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Soluble ICAM-1 Activates Lung Macrophages and Enhances Lung Injury

Hagen Schmal,* Boris J. Czermak,* 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, alveolar injury is neutrophil dependent and requires ICAM-1, CD11a/CD18 (LFA-1), CD11b/CD18 (Mac-1), and E- and L-selectin. Interestingly, in the lung a compartmentalized role for the adhesion has been found, with LFA-1 being important on the vascular side and Mac-1 being important on the airway side (10, 11). Although blocking of ICAM-1 in the vascular or in the distal airway compartment inhibited full development of lung injury, anti-CD11a was only protective when given i.v., and anti-CD11b was only protective when given intratracheally. These data suggest a role for ICAM-1 in the distal airway compartment (perhaps involving ICAM-1 on alveolar type II epithelial cells or on alveolar macrophages). ICAM-1 could function as a stimulus for alveolar macrophages via their β2 integrin content and in the vascular compartment as a central adhesion molecule required for neutrophil recruitment from the blood.

In the current study we investigated the ability of rat sICAM-1 to activate alveolar macrophages and to enhance lung injury. Production of TNF-α and MIP-2 by alveolar macrophages and PBMCs was monitored following exposure to sICAM-1. Both of these cytokines are known to play a role in IgG immune complex-mediated lung injury (12, 13). The intracellular signal transduction pathways of cells exposed to sICAM-1 were partially analyzed using inhibitors of NFκB activation and of tyrosine kinase activity. Binding of sICAM-1 to surfaces of alveolar macrophages and adhesion of alveolar macrophages to immobilized sICAM-1 were also demonstrated. Our results indicate that sICAM-1 binds to and activates macrophages in a CD18-dependent manner. The signaling induced by this interaction appears to be NFκB and tyrosine kinase dependent. In rat lungs undergoing injury during deposition of IgG immune complexes, the instillation of sICAM-1 significantly enhanced lung injury in a manner associated with enhanced recruitment of neutrophils and increased lung levels of TNF-α and MIP-2. Since sICAM-1 has been detected in body fluids of humans, it may have important biologic roles in vivo.

Materials and Methods

Chemicals and reagents

Except where noted, all reagents were purchased from Sigma (St. Louis, MO).

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‡Abbreviations used in this paper: sICAM-1, soluble ICAM-1; MIP-2, macrophage inflammatory protein-2; BAL, bronchoalveolar lavage; EMSA, electrophoretic mobility shift assay; PSL, proteosome inhibitor; IPTG, isopropyl β-D-thiogalactoside.
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-β-D-thiogalactoside (IPTG) (14). The ICAM-1 derivative consisted of the three N-terminal domains responsible for its interactions with β2 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 (61.1 ± 1.71 ng/ml) were produced by rat alveolar macrophages compared with background expression by macrophages (40.7 ± 1.34 ng/ml).

Northern blots

RNA (12 μg) extracted from whole lungs of IgG immune complex-injected rats (at 4 h) were electrophoretically fractionated in a 1% agarose formaldehyde gel and then transferred to a nylon membrane (Zetabind, Cuno, Meriden, CT). 32P-labeled dCTP probes were generated using the PCR primers specific for the partial length cDNA templates of ICAM-1. Radioactivity of the probes was determined by scintillation counting. Probes containing 1.5 × 107 cpm were applied to the blot, with hybridization occurring at 65°C for 16 h. Autoradiography of the blots was performed at −70°C for 24 to 48 h on Kodak X-OMAT-AR film (Eastman Kodak, Rochester, NY). Quantitation of autoradiographs was performed using an AMBIS Image Analysis System (San Diego, CA).

Isolation of PBMC

PBMC were isolated from the venous blood of rats by separation over Ficoll-Paque (Pharmacia, Uppsala, Sweden) followed by a lysis step to remove contaminating erythrocytes. The cells were then washed twice with 0.9% sodium chloride containing 0.1% BSA, resuspended in DMEM (Life Technologies, Gaithersburg, MD) containing 10% FBS, 1% L-glutamine, 0.1% BSA, and antibiotics, and resuspended in DMEM. The cells were analyzed by manual microcytometry. Neutrophils were easily distinguished from mononuclear cells (lymphocytes and macrophages). In each preparation the cells were 100% viable and contained only minor contaminating cells (>95% pure). For neutrophil cell counting, 5 ml of PBMC were instilled into the lung, withdrawn, and reinstalled repeatedly (three times). After centrifugation at 400 × g, the cell pellet underwent a lysis step to remove contaminating RBCs.

