Surfactant protein A differentially regulates peripheral and inflammatory neutrophil chemotaxis

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Submitted 26 April 2002; accepted in final form 27 August 2002

Surfactant protein A (SP-A) is a large multimeric protein found in the airways and alveoli of the lungs. SP-A is a member of the collectin protein family that is characterized by NH2-terminal collagen-like domains and COOH-terminal lectin, or carbohydrate-binding domains. These proteins are innate immune molecules, and SP-A’s best-characterized immunoregulatory interaction is with the resident pulmonary phagocyte, the alveolar macrophage (30). SP-A stimulates a variety of macrophage responses such as chemotaxis, actin polymerization, and phagocytosis (25, 30, 31). SP-A also regulates responses involved in the initiation and potentiation of inflammation by decreasing proinflammatory tumor necrosis factor-α production in response to LPS (14) and by accelerating the clearance of apoptotic neutrophils during the resolution of inflammation (17). In this way, SP-A is believed to help protect the delicate pulmonary tissue from potentially damaging effects of inflammation. Studies with mice made deficient for SP-A by homologous recombination support this hypothesis; these mice show a decreased ability to clear a pulmonary bacterial challenge and a large and persistent pulmonary infiltration of neutrophils (12).

In this study, we investigated the ability of SP-A to regulate neutrophil chemotaxis and found that although SP-A did not directly stimulate chemotaxis, it did regulate neutrophil chemotaxis toward known chemoattractants. This regulation was, however, dependent on the activation state of the neutrophil; peripheral neutrophils showed decreased chemotaxis in the presence of SP-A, and neutrophils isolated from inflammatory BAL neutrophils showed decreased chemotaxis in the presence of SP-A.

The directed migration or chemotaxis of neutrophils is essential to execution of an inflammatory response. A variety of molecules are known to stimulate neutrophil chemotaxis (7). These include microbial products such as the bacterial peptide formyl met-leu-phe (fMLP), activated complement (C5a), cytokines such as IL-8 and macrophage inhibitory protein 2 (MIP-2), and bioactive lipids such as leukotriene B4. All of these chemoattractants bind G protein-coupled receptors on the surface of neutrophils. Ligand binding of these receptors stimulates signaling events that result in cytoskeletal rearrangements and cell migration.

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In this study, we investigated the ability of SP-A to regulate neutrophil chemotaxis and found that although SP-A did not directly stimulate chemotaxis, it did regulate neutrophil chemotaxis toward known chemoattractants. This regulation was, however, dependent on the activation state of the neutrophil; peripheral neutrophils showed decreased chemotaxis in the presence of SP-A, and neutrophils isolated from inflammatory BAL neutrophils showed decreased chemotaxis in the presence of SP-A.

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flamed lungs showed increased chemotaxis in the presence of SP-A. We examined the ability of homologous proteins to mediate neutrophil chemotaxis and explored the mechanism by which SP-A is differentially regulating the migration of the two different neutrophil populations. This study provides evidence that SP-A indeed regulates neutrophil migration most likely via its collagen-like domain through some internal regulatory mechanism that remains to be identified.

MATERIALS AND METHODS

Reagents. Heteattract was produced by Abbott Laboratories (North Chicago, IL). DPBS, Gey's balanced salt solution (GBSS), and HBSS were obtained from Gibco Laboratories (Grand Island, NY). Iodo-Beads, desalting gel columns, and bichinchonic acid protein assays were all obtained from Pierce (Rockford, IL). Na\textsubscript{125}I was purchased from DuPont-NEN (Boston, MA). Human serum C1q was purchased from Advanced Research Technologies (San Diego, CA). IgG purified from normal rat serum was purchased from Sigma Chemical (St. Louis, MO). The IgG was reconstituted from lyophilized powder in saline and used without further treatment unless otherwise indicated.

Animals. Male Sprague-Dawley rats weighing \~200–400 g were obtained from Charles River (Raleigh, NC) or Taconic Farms (Germantown, NY).

