Impaired functional activity of alveolar macrophages from GM-CSF-deficient mice

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Paine, Robert, III, Susan B. Morris, Hong Jin, Steven E. Wilcoxen, Susan M. Phare, Bethany B. Moore, Michael J. Coffey, and Galen B. Toews. Impaired functional activity of alveolar macrophages from GM-CSF-deficient mice. Am J Physiol Lung Cell Mol Physiol 281: L1210–L1218, 2001.—We hypothesized that pulmonary granulocyte-macrophage colony-stimulating factor (GM-CSF) is critically involved in determining the functional capabilities of alveolar macrophages (AM) for host defense. To test this hypothesis, cells were collected by lung lavage from GM-CSF mutant mice (GM(−/−)) and C57BL/6 wild-type mice. GM(−/−) mice yielded almost 4-fold more AM than wild-type mice. The percentage of cells positive for the β2-integrins CD11a and CD11c was reduced significantly in GM(−/−) AM compared with wild-type cells, whereas expression of CD11b was similar in the two groups. The phagocytic activity of GM(−/−) AM for FITC-labeled microspheres was impaired significantly compared with that of wild-type AM both in vitro and in vivo (after intratracheal inoculation with FITC-labeled beads). Stimulated secretion of tumor necrosis factor-α (TNF-α) and leukotrienes by AM from the GM(−/−) mice was greatly reduced compared with wild-type AM, whereas secretion of monocyte chemoattractant protein-1 was increased. Transgenic expression of GM-CSF exclusively in the lungs of GM(−/−) mice resulted in AM with normal or supranormal expression of CD11a and CD11c, phagocytic activity, and TNF-α secretion. Thus, in the absence of GM-CSF, AM functional capabilities for host defense were significantly impaired but were restored by lung-specific expression of GM-CSF.

The pulmonal alveolar space is the largest interface of the host with the external environment. Alveolar macrophages (AM) are the resident inflammatory cells in the alveolar space (9, 11). These mobile cells reside in close proximity to alveolar epithelial cells. AM form the first line of defense against inhaled or aspirated microbes, engulfing and killing organisms that enter the lung in low numbers (3). However, AM also serve as sentinel cells, initiating a cascade of mediators to recruit and activate additional inflammatory cells (18).

AM are highly differentiated cells of monocyte lineage that differ from peripheral blood monocytes and from tissue macrophages from other sites (3). Complex factors in the alveolar environment are likely to be responsible for determining the particular functional characteristics of AM. A candidate molecule that is likely to play a major role in determining AM activity is granulocyte-macrophage colony-stimulating factor (GM-CSF). GM-CSF has important effects on mature mononuclear cells. It is both mitogenic (1, 17) and chemotactic (20) for AM and can activate macrophages to increase antimicrobial activity against a variety of pathogens (7, 13, 24). The lung is a rich source of GM-CSF. Alveolar epithelial cells, interstitial cells, and macrophages themselves all can produce GM-CSF upon appropriate stimulation (10). Recent studies have demonstrated that GM-CSF has an important role in homeostasis in the normal lung. Mice with a targeted deletion of the GM-CSF gene [GM(−/−)] mice have abnormalities in the turnover of pulmonary surfactant that eventually lead to a histological picture resembling human alveolar proteinosis (8). This defect is corrected by expression of GM-CSF solely in the lung (12, 33) or by chronic inhalation of recombinant GM-CSF (22).

Emerging evidence indicates that GM-CSF plays an important role in pulmonary host defense. Recent reports from this laboratory and elsewhere have described impaired host defense of GM(−/−) mice against pneumonia as a result of two specific pathogens, Pneumocystis carinii (21) and group B streptococci (13). GM-CSF has multiple effects on inflammatory and parenchymal cells and is likely to participate in determining the activity of cells in the alveolar environment. The present study has been undertaken to more fully determine the functional characteristics of AM from mice congenitally deficient in GM-CSF and of AM from mice in which GM-CSF is expressed exclusively in the lung.

