In vivo evidence for the role of GM-CSF as a mediator in acute pancreatitis-associated lung injury

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1Department of Surgery, Beth Israel Hospital Deaconess Medical Center, Harvard Medical School; 2Department of Adult Oncology, Dana-Farber Cancer Institute, Boston, Massachusetts 02115; and Divisions of 3Gastroenterology, 4Oncology, and 5Clinical Pathology, Geneva University Hospitals, 1211 Geneva 14, Switzerland

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FROSSARD, Jean Louis, Ashok K. Saluja, Nicolas Mach, Hong Sik Lee, Lakshmi Bhagat, Antoine Hadenque, Laura Rubbia-Brandt, Glenn Dranoff, and Michael L. Steer. In vivo evidence for the role of GM-CSF as a mediator in acute pancreatitis-associated lung injury. Am J Physiol Lung Cell Mol Physiol 283: L541–L548, 2002. First published May 3, 2002; 10.1152/ajplung.00413.2001.—Severe pancreatitis is frequently associated with acute lung injury (ALI) and the respiratory distress syndrome. The role of granulocyte-macrophage colony-stimulating factor (GM-CSF) in mediating the ALI associated with secretagogue-induced experimental pancreatitis was evaluated with GM-CSF knockout mice (GM-CSF−/−). Pancreatitis was induced by hourly (12×) intraperitoneal injection of a supramaximally stimulating dose of the cholecystokinin analog caerulein. The resulting pancreatitis was similar in GM-CSF−/− control animals and GM-CSF+/+ mice. Lung injury, quantitated by measuring lung myeloperoxidase activity (an indicator of neutrophil sequestration), alveolar-capillary permeability, and alveolar membrane thickness was less severe in GM-CSF−/− mice than in GM-CSF+/+ mice. In GM-CSF−/− mice, pancreas, lung and serum GM-CSF levels increase during pancreatitis. Lung levels of macrophage inflammatory protein (MIP)-2 are also increased during pancreatitis, but, in this case, the rise is less profound in GM-CSF−/− mice than in GM-CSF+/+ controls. Administration of anti-MIP-2 antibodies was found to reduce the severity of pancreatitis-associated ALI. Our findings indicate that GM-CSF plays a critical role in coupling pancreatitis to ALI and suggest that GM-CSF may act indirectly by regulating the release of other proinflammatory factors including MIP-2.

granulocyte-macrophage colony-stimulating factor; caerulein; inflammation; adult respiratory distress syndrome; adhesion molecules; neutrophils

ACUTE LUNG INJURY and the adult respiratory distress syndrome (ARDS) complicate many disease states and are central components of the systemic immune response syndrome (SIRS) (4, 17, 24). Although the mechanisms underlying these processes remain incompletely understood, the morphological and functional changes include sequestration of inflammatory cells within the lung (2, 7, 37), injury to the pulmonary microvascular surface (19), and extravasation of otherwise intravascular fluid across the microvascular endothelial barrier into the bronchoalveolar space (21). Acute lung injury and ARDS, along with other elements of SIRS, are frequently noted in patients with severe acute pancreatitis (30). Roughly 40% of patients who die during the early stages of severe pancreatitis succumb from lung injury and respiratory failure. Therefore a better understanding of the mechanism leading to lung injury is of importance.

Several models of acute pancreatitis in experimental animals have been shown to be accompanied by measurable lung injury (9, 14, 15, 37), and a number of recent studies have focused on identifying the factors that couple pancreatic inflammation to lung injury in those models (1, 2, 10, 26). In the current communication, we have utilized the secretagogue (caerulein)-induced model of acute pancreatitis (20) in mice to examine the role played by granulocyte-macrophage colony-stimulating factor (GM-CSF) in mediating acute lung injury in this model.

GM-CSF is a cytokine that promotes the growth of myeloid progenitor cells and the activation of mature neutrophils, eosinophils, and monocytes (23, 34). It also enhances complement- and antibody-mediated phagocytosis. GM-CSF as well as other proinflammatory cytokines, including tumor necrosis factor (TNF)-α and interleukin-1 (IL)-1, and chemokines, including macrophage inflammatory protein (MIP)-1α and MIP-2, have been reported to increase during numerous lung inflammatory conditions.

