Neutrophils induce apoptosis of lung epithelial cells via release of soluble Fas ligand

KARL L. SERRAO,¹ JAMES D. FORTENBERRY,¹,3 MARILYN L. OWENS,¹,3 FRANK L. HARRIS,² AND LOU ANN S. BROWN²

Divisions of ¹Critical Care and ²Neonatology, Department of Pediatrics, Emory University School of Medicine, and ³Division of Critical Care Medicine, Children’s Healthcare of Atlanta at Egleston, Atlanta, Georgia 30322

Received 3 December 1999; accepted in final form 7 September 2000

SERRAO, Karl L., James D. Fortenberry, Marilyn L. Owens, Frank L. Harris, and Lou Ann S. Brown. Neutrophils induce apoptosis of lung epithelial cells via release of soluble Fas ligand. Am J Physiol Lung Cell Mol Physiol 280: L298–L305, 2001.—Neutrophils release soluble Fas ligand (sFasL), which can induce apoptosis in certain Fas-bearing cell types (Liles WC, Kiener PA, Ledbetter JA, Aruffo A, and Klebanoff SJ. J Exp Med 184: 429–440, 1996). We hypothesized that neutrophils could induce alveolar epithelial apoptosis via release of sFasL. A549 pulmonary adenocarcinoma cells expressed surface Fas and underwent cell death (10 ± 7% viability) and DNA fragmentation (35 ± 98% of control cells) when incubated with agonistic CD95/Fas monoclonal antibody (P < 0.05). Coincubation with human neutrophils induced significant A549 cell death at 48 (51 ± 9% viability; P < 0.05) and 72 h (25 ± 10%; P < 0.05) and increased DNA fragmentation (178 ± 42% of control cells; P < 0.05), with morphological characteristics of apoptosis. The addition of antioxidants did not inhibit apoptosis. sFasL concentrations were maximally increased in coculture medium at 24 h (4.9 ± 0.7 ng/ml; P < 0.05). Neutrophil-induced A549 cell apoptosis was blocked by inhibitory anti-Fas (42 ± 6% of control cells; P < 0.05) and anti-FasL monoclonal antibodies (29 ± 3%; P < 0.05). Human neutrophils and Fas similarly affected murine primary alveolar epithelial cell bilayers, and caspase activation occurred in response to Fas exposure. We conclude that neutrophils undergoing spontaneous apoptosis induce A549 cell death and DNA fragmentation, independent of the oxidative burst, that is mediated by sFasL.

Fas antigen; alveolar epithelium

ACUTE LUNG INJURY IS MEDIATED by the interplay between inflammatory cells such as neutrophils and macrophages and epithelial cells lining the distal airways and alveoli (35). The role of these cellular interactions in alveolar cell death remains uncertain, particularly with regard to the induction of apoptosis, a form of programmed cell death characterized by nuclear condensation, endonuclease-mediated DNA fragmentation, and preservation of cell membrane integrity (16, 45). Aging neutrophils spontaneously undergo apoptosis and die within 72 h (3, 33). Likewise, apoptosis plays a role in cell turnover and inflammation of several epithelial cell types including alveolar epithelial type II cells (24).

The Fas antigen-Fas ligand (Fas-FasL) system comprises an important mechanism for regulating apoptotic cell death. Fas is a 45-kDa type I membrane protein member of the tumor necrosis factor (TNF)/nerve growth factor family that signals for apoptosis in certain cell types (8, 18, 23, 39). Fas acts as a receptor for the naturally occurring FasL (18, 29, 36). Membrane-bound FasL, a type II integral membrane protein of the TNF family (18, 36, 38), is cleaved by specific matrix metalloproteinases to produce its soluble form, sFasL (20). Fas is widely expressed on many cell lines and is highly expressed on neutrophils, whereas FasL cell expression is relatively restricted but is found on neutrophils (18, 25, 37). Apoptosis of Fas-bearing Jurkat cells can be triggered by cross-linking of Fas membrane receptors with agonistic anti-Fas monoclonal antibody (MAb), by purified FasL, or by expressed sFasL (36).

