Fas (CD95) Induces Alveolar Epithelial Cell Apoptosis *in Vivo*

Implications for Acute Pulmonary Inflammation

Gustavo Matute-Bello,* Robert K. Winn,[†]
Mechthild Jonas,[§] Emil Y. Chi,[§]
Thomas R. Martin,* and W. Conrad Liles[‡]

From the Divisions of Pulmonary and Critical Care Medicine* and Allergy and Infectious Diseases[‡] of the Department of Medicine, the Department of Surgery,[†] and the Department of Pathology,[§] University of Washington, Seattle, Washington

Activation of the Fas/FasL system induces apoptosis of susceptible cells, but may also lead to nuclear factor κ B activation. Our goal was to determine whether local Fas activation produces acute lung injury by inducing alveolar epithelial cell apoptosis and by generating local inflammatory responses. Normal mice (C57BL/6) and mice deficient in Fas (*lpr*) were treated by intranasal instillation of the Fas-activating monoclonal antibody (mAb) Jo2 or an irrelevant control mAb, and studied 6 or 24 hours later using bronchoalveolar lavage (BAL), histopathology, DNA nick-end-labeling assays, and electron microscopy. Normal mice treated with mAb Jo2 had significant increases in BAL protein at 6 hours, and BAL neutrophils at 24 hours, as compared to *lpr* mice and to mice treated with the irrelevant mAb. Neutrophil recruitment was preceded by increased mRNA expression for tumor necrosis factor- α , macrophage inflammatory protein-1 α , macrophage inflammatory protein-2, macrophage chemotactic protein-1, and interleukin-6, but not interferon- γ , transforming growth factor- β , RANTES, eotaxin, or IP-10. Lung sections from Jo2-treated normal mice showed neutrophilic infiltrates, alveolar septal thickening, hemorrhage, and terminal dUTP nick-end-labeling-positive cells in the alveolar septae and airspaces. Type II pneumocyte apoptosis was confirmed by electron microscopy. Fas activation *in vivo* results in acute alveolar epithelial injury and lung inflammation, and may be important in the pathogenesis of acute lung injury. (*Am J Pathol* 2001, 158:153–161)

The Fas/Fas ligand system plays a significant role in the regulation of apoptosis in many types of cells.¹ This system is comprised of the cell membrane surface receptor Fas (CD95) and its natural ligand, Fas-ligand (FasL).¹ Fas

is a 45-kd type I membrane protein that is a member of the tumor necrosis factor family of surface receptors.^{2,3} Fas is expressed on many cells, including lymphocytes, neutrophils, monocytes, and alveolar epithelial cells.^{4–7} Binding of FasL to Fas results in apoptosis of susceptible cells.⁸ Fas ligand is a 37-kd type II protein^{9,10} that exists as membrane-bound and soluble forms.⁸ Both forms are capable of inducing apoptosis when engaging Fas,^{2,11} although the membrane-bound form seems to be more efficient than the soluble form *in vitro*.¹¹

Neutrophil apoptosis is generally regarded as an anti-inflammatory process.^{12,13} However, recent studies have shown that binding of Fas to an agonist, in addition to triggering apoptosis, may also lead to activation of the nuclear factor κ B under certain circumstances.^{14,15} This cellular activation is in addition to its pro-apoptotic function and is probably independent of apoptosis.¹⁶ Nuclear factor κ B partially controls the expression of multiple inflammatory cytokines and cell surface receptors, including the chemokines interleukin-8 (IL-8) and GRO in humans, as well as the murine GRO analogs KC and macrophage inflammatory protein (MIP)-2.^{17–21}

The potential role of the Fas/FasL system as a dual pro-apoptotic/pro-inflammatory system becomes relevant with the finding that circulating levels of sFasL are present in certain human diseases.^{22–28} Furthermore, sFasL is found in bronchoalveolar lavage (BAL) fluid from patients with the acute respiratory distress syndrome (ARDS) at concentrations capable of inducing apoptosis of primary human small airway epithelial cells *in vitro*.²⁹ Destruction of the alveolar epithelium is one of the hallmarks of ARDS,^{30,31} but the mechanisms that account for the epithelial injury that occurs in ARDS remain unclear. Although it has been reported that repeated activation of Fas in the lungs of mice results in pulmonary fibrosis after 2 weeks,³² the acute effects of Fas activation in the lungs