GM-CSF is synthesized and secreted by a wide variety of activated cells, including T lymphocytes, macrophages, lung epithelial cells, and cytokine-activated endothelial cells (8, 32). It has been shown to increase
neutrophil chemotaxis, upregulate the intercellular adhesion molecule CD11b/CD18 and enhance the functional activity of mature cells (12, 29, 35). GM-CSF is secreted by lung epithelial cells, and GM-CSF injections, to humans, have been shown to induce 1) an increased neutrophil count, 2) neutrophil activation, 3) neutrophil degranulation, and 4) the release of IL-8 (33) as well as MIP-2, both cytokines known to play a key role in acute lung injury (9). On the basis of these observations and the fact that GM-CSF is secreted by lung epithelial cells, we postulate that it might mediate lung injury in diseases such as acute pancreatitis and that it might carry out this role both directly and indirectly by regulating release of other, downstream, proinflammatory factors such as MIP-2.

In this study, we have found that GM-CSF and MIP-2 are upregulated in the lung during caerulein-induced acute pancreatitis. We have compared the severity of the pancreatic and lung injury noted when pancreatitis is induced in control animals to the severity of these injuries when pancreatitis is induced in animals that are genetically incapable of generating GM-CSF [GM-CSF-deficient (GM-CSF−/−) mice]. We report that the absence of GM-CSF does not significantly reduce the severity of caerulein-induced pancreatitis, but it markedly reduces the severity of pancreatitis-associated lung injury in this model. We have also noted that the rise in lung levels of MIP-2, the mouse neutrophil chemoattractant mediator that seems to be comparable to IL-8 in primates (16), is blunted when pancreatitis is induced in GM-CSF−/− animals and that the severity of pancreatitis-associated lung injury can be reduced by administration of anti-MIP-2 antibodies. These observations lead us to conclude that GM-CSF plays an important role in regulating the severity of pancreatitis-associated lung injury but not in regulating the severity of pancreatitis in the secretagogue-induced model. We suggest that GM-CSF may regulate the severity of lung injury directly by increasing inflammatory cell infiltration into the lung and indirectly by modifying the magnitude with which other inflammatory mediators such as MIP-2 (i.e., IL-8) are released from neutrophils during pancreatitis.

MATERIALS AND METHODS

All experiments were performed according to protocols approved by the Institutional Animal Care and Use Committee of the Beth Israel Deaconess Medical Center. Homozygous GM-CSF−/− and wild-type Black 6 GM-CSF-sufficient mice (GM-CSF+/+) were provided by Dr. G. Dranoff. Animal genotyping was done before each experiment. Thus each GM-CSF−/− mouse was shown to be null for GM-CSF before the start of each set of experiments. We generated the GM-CSF−/− animals by gene-targeting techniques in embryonal stem cells (23). They were shown to have normal baseline and stressed hematoipoiesis, i.e., the number of circulating hematopoietic cells and the progenitor cell levels in hematopoietic organs were similar to those in GM-CSF+/+ mice. Because GM-CSF−/− mice are known to develop surfactant accumulation in the alveolar space mimicking pulmonary alveolar proteinosis at older ages, we used mice 4 wk of age or younger in our studies. At that age, no histological lung abnormalities are detectable. Mice were bred and housed in standard cages in a climate-controlled room with an ambient temperature of 23 ± 2°C and a 12:12-h light-dark cycle. They were fed standard laboratory chow, given water ad libitum, and randomly assigned to experimental groups. All animals were fasted overnight before each experiment, but water was not withheld. Caerulein, the decapeptide analog of the pancreatic secretagogue cholecystokinin, was purchased from Research Plus (Bayonne, NJ). Monoclonal antibodies to MIP-2 were obtained from R&D Systems (Abingdon, UK). All other chemicals and reagents were from sources noted or previously reported (28, 36).

Induction of pancreatitis. Randomly chosen male and female GM-CSF+/+ and GM-CSF−/− mice weighing 20–22 g were given hourly (12×) intraperitoneal injections (0.2 ml) containing caerulein at a concentration calculated to deliver a dose of 50 μg/kg with each injection. Control animals received 0.2-ml hourly injections of saline. For most determinations, two sets of experiments were performed. In the first, the animals were killed by administration of a lethal dose of pentobarbital 1 h after receiving the final caerulein or saline injection (i.e., 12 h after the first injection of caerulein or saline), whereas, in the second set of experiments, the mice were killed either 1 or 24 h after the start of caerulein administration.

