Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice

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Aerosolized GM-CSF ameliorates pulmonary alveolar proteinosis in GM-CSF-deficient mice. Am. J. Physiol. 276 (Lung Cell. Mol. Physiol. 20): L556–L563, 1999.—Surfactant proteins and phospholipids accumulate in the alveolar spaces and lung tissues of mice deficient in granulocyte-macrophage colony-stimulating factor (GM-CSF), with pathological findings resembling the histology seen in the human disease pulmonary alveolar proteinosis (PAP). Previous metabolic studies in GM-CSF-deficient [GM(-/-)] mice indicated that defects in surfactant clearance cause the surfactant accumulation in PAP. In the present study, GM(-/-) mice were treated daily or weekly with recombinant mouse GM-CSF by aerosol inhalation or intraperitoneal injection for 4–5 wk. Lung histology, alveolar macrophage differentiation, and surfactant protein B immunostaining returned toward normal levels in the GM-CSF aerosol-treated mice. Alveolar and lung tissue saturated phosphatidylcholine and surfactant protein B concentrations were significantly decreased after treatment with aerosolized GM-CSF. Cessation of aerosolized GM-CSF for 5 wk resulted in increased saturated phosphatidylcholine pool sizes that returned to pretreatment levels. In contrast, PAP did not improve in GM(-/-) mice treated daily for 5 wk with larger doses of systemic GM-CSF. Aerosolized GM-CSF improved PAP in the GM(-/-) mice, demonstrating that surfactant homeostasis can be influenced by local administration of GM-CSF to the respiratory tract.

granulocyte-macrophage colony-stimulating factor; surfactant; surfactant proteins; alveolar macrophage

GRANULOCYTE-MACROPHAGE colony-stimulating factor (GM-CSF) is a hematopoietic growth factor required for pulmonary surfactant homeostasis (5, 26). Although gene-targeted disruption of either the GM-CSF or the GM-CSF-receptor common β-subunit (βc) locus in mice failed to perturb hematopoiesis, surfactant proteins (SPs) and phospholipids accumulated in the alveolar spaces (5, 20, 24, 26). Lung histopathology in GM-CSF-deficient [GM(-/-)] and βc-deficient mice resembled that of the human disease known as pulmonary alveolar proteinosis (PAP). Pulmonary surfactant is a complex mixture of phospholipids and SP-A, -B, -C, and -D, which are synthesized and secreted primarily by alveolar type II epithelial cells (29). Approximately 80% of alveolar surfactant is cleared by type II cells, which recycle or catabolize SPs and lipids; most of the remaining 20% of surfactant is catabolized by macrophages (23, 30). Metabolic studies demonstrated that decreased surfactant clearance in GM(-/-) mice contributed to a progressive accumulation of surfactant (15, 22).

GM-CSF was selectively expressed in the lungs of the GM(-/-) mouse with pulmonary epithelial cell-specific promoter sequences of the human SP-C gene (11). Expression of the SP-C-GM-CSF (SPC-GM) transgene restored surfactant lipid and protein clearance and corrected the PAP typical of GM(-/-) mice (11, 14). Correction of PAP with the SPC-GM transgene was associated with increased numbers and altered histology of alveolar macrophages and type II cells, consistent with the concept that local production of GM-CSF influences differentiation and/or function of these pulmonary cell types (12).

Alteration of GM-CSF production or response has been recently associated with PAP in humans. Alveolar macrophages from a patient with PAP synthesized GM-CSF mRNA when stimulated with lipopolysaccharide in vitro but, unlike normal alveolar macrophages, did not release GM-CSF into the medium (27). Seymour et al. (25) demonstrated that a daily subcutaneous injection of GM-CSF improved oxygen saturation and exercise tolerance in one patient with acquired PAP, suggesting that therapy with GM-CSF may be useful for treatment of PAP.

In the present study, GM(-/-) mice were treated with injected or inhaled recombinant mouse GM-CSF. Analyses of lung tissues and bronchoalveolar lavage (BAL) fluid demonstrated that aerosolized GM-CSF significantly inhibited the progression of PAP in GM(-/-) after 4–5 wk of daily treatment, whereas neither weekly aerosol treatments nor daily intraperitoneal injections of GM-CSF altered PAP.