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Address reprint requests to W. Conrad Liles, M.D., Ph.D., Box 357185, Department of Medicine, University of Washington, Seattle, WA 98195. E-mail: foghorn@u.washington.edu.

are unclear. We hypothesized that sFasL in the airspaces may contribute to the pathogenesis of acute lung injury by inducing apoptosis of epithelial cells, as well as by stimulating the release of inflammatory cytokines via nuclear factor κ B activation.

The major goal of this study was to determine whether activation of Fas in the alveoli of the lungs would induce apoptosis of type II pneumocytes in the alveolar wall, and initiate an inflammatory response in the lungs. We treated normal mice or naturally occurring mutant mice deficient in Fas expression (*lpr* mice) with the monoclonal antibody (mAb) Jo2, which activates Fas on the surface of cells *in vitro* and *in vivo*.^{32,33} We identified apoptotic cells in the alveolar walls by DNA nick-end-labeling and electron microscopy, and the alveolar inflammatory response by quantitative histology, BAL, and cytokine ribonuclease protection assays (RPA).

Materials and Methods

Antibodies

The activating anti-Fas mAb Jo2 (Armenian hamster IgG) and a control mAb (Armenian hamster anti-TNP IgG) were purchased from PharMingen (San Diego, CA). Both antibodies were free of azide and endotoxin, as determined by the manufacturer. In addition, the antibodies were confirmed to contain <0.01 endotoxin U/ml by the limulus amebocyte assay (ECL-1000; Biowhittaker, Walkersville, MD).

Animal Preparation

Male mice, weighing 20 to 30 g, were briefly anesthetized with inhaled halothane. Either mAb Jo2 or control mAb at a dose of 2.5 μ g/g was administered to each mouse by intranasal instillation in a solution containing 1 mg/ml of mAb in sterile phosphate-buffered saline (PBS), as previously described.³⁴ The animals were allowed to recover from anesthesia, returned to their cages, and given free access to water and food. At the end of the experiment the animals were euthanized with ketamine and xylazine, the thorax was rapidly opened and the animal was exsanguinated by direct cardiac puncture. The lungs were dissected free and the trachea was cannulated to perform BAL, or to fix the lungs, or to extract mRNA. The protocol was approved by the animal care committee of the University of Washington.

Experimental Protocols

We used male mice weighing 20 to 30 g. The mice were either C57BL/6 mice, (B&K Universal, Seattle, WA), or naturally occurring mutant mice lacking the Fas receptor (*lpr* mice) (Jackson Laboratory, Bar Harbor, ME). The *lpr* mice are derived from the C57BL/6 mouse strain. The mice were treated with either the Fas-activating mAb Jo2 or an irrelevant mAb (hamster anti-TNP IgG) as described above, and euthanized at either 6 or 24 hours after the administration of the antibody. Studies with *lpr*

mice were performed to confirm that effects observed with mAb Jo2 in C57BL/6 mice were specific for Fas activation. As an additional comparison, C57BL/6 were treated with an irrelevant mAb (hamster anti-TNP IgG) and euthanized at either 6 or 24 hours.

BAL Protocol

BAL was performed by instilling 0.9% NaCl containing 0.6 mmol/L ethylenediaminetetraacetic acid in two separate 0.5 ml aliquots. The fluid was recovered by gentle suction and placed on ice for immediate processing. An aliquot of the BAL fluid was processed immediately for total and differential cell counts. Total cell counts were performed with a hemocytometer, whereas differential cell counts were performed on cytospin preparations stained with modified Wright-Giemsa stain (Diff-Quik; American Scientific Products, McGaw Park, IL). The remainder of the lavage fluid was spun at $200 \times g$ for 30 minutes, and the supernatant was removed aseptically and stored in individual aliquots at -70°C .