Protein purification. SP-A was purified from the bronchoalveolar lavage (BAL) fluid of patients with alveolar proteinosis as previously described (14). Briefly, SP-A was extracted from lavage fluid with butanol and sequential solubilization in octylglucoside and 5 mM Tris, pH 7.4. The SP-A was treated with polymyxin B agarose beads to reduce endotoxin contamination. All SP-A preparations had \textlessthan;0.1 pg endotoxin/\mu g SP-A by the Limulus amebocyte lysate assay QCL-1000 (BioWhittaker, Walkersville, MD). SP-A was stored in 5 mM Tris, pH 7.4, at \textdegree C for 10 min. Treatment of SP-A was carried out at 95\degree C for 10 min.

Mannose binding lectin (MBL) was purified from rat serum (Pel-​​Freeze, Rogers, AR) by affinity and gel filtration chromatography as previously described (24).

Iodination of SP-A. Purified SP-A was iodinated using N-chloro-benzencesulfonylamine oxidizing agent immobilized on Pierce Iodo-Beads (26). Free Na\textsubscript{125}I was separated from \textsuperscript{125}I-labeled protein on d-salt exocellulose GF-5 desalting gel columns. Fractions that contained radioactivity that was \textgreateq;85% precipitable by trichloroacetic acid were analyzed for protein concentrations by bichinchonic acid protein assay and stored at 4\degree C. Radiolabeled SP-A was used within 2 wk.

Neutrophil isolation. Peripheral neutrophils were isolated as previously described with minor modifications (23, 29). Briefly, rats were anesthetized by intramuscular injection of 0.1 mg acepromazine, 7 mg ketamine, and 29 mg xylazine per 250 g body wt, and the jugular was cannulated with a 23-gauge butterfly needle attached to a three-way stop cock. An exchange of 3–4 ml of 6% hetastarch solution with 100 U/ml heparin for 3–4 ml of blood was performed until 40 ml were exchanged per rat. The first 10 ml of blood were collected in a syringe containing 10 ml of the hetastarch solution. After the exchange, blood was collected until the rats died. Blood was allowed to settle 30–45 min at room temperature, and the red blood cell-depleted fraction was collected and centrifuged at 330 g for 10 min. Cells were resuspended in 2 ml of DPBS and underlayed with a five-step Percoll gradient. Step densities were 1.081, 1.085, 1.089, 1.093, and 1.097. Gradients were centrifuged at 500 g for 30 min at room temperature, and the fractions at the interfaces of 1.085–1.089 and 1.089–1.093 density layers were pooled, washed, and resuspended in the appropriate buffer. Neutrophil purities were 83 ± 3% as determined by hematoxylin differential stain (EM Science, Gibbstown, NJ). Other cell types present were 7 ± 1% eosinophils and 10 ± 3% mononuclear cells. Cells were 98 ± 1% viable as determined by trypan blue exclusion.

For isolation of inflammatory BAL neutrophils, rats received an intratracheal instillation of LPS (100 \mu g of O26: B6/kg rat in 350 \mu l of normal saline). Twelve hours later, the lungs were lavaged six times with PBS (pH 7.2) containing 0.2 mM EGTA. Cells were collected by centrifuging the BAL fluid for 10 min at 228 g. Cells were then suspended in 2 ml of DPBS per rat and underlayed with the same five-step Percoll gradient used for isolation of peripheral neutrophils. Gradients were centrifuged at 500 g for 30 min at room temperature, and the fractions at the interfaces of 1.085–1.089 and 1.089–1.093 density layers were pooled, washed, and resuspended in the appropriate buffer. Neutrophil purities were >97% as determined by hematoxylin differential stain.

Human peripheral neutrophils were isolated from whole blood using Mono-Poly Resolving Medium (ICN Biochemicals, Aurora, OH) according to the manufacturer’s specifications. Neutrophils were >95% pure.