METHODS

Animals. The GM(-/-) mice used were generated by targeted ablation of the GM-CSF gene locus as previously described (5). The GM(-/-) mutation was maintained in a C57BL/6/J background. Mice were bred and housed in microisolator cages and required no special care. Equal numbers of male and female mice were randomly divided into groups for aerosol inhalation or intraperitoneal injection of GM-CSF (Table 1). Mice were 6 wk old when treatments were initiated.

Aerosol treatment. Mice were placed in 50-ml conical centrifuge tubes with a hole in the tip and ventilation holes along the sides of each tube. A 4-liter container with lid was modified by drilling a 1.25-inch-diameter hole in one end to serve as an inlet and four 0.75-inch-diameter holes in the rear of the box for an outlet. Mice were positioned in the container to ensure exposure of the nasal area to the aerosol. A Puritan Bennett model US-1 clinical ultrasonic nebulizer was used to

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generate the aerosol at a flow rate of 1.5 l/min. Treatment time for each group was ~ 45 min.

Administration of aerosolized GM-CSF. To determine the volume of aerosol reaching the lungs of GM (−/−) mice, 100 µCi of a technetium-sulfur colloid in 12 ml of saline were nebulized to a group of five mice. Recovery of radioactivity was measured in the lung parenchyma to estimate the dosage of GM-CSF delivered by the aerosol. The volume of solution deposited in the lungs was ~ 1.5 µl in 45 min. Recombinant mouse GM-CSF was kindly provided by Immunex (Seattle, WA). The vehicle solution contained 0.1% mouse albumin fraction V from Sigma Diagnostics (St. Louis, MO) in 0.9% saline. GM-CSF was diluted in vehicle in a total volume of 12 ml immediately before nebulization. Mice treated for 4 wk inhaled an aerosol solution containing 1.3 µg/ml of GM-CSF, thus receiving an estimated dose of ~ 2 ng/mouse (equivalent to 0.07 ± 0.01 µg/kg), administered 5 days/wk. Mice treated for 5 wk inhaled an aerosol solution containing 2.6 µg/ml of GM-CSF, receiving an estimated dose of ~ 4 ng/mouse (equivalent to 0.14 ± 0.02 µg/kg), administered either 1 or 5 days/wk for 5 wk. Control mice received aerolized vehicle 5 days/wk for 5 wk. All mice described above were killed for analysis 48 h after the final treatment. Three groups of mice treated with aerosolized GM-CSF or vehicle for 5 wk were analyzed 5 wk after completion of the aerosol treatments.

Intraperitoneal injection of GM-CSF. GM-CSF was diluted in 1% serum-saline vehicle to a final concentration of 2 µg/ml. The serum used to prepare the vehicle was collected from untreated GM (−/−) mice and pooled. Mice were injected intraperitoneally with 100 µl of GM-CSF solution (200 ng of GM-CSF, equivalent to a dose of ~ 8 µg/kg body wt) or vehicle 5 days/wk for 5 wk.

Intratracheal injection of GM-CSF. GM (−/−) and GM-CSF-positive [GM (+/−)] mice were briefly anesthetized with inhaled methoxyflurane (Metofane, Pitman-Moore, Mundelein, IL) and intratracheally intubated with a 24-gauge animal feeding needle (Popper & Sons, New Hyde Park, NY). Three daily treatments of 15 ng of GM-CSF (equivalent to 6.3 µg/kg body wt) in 100 µl of saline or saline alone were instilled into the lungs via the intratracheal needle. On the third day of treatment, 0.1 µCi of 125I-labeled SP-C was added to the GM-CSF injection solution. Recombinant SP-C (Byk Gulden, Constance, Germany) was iodinated with 125I-labeled Bolton-Hunter reagent (ICN, Irvine, CA) as previously described (13). All mice were killed at 40 h after the final intratracheal injection. The lungs were lavaged and homogenized. 125I-SP-C content in BAL and lung homogenate samples was counted, and saturated phosphatidylcholine (Sat PC) pool sizes were measured as previously described (13, 15).

Processing and staining of tissues for histopathology. Mice were anesthetized with intraperitoneal pentobarbital sodium. The lungs were inflation fixed with 4% paraformaldehyde in phosphate-buffered saline (PBS), pH 7.4, for 24 h as previously described (11). The tissues were then washed in PBS, dehydrated in a series of alcohols, and embedded in paraffin. Hematoxylin and eosin staining was used for histo pathological analysis of paraffin sections of the lung, liver, and spleen.