Dysregulation of the Fas-FasL system may play a crucial role in states such as autoimmune disease, lymphoproliferative disorders, human immunodeficiency virus, and graft rejection (24, 32) by inducing excessive apoptosis and death of cell populations. The Fas-FasL system could also be important in regulating neutrophil-associated tissue injury. Liles et al. (25) demonstrated that neutrophils undergoing apoptosis release sFasL and, in a paracrine fashion, promote the death of Jurkat cells. A growing body of research also supports a role for the Fas-FasL system in alveolar epithelial lung injury. Fine et al. (10) demonstrated expression of Fas mRNA in isolated primary murine alveolar type II (ATII) cells and induction of apoptosis in mouse alveolar type II cells by activation of Fas. In contrast, treatment with an anti-Fas antibody did not induce apoptosis in primary murine alveolar type II cells (27). Agonistic Fas antibodies induced apoptosis in these cells, suggesting that Fas-mediated apoptosis could help regulate alveolar epithelial turnover. In a...
recent report (28), sFasL was demonstrated in bronchoalveolar lavage fluid (BALF) of adults with acute respiratory distress syndrome (ARDS); BALF samples induced apoptosis in distal lung epithelial cells, a process that could be blocked by inhibition of Fas-FasL binding.

Fas-FasL interactions could potentially play a role in the modulation of alveolar cell death and influence the progression and resolution of inflammation and acute lung injury. We hypothesized that human neutrophils undergoing spontaneous apoptosis would induce apoptotic cell death in coexistent alveolar epithelial cells and that this effect is mediated by neutrophil release of sFasL.

METHODS

Materials. Antibiotics, calf sera, methylene blue, Wright stain, propidium iodide, copper/zinc superoxide dismutase, catalase, and Histopaque-1077 were purchased from Sigma (St. Louis, MO). Calcium- and magnesium-free Dulbecco’s PBS, Iscove’s modified Dulbecco’s medium, and Kaighn’s modified F-12K nutrient mixture were obtained from Gibco BRL (Life Technologies, Grand Island, NY). Dextran was purchased from Spectrum (Gardenia, CA). A549 cells, an immortalized human lung adenocarcinoma cell line with ATI cell properties (24), were obtained from the American Type Culture Collection (Manassas, VA). Tissue culture plates were purchased from Corning Costar (Cambridge, MA). Terminal deoxynucleotidyltransferase-mediated dUTP nick end labeling (TUNEL) assay and annexin assay were purchased from Boehringer Mannheim (Indianapolis, IN). Fluorescent calcein-ethidium iodide assay (LIVE/DEAD Eukolight viability/cytotoxicity kit) was obtained from Molecular Probes (Eugene, OR). FITC-labeled anti-human APO-1/Fas monoclonal antibody (MAb) (mouse IgG1), cross-linking functional anti-human CD95/Fas MAb (mouse IgG1), and anti-human Fas MAb (NOK1, murine IgG1 MAb) were obtained from Pharmingen (San Diego, CA). Anti-human Fas MAb (murine HB4 IgG1 MAb) was purchased from Upstate Biotechnology (Lake Placid, NY). A human sFasL ELISA with an anti-FasL MAb (29) was obtained from OncoImmunin.

Neutrophil isolation and A549 and murine alveolar epithelial cell cultures. Whole blood was drawn by venipuncture from healthy adult volunteers after informed consent was obtained. Blood was heparinized and centrifuged over a layer of Histopaque-1077 followed by sedimentation in 6% dextran and hypotonic lysis of erythrocytes as previously described (2). Neutrophils were maintained in Iscove’s modified Dulbecco’s medium. Isolated preparations contained >95% neutrophils by Wright stain and differential count and demonstrated >95% trypan blue exclusion.

A549 cells were maintained in Kaighn’s modified F-12K nutrient mixture supplemented with 10% fetal bovine serum and an antibiotic-antimycotic solution composed of gentamicin, penicillin, streptomycin, and amphotericin B. Cells were grown to confluence at 37°C in a humidified CO2 incubator (5% CO2-95% air) and transferred for individual experiments.

For preliminary primary cell experiments, murine ATII cells were isolated as previously described (3). Briefly, the tracheae of anesthetized rats were cannulated, and the airways were filled with an elastase solution. The lung parenchyma was cut away from the large airways and minced in a solution of DNase and newborn calf serum. After sequential filtration through 100- and 20-μm nylon mesh, cells were plated onto IgG-coated bacteriological plates. After 1 h, alveolar macrophages and other immune cells remained adherent while nonadherent ATI cells were removed. Samples obtained by this method have shown 89 ± 3% ATI cells (3).