Anti-MIP-2 treatment. For experiments using anti-MIP2 antibody, 60 min before the start of caerulein administration, caerulein-treated animals received a single intravenous injection containing 5 mg/kg anti-MIP-2 antibody dissolved in 0.2 ml of saline (pH 7.4) as previously reported by others (8).

Quantitation of pancreatitis severity. We evaluated the severity of pancreatitis by quantitating serum amylase activity, sequestration of neutrophils within the pancreas (i.e., pancreas myeloperoxidase (MPO) activity), pancreatic edema (i.e., pancreatic water content), and morphological evidence of pancreatic acinar cell necrosis. Serum amylase activity was measured, in samples obtained at the time of death, as described by Pierre et al. (27) using 4,6-ethylidine (G1)−p-nitrophenyl (G1)−d-maltoheptaside (Sigma, St. Louis, MO) as the substrate. Pancreas MPO activity was measured as previously described (10) and expressed as a function of tissue wet weight. We quantitated pancreatic water content by weighing freshly obtained blotted samples and then weighing those samples after dessication (95°C, 12 h). The difference between wet and dry weight (i.e., water content) was calculated and expressed as a percentage of tissue wet weight. For morphological examination of the pancreas, samples obtained at the time of death were immediately fixed, embedded in paraffin, and sectioned (5 μm). They were stained with hematoxylin-eosin and examined by an experienced morphologist who was not aware of the sample identity. The extent of acinar cell necrosis was quantitated by computer-assisted morphometry as previously described (1) and expressed as a percentage of total acinar tissue.

Quantitation of lung injury severity. We evaluated the severity of pancreatitis-associated lung injury by quantitating lung water content as described above for pancreas water content, measuring the sequestration of neutrophils within the lung (i.e., lung MPO activity), evaluating capillary-alveolar membrane thickness, and characterizing pulmonary microvascular permeability (i.e., leakage of intravenously administered FITC-labeled albumin into the bronchoalveolar space). Lung MPO activity was measured, on samples obtained at the time of death, as described in Induction of pancreatitis and previously (13). MPO activity was expressed as a function of lung wet weight. For estimation of capillary-
alveolar membrane thickness, we distended the lung at the time of death by instilling 4% neutral buffered formalin at a hydrostatic pressure of 20 cmH2O. Portions of the formalin-
distended lungs were harvested, fixed, paraffin-embedded, sectioned (5 μm), stained with hematoxylin-eosin, and examined by a morphologist who was not aware of the sample identity. Capillary-alveolar membrane thickness was visually estimated on a scale ranging from 0 to 4, with 0 being the thinnest and 4 the thickest as observed in the course of these experiments. Leakage of intravenously administered FITC-labeled albumin into the bronchoalveolar space was evaluated as previously described (30). The ratio of FITC fluorescence in bronchoalveolar lavage fluid and blood was calculated and expressed as the permeability index.

**GM-CSF immunolocalization within pancreas and lung.**

Immunolocalization of GM-CSF was characterized by conventional light microscopy on cryostat sections from pancreas and lung samples taken from GM-CSF +/+ mice given either saline or caerulein injections. We performed immunohistochemical staining using primary antibodies to mouse GM-CSF (rat anti-mouse GM-CSF, dilution 1:50; Pharmingen) diluted in BSA-PBS. Briefly, frozen sections were mounted on silane-coated glass slides and pretreated with levamisole to block endogenous alkaline phosphatase activity. Negative control sections were incubated in BSA-PBS (without primary antibody) and showed no immunostaining. After incubation for 1 h at room temperature with the diluted primary antibodies, the sections were sequentially treated with secondary antibody (rabbit anti-rat IgG 1:100 in BSA-PBS) for 30 min, and reactions were revealed by the alkaline phosphatase complex method (Dako). Sections were counterstained with Mayer’s hematoxylin.

**Other assays.** Circulating leukocytes were quantitated with blood samples obtained 13 h after the start of saline or caerulein administration. For these measurements, total and differential cell counts were made with Giemsa-stained samples. We measured GM-CSF and MIP-2 levels in pancreas, serum, and lung with commercially available ELISA kits according to the manufacturers’ instructions (R&D Systems, Abingdon, UK).

**Analysis of data.** The results reported in this communication represent means ± SE values obtained from multiple determinations in three or more experiments for each set. In Figs. 2–4 and 6, vertical bars denote SE values, and the absence of such bars indicates that the SE is too small to illustrate. The significance of changes was evaluated by Student’s t-test when data consisted of only two groups or by ANOVA when three or more groups were compared. For comparison of capillary-alveolar thickness measurements, Wilcoxon’s nonparametric test was done using SPSS software package.