The total protein concentration in BAL fluid was measured using the bicinchoninic acid method (BCA assay; Pierce Co., Rockford, IL).

mRNA Extraction and RPA Protocol

Lung cytokine mRNA expression (RANTES, eotaxin, MIP-1 α , MIP-2, IP-10, macrophage chemotactic protein-1 (MCP-1), tumor necrosis factor- α , interleukin-6, interferon- γ , transforming growth factor- β) was measured by RPA. Three mice were treated by intranasal instillation of mAb Jo2 (2.5 μ g/g), and three control mice were treated with an irrelevant mAb. After 6 hours, the mice were euthanized and their lungs excised. Lung RNA was extracted with Triazol (Biotecx Laboratories, Inc., Houston, TX) according to the vendor's instructions. RPA was performed using an RPAII kit (Ambion Inc., Austin, TX), with MCK-3 and MCK-5 template sets (PharMingen, San Diego, CA) and [α -³²P]UTP according to the manufacturer's instructions. Samples were run under denaturing electrophoresis on 5% polyacrylamide gel, then imaged and analyzed in a Packard Cyclone Phosphorimager (Amersham Pharmacia Biotech, Piscataway, NJ). To control for relative differences in RNA loading between samples, the specific cytokine mRNA signals were normalized to the intensity of the respective glyceraldehyde-3-phosphate dehydrogenase signal. Results are expressed as relative mRNA expression (mean $\pm$ SEM), using the following equation: normalized Jo2 induced expression ($n = 3$)/normalized control expression ($n = 3$).

Histopathology Protocols

The lungs were fixed by inflation with 10% neutral-buffered formalin at a transpulmonary pressure of 15 cm H₂O and embedded in paraffin. Within 24 hours of fixation, lung sections were stained with hematoxylin and eosin for light microscopy, or by the DNA nick-end-labeling assay to evaluate apoptotic cells, or processed for transmission electron microscopy as described below.

Table 1. Quantitative Histopathology Score of Lung Injury

Tissue	0	1	2	3
Alveolar septae	All septae are thin and delicate	Congested alveolar septae in less than 1/3 of the field	Congested alveolar septae in 1/3 to 2/3 of the field	Congested alveolar septae in greater than 2/3 of the field
Alveolar hemorrhage	No hemorrhage	At least 5 erythrocytes per alveolus in 1 to 5 alveoli	At least 5 erythrocytes per alveolus in 5 to 10 alveoli	At least 5 erythrocytes per alveolus in more than 10 alveoli
Intra-alveolar fibrin	No intra-alveolar fibrin	Fibrin strands in less than 1/3 of the field	Fibrin strands in 1/3 to 2/3 of the field	Fibrin strands in greater than 2/3 of the field
Intra-alveolar infiltrates	Less than 5 intra-alveolar cells per field	5 to 10 intra-alveolar cells per field	10 to 20 intra-alveolar cells per field	More than 20 intra-alveolar cells per field

DNA Nick-End-Labeling Assay

The slides were submerged in 10% neutral-buffered formalin for 10 minutes, followed by 70% ethanol for 5 minutes. The slides were rehydrated for 10 minutes in PBS and treated with 0.002% proteinase K (Sigma, St. Louis, MO.) in double-distilled water for 5 to 15 minutes at room temperature. Endogenous peroxidase was quenched by placing the slides in 2% hydrogen peroxide for 5 minutes. For equilibration, the slides were treated in Klenow labeling buffer (TACS *In situ* Apoptosis Detection Kit; Trevigen Inc., Gaithersburg, MD) for at least 1 minute and then incubated for 60 minutes at 37°C with Klenow enzyme and Klenow dNTP mix in Klenow labeling buffer (all reagents from Trevigen, Inc.) prepared according to instructions from the manufacturer. Negative control slides were incubated with the labeling mixture without the Klenow enzyme. After incubation the slides were completely submerged in Klenow Stop buffer (Trevigen Inc.) for 5 minutes at room temperature and rinsed in PBS for 2 minutes. The samples were then treated for 15 minutes with streptavidin-horseradish peroxidase detection solution (Trevigen Inc.), washed twice for 2 minutes in PBS and incubated in diaminobenzidine (Trevigen Inc.) for 7 minutes at room temperature. The samples were then rinsed twice in distilled water and stained with 1% methyl green in 0.1 mol/L sodium acetate (pH 4.0) for 5 minutes, quickly dehydrated in 95% and 100% ethanol, cleared in xylene, and mounted with Permount (Fisher Scientific, Pittsburgh, PA).

