GM-CSF regulates protein and lipid catabolism by alveolar macrophages

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Yoshida, Mitsuhiro, Machiko Ikegami, Jacquelyn A. Reed, Zissis C. Chroneos, and Jeffrey A. Whitsett. GM-CSF regulates protein and lipid catabolism by alveolar macrophages. Am J Physiol Lung Cell Mol Physiol 280: L379–L386, 2001.—Metabolism of surfactant protein (SP) A and dipalmitoylphosphatidylcholine (DPPC) was assessed in alveolar macrophages isolated from granulocyte-macrophage colony-stimulated factor (GM-CSF) gene-targeted [GM(−/−)] mice, wild-type mice, and GM(+/−) mice expressing GM-CSF under control of the SP-C promoter element (SP-C-GM). Although binding and uptake of 125I-SP-A were significantly increased in alveolar macrophages from GM(−/−) compared with wild type or SP-C-GM mice, catabolism of 125I-SP-A was markedly decreased in GM(−/−) mice. Association of [3H]DPPC with alveolar macrophages from GM(−/−), wild-type, and SP-C-GM mice was similar; however, catabolism of DPPC was markedly reduced in cells from GM(−/−) mice. Fluorescence-activated cell sorter analysis demonstrated decreased catabolism of rhodamine-labeled dipalmitoylphosphatidylethanolamine by alveolar macrophages from GM(−/−) mice. GM-CSF deficiency was associated with increased SP-A uptake by alveolar macrophages but with impaired surfactant lipid and SP-A degradation. These findings demonstrate the important role of GM-CSF in the regulation of alveolar macrophage lipid and SP-A catabolism.

Surfactant homeostasis is maintained by mechanisms controlling surfactant synthesis, secretion, uptake, catabolism, and recycling. Surfactant lipids are synthesized, secreted, catabolized, and recycled by type II epithelial cells. Catabolism of surfactant lipids is also mediated by alveolar macrophages that account for the clearance of ~20% of the phospholipids from the air spaces in normal adult rabbits (21). Because GM-CSF affects both proliferation and differentiation of type II cells and alveolar macrophages (10), it remains unclear whether the PAP associated with the deficiency of GM-CSF signaling is mediated primarily by changes in respiratory epithelial or alveolar macrophage cell function in vivo. Furthermore, it is unclear whether binding, uptake, or degradation of surfactant components by alveolar macrophages are influenced by GM-CSF. Therefore, we hypothesized that GM-CSF plays a role in the regulation of binding, uptake, and catabolism of protein and lipids by alveolar macrophages.
METHODS

Transgenic mice. GM(−/−) mice were generated by gene-targeted ablation of the GM-CSF locus previously described by Dranoff et al. (7) and have been maintained in the C57BL/6 background for several years. Bitransgenic mice bearing a chimeric gene consisting of the 3.7-kb human SP-C gene promoter that drives expression of mouse GM-CSF were maintained in the GM(−/−) mutant background (SP-C-GM), generating mice in which GM-CSF was selectively expressed at increased levels in the lungs of GM(−/−) mice, the PAP in the GM(−/−) mutant being corrected by the SP-C-GM transgene (10). Control C57BL/6 mice, termed wild type (WT), were purchased from Jackson Laboratories (Bar Harbor, ME). All of the mice used were housed and studied in the Animal Facility of the Children’s Hospital Research Foundation (Cincinnati, OH) under approved procedures of the Institutional Animal Care and Use Committee. Mice were used between 8 and 10 wk of age. The animals were maintained in a pathogen-free barrier facility, and analysis of sentinel mice revealed no evidence of pathogens.

Alveolar macrophage isolation. Alveolar cells were obtained by bronchoalveolar lavage by instilling ten 1-ml aliquots of phosphate-buffered saline (PBS) containing 0.5 mM EDTA. Alveolar cells obtained from two to eight mice from each group were pooled and used for analysis. After centrifugation at 1,000 g for 5 min, the cell pellet was resuspended in 15% bovine serum albumin (BSA), PBS, and 10 mM EDTA followed by centrifugation for removal of the surfactant lipid and SPs (8, 23). The cells were then resuspended in Dulbecco’s modified Eagle’s medium (DMEM) containing 0.1% BSA, cultured at a density of 1 × 10⁶ cells/well in flat-bottom, 96-well tissue culture plates, and allowed to adhere for 1 h at 37°C. The nonadherent cells were removed, and the adherent cells were washed three times with DMEM containing 0.1% BSA. Peritoneal macrophages were isolated by peritoneal lavage in GM(−/−), SP-C-GM, and WT mice.

