistein (30 μg/ml for 15 min at 37°C) (23), which caused diminished production of both MIP-2 and TNF-α (Fig. 7). Positive controls were incubated with sICAM-1 at a concentration of 500 ng/ml in the presence of DMSO. In the presence of sICAM-1, MIP-2 levels rose nearly threefold above negative control values, to 134 ± 7.50 ng/ml. In the presence of genistein, MIP-2 levels dropped 64% (p < 0.02), to 76.5 ± 5.52 ng/ml. The TNF-α level in the positive controls was 1276 ± 124 pg/ml. Pretreatment with genistein reduced TNF-α levels by 89% (p < 0.004), to 512 ± 77 pg/ml (Fig. 7). This experiment demonstrates that MIP-2 and TNF-α production stimulated by sICAM-1 is genistein sensitive.

Effect of cross-linking of alveolar macrophage CD18 on cytokine production

It has been demonstrated that monocyte integrin cross-linking with Abs can mimic ligand binding, resulting in cell activation (24).

![FIGURE 5. NF-κB induction in alveolar macrophages activated by sICAM-1, as determined by EMSA. NF-κB translocation was inhibited in the presence of PSI.](image)

Similar results were observed in alveolar macrophages incubated with anti-CD18 (WT-3; Fig. 8). At anti-CD18 doses of 10 and 20 μg/ml, MIP-2 levels in the supernatant rose to 189 ± 15 and 469 ± 19 ng/ml, respectively. These values were significantly different (p < 0.05) compared with the effect of the subclass-matched, control MOPC-21 Ab. MIP-2 concentrations in the supernatant fluids of macrophages treated with 10 and 20 μg/MOPC-21/ml were 115 ± 4.07 and 139 ± 16.2 ng/ml, respectively, values close to background MIP-2 expression (108 ± 4.78 ng/ml). If activation of macrophages is a model for ligand binding, intracellular signaling induced by an antagonist of anti-CD18 should be similar to that caused by sICAM-1, as described above. Accordingly, the inhibitors that blocked responses to sICAM-1 were applied to macrophages undergoing activation with anti-CD18, as described in Figure 9. As expected, addition of the proteasome inhibitor in a concentration of 30 μg/ml resulted in a 39% reduction in MIP-2 levels in supernatant fluids of macrophages that had been activated with 20 μg/ml anti-CD18 (with DMSO, the vehicle for PSI). Positive controls generated 187 ± 8.64 ng MIP-2/ml, while PSI-treated cells produced 132 ± 9.77 ng/ml; background MIP-2 expression was 31.4 ± 2.74 ng/ml. The addition of genistein (at a concentration of 30 μg/ml) decreased anti-CD18-induced MIP-2 production by 43% (to 126 ± 9.77 ng/ml). With respect to TNF-α levels, addition of anti-CD18 caused the production of 1423 ± 46.0 pg TNF-α/ml, significantly more than that by cells coincubated with the same amount of MOPC 21 (216 ± 7.00 pg/ml) or with no treatment (249 ± 2.00 pg/ml). Pretreatment

![FIGURE 6. Inhibition of MIP-2 (A) and TNF-α production (B) in macrophages stimulated with sICAM-1 in the presence of PSI.](image)
of macrophages with PSI followed by addition of anti-CD18 resulted in a 72% drop (to 575 ± 194 pg/ml), while addition of genistein caused a 47% reduction (to 870 ± 98 pg/ml; Fig. 9). These data indicate that stimulation of alveolar macrophages with anti-CD18 causes cytokine production in a manner that is susceptible to inhibitors that also suppress sICAM-1-induced production of MIP-2 and TNF-α.

Effects of sICAM-1 in vivo on lung injury

Since sICAM-1 can bring about activation of alveolar macrophages in vitro, we sought to determine whether sICAM-1 would enhance the inflammatory response in IgG immune complex-induced lung injury. The lung permeability index of the negative control animals was 0.23 ± 0.03 (n = 5; Fig. 10A). When 50 μg of sICAM was instilled into rat lungs, the lung permeability index (0.25 ± 0.04; n = 5) was not statistically different from that for the negative control group. Positive control animals injured with IgG immune complexes together with heat-inactivated sICAM-1 had a vascular permeability index of 0.37 ± 0.03 (n = 10). Instillation of IgG immune complexes together with 50 μg of unheated sICAM-1 resulted in a 62% increase in the lung permeability index, to 0.46 ± 0.03 (p < 0.04; n = 10), compared with that in positive controls. The number of BAL neutrophils in these experimental groups was also assessed. In negative control animals the total number of BAL neutrophils was 2.2 ± 0.25 × 10⁵ (Fig. 10B). Administration of sICAM-1 alone (50 μg) caused increased (p < 0.03) numbers of neutrophils, to 4.2 ± 0.45 × 10⁵ cells. Deposition of IgG immune complexes in the presence of heat-inactivated sICAM-1 in lung resulted in 5.6 ± 1.5 × 10⁵ neutrophils. The coinflation of sICAM-1 with anti-BSA further increased the neutrophil count in BAL fluids by 131%, to 13 ± 2.2 × 10⁵ cells (p < 0.02).