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MATERIALS AND METHODS

Animals. Wild-type C57BL/6 mice were obtained from Jackson Laboratory (Bar Harbor, ME). GM(-/-) mice were generated by Dranoff et al. (8) by targeted interruption of the GM-CSF gene and expressed no detectable GM-CSF. These mice have been extensively backcrossed against C57BL/6 mice. GM(-/-) mice were used at 6–8 wk of age. Bitransgenic mice, in which GM-CSF is expressed exclusively in the lung, were generated from GM(-/-) mice by transgenic expression of a chimeric gene containing GM-CSF under the surfactant protein C (SP-C) promoter (SP-C-GM mice; see Ref. 12). The specificity of the SP-C promoter results in targeted expression of GM-CSF exclusively by type II alveolar epithelial cells of these SP-C-GM mice. Founder GM(-/-) and SP-C-GM mice were kindly provided by Dr. J. Whitsett (Children’s Hospital, Cincinnati, OH). All mice were housed in microisolator cages under laminar flow hoods in an isolation room of the Animal Care Facilities at the University of Michigan and the Ann Arbor Veterans Affairs Medical Center.

Whole lung lavage and differential cell counting. AM were recovered by whole lung lavage, as described previously (21). Mice were killed with intraperitoneal pentobarbital sodium. The trachea was cannulated, and the lungs were lavaged with a total of 5 ml of PBS (containing 0.5 mM EDTA) in aliquots of 0.5 ml. The lavage aliquots for each animal were pooled, and the cell pellet was collected by centrifugation. Differential cell counts were performed on >200 cells/mouse stained with hematoxylin and eosin, as described previously (2).

AM adherence. AM were collected from wild-type, GM(-/-), and SP-C-GM mice by whole lung lavage and were suspended in DMEM (GIBCO BRL) supplemented with penicillin-streptomycin (1 × 10^6 cells/ml). The cells were plated in four-well Lab-Tek tissue culture-treated plastic chamber slides (Nunc, Naperville, IL) at 2.5 × 10^5 cells/well. After 30 min, the wells were washed three times with warm PBS, fixed with paraformaldehyde (0.5%), and then viewed on a phase-contrast microscope (×20 objective). The adherent cells were counted in eight random fields.

Flow cytometry of AM. AM were collected from groups of three wild-type, GM(-/-), and SP-C-GM mice and pooled. The cell pellets were washed three times with PBS. After each wash, the cells were collected by centrifugation at 5 min at 400 g. The cells were suspended in staining buffer (PBS with 2% FCS and 0.1% sodium azide) at 1 × 10^6 cells/ml. Aliquots containing 1 × 10^5 cells were processed as follows. All incubations were for 30 min at 4°C on ice. Cells were first incubated with anti-murine FCyRII/III (CD16/CD32; PharMingen, San Diego, CA) and then incubated with primary antibodies, including phycoerythrin-conjugated rat anti-murine CD11a and anti-rat-murine CD11b (both 4 μg/ml), FITC-conjugated hamster anti-murine CD11c (5 μg/ml), and isotype-matched controls. In separate experiments, lymphocytes were identified by staining with rat anti-murine CD19 (pan B cell), rat anti-murine CD3 (T cells), or rat anti-murine Gr-1 (Ly-6G, staining neutrophils). All antibodies were from PharMingen. In selected experiments, cell viability was determined by flow cytometric analysis of cells stained with propidium iodide (0.25 μg/1.5 × 10^5 cells in 100 μl; PharMingen) to identify dead cells. Cells also were stained with FITC-conjugated Bandeiraea simplicifolia (BS-1) lectin (Sigma, St. Louis, MO). After being stained, the cells were washed two times in FA buffer (Difco, Detroit, MI) and then fixed with 0.5% paraformaldehyde. Flow cytometry was performed on a FACScan flow cytometer (Becton Dickinson, Mountain View, CA). Data were analyzed using the Cell Quest software package (Becton Dickinson). Thresholds for positive staining were determined from the isotype-matched control samples.

In vivo and in vitro phagocytosis assays. For the in vitro assay, AM were first obtained from wild-type C57BL/6, GM(-/-), and SP-C-GM mice by whole lung lavage. AM (10^5/well in DMEM without FCS) were adhered for 30 min in wells of eight-well tissue culture-treated plastic slides (Lab-Tek). The cells were washed gently, and latex microbeads labeled with FITC (1.7 μm in diameter; Polysciences) were added to the wells (8 × 10^6 beads/well). After 1 h of incubation at 37°C, the wells were washed gently with PBS, fixed with methanol at −20°C for 20 min, and washed extensively. The cells were viewed by a blinded observer using a Nikon Labphot 2 microscope equipped with epifluorescence. In each well, the fraction of cells containing labeled beads and the phagocytic index were determined by microscopy counting of at least 200 cells in random high-power fields. The phagocytic index (PI) was calculated as follows

\[ PI = \frac{\text{fraction of AM with beads}}{\text{mean number of beads per positive AM}} \]

Four replicate wells were evaluated for each condition.