SP-A binding, uptake, and degradation by macrophages. SP-A (a generous gift from Dr. G. Ross, Children’s Hospital, Cincinnati, OH) was isolated from the lung lavage fluid of patients with alveolar proteinosis (9). The SP-A used here had no detectable endotoxin (<0.06 endotoxin unit/ml) as tested with the *Limulus* amebocyte lysate assay (Sigma, St. Louis, MO). SP-A was iodinated with chloramine T (3). The specific activity of the SP-A preparations was between 400 and 600 counts/min and 5000 DPPC radioactivity. To measure the binding of [3H]DPPC, the cells were incubated for 4 h at 4°C with the medium containing the labeled natural surfactant. After incubation, the supernatant was removed, and the cells were lysed with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40, 0.1% sodium deoxycholate, and 5 mM EDTA) to measure the cell-associated radioactivity. To determine the degradation of [3H]DPPC, the cells were incubated for 4 h at 4°C with the [3H]DPPC-labeled surfactant in culture medium. At each time point, the supernatant was collected, and the cells were washed three times with DMEM containing 0.1% BSA followed by cell lysis with radioimmunoprecipitation assay buffer. The lipid and aqueous fractions were extracted from both the supernatants and cells according to Bligh and Dyer (1). The degradation of [3H]DPPC by alveolar macrophages was estimated by measuring the generation of radioactive products partitioning in the water-methanol phase during the extraction. Background counts in the absence of the cells were subtracted. Results are expressed as counts per minute per microgram of cellular protein.

Fluorescently labeled surfactant uptake by macrophages. N-rhodamine dipalmitoylphosphatidylethanolamine (R-DPPE; Avanti Polar Lipids, Alabaster, AL) was mixed with natural surfactant at a ratio of 1:1 (wt/wt). The final suspension in the medium contained 100 µg/ml of phospholipid with 10 µg/ml of [3H]DPPC radioactivity. To measure the binding of [3H]DPPC, the cells were incubated for 4 h at 4°C with the medium containing the labeled natural surfactant. After incubation, the supernatant was removed, and the cells were lysed with radioimmunoprecipitation assay buffer (50 mM Tris-HCl, pH 8, 150 mM NaCl, 1% Nonidet P-40, 0.1% sodium deoxycholate, and 5 mM EDTA) to measure the cell-associated radioactivity. To determine the degradation of [3H]DPPC, the cells were incubated for 4 h at 37°C with the [3H]DPPC-labeled surfactant in culture medium. At each time point, the supernatant was collected, and the cells were washed three times with DMEM containing 0.1% BSA followed by cell lysis with radioimmunoprecipitation assay buffer. The lipid and aqueous fractions were extracted from both the supernatants and cells according to Bligh and Dyer (1). The degradation of [3H]DPPC by alveolar macrophages was estimated by measuring the generation of radioactive products partitioning in the water-methanol phase during the extraction. Background counts in the absence of the cells were subtracted. Results are expressed as counts per minute per microgram of cellular protein.
trypsinized, and assessed by FACS analysis. The cells incubated with 100 μg/ml of nonlabeled natural surfactant were used as controls. Samples of labeled cells were also imaged by fluorescence microscopy and photographed.

Statistical analyses. Results are expressed as means ± SE and evaluated for significance by analysis of variance. The level of significance was taken at $P < 0.05$.