Coculture experiments. A549 cells were first seeded onto tissue culture plates in F-12K medium supplemented with 10% fetal bovine serum and antibiotic-antimycotic solution. Cells were incubated for 24 h to allow attachment and formation of a confluent monolayer. Medium was then removed and replaced with serum-deprived F-12K nutrient mixture with antibiotic-antimycotic solution. Freshly isolated human neutrophils (2.5 × 106 cells/ml) in Iscove’s modified Dulbecco’s medium were seeded onto the upper chamber of a Transwell polycarbonate microporous insert (0.3-μm membrane pore size) above an A549 monolayer (Fig. 1), allowing for potential diffusion of soluble mediators but preventing cell-cell contact. The neutrophils and A549 cells were then cocultured at 37°C in 5% CO2-95% air for 24, 48, or 72 h. A549 monolayers without exposure to isolated neutrophils served as negative controls. After the coculture period, A549 monolayers were evaluated for viability, and flow cytometric evaluation of DNA fragmentation at each time period was performed.

Murine ATI cells were seeded in a fashion similar to A549 cells on the surface of the lower chamber of Transwell plates. Freshly isolated human neutrophils (2.5 × 106 cells/ml) in Iscove’s modified Dulbecco’s medium were seeded onto the upper chamber above the ATI cells. Murine neutrophils were not used due to difficulties in isolation procedures.

Assessment of cell death. A549 cells were assessed for viability with a standard fluorescence calcein-ethidium homodimer assay (11, 26). Intracellular esterase activity in viable cells induces green fluorescence of cell-permeant calcein, whereas ethidium homodimer crosses permeable membranes of nonviable cells and provides a red fluorescence. Cells were counted from three high-power fields by fluorescence microscopy and averaged and are expressed as percentage of viable (calcein-positive) cells per total calcein- and ethidium-positive cells.

Assessment of DNA fragmentation and apoptosis. DNA fragmentation was quantified with a standard TUNEL assay. Terminal deoxynucleotidyltransferase catalyzes the polymerization of fluorescein-labeled dUTP nucleotide to label the 3’-OH DNA nick ends that result from DNA fragmentation (11–13). A549 cells were removed from tissue culture
plates after each experiment, washed, fixed with 4% paraformaldehyde, permeabilized with 0.1% Triton X-100, and incubated with fluorescein-labeled dUTP. Nick end labeling was quantified by flow cytometry (FACScan, Becton Dickinson, Mountain View, CA) and recorded as mean fluorescence intensity (MFI).

For later antibody blocking studies, an annexin assay was used instead of TUNEL as a more sensitive and specific marker of early apoptosis (17, 21, 44). FITC-conjugated annexin V binds to surface phosphatidylserine translocated from the intra- to the extracellular plasma membrane early in apoptosis (7, 9). Cells were simultaneously stained with propidium iodide to discriminate membrane-permeable necrotic cells from FITC-labeled apoptotic cells. A549 monolayers were incubated in HEPES buffer (10 mM HEPES-NaOH, pH 7.4, 140 mM NaCl, and 5 mM CaCl₂) with annexin V-FLUOS labeling reagent and propidium iodide; then they were washed and examined with fluorescence microscopy. To facilitate cell counting, background staining of A549 cell nuclei was done with 0.1% methylene blue dye. Cells were counted and averaged from three high-power fields and are expressed as a ratio of annexin-positive (apoptotic) cells minus propidium iodide-stained (necrotic) cells per total number of cells. Cells were also examined under phase microscopy to evaluate the morphological characteristics of apoptosis.

Assessment of neutrophil-derived reactive oxidant species in A549 injury. To determine if neutrophil-associated A549 apoptosis was due to oxidant radical production, coculture experiments were performed in the presence and absence of copper/zinc superoxide dismutase (SOD; 300 U/ml), catalase (1,000 U/ml), or a combination of SOD and catalase. Cells were cocultured for 72 h and assessed for viability and DNA fragmentation. Supernatants were also assayed for the presence of hydrogen peroxide by quantitative spectrophotometric assay based on the conversion of ferrous to ferric iron species by hydrogen peroxide to a peroxyl radical in the presence of xylenol orange dye.

Fas surface levels and effects of Fas on A549 and murine ATII cells. A549 monolayers and freshly isolated neutrophils were incubated with FITC-labeled anti-human MAb to Fas for 60 min at 4°C and then fixed with paraformaldehyde (4% in PBS, pH 7.4). Negative control cultures were prepared in a similar fashion with irrelevant IgG1-FITC antibody. Fluorescence intensity was measured by flow cytometry, and the presence of surface Fas is expressed as MFI minus negative control MFI.