Immunohistochemistry. Paraffin sections (5 µm) sampling all five lobes of lung tissue were stained for SP-B in each mouse. Anti-SP-B, a rabbit anti-bovine polyclonal antibody that recognizes mature SP-B, was detected with the Vectastain ABC anti-rabbit immunohistochemical horseradish peroxidase kit from Vector Laboratories (Burlingame, CA) as previously described (1). The tissues were counterstained with Tris-cobalt and nuclear fast red and qualitatively assessed for relative SP-B immunostaining. In addition, 5 random fields in 3 vehicle-treated and 5 GM-CSF-treated mice were examined under high magnification ( × 40), and 31-56 alveoli were counted per field. Alveoli with and without immunostained proteinosis material were counted to determine the percentage of alveoli containing stained material.

BAL. The trachea was cannulated, and the lungs were washed three times with five 1-ml aliquots of PBS (15). BAL fluids for each animal were pooled, measured for volume, and divided into aliquots for phospholipid or protein measurement. The lung tissue was homogenized for Sat PC measurement as previously described (15). SP-B was measured by

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**Table 1. GM-CSF treatment groups**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>Dose of GM-CSF</th>
<th>Mode of Delivery</th>
<th>Treatment Schedule</th>
<th>Treatment Period</th>
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<td>Daily</td>
<td>4 wk</td>
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<td>Aerosol</td>
<td>Daily</td>
<td>4 wk</td>
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<tr>
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<td>Daily</td>
<td>5 wk</td>
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<tr>
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<td>Aerosol</td>
<td>Daily</td>
<td>5 wk</td>
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<tr>
<td>GM-CSF 4 ng</td>
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<td>Weekly</td>
<td>5 wk</td>
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<tr>
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<td>Aerosol</td>
<td>Weekly</td>
<td>5 wk</td>
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<tr>
<td>GM-CSF 4 ng</td>
<td>Aerosol</td>
<td>Daily</td>
<td>5 wk + 5-wk rest</td>
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<tr>
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<td>Aerosol</td>
<td>Daily</td>
<td>5 wk + 5-wk rest</td>
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<tr>
<td>GM-CSF 200 ng</td>
<td>ip injection</td>
<td>Daily</td>
<td>5 wk</td>
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<tr>
<td>Vehicle 0</td>
<td>ip injection</td>
<td>Daily</td>
<td>5 wk</td>
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GM-CSF, granulocyte-macrophage colony-stimulating factor; daily, 5 days/wk; weekly, 1 day/wk; ip, intraperitoneal; it, intratracheal.
enzyme-linked immunosorbent assay (ELISA) with bovine SP-B as a standard as previously described (17). The total alveolar pool size was calculated and divided by body weight. Replicate SP-B concentrations for each sample are expressed as a percentage of the control values measured on the same ELISA plate to control for plate-to-plate variability.

α-Naphthyl acetate esterase detection. Cytospins of BAL cells were prepared from an aliquot of BAL fluid and stained for α-naphthyl acetate esterase (α-NAE) activity with an α-NAE staining kit (Sigma Diagnostics, St. Louis, MO) according to the protocol provided in the package insert. Differential cell counts of at least 250 cells/slide were performed to determine the percentages of stained and unstained alveolar macrophages.

Statistical analysis. Values are expressed as means ± SE of n observations. Statistical analyses of the data were performed by ANOVA or χ² test; P < 0.01 was considered significant.

RESULTS

Aerosolized GM-CSF ameliorated PAP. Histological examination of the lungs from GM(−/−) mice treated with aerosolized GM-CSF for 4 or 5 wk demonstrated a marked improvement in PAP compared with that in vehicle-treated mice (Fig. 1). Improvement in PAP was observed after treatment for 4 wk with 2 ng or 5 wk with 4 ng of GM-CSF daily. However, aerosol treatments were consistently more effective in the mice receiving the higher concentration of GM-CSF daily for 5 wk. Perivascular and peribronchiolar mononuclear cell infiltrates were also decreased in mice treated daily with aerosolized GM-CSF. PAP was not improved in GM(−/−) mice treated 1 day/wk with 4 ng of GM-CSF for 5 wk; histology of these lungs resembled that in vehicle-treated or untreated GM(−/−) control mice (data not shown).