In experiments to measure the phagocytosis of microbeads in vivo, mice first were lightly anesthetized with pentobarbital sodium by intraperitoneal injection. The trachea was exposed, and 50 μl of FITC-labeled microbeads (5 μl of beads suspended in a total volume of 45 μl of PBS for a total of 5 × 10^7 beads) were injected into the trachea under direct visualization. The mice were allowed to recover from anesthesia, with their position changed frequently until ambulation. One hour after the initial instillation of the beads, the mice were killed, and alveolar cells were recovered by whole lung lavage. The percentage of AM that had engulfed beads and the phagocytic index were determined by microscopy counting of cytocentrifugation preparations, as described above.

Measurement of AM leukotriene production in vitro. AM were collected by whole lung lavage of wild-type, GM(-/-), and SP-C-GM mice by instilling 1-ml aliquots of PBS containing 5 mM EDTA to a total volume of 5 ml. Cells were pooled from three mice in each instance. The cells were plated in triplicate at 0.2 × 10^9/200 μl in 96-well plates for 1 h. The nonadherent cells were washed gently, and the adherent cells were stimulated with A-23187 (1 μM) for 30 min. The culture supernatants were harvested and frozen at −70°C for future analysis for peptidoleukotriene content by enzyme immunoassay (EIA; Cayman Chemical, Ann Arbor, MI).

ELISAs for tumor necrosis factor-α production by AM in vitro and monocyte chemotactic protein-1 in bronchoalveolar lavage fluid. AM were obtained from the lungs of wild-type C57BL/6, GM(-/-), and SP-C-GM mice by whole lung lavage as described above and placed in culture in 96-well plates (10^5 cells/well). After the AM had adhered for 30 min, the plates were washed gently, and lipopolysaccharide (LPS; Escherichia coli 026:B6; Sigma) was added to the wells in DMEM with or without 10% FCS. The cells were incubated for 24 h at 37°C, the medium was harvested, and the concentration of tumor necrosis factor-α (TNF-α) or monocyte chemokine protein (MCP)-1 in the cell-free supernatant was determined using ELISA kits (both from R&D Systems, Minneapolis, MN).
Minneapolis, MN) following the manufacturer's recommendations. For each data point, supernatants from quadruplicate wells obtained from a single animal [GM(−/−) or SP-C-GM mice] or pooled from two to three mice (wild-type mice) were measured.

To determine the concentration of MCP-1 in bronchoalveolar lavage (BAL) fluid, whole lung lavage was performed as described above, and the cells were collected by centrifugation. MCP-1 in the BAL fluid cell-free supernatant was determined by ELISA, following the manufacturer's instructions.

Statistical methods. Data are expressed as means ± SE and were compared by one-way ANOVA with the Tukey-Kramer multiple comparisons test using the InStat software program (version 3.01 for Windows 95; GraphPad Software, San Diego, CA). Data were considered statistically significant if P values were <0.05.

RESULTS

Number and appearance of alveolar cells recovered by BAL. To examine the effects of congenital absence of GM-CSF on the population of alveolar inflammatory cells, BAL was performed on wild-type C57BL/6 mice and GM(−/−) mice aged 6 wk. Alveolar cells also were harvested from SP-C-GM mice, which lack GM-CSF except for expression in type II alveolar epithelial cells. GM-CSF is expressed in higher concentrations in the lungs of SP-C-GM mice than in wild-type mice (12, 21). Significantly more cells per mouse were recovered by lung lavage from GM(−/−) mice and SP-C-GM mice than from wild-type controls (Fig. 1). Differential cell counting of Diff-Quik-stained BAL fluid cells identified 89% of the cells from wild-type mice as AM, with 8.5% lymphocytes and 1.2% neutrophils. Cells from GM(−/−) mice and SP-C-GM mice were 99% monocyte/macrophage in appearance (Fig. 2). To better characterize these cells, forward and side light scatter of alveolar cells collected by BAL were determined by flow cytometry. As shown in Fig. 3, there was a slight increase in the number of smaller cells from the GM(−/−) mice compared with wild-type mice. Trypan blue exclusion demonstrated >95% viability of BAL fluid cells from all three strains of mice. In selected experiments, the fraction of cells stained with propidium iodide was determined by flow cytometry as an indication of cell death. Only 1–4% of the BAL fluid cells from GM(−/−) mice stained with propidium iodide, confirming results from trypan blue exclusion.