**RESULTS**

**Effects of secretagogue-induced pancreatitis on GM-CSF levels.** The levels of GM-CSF in pancreas, serum, and lung of GM-CSF +/+ animals 12 h after the start of caerulein administration are shown in Table 1. Within 12 h of the start of caerulein administration, pancreas GM-CSF levels are slightly, but significantly, elevated. GM-CSF levels in serum are elevated to an even greater extent, whereas those in lung exceed even those noted in serum.

The identity of pancreas and lung cells expressing GM-CSF during caerulein-induced pancreatitis was evaluated by immunolocalization using anti-GM-CSF antibody (Fig. 1). GM-CSF was not detected in either tissue when the primary antibody was omitted (not

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<th>Control</th>
<th>Caerulein</th>
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<tr>
<td>Pancreas</td>
<td>6.5 ± 3</td>
<td>13 ± 1*</td>
</tr>
<tr>
<td>Lung</td>
<td>110.6 ± 29</td>
<td>880.1 ± 41*</td>
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<tr>
<td>Serum</td>
<td>28.3 ± 17</td>
<td>437.4 ± 33*</td>
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Results represent means ± SE values for 5 or more animals in each group. Mice were given 12 hourly injections of caerulein (50 μg/kg) or saline and killed 1 h later. Granulocyte-macrophage colony-stimulating factor (GM-CSF) levels were quantitated by ELISA. *P < 0.05 compared with control values.

![Fig. 1. Granulocyte-macrophage colony-stimulating factor (GM-CSF) immunolocalization within pancreas and lung.](http://ajplung.physiology.org/)

Cryostat sections were treated as described in MATERIALS AND METHODS. The presence of GM-CSF in the pancreas from either control animals or animals receiving caerulein was not detected in the acinar cells (A), but there were some inflammatory cells positive for GM-CSF (arrows, B). Bronchial epithelial cells and macrophages in the lungs from control animals (C) were positive for GM-CSF. In animals given caerulein, the number of immunopositive cells as well as the staining intensity for GM-CSF increased significantly compared with controls (D). Magnification ×400. Pictures are representative of at least 3 experiments.
administration, total circulating leukocyte counts had increased more than threefold in the GM-CSF +/+ group. A nearly identical rise was noted at this time in the GM-CSF −/− group. The rise in circulating leukocyte count in both groups was similar and attributable to an increase in circulating neutrophil and nonneutrophil leukocytes (Fig. 2). Twelve hours later, i.e., 12 h after administration of the last dose of caerulein, the total circulating leukocyte count in both the GM-CSF +/+ and GM-CSF −/− groups had fallen to a similar extent toward, but not reaching, the precaerulein level (not shown).

Effects of GM-CSF deletion on the severity of caerulein-induced pancreatitis. The severity of caerulein-induced pancreatitis was evaluated 12 h after the start of caerulein administration (Fig. 3). At this time, a marked and similar increase in serum amylase activity, pancreatic water content, and extent of acinar cell necrosis was noted in both the GM-CSF +/+ and GM-CSF −/− groups. Pancreas MPO activity was also markedly increased in both groups, but, in this case, the rise noted in the GM-CSF −/− group was significantly less than that noted in the GM-CSF +/+ group.

Effects of GM-CSF deletion on the severity of pancreatitis-associated lung injury. Guice et al. (14) have previously noted that, in the secretagogue-induced model of pancreatitis, pancreatic injury precedes the development of lung injury. For that reason, we evaluated the effect of GM-CSF deletion on the severity of pancreatitis-associated lung injury both 12 and 24 h after the start of caerulein administration (Fig. 4). We evaluated lung injury by quantitating neutrophil sequestration in the lung (i.e., lung MPO activity) and pulmonary microvascular permeability (i.e., leakage of intravenously administered FITC-labeled albumin into the bronchoalveolar fluid). In the GM-CSF +/+ group, lung MPO activity was increased at 12 and 24 h after

![Graph](http://ajplung.physiology.org/)
the start of caerulein administration, and this rise in lung MPO activity was markedly blunted in the GM-CSF −/− group at both times. Pulmonary microvascular permeability was also markedly increased both 12 and 24 h after the start of caerulein administration in the GM-CSF +/+ group, and, at both times, a marked blunting of this response was noted in the GM-CSF −/− group. Capillary-alveolar membrane thickness was slightly increased 12 h after the start of caerulein administration, and this parameter of lung injury was more markedly increased 24 h after the start of caerulein administration (Figs. 4 and 5). At this later time, the changes noted in the GM-CSF −/− animals are markedly reduced in the GM-CSF +/+ animals.