To assess the effect of Fas antigen in inducing alveolar epithelial cell death, A549 monolayers were incubated in the absence and presence of cross-linking functional agonistic anti-Fas MAB (20 μg/ml) for 48 h. Cell viability was evaluated by fluorescence viability/cytotoxicity assay, and DNA fragmentation was measured with TUNEL assay.

Caspase activity was measured to assess a possible mechanism of Fas-mediated apoptosis in murine ATII cells. After treatment with FasL, cells were scraped, resuspended in a hypotonic lysis buffer, and homogenized to disrupt the cell membrane. After centrifugation, the cytosolic fraction (20 μg of protein) was incubated with a fluorogenic caspase-3 substrate (10 μM) at 4°C. Flow cytometry with excitation at 488 nm was performed, and results are expressed as relative fluorescence units per cell.

sFasL assay. Neutrophil supernatants were collected 24, 48, and 72 h after isolation, and sFasL at each time point was measured by sFasL ELISA. The time of maximal sFasL concentration also determined select optimal timing of neutrophil medium and anti-FasL antibody administration in subsequent experiments.

Effects of sFasL blocking studies. To determine the effects of sFasL on neutrophil-induced A549 cell death, blocking studies were performed with functional inhibitory antibodies. Isolated human neutrophils were cultured on microporous polycarbonate membrane inserts in serum-free F-12K mixture. Based on previous ELISA results, the neutrophil medium was removed at 24 h and added to A549 cell monolayers in separate tissue culture plates. A549 monolayers with neutrophil medium were then incubated in the presence and absence of anti-human FasL MAB (10 μg/ml), a functional antibody binding to and inhibiting FasL. In similar experiments, A549 cells were incubated in the presence and absence of a functional inhibitory anti-human Fas MAB (1 μg/ml). A549 cells with neutrophil medium (without blocking antibody) served as positive controls, and cells with F-12K nutrient medium not exposed to neutrophils served as negative controls. At 24 h, samples were analyzed with the annexin assay.

Statistical analysis. Results are means ± SD. Statistical analysis was performed with multiple analysis of variance testing. Multiple comparisons were made with the Student-Newman-Keuls test. The Mann-Whitney rank sum test was used for nonparametric comparisons. Statistical significance was defined as P < 0.05.

RESULTS

Effects of neutrophil exposure on A549 cell death and DNA fragmentation. Viability of A549 cells exposed to neutrophils in coculture experiments decreased in a time-dependent manner at 48 and 72 h (P < 0.05; Fig. 2A) as assessed by viability/cytotoxicity assay (Fig. 3). Exposed A549 cells also demonstrated increasing time-dependent DNA fragmentation by TUNEL assay (Fig. 2B). DNA fragmentation was not significantly increased at 24 h (MFI, 125 ± 25.5% of control value; n = 8 cultures). A549 cell apoptosis was also increased within 24 h after the onset of incubation with neutrophil-exposed medium alone (68 ± 7% of cells counted; P < 0.05 vs. control values; n = 4 cultures).

By annexin assay, A549 cell apoptosis was seen earlier, with significant increases in apoptosis in cocultured A549 cells within 24 h (71.3 ± 8.1% of cells counted; P < 0.05 vs. control values; n = 4 cultures). A549 cell apoptosis was also increased within 24 h after exposure to human neutrophils in medium alone (68 ± 7% of cells counted; P < 0.05 vs. control values; n = 4 cultures).

Exposure to human neutrophils for 72 h increased murine ATII cell apoptosis as assessed by TUNEL (114% of control value; Fig. 4). A549 cell DNA fragmentation in the presence of antioxidants was also unchanged (Fig. 4B). Hydrogen peroxide concentrations were not increased in neutrophil-exposed A549 cells (4.7 ± 0.2 μM) compared with those in A549...
Cross-linking functional anti-CD95/Fas MAb induced A549 cell death 48 h after initial exposure (10 ± 7% viability vs. 98 ± 1% viability in control cultures; P < 0.05; n = 7 cultures; Fig 3). By TUNEL, DNA fragmentation was significantly increased in A549 cells exposed to anti-CD95/Fas MAb (354 ± 98% of control MFI; P < 0.05; n = 7 cultures). Addition of cross-linking Fas ligand MAb for 72 h to murine ATII cells also increased MFI (137% of control value; Fig 4), consistent with increased DNA fragmentation and apoptosis. Caspase-3 activity increased significantly in FasL-treated murine ATII cells (367 ± 48 relative fluorescence units/cell) compared with control ATII cells alone (120 ± 23 relative fluorescence units/cell; P < 0.05; n = 4 cultures/group).