RESULTS

Binding, uptake, and degradation of $^{125}$I-SP-A. Binding of $^{125}$I-SP-A to purified alveolar macrophages from GM(−/−), SP-C-GM, and WT mice was compared in vitro. Binding was time dependent, complete by 3–4 h of incubation, and saturable. Unexpectedly, binding of $^{125}$I-SP-A to alveolar macrophages from GM(−/−) mice was significantly greater than that in WT mice, whereas the number of SP-A binding sites on alveolar macrophages from SP-C-GM mice was significantly less than that in WT mice (Fig. 1). Scatchard analysis revealed the presence of a single class of binding sites in WT mice (Fig. 2), with a $K_d$ of 14.8 ± 3.4 nM (Table 1). The number of binding sites per cell (1.1 × 10^6 ± 0.12 × 10^6) estimated from the Scatchard plot (Table 1) in macrophages from the WT mouse was similar to the value of 0.97 × 10^6 ± 0.29 × 10^6 previously reported in rat alveolar macrophages (3). Human SP-A bound to alveolar macrophages from SP-C-GM mice with a similar $K_d$; however, the number of binding sites per cell was significantly decreased in SP-C-GM (~60%) mice compared with that in WT mice (Table 1). In contrast to findings in WT and SP-C-GM mice, the binding constants for SP-A binding to alveolar macrophages from GM(−/−) mice could not be reliably estimated by Scatchard analysis because the shape of this curve was nearly flat (Fig. 2A). To further evaluate the binding characteristics of SP-A to macrophages, the data were analyzed according to the Hughes-Klotz double-reciprocal equation (19). As shown in Fig. 2B, the binding data from both WT and GM(−/−) mice were fit to a straight line. The $K_d$ and the number of binding sites estimated by the Hughes-Klotz equation for binding of SP-A to WT macrophages were similar to the results obtained by Scatchard analysis (Table 1). Analysis of the data from the GM(−/−) macrophages revealed a large number of low-affinity binding sites, with a $K_d$ of 167 ± 62.5 nM and 4.54 × 10^6 ± 1.46 × 10^6 sites/cell in this model. Interestingly, the data from SP-C-GM macrophages were not well fit to a straight line (Fig. 2B). The convex shape of this curve may indicate negative cooperativity or multiple classes of binding sites in the cells from SP-C-GM mice. Because alveolar macrophages from GM(−/−) mice express a single class of low-affinity binding sites, it is suggested that the high-affinity binding site in WT alveolar macrophages may be distinct from those affinity sites or perhaps is composed of more than one subunit in which affinity is influenced by GM-CSF. The uptake of $^{125}$I-SP-A by alveolar macrophages was significantly increased in cells from GM(−/−) mice compared with that in WT mice.

![Fig. 1. Binding of surfactant protein (SP) A by alveolar macrophages. Alveolar macrophages (1 × 10^5/well) were incubated with various concentrations of $^{125}$I-SP-A at 4°C for 3 h. ○, Granulocyte-macrophage colony-stimulating factor (GM-CSF)-deficient [GM(−/−)] mice; ●, wild-type (WT) mice; □, GM(−/−) mice expressing GM-CSF under control of the SP-C promoter element (SP-C-GM). Values are means ± SE; n = 6 experiments. SP-A binding to alveolar macrophages from GM(−/−) mice was significantly greater than that in WT mice, whereas SP-A binding to alveolar macrophages from SP-C-GM mice was significantly less than that in WT mice. *$P < 0.05$ compared with WT mice.](http://ajplung.physiology.org/)

![Fig. 2. Analysis of $^{125}$I-SP-A binding data. The binding data from the saturation curves depicted in Fig. 1 were analyzed according to Scatchard analysis (A) and the Hughes-Klotz equation (B). The binding constants for binding of SP-A to alveolar macrophages from the WT (○) and SP-C-GM (□) mice shown in Table 1 were derived from the slopes and intercepts of the linear curves in A. The binding constants for binding to alveolar macrophages from the GM(−/−) mice (●) were derived from the linear curve in B (19). Values are means ± SE; n = 6 experiments.](http://ajplung.physiology.org/)

<table>
<thead>
<tr>
<th>Genotype</th>
<th>$K_d$, nM</th>
<th>Sites/Cell, $\times 10^6$</th>
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<tr>
<td>GM(−/−)</td>
<td>167 ± 62.5*</td>
<td>4.54 ± 1.46*</td>
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<tr>
<td>WT</td>
<td>14.8 ± 3.4</td>
<td>1.08 ± 0.13</td>
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<tr>
<td>SP-C-GM</td>
<td>10.4 ± 1.1</td>
<td>0.435 ± 0.033*</td>
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Values are means ± SE. $K_d$, multimeric dissociation constant or affinity constant. Binding constants were estimated by graphical analysis of data according to the Hughes-Klotz equation (Fig. 2B) from surfactant protein (SP) A binding data with alveolar macrophages from granulocyte-macrophage colony-stimulating factor (GM-CSF)-deficient (GM(−/−)) mice. Binding constants were estimated by graphical analysis according to Scatchard analysis (Fig. 2A) for SP-A binding data from wild-type (WT) mice and GM (−/−) mice expressing GM-CSF under control of the SP-C promoter element (SP-C-GM). *$P < 0.01$ compared with WT mice.
mice and significantly decreased in cells from SP-C-GM mice, suggesting a relationship between internalization and the density of binding sites for SP-A on the macrophage surface (Fig. 3).