In the same model we assessed the effects of sICAM-1 on TNF-α and MIP-2 levels in BAL fluids. The TNF-α level in negative controls was 519 ± 29.1 pg/ml. Instillation of sICAM-1 alone into otherwise normal lungs did not affect TNF-α production (698 ± 227 pg/ml), but significantly increased TNF-α production occurred when sICAM-1 was coinflation with the anti-BSA, rising...
by 97% to 25,765 ± 4,741 pg/ml (Fig. 11A). Immune complexes alone caused a TNF-α level of 14,857 ± 2,423 pg/ml. There was virtually no MIP-2 present in lavage fluid of negative control animals (3.35 ± 2.7 ng/ml). The sICAM-1 alone increased MIP-2 levels to 173 ± 11.6 ng/ml (Fig. 11B). In the positive controls also given heat-inactivated sICAM-1 (Fig. 11B), the MIP-2 level rose to 304.9 ± 25.2 ng/ml. When immune complexes and unheated sICAM-1 were coinstilled, MIP-2 levels increased by an additional 29%, to 393.5 ± 31.78 ng/ml (*p*, 0.04). These data demonstrate that sICAM-1 enhances IgG immune complex-induced lung injury, associated with increased lung production of MIP-2 and TNF-α and greater accumulation of neutrophils, and intensifies the level of lung injury.

**Induction of MIP-2 and TNF-α by fluid phase and solid phase sICAM-1**

Alveolar macrophages were incubated with sICAM-1 in fluid phase or in solid phase, and the expressions of MIP-2 and TNF-α were compared. The results are summarized in Table III. Both fluid phase and solid phase sICAM-1 induced MIP-2 responses in a dose-dependent manner. The responses to solid phase sICAM-1 appeared to be consistently higher than those responses to fluid phase sICAM-1, although statistical significance between the two responses was only reached at the highest dose (2000 ng/ml). There was a dose-response relationship for MIP-2 production for both fluid phase and solid phase presentation of sICAM-1. In the case of TNF-α responses, the response to 1000 ng of sICAM-1 (fluid phase) was twofold above background; at the same dose of solid phase sICAM-1, the response was nearly eightfold greater.

**FIGURE 10.** In vivo enhancement of lung injury by intratracheally administered sICAM-1. Vascular permeability (A) and neutrophil content in BAL fluids (B) were measured after intratracheal instillation of 50 µg of sICAM-1 alone or in the presence of IgG immune complex.

**FIGURE 11.** Effects of sICAM-1 on TNF-α (A) and MIP-2 (B) levels in BAL fluids after intrapulmonary deposition of IgG immune complexes.

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Cytokine Response</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>MIP-2 (ng/ml)</td>
</tr>
<tr>
<td>None</td>
<td>61.1 ± 15.1</td>
</tr>
<tr>
<td>Fluid phase sICAM-1</td>
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</tr>
<tr>
<td>125</td>
<td>90.1 ± 5.67</td>
</tr>
<tr>
<td>250</td>
<td>138 ± 14.2</td>
</tr>
<tr>
<td>500</td>
<td>178 ± 9.08</td>
</tr>
<tr>
<td>1000</td>
<td>276 ± 7.91</td>
</tr>
<tr>
<td>2000</td>
<td>352 ± 13.8</td>
</tr>
<tr>
<td>Solid phase sICAM-1</td>
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</tr>
<tr>
<td>125</td>
<td>102 ± 11.3</td>
</tr>
<tr>
<td>250</td>
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<td>1000</td>
<td>303 ± 26.0</td>
</tr>
<tr>
<td>2000</td>
<td>434 ± 15.3*</td>
</tr>
</tbody>
</table>

*p value < 0.05 when compared to corresponding amount of fluid phase sICAM-1.
Adhesion of alveolar macrophages to solid phase sICAM-1

These experiments were designed to determine whether alveolar macrophages would adhere to solid phase sICAM-1 and to assess whether such adherence was CD18 dependent. The sICAM-1 was immobilized on plastic surfaces as described above, and alveolar macrophages were exposed for 10 min to coated plates. As shown by the data in Figure 12A, macrophages adhered to plates treated with 0.5 to 2.0 µg of sICAM-1 when compared with adhesion in the absence of immobilized sICAM-1. Approximately 56% of macrophages bound to surfaces exposed to 2 µg of immobilized sICAM-1 compared with binding of 18% of macrophages to uncoated plates. Under the same conditions, the effect of anti-CD18 on adherence of macrophages to immobilized sICAM-1 adhesion was evaluated. Macrophages underwent 15 min of preincubation with anti-CD18 10 µg/ml (clone WT-3, Endogen, Woburn, MA) or with an isotype-matched mouse IgG1 (MOPC-21) at the same concentration. Using 1.0 or 2.0 µg of sICAM-1 for immobilization, the presence of anti-CD18 totally abolished the response to 1.0 µg of sICAM and reduced by 94% the adhesion response to wells exposed to 2.0 µg of sICAM-1 (Fig. 12B). Thus, adhesion of alveolar macrophages to immobilized sICAM-1 requires CD18.