sFasL levels and effects of functional FasL inhibition. Concentrations of sFasL in neutrophil medium supernatants were increased at 24, 48, and 72 h by ELISA (Fig. 5). Maximal sFasL concentration was seen at 24 h (4.9 ± 0.7 ng/ml; P < 0.05 vs. control cultures; n = 6 cultures cell). Addition of anti-sFasL MAb to neutrophil supernatant-exposed medium inhibited A549 apoptosis 24 h after initiation compared with neutrophil supernatant-exposed medium alone (Fig. 6) as assessed by annexin assay (19 ± 6% vs. 68%; P < 0.05; n = 4 cultures). The addition of anti-Fas MAb to exposed medium produced similar inhibition of A549 apoptosis (Fig. 6). Apoptosis was inhibited by sFasL and Fas antibodies to levels similar to those in control A549 cell apoptosis.

DISCUSSION

These studies confirmed previous findings in other alveolar cell types (10) that Fas expression occurs in ATII cells and that alveolar cell death and DNA fragmentation can be triggered by agonistic anti-Fas MAb. In addition, these studies showed that neutrophils induced ATII cell death and DNA fragmentation in vitro, consistent with apoptosis. Aging neutrophils spontaneously undergoing apoptosis released sFasL, and neutrophil induction of ATII cell apoptosis was in part mediated by sFasL- and Fas-mediated interactions. These findings correlate well with a previous study by Liles et al. (25) but are in contrast with other findings (27). Differences in results may be related to the type of cell line chosen for study or a result of the specific anti-Fas antibody used, which was not delineated in the previous study (27).

Other potential pathways of injury by neutrophils exist, such as the release of cytokines, proteases, and reactive oxygen species (ROS). Previous investigators (19, 25) have found that ROS such as hydrogen peroxide and superoxide anion are required for spontaneous and Fas-mediated apoptosis in neutrophils. We were unable, however, to demonstrate that ROS played a mechanistic role in neutrophil-promoted apoptotic cell death in this model. It is possible that inefficiency of cellular uptake of antioxidants produced a lack of effect by SOD or catalase. A previous study (46) that used similar concentrations of SOD and catalase was able to...
demonstrate inhibition of apoptosis in A549 cells exposed to chromium(VI), a potent oxidant, implying the ability to inhibit oxidant-mediated effects, if present, in our model. Furthermore, no significant differences were seen in hydrogen peroxide concentrations with the addition of neutrophils. Thus inhibition of hydrogen peroxide would not be expected to have any effect on apoptosis in this model.

A549 cells, as a tumor cell line, may not behave or appropriately represent the responses of primary human lung alveolar epithelial cells in vitro or in vivo. A549 cells are a human adenocarcinoma cell line with epithelial-like morphology (24); they can synthesize lecithin but do not differentiate into alveolar type I cells as primary type II cells are able to do. Pulmonary adenocarcinomas, including the A549 cell line, were found in one study (30) to have varied surface Fas expression that might make them more resistant to Fas-mediated apoptosis. The positive results in our study with A549 cells may have been in part due to differences in the cross-linking Fas antibodies used. Our results in relatively resistant A549 cells could imply that primary alveolar epithelial cells would be even more sensitive to the FasL-mediated effects of neutrophils.

Our preliminary results with murine ATII cells suggest that neutrophil effects may also extend to primary alveolar epithelial cells, but several weaknesses preclude firm conclusions. Difficulties isolating rat neutrophils led to the use of human neutrophils and the questions inherent in cross-species experiments. We have also been unable to locate a murine-specific sFasL antibody to determine the effects of blocking primary cell-FasL interactions. Further studies are ongoing. Present advances in our work to isolate primary human ATII cells will also allow more representative studies.

The TUNEL assay has previously been considered to correlate well with the histological findings of apoptosis (12, 13), but recent studies (14, 43) have suggested that TUNEL may not always be specific for apoptosis, especially in autopsy material or with prolonged periods of fixation. A549 cell samples used in these studies were rapidly fixed and immediately analyzed, making such artifacts unlikely. Increased DNA fragmentation by TUNEL also correlated well with the detection of apoptosis by annexin assay.