Electron Microscopy

Lung tissue was fixed for electron microscopy by immersion in 6.25% glutaraldehyde, 2% paraformaldehyde in 0.1 mol/L sodium cacodylate buffer for 2 hours. The tissue was postfixed in 2% potassium-ferrocyanide in distilled water for 4 hours at room temperature, rinsed with distilled water, stained with 0.5% uranyl acetate for 20 minutes, and then rinsed in distilled water. The samples were dehydrated in a graded series of ethanol solutions and embedded in Eponate 12 (Ted Pella Inc., Redding, CA). Thin sections were cut from two randomly selected blocks with a diamond knife using an LKB Nova ultramicrotome and collected on parlodion-coated 200

mesh copper grids (Electron Microscopy Sciences, Fort Washington, PA.). The sections were stained with uranyl acetate and lead citrate and examined with a JEOL TEM 1200 EX at a magnification of $\times 3000$ or higher. Only cells with a recognizable nucleus were included in the analysis. For each sample several sections from at least two electron microscopic blocks were used for evaluation.

Lung Injury Score

Each slide was evaluated by two separate investigators (GMB and WCL) in a blinded manner. To generate the lung injury score, a total of 300 alveoli were counted on each slide at $\times 400$ magnification. Within each field, points were assigned according to predetermined criteria (Table 1). All of the points for each category were added and weighted according to their relative importance. The injury score was calculated according to the following formula: injury score = [(alveolar hemorrhage points/no. of fields) + 2 $\times$ (alveolar infiltrate points/no. of fields) + 3 $\times$ (fibrin points/no. of fields) + (alveolar septal congestion/no. of fields)]/total number of alveoli counted.

Statistical Analysis

Comparisons between two groups were made with the two-tailed Fisher's exact *t*-test. Comparisons between multiple groups were made with the Kruskal-Wallis analysis of variance and with factorial analysis of variance.³⁵ For post hoc analysis, the Fisher's test was used. A *P* value < 0.05 was considered significant.

Results

Effect of Activation of the Fas System on BAL Fluid Total Protein Concentration, Polymorphonuclear Leukocytes (PMN) Recruitment, and Cytokine Production

Six hours after intranasal instillation of mAb Jo2, the BAL total protein concentration was significantly increased in C57BL/6 mice ($n = 5$) as compared to the *lpr* mice ($n = 5$), or to the C57BL/6 mice treated with an irrelevant

Figure 1. Total BAL protein at 6 hours (A) or 24 hours (B) after the intranasal administration of either an irrelevant control mAb or the Fas-activating mAb Jo2. C57, wild-type mice; *lpr*, Fas-deficient mice. Data are shown as means $\pm$ SEM.

control mAb ($n = 5$) (Figure 1A). At 24 hours, all animal groups had similar concentrations of total protein in the BAL fluid (Figure 1B).

In contrast to the early increase in BAL fluid total protein, the BAL fluid neutrophil response was delayed. Six hours after Jo2 instillation, there was trend toward more BAL fluid PMN in the C57BL/6 mice treated with the mAb Jo2, but this did not reach statistical significance (Figure 2A). At 24 hours, the number of BAL fluid PMN was significantly higher in C57BL/6 mice treated with mAb Jo2 ($n = 5$), as compared to the *lpr* group ($n = 5$), and to C57BL/6 animals treated with the control mAb ($n = 5$) (Figure 2B).