Chemotaxis assay. Chemotaxis assays were performed using a Neuro Probe 48-well microchemotaxis chamber (Cabin John, MD) (31). Lower wells were filled with the chemotactic test solution diluted in chemotaxis buffer (GBSS + 2% BSA). Poretics polyvinylpyrroldone-free polycarbonate filters with 2-\mu m pores (Osmonics, Livermore, CA) were used. The assembly was incubated at 37\degree C, 5% CO\textsubscript{2}, for 10 min before cell suspensions (2.5 × 10\textsuperscript{5} cells/ml chemotaxis buffer) were added. After a 30-min incubation at 37\degree C, 5% CO\textsubscript{2}, the filter was stained by hematoxylin differential stain and analyzed by light microscopy for the number of cells that had migrated through the filter. Ten randomly selected oil immersion fields were counted at ×100 magnification. Chemotactic stimuli were analyzed in triplicate and averaged for each experiment.

Phagocytosis assay. Fluorescein beads were conjugated with BSA according to the manufacturer’s directions in the Polysciences Carbodiimide kit (Warrington, PA). Neutrophils at 10\textsuperscript{5} cells/ml were coincubated with 2 \mu g of polyclonal antibodies to human IgG (Sigma, St. Louis, MO) according to the manufacturer’s instructions. Neutrophils were then washed three times and fixed with 1% formaldehyde before analysis by flow cytometry.

Iodinated SP-A binding assay. To measure binding of iodinated SP-A, neutrophils were suspended at 5 × 10\textsuperscript{5} cells/ml in binding buffer (DPBS with 0.1% BSA and either 0.9 mM CaCl\textsubscript{2} + 0.5 mM MgCl\textsubscript{2} or 1 mM EDTA as indicated). Assay tubes were precoated with 1% BSA overnight at 4\degree C. Iodinated SP-A was coincubated with 2 × 10\textsuperscript{5} cells in 0.5 ml of buffer for 4 h at 4\degree C with gentle rotation. To minimize nonspecific absorption of radioactivity to the tubes, cells were washed once with cold binding buffer lacking BSA and transferred to new tubes. Cells were then washed twice more and lysed (50 mM sodium phosphate buffer, pH 7.2, 150 mM NaCl, 2 mM EDTA, and 0.5% Nonidet P-40). All washes were done at 4\degree C. Incorporated radioactivity was analyzed by gamma counting, and radioative signal was normalized to

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total cellular protein recovered (quantitated by bicinchoninic acid protein assay). Background was determined by performing the assay in the absence of cells and subtracting the background counts from the samples with cells.

Quantitation of fMLP receptor expression. fMLP receptor expression was measured using the fluorescein-conjugated formyl peptide formyl-Nle-Leu-Phe-Nle-Tyr-Lys (Molecular Probes, Eugene, OR) (1). Varying concentrations of peptide (1, 2, 5, 10, and 20 nM) were incubated with 2.5 × 10⁶ neutrophils/ml binding buffer (HBSS + 1% BSA + 0.1% azide) for 30 min on ice. Cells were then washed and resuspended in fixative (1% formaldehyde in DPBS) for analysis by flow cytometry. Cells were analyzed for the mean relative fluorescence as an indication of the relative quantity of formyl peptide bound. For measurement of the effect of SP-A on formyl peptide binding, 25 μg/ml SP-A were added to the binding reaction. For measurement of the effect of SP-A on fMLP receptor expression, cells were incubated in the chemotaxis buffer plus 25 μg/ml SP-A for 30 min at 37°C with gentle shaking. Cells were then washed with DPBS without calcium or magnesium + 1 mM EDTA + 1% BSA and resuspended in binding buffer, and formyl peptide binding was measured as described.

CD11b expression. Neutrophils at 2 × 10⁶ cells/ml HBSS + 0.1% BSA were incubated with FITC-conjugated CD11b (PharMingen, San Diego, CA) for 5 min at 37°C and then stimulated with SP-A (25 μg/ml), fMLP (0.1 nM), or SP-A + fMLP for 10 min at 37°C (3). Cells were immediately washed with 1 ml of cold HBSS + 0.1% BSA and suspended in fixative for analysis by flow cytometry.