Fig. 4. Effects of GM-CSF deficiency on the severity of secretagogue-induced pancreatitis-associated lung injury. Animal groups and bar description are as described in Fig. 3 legend. Lung MPO (A), lung permeability index (B), lung water content (C) and alveolar thickness (D) were quantitated as described in MATERIALS AND METHODS. FITC-labeled albumin was administered intravenously 2 h before animals were killed, and leakage of FITC-labeled albumin into the bronchoalveolar space was quantitated as described in Quantitation of lung injury severity. Results shown are means ± SE for 10 animals in each group. *P < 0.01 when GM-CSF −/− animals were compared with GM-CSF +/+ animals.

Fig. 5. Lung morphology. GM-CSF +/+ mice were given either saline (A) or caerulein (B and C) for 12 h. They were killed 12 (B) or 24 h (C) after the start of caerulein administration. Identical studies were performed on GM-CSF −/− mice given either saline (D) or caerulein (E and F), which were killed 12 (E) or 24 h (F) after the start of caerulein administration. Alveolar hemorrhage was more pronounced 12 h after the start of caerulein in GM-CSF +/+ mice (B) than in GM-CSF −/− mice (E), and thickening of the alveolar membrane was dramatically reduced in −/− mice at either 12 (E) or 24 h (F) compared with GM-CSF +/+ mice (B and C). Magnification is ×150 for lung.
MIP-2 level was markedly blunted in the GM-CSF group compared with that observed in the GM-CSF knockout mice (GM-CSF −/−).

**Effect of GM-CSF deletion on MIP-2 levels.** Twelve hours after the start of caerulein administration, pancreas, serum, and lung MIP-2 levels in GM-CSF +/+ animals were markedly elevated (Table 2). A similar rise in pancreas MIP-2 level was observed in the GM-CSF −/− group, but the rise in both serum and lung MIP-2 level was markedly blunted in the GM-CSF −/− group compared with that observed in the GM-CSF +/+ animals.

**Effects of anti-MIP2 treatment on caerulein-induced lung injury.** As shown in Fig. 6, anti-MIP-2 antibody administration in mice receiving caerulein resulted in a significant decrease of all parameters that characterize the severity of pancreatitis-associated lung injury.

**DISCUSSION**

Acute lung injury, ARDS, and respiratory failure are clinically important complications of severe pancreatitis. They account for significant morbidity and mortality in patients with this disease (31). Recent studies, most of which have employed experimental models of pancreatitis, have indicated that a number of inflammatory factors regulate the coupling of pancreatic to lung injury (9, 26). Those studies have shown that neutrophils (2, 10), T lymphocytes (10, 22), adhesion molecules (10), platelet-activating factor (18), TNF-α (13, 14), substance P (1), and chemokines acting via the CCR-1 receptor (13) are each involved in this process.

The currently reported studies have examined the role of GM-CSF in regulating the severity of pancreatitis-associated lung injury. For these studies, the secretagogue-induced model of pancreatitis in mice was used, and genetically modified animals unable to generate GM-CSF were employed to evaluate the role of GM-CSF.

GM-CSF is a proinflammatory cytokine that is synthesized by several types of cells, including monocytes, T lymphocytes (5, 10, 34), lung epithelial cells (23), activated eosinophils (25), and cytokine-activated endothelial cells in vitro (8). It promotes the growth and maturation of granulocyte and monocyte progenitors and the activation of mature neutrophils, eosinophils, and monocytes (34). It primes neutrophils, making them more receptive to activation by secondary stimuli (3, 33). GM-CSF has been shown to increase neutrophil chemotaxis, upregulate the expression of intracellular adhesion molecule CD11b/CD18 (22), and enhance the functional activity of mature cells (35). In humans, injection of GM-CSF has been shown to activate and degranulate neutrophils and to cause release of IL-8 (33).

The studies reported in this communication indicate that pancreas and serum GM-CSF levels are moder-
ately increased during caerulein-induced pancreatitis. Of perhaps more importance, however, is our observation that lung GM-CSF levels are even more markedly increased during caerulein-induced necrotizing pancreatitis (Table 1).