To assess cytokine message expression we performed RPAs on mRNA extracted from the lungs of C57BL/6 mice 6 hours after instillation of either Jo2 mAb ($n = 3$) or control mAb ($n = 3$) (Figure 3). The cytokine expression was normalized to the GAPDH signal to control for loading differences. Jo2-treated mice showed a sixfold increase in MIP-2 mRNA, a twofold increase in MIP-1 α mRNA, a fourfold increase in MCP-1 mRNA, a threefold increase in tumor necrosis factor- α mRNA, and a 60-fold increase in interleukin-6 mRNA. In contrast, there was no increase in message for transforming growth factor- β ,

Figure 2. Total BAL PMN at 6 hours (A) or 24 hours (B) after the intranasal administration of either an irrelevant control mAb or the Fas-activating mAb Jo2. C57, wild-type mice; *lpr*, Fas-deficient mice. Data are shown as means $\pm$ SEM.

eotaxin, or IP-10. The relative expression of RANTES and interferon- γ were significantly decreased.

Histopathological Evidence of Lung Injury

At 24 hours, C57BL/6 mice treated with mAb Jo2 ($n = 6$) had a significantly higher lung injury score than animals treated with the control mAb ($n = 7$; $P < 0.01$) (Figure 4). The lung injury was characterized by patchy areas of neutrophilic infiltrates with thickening of the alveolar septae and areas of hemorrhage (Figure 5a). The majority of the cells infiltrating the airspaces, as well as cells in the alveolar septae, showed densely condensed nuclei suggesting apoptosis in areas of injury (Figure 5b). No histopathological abnormalities were present in animals that were treated with the control mAb (Figure 6, a and b).

DNA nick-end-labeling assays confirmed the presence of apoptosis in cells infiltrating the airspaces (Figure 5, c and d). In addition, positive cells were present in the alveolar septae of areas of injury. No positive cells were seen in the animals treated with the control mAb (Figure 6, c and d).

By electron microscopy, the lungs of a mouse treated with mAb Jo2 showed features characteristic of apoptosis in alveolar type II cells. These features included increased electron density of the cytoplasm, condensation

Figure 3. RPA analysis of cytokine mRNA expression in the lungs of mice, 6 hours after the intranasal administration of the mAb Jo2. RPA analysis was performed as described in the Methods section. Results are expressed as relative mRNA expression (mean $\pm$ SEM), which represents normalized Jo2-induced expression ($n = 3$ mice)/normalized control expression ($n = 3$ mice) for each cytokine. *, Value significantly greater than control ($P < 0.05$). **, Value significantly less than control ($P < 0.05$).

of the chromatin, and vacuolization of the nuclear envelope (Figure 7).^{36–38} Interestingly, there were also mitochondrial abnormalities. Changes consistent with apoptosis were not seen in any of the endothelial cells that were identified.

Discussion

The primary goal of this study was to determine whether activation of the Fas system *in vivo* results in damage to cells in the alveolar walls, and whether this event initiates an acute inflammatory response. We found that C57BL/6 mice developed patchy neutrophilic infiltrates, areas of alveolar hemorrhage, thickening of the alveolar septae, and apoptosis of type II pneumocytes within 24 hours after treatment with Fas-activating mAb Jo2. These histopathological changes were associated with an early increase in BAL fluid protein and early induction of cytokine gene expression, followed by a later increase in BAL fluid neutrophils. Similar changes did not occur in mice lacking Fas (*lpr*), or in mice treated with an irrelevant antibody. These findings provide clear evidence that the induction of apoptosis in cells of the alveolar wall initiates a sustained inflammatory response in the lungs.