Although the number of SP-A binding sites was markedly increased in the GM(−/−) mice, the degradation of 125I-SP-A as determined by the generation of TCA-soluble fragments at 37°C was markedly decreased in alveolar macrophages from the GM(−/−) mice (Fig. 4). Degradation of 125I-SP-A by alveolar macrophages from GM(−/−) mice was approximately fivefold less than that in either WT or SP-C-GM mice. There were no significant differences in the degradation of 125I-SP-A by alveolar macrophages from WT mice compared with that in SP-C-GM mice (Fig. 4).

Cell association and degradation of DPPC by alveolar macrophages. To assess whether GM-CSF influenced cell association or uptake or degradation of surfactant lipids, natural surfactant lipids were labeled with tracer quantities of [3H]DPPC. Initial studies determining the association of the labeled lipid with alveolar macrophages at 4°C in vitro showed that [3H]DPPC was associated with alveolar macrophages in a time-dependent manner, which plateaued by 4 h. The amount of cell-associated [3H]DPPC was similar in alveolar macrophages from WT and SP-C-GM mice. Association of [3H]DPPC with alveolar macrophages from GM(−/−) mice at 4°C was significantly decreased compared with that in WT or SP-C-GM mice, although these differences were relatively small (Fig. 5). As seen in the studies with 125I-SP-A, the catabolism of [3H]DPPC by alveolar macrophages from GM(−/−) mice was markedly decreased compared with that in WT or SP-C-GM mice (Fig. 6). Degradation of [3H]DPPC by alveolar macrophages from the GM(−/−) mice was significantly less than that in WT or SP-C-GM mice. *P < 0.05 compared with WT mice.
approximately fourfold less than that from WT mice. The degradation of $[^{3}H]$DPPC by alveolar macrophages from SP-C-GM transgenic mice was significantly increased compared with that in WT mice (Fig. 6).

**DPPC degradation by peritoneal macrophages.** To determine whether the observed catabolic defect in alveolar macrophages from GM($^{-/-}$) mice was restricted to the lung, catabolism of $[^{3}H]$DPPC by peritoneal macrophages was compared in GM($^{-/-}$), WT, and SP-C-GM mice. In WT mice, degradation of $[^{3}H]$DPPC by peritoneal macrophages was less than that by alveolar macrophages (Fig. 7). Degradation of $[^{3}H]$DPPC by peritoneal macrophages from both GM($^{-/-}$) and SP-C-GM mice was approximately twofold less than that by peritoneal macrophages from WT mice (Fig. 7). Interestingly, there were no differences in the degradation of $[^{3}H]$DPPC by peritoneal macrophages from GM($^{-/-}$) mice compared with that in SP-C-GM mice, supporting the concept that local production of GM-CSF influences DPPC degradation by macrophages.

**Uptake and degradation of R-DPPE.** Uptake and degradation of fluorescence R-DPPE surfactant by alveolar macrophages were studied by FACs and fluorescence microscopy. Images observed by fluorescence microscopy showed that there was cellular heterogeneity in the level of uptake by alveolar macrophages but that the amount of uptake of R-DPPE by alveolar macrophages from GM($^{-/-}$), WT, and SP-C-GM mice was similar (Fig. 8). Likewise, FACs analysis consistently demonstrated that the fluorescence intensity of labeled alveolar macrophages 30 min after incubation with the labeled lipid was similar in all strains of mice. However, after 4.5 h of continued incubation at 37°C, fluorescence of R-DPPE was decreased in alveolar macrophages from WT and SP-C-GM mice but persisted in cells from GM($^{-/-}$) mice, consistent with the observed decreased catabolism of the radiolabeled lipid by alveolar macrophages from GM($^{-/-}$) mice (Fig. 9).

**DISCUSSION.**

Catabolism but not binding and uptake of SP-A and phospholipid was markedly impaired in alveolar macrophages from GM-CSF($^{-/-}$) mice. Prolonged local expression of GM-CSF in the lungs of SP-C-GM mice...
restored catabolic activity in alveolar macrophages but not in peritoneal macrophages, demonstrating a critical role for local GM-CSF in the regulation of surfactant degradation by alveolar macrophages. Unexpectedly, SP-A uptake was significantly increased in the alveolar macrophages from GM(−/−) mice and was reduced in SP-C-GM mice compared with WT control mice. The affinity and number of SP-A binding sites on the alveolar macrophages were also influenced by GM-CSF genotype. Taken together, the present findings support the concept that the decreased clearance of SPs and lipids in GM(−/−) mice is mediated, at least in part, by the decreased catabolism of surfactant components by alveolar macrophages.