These studies were performed with quiescent neutrophils, and their effect on type II cells may have differed if stimulated. In vitro and in vivo studies have
shown that proinflammatory cytokines prolong the neutrophil life span by inhibiting neutrophil apoptosis and delaying the resolution of the inflammatory response (4, 6, 40), whereas anti-inflammatory cytokines may have the opposite effect (5). Further studies to examine the effect of neutrophil activation on the timing and magnitude of lung cell apoptosis would be beneficial. These in vitro results may not be representative of more complex in vivo interactions.

Direct cellular interaction could also alter these in vitro results. Apoptotic neutrophils are cleared from the cellular milieu both in vitro and in vivo by circulating macrophages (6, 32, 34). The absence of macrophage-neutrophil interaction may have produced several effects. Apoptotic cells that have not been phagocytized and removed progress to secondary necrosis with loss of cell membrane integrity and the release of cytoplasmic contents (32, 34). This secondary necrosis phenomenon may account for the second peak of sFasL observed at 72 h. Lysis of neutrophil cell membranes may have caused the cleavage and release of membrane-bound FasL. Neutrophils undergoing apoptosis that are not removed by macrophages eventually disintegrate and release elastase and myeloperoxidase, resulting in further surrounding tissue necrosis and chemotactic inflammatory response (32, 34). Altered fibroblasts that emerge during fibrotic lung injury also appear capable of inducing alveolar epithelial cell death, perhaps by release of an undetermined soluble factor (42).

Fig. 4. Effects of PMN and Fas exposure on murine primary alveolar epithelial cell apoptosis. Flow cytometric histograms from TUNEL assay demonstrate increased MFI associated with DNA fragmentation and apoptosis in murine alveolar type II (ATII) cells coincubated with PMN and with Fas ligand compared with that in control cells.

Fig. 5. Soluble Fas ligand (sFasL) concentration in coculture medium supernatants as measured by ELISA. Values are means ± SD; n = 6 cultures. sFasL in PMN cocultures was elevated at all time intervals compared with baseline control cultures, with maximum levels seen at 24 h. *P < 0.05 compared with controls by ANOVA and Student-Newman-Keuls test.

Fig. 6. Effects of Fas and sFasL inhibition on A549 cell apoptosis. Addition of anti-human sFasL monoclonal antibody (MAb) or anti-human Fas MAb to A549 cells coincubated with PMN supernatant for 24 h inhibited A549 cell apoptosis as expressed by percent annexin-positive cells. Values are means ± SD; n = 4 cultures/group. *P < 0.05 compared with control cells. **P < 0.05 compared with A549 cells plus PMN supernatant by ANOVA and Student-Newman-Keuls test.
Our findings suggest that the Fas-FasL interaction could be important in neutrophil-induced lung injury. Matute-Bello et al. (28) found that sFasL is upregulated in BALF from adult patients with ARDS compared with that in normal control subjects, with the highest levels occurring during the first week of illness. BALF cells of ARDS patients released sFasL spontaneously and on stimulation with bacterial endotoxin. BALF from these ARDS patients induced apoptosis of small epithelial airway cells, a process that was blocked by antagonistic anti-Fas MAAb, anti-FasL MAAb, and Fas-Ig fusion protein (28). Thus in ARDS, sFasL is released by inflammatory cells and may act as a death-promoting mediator.

Overexpression of the Fas-FasL system could also be a predisposing factor in fibroblast proliferation in ARDS (22). Excessive alveolar epithelial cell death could overwhelm the necessary elimination of proliferating mesenchymal and inflammatory cells from the alveolar air space or alveolar walls after lung injury (31), prolonging inflammation and interfering with reepithelialization, leading to overgrowth of lung fibroblasts (15, 31). Alveolar epithelial apoptosis could also directly induce the proliferation of lung fibroblasts through the loss of normal inhibition (1). Evidence for the Fas-FasL link in these effects is provided by a report that showed that inhaled aerosolized agonistic Fas MAAb in mice induced alveolar apoptosis and pulmonary fibrosis (15).

Manipulating the Fas-FasL system and thereby modulating the inflammatory response in acute lung injury may prove to be therapeutically useful. Tsuyuki et al. (41) demonstrated that administration of agonistic anti-Fas MAAb to murine lungs after induction of lung injury may prove to be therapeutically useful. Tsuyuki et al. (41) demonstrated that administration of agonistic anti-Fas MAAb to murine lungs after induction of lung injury may prove to be therapeutically useful.

REFERENCES