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 microcytometry. Neutrophils were easily distinguished from mononuclear cells (lymphocytes and macrophages). In each preparation the cells were 100% viable and contained only minor contaminating cells (>95% pure). For neutrophil cell counting, 5 ml of PBMC were instilled into the lung, withdrawn, and reinstalled 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 used 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 streptavidin-peroxidase-conjugated Ab (Pierce, Rockford, IL), the assay was developed by addition of the substrate 4-chloronaphthol. The developing reaction was stopped by adding 50 μl 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 rat polyclonal IgG (1.25 mg) rich in Ab to BSA (anti-BSA) in the presence of or absence of added sICAM-1 in a volume of 300 μl of PBS via an intratracheal catheter during inspiration. Immediately thereafter, 10 μg of BSA with trace amounts of [125I]BSA (as a quantitative marker of permeability) 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 (1.25 μg/120 μl [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.

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). WeHI cells (WeHI 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 TNF-α (Biosource, Camarillo, CA; starting at a concentration of 100 pg/ml) was added. The cells were incubated at 37°C for 20 h, then 20 μl of 3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyltetrazolium bromide from a 5 mg/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 × 10⁷ 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
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 ± 15.2 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

![Figure 3](http://www.jimmunol.org/)

**FIGURE 3.** Cell-based ELISA to demonstrate binding of sICAM-1 to macrophages.

![Figure 4](http://www.jimmunol.org/)

**FIGURE 4.** Production of MIP-2 (A) and TNF-α (B) by rat alveolar macrophages after exposure to increasing concentrations of sICAM-1 (heated or unheated) or to a single dose of LPS.

**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.*

**Additions**

- native protein
- heat denatured protein

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sICAM-1 AND LUNG INJURY

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Table II. Failure of sICAM-1 to induce MIP-2 and TNF-α production by PBMC

<table>
<thead>
<tr>
<th>Additions</th>
<th>MIP-2 (ng/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>24.8 ± 5.00</td>
<td>5,620 ± 1,173</td>
</tr>
<tr>
<td>LPSa</td>
<td>154 ± 9.23</td>
<td>88,192 ± 8,761</td>
</tr>
<tr>
<td>sICAM-1 500 ng/ml</td>
<td>35.7 ± 5.32</td>
<td>1,046 ± 309</td>
</tr>
<tr>
<td>sICAM-1 2,000 ng/ml</td>
<td>44.0 ± 1.69</td>
<td>1,079 ± 410</td>
</tr>
<tr>
<td>500 ng/ml, heated</td>
<td>40.9 ± 5.32</td>
<td>660 ± 183</td>
</tr>
<tr>
<td>2,000 ng/ml, heated</td>
<td>35.3 ± 0.94</td>
<td>1,295 ± 196</td>
</tr>
</tbody>
</table>

a For the MIP-2 study, 10 ng LPS/ml were used, while for the TNF-α study, 100 ng LPS/ml were used.

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).

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 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.

FIGURE 6. Inhibition of MIP-2 (A) and TNF-α production (B) in macrophages stimulated with sICAM-1 in the presence of PSI.
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 mg 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 coinstitution 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 coinstituted 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.

**Table III. In vitro cytokine responses to fluid phase or solid phase sICAM-1**

<table>
<thead>
<tr>
<th>Material Present</th>
<th>Amount (ng/ml)</th>
<th>MIP-2 (ng/ml)</th>
<th>TNF-α (pg/ml)</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>125</td>
<td>61.1 ± 15.1</td>
<td>2,190 ± 390</td>
</tr>
<tr>
<td>Fluid phase sICAM-1</td>
<td>90.1 ± 5.67</td>
<td>138 ± 14.2</td>
<td>178 ± 9.08</td>
</tr>
<tr>
<td>250</td>
<td>276 ± 7.91</td>
<td>325 ± 13.8</td>
<td>303 ± 26.0</td>
</tr>
<tr>
<td>500</td>
<td>303 ± 26.0</td>
<td>34,490 ± 2,875*</td>
<td></td>
</tr>
<tr>
<td>1000</td>
<td>34,490 ± 2,875*</td>
<td>34,490 ± 2,875*</td>
<td></td>
</tr>
<tr>
<td>2000</td>
<td>434 ± 15.3*</td>
<td>434 ± 15.3*</td>
<td></td>
</tr>
</tbody>
</table>

* p value < 0.05 when compared to corresponding amount of fluid phase sICAM-1.
by the data in Figure 12, macrophages adhered to plates treated with the sICAM-1 immobilized on plastic surfaces as described above, and alveolar macrophages were exposed for 10 min to coated plates. As shown 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 inflammatory 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 cytokine production (3, 25, 26). Steric changes in immobilized sICAM-1 may provide better access to β2 integrins on surfaces of alveolar macrophages. We have 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 TNF-α 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 sICAM-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.

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References