RESULTS

SP-A does not directly stimulate neutrophil chemotaxis. In contrast to its effects on macrophage chemotaxis, SP-A did not directly stimulate peripheral or inflammatory BAL neutrophil migration above buffer alone (GBSS) at a range of concentrations tested (0.5–25 μg/ml) (Fig. 1). The bacterial peptide fMLP (10 nM) stimulated chemotaxis, and inflammatory BAL neutrophils were significantly more responsive to fMLP than peripheral neutrophils.

SP-A regulates neutrophil chemotaxis stimulated by chemoattractants. Although SP-A alone did not directly stimulate neutrophil migration, we analyzed its ability to affect neutrophil migration toward several different chemoattractants. We measured the effect of 25 μg/ml SP-A on neutrophil chemotaxis toward MIP-2, activated complement protein C3b in zymosan-activated serum (ZAS), and fMLP. SP-A significantly altered both peripheral and inflammatory BAL chemotaxis toward these chemoattractants. However, the effect was dependent on the activation state of the neutrophil. SP-A significantly inhibited peripheral neutrophil chemotaxis toward MIP-2 and fMLP and significantly enhanced inflammatory BAL chemotaxis toward each chemoattractant tested (Fig. 2). Significant inhibition of peripheral neutrophil chemotaxis toward ZAS was not observed at the concentration tested (5% ZAS) (Fig. 2).

To further substantiate these observations and because human SP-A was used in these experiments with rat cells, we tested SP-A’s effect on human peripheral neutrophil migration toward IL-8. As observed with rat peripheral neutrophil chemotaxis toward fMLP and MIP-2, human peripheral neutrophil chemotaxis toward IL-8 was significantly reduced in the presence of 25 μg/ml SP-A (Fig. 3).

To assess whether SP-A’s ability to regulate neutrophil chemotaxis was dose dependent, we examined the effect of a range of SP-A doses on neutrophil chemotaxis toward fMLP. SP-A at 25 and 50 μg/ml significantly altered both peripheral and inflammatory BAL neutrophil chemotaxis toward 10 nM fMLP (Fig. 4). In the presence of 25 μg/ml SP-A, inflammatory BAL neutrophil chemotaxis toward fMLP was 185 ± 41% chemotaxis toward fMLP alone, whereas peripheral neutrophil chemotaxis toward fMLP was reduced to 66 ± 8% in the presence of SP-A.
BAL neutrophils were exposed to LPS in vivo before isolation. Because LPS is such a potent stimulus for neutrophils, it seemed possible that LPS exposure was responsible for the difference in functional responsiveness between the peripheral and BAL neutrophils. To test this theory, peripheral neutrophils were incubated in the presence of 100 ng/ml LPS (LCD 25, List Biologicals) for 1 h at 37°C, 5% CO2. Cells were then assayed for their chemotactic responsiveness to 10 nM fMLP in the presence and absence of 25 μg/ml SP-A. Cells pretreated with LPS exhibited a stronger chemotactic response to fMLP (147 and 163% chemotaxis toward fMLP vs. untreated control, data from two independent experiments). However, SP-A inhibited chemotaxis of the LPS-treated peripheral neutrophils to a similar extent as the untreated peripheral neutrophils (63 and 54% and 67 and 33%, respectively, data from two independent experiments).

**SP-A regulates neutrophil phagocytosis.** To explore whether SP-A may regulate another aspect of neutrophil function, neutrophil phagocytosis was examined. Peripheral and inflammatory BAL neutrophils were incubated with BSA-conjugated fluorescein beads in the presence and absence of 25 μg/ml SP-A. SP-A significantly reduced peripheral neutrophil phagocytosis to 70 ± 9% vs. control, whereas SP-A enhanced BAL neutrophil phagocytosis to 143 ± 35% vs. control (Fig. 5). Although enhancement of BAL neutrophil phagocytosis was not statistically significant, SP-A clearly had different effects on peripheral and BAL neutrophils.