The precise receptors or mechanisms mediating SP and lipid binding, uptake, and catabolism remain relatively poorly understood. The increased binding and uptake of SP-A observed in the alveolar macrophages from GM(−/−) mice were associated with changes in both the affinity and increased number of SP-A binding sites, supporting a role for GM-CSF-dependent pathways in the regulation of SP-A binding sites on alveolar macrophages. These findings support previous observations (4) that binding of SP-A decreased during GM-CSF-induced differentiation of monocytes in vitro. Although Scatchard analysis suggests the presence of a single class of SP-A binding sites on alveolar macrophages from WT mice, heterogeneity and/or changes in cooperativity were observed in binding studies with cells from GM(−/−) mice. An increased number of relatively low-affinity 125I-SP-A binding sites was correlated with increased SP-A uptake by alveolar macrophages from GM(−/−) mice, suggesting a relationship between binding site number and the uptake process. Because prolonged expression and administration of GM-CSF were required for correction of PAP in GM(−/−) mice, changes in surfactant homeostasis by GM-CSF are likely dependent on the effects of GM-CSF in alveolar macrophage differentiation that, in turn, influence SP-A binding and surfactant catabolism. GM(−/−) mice develop severe PAP associated with marked increases in alveolar SP-A concentrations [increased >10-fold in adult GM(−/−) mice] in the absence of changes in SP-A mRNA, supporting an important role for surfactant clearance in the pathogenesis of the disorder (7). Thus alveolar macrophages in GM(−/−) mice have been exposed to high prevailing concentrations of SPs and lipids that may also influence SP-A binding site number and affinity. It is intriguing to speculate that the increased number of binding sites may represent a response to the increased concentration of SP-A in the air spaces of GM(−/−) mice. The number of binding sites and affinity for SP-A observed in the GM-CSF-replete alveolar macrophages in the present study are consistent with those in previous studies (3, 18). Whether the characteristics of the SP-A binding sites detected in the present study represent single or multiple classes of SP-A receptor(s) or binding sites and whether the SP-A binding sites represent clearance receptors, lack high-affinity binding signaling receptors, or both remain unclear. Alveolar macrophages are highly responsive to SP-A, the polypeptide influencing phagocytosis, viral and bacterial clearance, oxidant burst, and cytokine production in vivo (12–15). The present findings that the local expression of GM-CSF in SP-C-GM mice reduced the number of SP-A binding sites on alveolar macrophages provide support for the concept that GM-CSF or GM-CSF-dependent signaling pathways regulate SP-A binding sites on alveolar macrophages.

Interpretation of quantitative data is complicated by differences in binding site affinity, cell size, and cell adherence in the assays and imprecision regarding the precise size of SP-A oligomers binding to the cell surface.

Consistent with the increased number of SP-A binding sites on alveolar macrophages, 125I-SP-A uptake by alveolar macrophages from GM(−/−) mice was significantly greater than that in WT or SP-C-GM mice. Although SP-A binding sites were increased in cells from GM(−/−) mice, substantive differences in [3H]DPPC binding by alveolar macrophages from GM(−/−), WT, and SP-C-GM mice were not observed. FACS studies also demonstrated that the uptake of R-DPPE-labeled surfactant by alveolar macrophages from mice of all genotypes was approximately similar. Thus despite increased binding or uptake activity of surfactant components by alveolar macrophages, catabolism of both 125I-SP-A and [3H]DPPC was markedly impaired in alveolar macrophages from GM(−/−) mice. Differences in uptake and degradation were not likely related to cell viability because the cells were isolated by adherence to plastic and cell viability was similar in cells from each genotype.