The alveolar spaces of untreated GM(−/−) mice contained abundant SP-B staining of PAP material as previously described (5, 11, 12). SP-B staining in Fig. 2. Surfactant protein (SP) B immunostaining decreased with aerosolized GM-CSF. GM(−/−) mice were treated with 2 ng of aerosolized GM-CSF daily for 4 wk (n = 2; A), 4 ng daily for 5 wk (n = 5; B), or vehicle alone (n = 4; C). Lung sections were stained with antibodies to SP-B. SP-B immunostaining decreased in mice treated for 4 wk (A) but was markedly decreased with 5 wk of daily aerosolized GM-CSF treatments (B). Mice treated weekly with 4 ng of aerosolized GM-CSF for 5 wk (data not shown) did not differ from mice treated with vehicle alone (C). Original magnification, ×140.

SP-B decreased in bronchoalveolar lavage (BAL) fluid from mice treated with aerosolized GM-CSF daily for 4 wk (n = 3), 4 ng daily or weekly for 5 wk (n = 6), or vehicle alone for 5 wk (n = 6). SP-B was measured in BAL fluid collected from treated mice and compared with that from untreated wild-type mice. Solid bars, means. Although SP-B levels did not reach range measured in untreated wild-type mice, SP-B was significantly decreased in mice treated daily with aerosolized GM-CSF. Concentration of SP-B in BAL fluid from mice treated weekly with GM-CSF did not differ from that in mice treated with vehicle alone. *P < 0.004 and **P < 0.001 compared with daily vehicle-treated GM(−/−) mice.

Saturated phosphatidylcholine (Sat PC) alveolar and tissue pool sizes were measured in aerosolized GM-CSF- and vehicle-treated mice (n = 6). Sat PC alveolar and tissue pool sizes decreased in mice treated with aerosolized GM-CSF daily for 5 wk (hatched bars) but not with weekly GM-CSF (open bars) compared with mice treated with vehicle alone (solid bars). Decreases in pool sizes of GM-CSF-treated GM(−/−) mice were significant compared with those in vehicle-treated GM(−/−) mice, *P < 0.001.
GM(−/−) mice treated daily with aerosolized vehicle did not differ from that in untreated GM(−/−) mice. In contrast, SP-B staining was markedly decreased in the alveolar spaces of lung tissue sections from GM(−/−) mice receiving daily aerosolized GM-CSF (Fig. 2). Only scant, scattered SP-B staining was observed in the alveolar spaces of aerosolized GM-CSF-treated GM(−/−) mice, whereas cell-associated staining for SP-B appeared to be increased. The number of alveoli containing SP-B-immunostained proteinosis material was counted in lung sections from mice treated with daily aerosolized GM-CSF or vehicle; the percentage of alveoli containing immunostaining was 9.6 ± 1.5% in GM-CSF-treated mice compared with 42.3 ± 2.6% in vehicle-treated mice (data not shown). Consistent with the histological assessment of PAP by hematoxylin and eosin staining, mice treated for 5 wk with 4 ng of GM-CSF had less alveolar SP-B staining than mice treated for 4 wk with 2 ng of GM-CSF. Intense SP-B staining was observed in the alveolar spaces of GM(−/−) mice treated 1 day/wk with aerosolized GM-CSF (data not shown), these lungs being indistinguishable from those of vehicle-treated or untreated GM(−/−) mice.

SP-B concentrations decreased significantly in mice treated with daily aerosolized GM-CSF (Fig. 3). The SP-B concentration in BAL fluid from mice treated daily for 4 wk with aerosolized GM-CSF (2 ng) was 63 ± 5% (SE) of that from mice treated with vehicle. SP-B was observed in 60% of alveolar macrophages from GM-CSF-treated mice daily for 5 wk with GM-CSF (4 ng) was 23 ± 3% of that from mice treated with vehicle. BAL fluid SP-B concentrations in mice treated weekly with aerosolized GM-CSF were unchanged, being 105 ± 5% of that in GM(−/−) mice treated with vehicle alone. SP-B concentrations in BAL fluid from GM-CSF-treated mice remained approximately four- to fivefold higher than those recovered from untreated wild-type mice.

Sat PC, the major component of surfactant, was measured in mice treated with GM-CSF for 5 wk (Fig. 4). Daily aerosolized GM-CSF (4 ng) significantly decreased alveolar and lung tissue Sat PC pool sizes in GM(−/−) mice compared with that in GM(−/−) mice treated daily with vehicle. However, alveolar and lung Sat PC pool sizes in GM-CSF-treated mice remained approximately fivefold and twofold higher, respectively, than levels measured in untreated wild-type mice (data not shown). Treatment with aerosolized GM-CSF 1 day/wk did not improve Sat PC pool sizes in the GM(−/−) mice.