**Specificity of SP-A’s effect on neutrophil chemotaxis.** To determine if SP-A’s effect was specific, we examined the ability of other proteins to regulate neutrophil migration toward fMLP. IgG, which does not share any structural homology to SP-A but is a soluble immunoregulatory protein, did not alter peripheral or inflammatory BAL neutrophil chemotaxis toward fMLP (Table 1). MBL, a serum member of the collectin protein family, and complement protein C1q both share significant structural homology to SP-A. In contrast to SP-A, neither MBL nor C1q (at 25 μg/ml) significantly altered peripheral or inflammatory BAL neutrophil chemotaxis toward fMLP (Table 1). None of these proteins alone at 25 μg/ml significantly stimulated neutrophil chemotaxis (data not shown).

**Table 1. Effect of immune proteins on neutrophil chemotaxis toward fMLP**

<table>
<thead>
<tr>
<th>Immune Protein</th>
<th>Peripheral (n)</th>
<th>BAL (n)</th>
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<tbody>
<tr>
<td>fMLP</td>
<td>100</td>
<td>100</td>
</tr>
<tr>
<td>SP-A</td>
<td>63 ± 8* (7)</td>
<td>181 ± 27* (13)</td>
</tr>
<tr>
<td>IgG</td>
<td>92 ± 19 (3)</td>
<td>136 ± 49 (3)</td>
</tr>
<tr>
<td>MBL</td>
<td>125 ± 19 (4)</td>
<td>178 ± 32 (3)</td>
</tr>
<tr>
<td>C1q</td>
<td>105 ± 24 (4)</td>
<td>136 ± 25 (4)</td>
</tr>
</tbody>
</table>

Relative neutrophil chemotaxis = [chemotaxis toward fMLP + SP-A (chemotaxis toward fMLP alone)] × 100). Means ± SE; BAL: n = 5; peripheral: 0 and 25 μg/ml, n = 7; and 2, 10, and 50 μg/ml, n = 3. *P < 0.005 vs. BAL neutrophil chemotaxis toward fMLP alone. †P < 0.005 vs. peripheral neutrophil chemotaxis toward fMLP alone.
Because the collagen-like domain of SP-A is similar to type IV collagen, the ability of type IV collagen to regulate neutrophil migration toward fMLP was examined. Surprisingly, collagen had the same effect as SP-A: it inhibited peripheral neutrophil chemotaxis toward fMLP (Table 2). Also, similar to SP-A, collagen alone did not stimulate chemotaxis (data not shown). To determine if the collagen domain of SP-A was responsible for its regulation of neutrophil chemotaxis, we examined the effect of heat treatment on SP-A’s function. Heat treatment abrogated the ability of collagen and SP-A to regulate peripheral and inflammatory BAL neutrophil chemotaxis (Table 2).

**Binding of SP-A to peripheral vs. inflammatory BAL neutrophils.** Because of the differential effect of SP-A on peripheral and inflammatory BAL neutrophil chemotaxis, we examined if there was a difference in binding of SP-A to the two neutrophil populations. Iodinated SP-A at 2, 5, and 10 μg/ml was incubated with the neutrophils for 4 h at 4°C. There was no apparent difference in binding of SP-A to peripheral and inflammatory BAL neutrophils (Fig. 6). Higher doses of SP-A were tested, but background levels of radioactivity (as assessed by radioactivity that pelleted in the absence of cells) became significant, suggesting that the iodinated protein was aggregating at concentrations equal to or greater than 25 μg/ml (data not shown).

The calcium dependence of SP-A binding to neutrophils was also examined by measuring binding in the absence of calcium and the presence of 1 mM EDTA (Fig. 6B). Although SP-A binding was slightly reduced in the absence of calcium (more so in peripheral than in BAL neutrophils), there was no significant difference in the binding between the peripheral and inflammatory BAL neutrophils in the absence of calcium.