In humans, acute lung injury is characterized by epithelial and endothelial injury, neutrophilic alveolitis, and hyaline membrane formation. The primary event leading to lung injury in ARDS has not been established. A prevalent hypothesis is that the neutrophil mediates lung injury in ARDS.³⁹ In this paradigm, uncontrolled neutrophil activation leads to the accumulation of oxidants and proteases in the lungs, which cause damage to the cells of the alveolar environment. However, neutrophils can migrate into the lungs of humans without causing injury, and large numbers of neutrophils can migrate into the lungs of sheep without causing injury to the tight epithelial barrier.^{40,41} Blockade of PMN chemoattractants or systemic PMN depletion blocks lung injury in some, but not all

Figure 4. Histopathological lung injury score for C57 mice 24 hours after receiving either an irrelevant control mAb ($n = 7$) or the Fas-activating mAb Jo2 ($n = 6$). The score represents the average of two independent investigators who read each H&E-stained slide in a blinded manner. The categories used to generate the score were alveolar septal congestion, alveolar hemorrhage, intra-alveolar fibrin deposition, and intra-alveolar infiltrates (see text for further explanation). *, $P < 0.01$.

animal models.^{42–44} Although PMN may contribute to lung injury in some circumstances, it is not clear whether the first event leading to lung injury involves PMN migration, or whether epithelial injury occurs before leukocytes are involved.⁴⁵ Answering this question is critical to designing specific therapeutic strategies for ARDS.

The present study shows that activation of the Fas system *in vivo* in the lungs of mice leads to a primary epithelial injury. We found that intranasal administration of the Fas-activating mAb Jo2 resulted in an early increase in the BAL fluid total protein concentration, followed later by PMN migration into the airspaces. The cells of the alveolar wall became apoptotic, as detected by DNA end-nick-labeling assays and electron microscopy. Thus in this model, damage to the alveolar epithelial barrier occurred first, before PMN recruitment. The possibility that the Fas system might be involved in epithelial damage during acute lung injury was raised by our previous findings that soluble FasL is elevated in BAL fluid from patients with ARDS at concentrations capable of inducing apoptosis of normal human distal lung epithelial cells *in vitro*, and that higher concentrations of soluble FasL in BAL fluid from patients on day 1 of ARDS are associated with increased mortality.²⁹ An earlier study had demonstrated that a trypsin-sensitive pro-apoptotic factor (or factors) was present in BAL fluid obtained from patients during the late stage of ARDS, and that this factor was active for fibroblasts and endothelial cells.⁴⁶

Several lines of evidence suggest that the Fas system causes apoptosis of alveolar epithelial cells. The human neoplastic alveolar epithelial cell line A549 expresses Fas on its surface and undergoes apoptosis when exposed to activating anti-Fas IgM.⁷ Furthermore, we have found that normal human distal lung epithelial cells express Fas and become apoptotic when exposed to recombinant human soluble FasL.²⁹ These results suggest that Fas-mediated apoptosis of the alveolar epithelium could be an initial event in the development of some forms of lung injury.

Figure 5. H&E preparation and DNA nick-end-labeling assay from consecutive sections of the caudal lung lobe of a wild-type mouse 24 hours after intranasal instillation of Fas activating mAb Jo2. The H&E preparation shows inflammatory infiltrates and areas of hemorrhage [original magnifications: $\times 100$ (**a**); $\times 400$ (**b**)]. The DNA nick-end-labeling assay shows positive cells in the inflammatory exudate [original magnification, $\times 100$ (**c**)] and in the alveolar walls (**arrows**) [original magnification, $\times 400$ (**d**)]. Negative controls for the DNA nick-end-labeling assay are shown in **e** and **f**.