To investigate the effects of GM-CSF treatment on alveolar macrophage morphology, cytospins of BAL cells were prepared from mice treated with aerosolized GM-CSF for 5 wk. The number of cells from GM-CSF-treated mice did not differ from that recovered from vehicle-treated or untreated GM(−/−) mice (data not shown). The cells were stained for fluoride-resistant α-NAE activity, an indicator of tissue macrophage differentiation (18, 31). In untreated or vehicle-treated GM(−/−) mice, the majority of alveolar macrophages were enlarged, containing a foamy cytoplasm with abundant lipid-filled vacuoles (Fig. 5). Fluoride-resistant α-NAE staining was observed in only 11.3 ± 2.5% (SE) of alveolar macrophages from untreated vehicle-treated GM(−/−) mice. In contrast, significantly fewer of the alveolar macrophages recovered from GM-CSF-treated mice had the characteristic foamy, lipid-laden cytoplasm observed in vehicle-treated or untreated GM(−/−) mice, and 60.0 ± 4.6% (SE) of alveolar macrophages stained positively for fluoride-resistant α-NAE activity.

Recurrence of PAP after discontinuing GM-CSF. To investigate the reversibility of the effects of GM-CSF on PAP, mice were treated with GM-CSF (4 ng) aerosol for 5 wk and analyzed 5 wk after cessation of treatment. SP-B concentrations were higher than those measured immediately after 5 wk of aerosolized GM-CSF but remained lower than those in vehicle-treated or untreated age-matched control GM(−/−) mice (Fig. 6A). Immunostaining of SP-B in the alveolar spaces increased in GM(−/−) mice after the cessation of treatment, consistent with a recurrence of PAP (data not shown). Alveolar, tissue, and total lung Sat PC pool sizes increased in the lungs of GM(−/−) mice 5 wk after treatment with aerosolized GM-CSF was discontinued, reaching levels similar to those in vehicle-treated mice (Fig. 6B).

Systemic GM-CSF did not improve PAP. Systemic administration of GM-CSF had no effect on PAP in GM(−/−) mice. Histological analyses and anti-SP-B

Fig. 5. Alveolar macrophage morphology was altered with aerosolized GM-CSF. Cytospins of BAL cells were stained for α-naphthyl acetate esterase (α-NAE) activity with fluoride inhibition. Normal alveolar macrophages from wild-type mice retained high levels of staining for α-NAE activity in presence of fluoride (data not shown). Fluoride-resistant α-NAE staining was observed in 60% of alveolar macrophages from GM(−/−) mice treated with 4 ng of aerosolized GM-CSF daily for 5 wk (left) compared with 11.3% in vehicle-treated mice (P < 0.001; right). In addition, fewer cells from GM-CSF-treated mice had foamy cytoplasm (arrowhead). Alveolar macrophages from vehicle-treated mice were similar to those from untreated GM(−/−) mice (data not shown), with enlarged foamy cytoplasm in most but not all cells (*). Original magnification, ×960.
immunostaining demonstrated widespread PAP in GM(-/-) mice injected intraperitoneally with 200 ng of GM-CSF daily for 5 wk, similar to findings in vehicle-treated or untreated GM(-/-) mice (Fig. 7). Likewise, SP-B concentrations measured by ELISA in BAL fluid and Sat PC pool sizes in mice treated with injected GM-CSF did not differ from vehicle-treated or untreated GM(-/-) mice (Fig. 8).

High doses of intratracheal GM-CSF for 3 days did not alter PAP in GM(-/-) mice. GM-CSF (15 ng) delivered directly to the lungs daily for 3 days did not alter Sat PC or the clearance of exogenous SP-C. At 40 h after the third and final treatment, Sat PC pool sizes in GM(-/-) and GM(+/+) mice treated with GM-CSF remained similar to those in mice treated with saline (Fig. 9A). Likewise, the 125I-SP-C recovery from treated and untreated GM(-/-) mice was unchanged (Fig. 9B). 125I-SP-C recovery from treated and untreated GM(+/+) control mice also did not differ.