**Effect of SP-A on fMLP receptor expression.** To determine if SP-A’s ability to regulate neutrophil chemotaxis is due to an alteration in fMLP receptor expression, we measured receptor expression on the peripheral vs. inflammatory BAL neutrophils. Fluorescein-conjugated formyl peptide (formyl-Nle-Leu-Phe-Nle-Tyr-Lys) at 1, 2, 5, 10, and 20 nM was incubated with peripheral and inflammatory BAL neutrophils for 30 min on ice. Formyl peptide binding to neutrophils was dose dependent and saturated between 5 and 10 nM (Fig. 7). As has been reported for LPS-stimulated peripheral neutrophils (1), we found that fMLP receptor expression was greater for activated inflammatory BAL neutrophils than for peripheral neutrophils (Fig. 7). To determine if the presence of SP-A could alter peptide binding or receptor expression, we added SP-A to the binding reaction or pretreated the neutrophils with SP-A (data not shown) altered formyl peptide binding to the peripheral or inflammatory BAL neutrophils.

**Effect of SP-A on fMLP-stimulated CD11b levels.** Besides stimulating chemotaxis, fMLP also upregulates CD11b expression on the neutrophil surface by stimulating release of secondary and tertiary granules and secretory vesicles where this β2-integrin is stored (4, 10). Consistent with the literature, we found that fMLP stimulated a significant increase in CD11b on the surface of peripheral neutrophils (Fig. 8). This was also observed for inflammatory BAL neutrophils, although the percent increase in levels was less in the

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Table 2. Role of collagen in neutrophil chemotaxis toward fMLP

<table>
<thead>
<tr>
<th>fMLP + Treatment</th>
<th>Relative Neutrophil Chemotaxis (Toward fMLP)</th>
</tr>
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<tbody>
<tr>
<td></td>
<td>Peripheral (n)</td>
</tr>
<tr>
<td>fMLP</td>
<td>100</td>
</tr>
<tr>
<td>SP-A</td>
<td>68 ± 7* (9)</td>
</tr>
<tr>
<td>Collagen</td>
<td>68 ± 8* (6)</td>
</tr>
<tr>
<td>Heat-treated SP-A</td>
<td>131, 105</td>
</tr>
<tr>
<td>Heat-treated collagen</td>
<td>163, 100</td>
</tr>
</tbody>
</table>

Relative neutrophil chemotaxis = [(chemotaxis toward fMLP + treatment) - (chemotaxis toward fMLP alone)] x 100. fMLP (10 nM), SP-A (25 μg/ml), collagen (type IV, 25 μg/ml). Heat treatment was at 95°C for 10 min. Experiments were done in triplicate. Means ± SE. The number of experiments (n) is shown next to each condition, except where indicated for heat-treated protein. *P ≤ 0.006 vs. fMLP alone.
BAL neutrophils than in the peripheral neutrophils (Fig. 8). This is likely due to the already high basal CD11b levels on the inflammatory BAL neutrophils (Fig. 8A). SP-A alone had no effect on levels of CD11b. However, SP-A subtly, yet significantly, reduced fMLP-stimulated CD11b levels in the peripheral neutrophils (Fig. 8B). SP-A had no significant effect on fMLP-stimulated CD11b expression on inflammatory BAL neutrophils.

DISCUSSION

These studies describe for the first time SP-A’s ability to alter neutrophil responsiveness to chemoattractants. We show that SP-A enhances chemotaxis of inflammatory BAL neutrophils toward fMLP, MIP-2, and ZAS, and SP-A reduces peripheral neutrophil chemotaxis toward fMLP and MIP-2. Because of the inherent limitations of in vitro chemotaxis assays (reviewed in Ref. 28), we also investigated the effects of SP-A on other neutrophil functions, including phagocytosis and expression of CD11b. Although the effects of SP-A were more pronounced for chemotaxis than for other functions, we found that SP-A differentially regulates a variety of peripheral and inflammatory neutrophil functions. These studies suggest that SP-A may play an important role in regulating neutrophil functions in the lung. It was surprising that SP-A did not directly stimulate neutrophil chemotaxis, as has been observed by alveolar macrophages. A comparison of SP-A binding to alveolar macrophages vs. neutrophils (Fig. 6) suggests that neutrophils have one-fifth the number of SP-A binding sites as has been reported for alveolar macrophages (15). This suggests that neutrophils may lack a receptor for SP-A chemotactic stimulation. One study reported the ability of SP-A to stimulate peripheral neutrophil chemotaxis (6). The ability of SP-A to stimulate in their assays may be due to subtle assay differences or differences in SP-A prepa-