As shown in Fig. 3, caerulein administration to both GM-CSF +/+ and GM-CSF −/− mice results in severe pancreatitis, which is characterized by marked and similar increases in serum amylase levels, pancreatic edema, and acinar cell necrosis in both animal groups. Pancreas MPO activity, an index of the extent of neutrophil sequestration in the pancreas, is also increased in both groups, but, in this case, the increase noted in the GM-CSF −/− group is less marked than that noted in the GM-CSF +/+ group. The significance of this isolated difference among the various parameters used to quantitate the severity of pancreatitis is not known, but it may reflect the recognized importance of GM-CSF in the process of neutrophil activation and the previously reported observation that neutrophil-independent events play pivotal roles in regulating the severity of pancreatic injury (10, 13).

In contrast to its marginal effect on the pancreatic injury in the caerulein model, the lack of GM-CSF in the GM-CSF −/− group of mice markedly reduces the severity of pancreatitis-associated lung injury. Twelve hours after the start of caerulein administration, the increase in lung MPO activity, as well as the increase in pulmonary microvascular permeability that occurs in the GM-CSF +/+ group, is markedly depressed in the GM-CSF −/− group of animals. The protection against increased microvascular permeability that accompanies GM-CSF deletion persists for at least an additional 12 h, and 24 h after the start of caerulein administration, GM-CSF −/− mice have much less capillary-alveolar membrane thickening than do the GM-CSF +/+ animals.

Together, these observations indicate that GM-CSF plays an important proinflammatory role in regulating the severity of pancreatitis-associated lung injury but that it does not play a critical role in regulating the severity of the pancreatic injury. This action of GM-CSF does not appear to reflect its known ability to regulate the growth and maturation of inflammatory cells systemically, since circulating leukocyte and neutrophils counts are similar in GM-CSF +/+ and GM-CSF −/− animals both before and after caerulein administration has induced pancreatitis-associated lung injury.

It is tempting, therefore, to speculate that GM-CSF exerts its proinflammatory effect on pancreatitis-associated lung injury by upregulating inflammatory cell function rather than number. Support for this speculation comes from our findings that the rise in lung MIP-2 levels seen during pancreatitis-associated lung injury in GM-CSF +/+ animals is substantially blunted in GM-CSF −/− mice and that the severity of pancreatitis-associated acute lung injury in GM-CSF +/+ animals can be reduced by administration of anti-MIP-2 antibodies. MIP-2 is believed by most observers to represent the mouse equivalent of IL-8, a potent neutrophil chemotactic cytokine, but the equivalence of MIP-2 and IL-8 is still speculative. However, our studies using anti-MIP-2 antibodies now provide strong evidence for the central role played by MIP-2 in translating pancreatic inflammation to the lung. Further support for our suggestion that GM-CSF promotes pancreatitis-associated lung injury via its effect on neutrophil function comes from recent observations, by others, indicating that GM-CSF levels rise in other models of lung injury (5) and that GM-CSF exerts a potent antiapoptotic effect on neutrophils in vitro in areas of inflammation (25). Presumably, the delay in neutrophil apoptosis induced by local generation of GM-CSF might favor persistence and worsening of the local (i.e., pulmonary in this case) inflammatory process, a feature recently suggested by O’Neill et al. (25) in neutrophils from patients with acute pancreatitis. Regardless of the mechanisms responsible for the proinflammatory effects of GM-CSF in pancreatitis-associated lung injury, the observations reported in this communication suggest that therapies designed to reduce or abort the rise in lung GM-CSF or prevent its signaling during pancreatitis may prove beneficial in reducing the severity of pancreatitis-associated lung injury.

One might speculate that the effects of GM-CSF deficiency noted in our studies might be due to the lifelong absence of GM-CSF in our knockout mice and not the absence of an acute GM-CSF-mediated response. The use of knockout mice devoid of active pro- or anti-inflammatory mediators allows examination of the effects of a specific cytokine without the drawbacks induced by pharmacological manipulations (26), but one must keep in mind that the specific mutation has been present in the mouse from the time of its conception. This may result in phenotypic changes due to the mutation itself, but it may also result in changes caused by adaptation to and compensation for the mutation (26). This latter possibility would seem unlikely, however, in our studies, in view of our observation that deletion of GM-CSF alters neither lung morphology nor circulating leukocyte levels at 3 mo of age.

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REFERENCES


L548 GM-CSF AND PANCREATITIS-ASSOCIATED LUNG INJURY