**DISCUSSION**

Surfactant phospholipids and SPs, accompanied by enlarged, foamy alveolar macrophages and perivascular or peribronchiolar mononuclear cell infiltrates, accumulate in the lungs of GM(-/-) mice. Histological findings in GM(-/-) mice resemble the lung pathology seen in humans with PAP. The present study demonstrated the efficacy of inhaled GM-CSF for treatment of PAP. Mice were treated with aerosolized recombinant mouse GM-CSF daily for 4 or 5 wk or with systemic GM-CSF daily for 5 wk. PAP was improved in GM(-/-) mice receiving daily GM-CSF aerosol treatments but not in mice receiving larger doses of systemic GM-CSF. Additionally, intratracheal treatment of GM(-/-) mice for 3 days with 15 ng of GM-CSF daily did not alter phospholipid or SP-C clearance from the lungs.

The finding that aerosolized GM-CSF improves PAP in GM(-/-) mice supports the concept that GM-CSF influences surfactant homeostasis through local signaling pathways within the lung. Previous studies (3, 19) demonstrated that GM-CSF signaling in hematopoietic cells occurs through both the JAK-STAT and Ras-Raf pathways. STAT5A plays a role in GM-CSF-induced proliferation and gene expression in hematopoietic cells; however, PAP was not evident in STAT5A-deficient mice (7). The precise identity of the path-
way(s) involved in the resolution of PAP remains unclear, but it may regulate expression levels of genes encoding proteases or phospholipases required for surfactant catabolism in alveolar macrophages and type II cells. Clearance of surfactant from the alveolar spaces was markedly impaired in GM(−/−) mice and corrected by gene replacement in which GM-CSF was expressed at high levels in respiratory epithelial cells of the lung (14, 15). Alveolar macrophages and type II cells clear the majority of surfactant from the alveolar spaces and are known to express GM-CSF receptors (12). Thus GM-CSF appears to have a role in modulating catabolic pathways in alveolar macrophages and/or type II cells that contribute to the disordered surfactant homeostasis seen in PAP.

Fig. 8. Systemic GM-CSF did not alter SP-B or Sat PC concentrations. GM(−/−) mice were injected intraperitoneally with 200 ng of GM-CSF or vehicle alone daily for 5 wk (n = 6). Lungs from treated and untreated GM(−/−) mice were lavaged and analyzed for SP-B and Sat PC. A: no changes in SP-B concentration were observed by ELISA of BAL fluid from mice treated with systemic GM-CSF compared with vehicle-treated or untreated mice. Solid bars, means. Differences between groups were not significant as assessed by ANOVA. B: alveolar and tissue Sat PC pools in mice treated with systemic GM-CSF (hatched bars) did not differ from those in vehicle-treated (solid bars) or untreated (open bars) mice. Differences between groups were not significant as assessed by ANOVA.

Fig. 9. High doses of GM-CSF delivered to the lung for 3 days did not alter Sat PC or clearance of exogenous SP-C. GM(−/−) and GM-CSF-positive [GM(+/+)] mice were treated with 15 ng of GM-CSF delivered in 100 µl of saline or saline only injected into the trachea daily for 3 days (n = 8). 125I-SP-C was added to the 3rd GM-CSF treatment. Mice were killed at 40 h after final dose of GM-CSF plus 125I-SP-C. A: Sat PC pool sizes. No differences were observed between GM(−/−) mice treated with GM-CSF (hatched bars) and those receiving saline control (solid bars). Likewise, no differences were found between treated (crosshatched bars) and untreated (open bars) GM(+/+) mice. B: recovery of 125I-SP-C in each group. 125I-SP-C recovery from GM(−/−) mice treated with GM-CSF or saline did not differ. Similarly, 125I-SP-C recovery in GM(+/+) mice was the same with GM-CSF or saline treatment. Differences between GM-CSF- and saline-treated groups (within genotypes) were not significant as assessed by ANOVA.
In previous studies of GM(−/−) mice (12, 32), type II cell proliferation was increased in response to continuous exposure to GM-CSF, suggesting that increases in type II cell number may have contributed to resolution of PAP. However, in the present study, type II cell proliferation or number, as assessed by proliferating cell nuclear antigen staining and morphology, did not appear to increase (data not shown). GM-CSF levels are likely increased only transiently after aerosolized GM-CSF, in contrast to the continuous levels of GM-CSF produced throughout development in SPC-GM transgenic mice, and may account for the absence of type II cell proliferation in the present study (11, 12). With aerosolized GM-CSF, improvement in PAP occurred in the absence of changes in the numbers of type II cells, suggesting that the growth stimulatory effects of GM-CSF were not required for resolution of PAP in GM(−/−) mice.