Discussion

It is becoming apparent that adhesion pathways not only provide a mechanism for translocation of leukocytes from blood to inflam-

atory sites but also participate in intracellular signaling of leukocytes, which can result in cytokine production (3, 25, 26). Several reports have suggested that inflammatory conditions are associated with shedding of adhesion molecules into body fluids; the levels correlate with the inflammatory status (27, 28). Only a few studies suggest that soluble adhesion molecules can induce signaling and cell activation (6, 29). Most such studies have concentrated on T cells or monocytes rather than macrophages. In this study we demonstrate a binding interaction between alveolar macrophages and sICAM-1, which results in generation of MIP-2 and TNF-α. Both the binding interactions and cytokine production appear to be CD18 dependent. In contrast to the responses of macrophages, PBMCs were not stimulated by sICAM-1, although production of MIP-1α after cell exposure to endothelial cell ICAM-1 has been described (3). Under the experimental conditions employed in the current studies, alveolar macrophages were considerably more responsive to sICAM-1 than were PBMCs. There were suggestions that the cytokine responses of macrophages to sICAM-1 may be optimized with the use of immobilized sICAM-1, especially at the higher concentrations. Steric changes in immobilized sICAM-1 may provide better access to β2 ligands on surfaces of alveolar macrophages. We have also obtained insights into the relevant intracellular pathways triggered by sICAM-1 binding. It has been suggested that NF-κB translocation might be an essential step in cell activation following adherence (30, 31). It is known that the promoter regions for both MIP-2 and TNF-α contain a consensus motif for NF-κB. In alignment with these reports, MIP-2 and TNF-α production by alveolar macrophages stimulated with sICAM-1 was associated with NF-κB activation. Interference with NF-κB activation using the aldehyde peptide inhibitor suppressed activation of NF-κB as well as cytokine formation. Our data also suggest that tyrosine kinases may play a role in intracellular signaling following binding of soluble ICAM-1 to β2 integrins of macrophages. This would be consistent with the conclusions of others (32, 33). Cross-linking of β2 integrins with Abs has been shown to stimulate cells and mimic receptor/ligand interactions (24). In our studies, exposure of macrophages to sICAM-1 or to monoclonal anti-CD18 Ab (WT-3) also induced generation of MIP-2 and TNF-α. As expected, inhibition of NF-κB translocation by the aldehyde peptide inhibitor of proteasome and blocking of tyrosine kinase activity by genistein inhibited the cytokine responses induced by anti-CD18.

What was of special interest in these studies was whether sICAM-1 would demonstrate proinflammatory effects in lung. To evaluate this question, we used the rat model of IgG immune complex-induced lung injury. MIP-2 and TNF-α have been shown to play a vital role in the development of inflammatory lung injury, being necessary for neutrophil recruitment (13, 34–36). In the current studies, airway instillation of sICAM-1 led to a small accumulation of BAL neutrophils, but did not cause evidence of lung injury (as determined by extravascular leak of albumin). Under the same conditions no production of TNF-α was measurable in BAL fluids, while small amounts of MIP-2 could be measured. In contrast, intratracheal coadministration of sICAM-1 with the anti-BSA intra-alveolarly significantly increased neutrophil accumulation, which was associated with enhanced lung injury, as defined by intensified vascular permeability. These effects could be directly linked to augmented levels of MIP-2 (by 29%) and TNF-α (by 97%) levels in BAL fluids. Our experiments also clearly showed that sICAM-1 can directly induce macrophage activation in vitro, causing NF-κB activation and generation of cytokines. Since sICAM-1 can be shed from surfaces of endothelial cells and released into the vascular compartment and may be shed from surfaces of alveolar epithelial cells and macrophages (both of which
are known to express ICAM-1 on their surfaces); this raises the question of the biologic relevance of this phenomenon. Recent reports have suggested that sICAM-1 and E-selectin can interfere with leukocyte binding to counter-receptors on endothelial cells or synovial cells (37, 38). This suggests that sICAM-1, if present in adequate amounts, may function as an anti-inflammatory component, especially by limiting leukocyte adhesion to endothelial cells. Thus, sICAM-1 shed into the vascular compartment may lead to a self-limiting mechanism of the inflammatory response. Alternatively, shedding of sICAM-1 into the distal airway compartment may, in the presence of an already activated inflammatory response, cause intensified injury due to enhanced production of cytokines by lung macrophages. In general, these data suggest that in lung, sICAM-1 may function as a proinflammatory or anti-inflammatory mediator depending on the anatomic location of the shed sICAM-1.

Acknowledgments
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References