The activation of Fas *in vivo* also caused a sustained inflammatory response characterized by initial activation of pro-inflammatory cytokine genes, followed by neutrophil accumulation in the airspaces. Leukocyte recruitment was seen at 24 hours, and was preceded at 6 hours by increased mRNA expression for the pro-inflammatory cytokines MIP-2, MIP-1 α , MCP-1, interleukin-6, and tumor necrosis factor- α . The expression of mRNA for the predominantly T-lymphocyte cytokines, RANTES, eotaxin, and interferon- γ , was not increased. Transforming growth factor- β mRNA expression was also not increased, which was surprising given the importance of the Fas/FasL system in the pathogenesis of pulmonary fibrosis.^{32,47} All of the pro-inflammatory cytokines whose mRNA expression was increased can be produced by activated monocytes/macrophages. We have recently found that Fas

transmits activation signals in normal human monocytes and macrophages under conditions in which apoptosis is inhibited (unpublished observations), strengthening the idea that Fas activation can directly lead to monocyte/macrophage activation and induction of inflammation. The late recruitment of PMN after Fas activation stands in contrast to a similar murine model of intranasal lipopolysaccharide instillation, in which PMN recruitment occurred as early as 4 hours after lipopolysaccharide administration.³⁴ Thus, the main role of Fas ligand in the lung inflammatory response might be in contributing to sustaining inflammation, rather than triggering the initial, early response.

Our data suggest that the Fas/FasL system plays a more complex role in the inflammatory response and in the pathogenesis of lung injury than previously appreci-

Figure 6. H&E preparation and DNA end-nick-labeling assay from consecutive sections of the caudal lung lobe of a wild-type mouse 24 hours after intranasal instillation of an irrelevant control mAb. The H&E preparation shows normal lung architecture [original magnifications, $\times 100$ (**a**); $\times 400$ (**b**)], whereas the DNA nick-end-labeling assay shows no positive cells [original magnifications, $\times 100$ (**c**); $\times 160$ (**d**)]. Negative controls for the DNA nick-end-labeling assay are shown in **e** (original magnification, $\times 100$) and **f** (original magnification, $\times 160$).

ated. Apoptosis has traditionally been considered as a mechanism promoting resolution of inflammation.¹³ However, recent findings suggest that Fas activation can result in an inflammatory response because of the release of inflammatory cytokines.^{14,15} Studies by Miwa and colleagues¹⁶ demonstrated that membrane-bound FasL can induce PMN infiltration in mice. Furthermore, soluble FasL itself has been reported to serve as a chemotactic factor for human and murine PMN, but the mechanism of this effect remains uncertain.⁴⁸

The relative contribution of pro-apoptotic and pro-inflammatory activities is likely to depend on other mediators present in the microenvironment, as well as local concentrations of soluble FasL. Fas-induced PMN apoptosis is suppressed by granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-

stimulating factor (GM-CSF).⁶ During the early phase of ARDS, PMN apoptosis is inhibited by G-CSF and GM-CSF present in the airspaces.⁴⁹ During acute inflammation, the pro-apoptotic activity of FasL on PMN may be counteracted by G-CSF and GM-CSF, whereas its pro-inflammatory properties may contribute to PMN recruitment. However, epithelial cells, which express Fas but may lack corresponding anti-apoptotic receptors, undergo apoptosis which results in injury to the alveolar-epithelial barrier. Thus, the Fas/FasL system may serve a dual role in the pathogenesis of acute inflammatory injury by causing direct damage to the alveolar-epithelial barrier and by recruiting PMN to the site of injury.

In summary, the data show that Fas activation *in vivo* can lead to apoptosis of alveolar epithelial cells with changes in epithelial permeability, expression of inflam-

Figure 7. Electron micrographs from the lungs of a wild-type mouse 24 hours after intranasal instillation of Fas activating mAb Jo2, showing alveolar type II cells with very electron dense cytoplasm, and swollen mitochondria (M) with irregular cristae. In **b** the nuclear chromatin is condensed at the periphery of the nucleus. Some of the lamellar bodies (LB) are degranulating (arrows). N nucleus. Original magnification, $\times 13,500$.

matory cytokines, and recruitment of PMN into the alveoli. The data also show that epithelial injury precedes leukocyte infiltration when Fas is activated. These findings are consistent with a dual role for the Fas/FasL system, as both a pro-inflammatory and a pro-apoptotic system in the lungs. The findings suggest a new paradigm for some forms of acute lung injury in which alveolar epithelial death is the critical initial factor that initiates inflammatory responses in the lungs.

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