rations. To rule out the possibility of the lack of effect being due to the species discrepancy between the SP-A and cells used, we also examined whether human SP-A could stimulate human peripheral neutrophil chemotaxis. No chemotaxis was observed at 5 μg/ml (data not shown) or 25 μg/ml SP-A (Fig. 3). The inability of SP-A to directly stimulate chemotaxis is supported by a study that reports that exogenous surfactant is unable to stimulate neutrophil chemotaxis (8). Importantly, it should be noted that the BAL neutrophils have been previously exposed to SP-A. Thus, any regulatory effects or the lack thereof that are a consequence of this exposure cannot be controlled for.

The finding that SP-A differentially regulates peripheral and inflammatory BAL neutrophil chemotaxis toward fMLP, MIP-2, and ZAS is consistent with previous reports that exudated cells differ in many respects from peripheral blood cells (5, 16, 18, 27). The findings are also consistent with previous reports that SP-A has differential effects on activated vs. resting macrophages (9, 22). Neutrophils are rarely found in the alveolar space of the noninflamed lung, but when neutrophils are recruited during inflammation, migration into the alveolar space and in contact with SP-A would give them the capacity to respond more rapidly to chemoattractants that are present during inflamma-

![Fig. 7. Formylated peptide binding to neutrophils.](http://www.ajplung.org)
tion. A role for SP-A in regulating neutrophil migration is supported by data obtained from studies with SP-A knockout mice, which show that once pulmonary inflammation is initiated, there is a large persistent infiltration of neutrophils into the pulmonary space (12) in the lungs of the knockout mice. However, it is important to note that the increased neutrophil migration in these models may be due to factors other than the absence of SP-A, including differences in cytokine levels and regulation of production of oxidant species.

Extracellular matrix proteins have been reported to affect leukocyte trafficking. Fibrin degradation products have been implicated in stimulating neutrophil chemotaxis, and chemotaxis was enhanced by the presence of LPS (11). Also, collagen has been reported to stimulate neutrophil chemotaxis (19). However, it has been reported that extracellular matrix produced by cultured endothelial cells has no neutrophil chemotactic activity yet did inhibit neutrophil activation by fMLP (13).

It does not seem likely that SP-A is altering chemotactic interactions at the neutrophil plasma membrane. FITC-formyl peptide Nle-Leu-Phe-Nle-Tyr-Lys is a marker for fMLP receptors (1), and the presence of SP-A did not affect peptide binding (Fig. 7). Also, when neutrophils were pretreated with SP-A under chemotaxis assay conditions and receptor expression was examined, there was no difference in peptide binding (data not shown). All of the attractants tested stimulated neutrophils through G protein-coupled receptors. A phenomenon of these receptors is desensitization. During desensitization, after the ligand binds the receptor, the receptor is internalized, which leads to a reduced ability of the cells to respond to subsequent chemoattractant stimulation (reviewed in Ref. 2). An exciting possible implication of the data reported here is that SP-A may be altering this desensitization process. Future studies may determine if this is the case.

Overall, these studies add to the growing body of data describing SP-A’s immunoregulatory role in the pulmonary tissue. These data show that this regulation is not only dependent on the type of leukocyte examined but also the activation state of the leukocyte. The ability of SP-A to differentially regulate different leukocyte populations supports the model that SP-A plays a significant role in tipping the balance of inflammation in favor of the beneficial vs. the damaging effects. In this way, SP-A is helping protect the delicate pulmonary epithelium while facilitating pathogen clearance.

We thank H. Garner and P. Keating for purifying the SP-A used in these studies. We also thank Dr. T. R. Martin, University of Washington, Seattle, Washington, for helpful discussions and encouragement.

This work was supported by National Heart, Lung, and Blood Institute Grant RO1-HL-51134.

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