Alveolar macrophages from untreated GM(−/−) mice have a characteristic morphology, with enlarged, foamy cytoplasm and lipid-filled vacuoles thought to result from a defect in surfactant catabolism (5, 15, 26). Similar alterations in alveolar macrophage morphology and function have been observed in alveolar macrophages from humans with PAP (9, 10). It is unclear, however, whether the alterations in macrophage morphology are directly related to a lack of GM-CSF during differentiation in the lung per se. Morphological changes seen in alveolar macrophages in PAP may also represent changes secondary to the increased surfactant concentrations. In the present study, alveolar macrophage morphology was improved by aerosolized GM-CSF. In addition, perivascular and peribronchiolar mononuclear cell infiltrates were reduced. It is not known whether mature alveolar macrophages obtained from the GM-CSF-treated mice were derived by correction of the foamy alveolar macrophages or by recruitment and differentiation of precursor cells, although the latter hypothesis seems more likely.

Induction of alveolar macrophage maturation is likely an important factor in the resolution of PAP by aerosolized GM-CSF. Pauicity of fluoride-resistant α-NAE staining in alveolar macrophages from untreated GM(−/−) mice suggests impaired or delayed maturation of these cells. Although the number of macrophages did not change with GM-CSF, the proportion of these cells staining for fluoride-resistant α-NAE was increased by aerosolized GM-CSF. Previous studies (2, 21, 32) associated alveolar macrophage differentiation or function with resolution of PAP in mice. The length of time (4–5 wk) required for improvement in PAP in response to GM-CSF is consistent with the time for immature mononuclear cells to be recruited to the lung and/or stimulated to differentiate. Monocytic precursor cells generally require 2–3 wk of GM-CSF stimulation in vitro to promote alveolar macrophage differentiation (6, 16). This time requirement for cell differentiation and restoration of macrophage morphology and activity in the lungs of GM(−/−) mice is also consistent with the estimated 21- to 28-day turnover of alveolar macrophages in mouse lungs (8, 28). The finding that acute intratracheal treatment with a relatively high concentration of GM-CSF (15 ng) did not alter lung Sat PC or SP-C clearance supports the concept that an extended period of treatment, perhaps dependent on alveolar macrophage differentiation, is necessary for the improvement in PAP in this model.

Improvement in PAP after aerosolized GM-CSF was associated with macrophage maturation, supporting the concept that GM-CSF plays an important role in restoring alveolar macrophage function in GM(−/−) mice. However, changes in alveolar macrophage function after daily aerosolized GM-CSF may not completely explain the improvement in PAP. Because type II cells clear ~80% of alveolar Sat PC in the rabbit, it is reasonable to predict that disordered type II cell function may play a role in PAP (23, 30). In the present study, aerosolized GM-CSF was more effective in restoring tissue rather than alveolar pools of surfactant lipid. The alveolar Sat PC pool decreased by ~50% after GM-CSF treatment but remained eightfold higher than that seen in wild-type mice. In contrast, lung tissue Sat PC was decreased to ~30% of that in the vehicletreated GM(−/−) mice, remaining twofold higher than that seen in wild-type mice. Of interest, staining of type II cells for SP-B was more intense after aerosolized GM-CSF treatment. Taken together, these results suggest that the aerosolized GM-CSF increased surfactant reuptake or catabolism in type II cells in addition to its effects on macrophage-mediated clearance.

Histological similarities in the pathology of GM(−/−) mice and humans with PAP suggest that findings in GM(−/−) mice may be relevant to human disease. Impairment of expression or function of GM-CSF was recently associated with PAP in humans (25, 27). The present study demonstrating that exogenous GM-CSF delivered to the lung improves PAP in GM(−/−) mice supports the use of GM-CSF as a potential therapeutic agent for PAP in humans. PAP has also been associated with decreased GM-CSF-receptor binding and a point mutation in the GM-CSF-receptor β subunit (4). However, it is unlikely that GM-CSF will be effective unless the GM-CSF receptor and its signaling pathways are functional, thus limiting its application to those cases where the putative cause of PAP is in the GM-CSF ligand rather than in the receptor or signal transduction pathway.

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