The production of GM-CSF in the lungs of GM(−/−) mice with the SP-C-GM transgene corrected alveolar proteinosis in transgenic mice in vivo, demonstrating the importance of local expression of GM-CSF (10). Consistent with those findings, the present data demonstrate that degradation of SP-A and lipids by alveolar but not by peritoneal macrophages was restored in SP-C-GM mice. With advancing age, alveolar macrophages from both GM(−/−) and common β-chain-deficient mice develop an increasingly foamy appearance associated with an accumulation of both lipids and SPs in the air spaces and within alveolar macrophages. Although phagocytosis of group B streptococcus was unaltered, production of superoxide radicals was markedly deficient in macrophages from GM(−/−) mice (16). The present findings are consistent with these observations and demonstrate the ability of alveolar macrophages from GM(−/−) mice to bind and take up but not to degrade surfactant components. Increased lipid content and a marked decrease in clearance of DPPC from the lungs of GM(−/−) mice are likely to be caused, at least in part, by a deficiency of GM-CSF-dependent pathways controlling phospholipid catabolism by alveolar macrophages.

Because alveolar macrophages in GM(−/−) mice become progressively foamy with age, it is possible that secondary abnormalities in macrophage function may contribute to the defect in surfactant catabolism in
GM(−/−) mice. To determine whether the defect in surfactant degradation by alveolar macrophages from GM(−/−) mice was limited to the lung or was influenced by accumulation of surfactant components in the cells, surfactant lipid degradation was compared in peritoneal macrophages isolated from GM(−/−), WT, and SP-C-GM mice. Degradation of [3H]DPPC by peritoneal macrophages was also impaired in GM(−/−) and SP-C-GM mice. Because GM-CSF expression in SP-C-GM mice (these mice are GM(−/−)) is restricted to the lung, these findings demonstrate that local production of GM-CSF is required for correction of the defect in lipid catabolism in GM(−/−) mice. Interestingly, although the present study demonstrates a defect in lipid catabolism in peritoneal macrophages, phenotypic changes in organ pathology are restricted to the lung in GM(−/−) mice. These findings support the concept that GM-CSF is required for DPPC clearance by macrophages in general but that the marked PAP seen in GM(−/−) mice reflects the unique role played by alveolar macrophages in phospholipid homeostasis in the lung.

Although cell association of labeled lipid was approximately similar in alveolar macrophages from all genotypes, the catabolism of surfactant lipids by alveolar macrophages from SP-C-GM mice was significantly increased compared with that in WT mice. However, the uptake of R-DPPE as assessed by fluorescence microscopy and FACS analysis demonstrated some heterogeneity in the ability of cells to take up and degrade labeled lipids. Differences in lipid uptake or degradation in WT, GM(−/−), and SP-C-GM-replete mice may reflect a change in the proportion of cells able to rapidly take up and degrade lipids. The findings that the percentage of cells actually degrading surfactant lipids is increased in the GM-CSF-sufficient mice supports the concept that GM-CSF influences cell differentiation of alveolar macrophages (or progenitors), producing cells with an increased ability to catabolize surfactant components. Because chronic expression of GM-CSF in the lungs also increased the number of alveolar macrophages in vivo (10), changes in the absolute number of active macrophages may also influence surfactant catabolism, providing yet another mechanism by which steady-state lipid and surfactant concentrations may be modulated by GM-CSF in vivo.

Human PAP is a heterogeneous disorder of acquired or genetic etiology. The pathogenesis of most cases is uncertain. Previous reports suggested that some cases of PAP were caused by defects in GM-CSF signaling (6, 24) or decreased production or secretion of GM-CSF (28). A recent study (26) demonstrated that PAP may also be caused by factors that neutralize GM-CSF activity in bronchoalveolar lavage fluid.

GM-CSF influences binding, uptake, and degradation of SFs and lipids by alveolar macrophages, likely reflecting the importance of GM-CSF in the differentiation of alveolar macrophages in vivo. The striking defect in SP-A and lipid catabolism observed in alveolar macrophages from GM(−/−) mice was restored by chronic, local replacement of GM-CSF. The present findings are consistent with the importance of alveolar macrophage differentiation and function in the pathogenesis of PAP and support previous studies (5, 10, 20, 30) that demonstrated that bone marrow transplantation of normal hematopoietic cell precursors improved PAP in common β-chain-deficient mice in vivo. There is a possibility that GM-CSF also affects surfactant metabolism by type II cells. Type II cells express GM-CSF receptors, and GM-CSF enhances type II cell proliferation in vivo (10). It remains unclear whether PAP in GM-CSF deficiency is caused primarily by alterations in the activity of type II cells or alveolar macrophages or by contributions from both cell types. Because PAP in humans has been associated with defects in GM-CSF production, function, or receptor activity, strategies focused on enhancing mechanisms by which alveolar macrophages degrade surfactant may provide new therapeutic approaches for the life-threatening disorder PAP.

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