A high proportion of rodent AM have been shown to bind the lectin BS-I (30). Flow cytometric analysis of BS-I-stained AM from wild-type and GM(−/−) mice was used to determine whether AM from GM(−/−) mice also were stained by this lectin. Although 72.6 ± 2.8% of wild-type AM were stained with BS-I, only 24.5 ± 1.0% of the AM from GM(−/−) stained positively for the lectin. AM from SP-C-GM mice were 93.4 ± 1.0% positive for BS-I staining. The BS-I-positive cells had light scatter characteristics similar to the BS-I-negative cells. Flow cytometric analysis of BAL fluid cells from wild-type, GM(−/−), and SP-C-GM mice by whole lung lavage was performed as described above, and the cells were collected by centrifugation. MCP-1 in the BAL fluid cell-free supernatant was determined by ELISA, following the manufacturer's instructions.

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fluid cells stained with anti-CD3 (T cells) and anti-CD19 (B cells) confirmed that the mononuclear cells collected from GM(−/−) mice were not activated lymphocytes. Less than 1% of the GM(−/−) BAL fluid cells proved to be lymphocytes. Staining for Gr-1 indicated that the fraction of neutrophils in the GM(−/−) BAL fluid population ranged between 1 and 5%. Thus, in the absence of GM-CSF, there were increased numbers of cells recovered by BAL. These GM(−/−) AM were somewhat smaller than wild-type AM and were less frequently positively stained for BS-I. Targeted expression of GM-CSF in the lungs of mice lacking GM-CSF at other sites led to increased staining of AM for BS-I, reversing the decrement demonstrated in GM(−/−) mice.

Decreased in vitro adhesion of AM from GM(−/−) mice. Normal AM readily adhere to tissue culture-treated plastic. The adhesion of AM from GM(−/−) mice was significantly less than that of AM from wild-type mice (Fig. 4). Interestingly, AM from SP-C-GM adhered to plastic dishes at a rate equivalent to that of wild-type AM. Thus, in the absence of GM-CSF, there was defective AM adherence to tissue culture-treated plastic, and this defect was reversed when GM-CSF was expressed in the lungs of otherwise GM-CSF-deficient mice.

Expression of β₂-integrins is altered in AM from GM(−/−) mice. Macrophage interactions with endothelial and epithelial cells are mediated in part by β₂-integrins expressed on the AM surface. AM from GM(−/−), wild-type, and SP-C-GM mice were collected by BAL and stained for the β₂-integrins CD11a, CD11b, and CD11c. The proportion of cells staining positively for these integrins was determined by flow cytometry (Fig. 5). The fractions of AM expressing CD11a and CD11c were reduced significantly in AM from GM(−/−) mice compared with those from wild-type mice. In contrast, expression of CD11b was similar in AM from GM(−/−) and wild-type mice. Interestingly, AM from SP-C-GM mice demonstrated higher expression of all three integrins compared with either GM(−/−) or wild-type mice. Thus GM-CSF has pronounced effects on integrin expression by AM, with diminished expression in the absence of GM-CSF and supranormal expression when GM-CSF was overexpressed in the lung.
Impaired phagocytic activity of AM from GM(−/−) mice in vitro and in vivo. To directly explore the consequences of congenital absence of GM-CSF for AM function, FITC-labeled latex microspheres were incubated with AM collected by BAL from the three strains of mice. After 1 h, the cells were fixed, and the phagocytic activity of the AM was determined by microscopy counting of microspheres associated with AM. AM from GM(−/−) mice were significantly less effective in binding/phagocytosis of microspheres compared with wild-type AM. Both the percentage of AM that had bound/internalized beads and the phagocytic index were reduced in the GM(−/−) AM (Fig. 6). Long-term exposure to GM-CSF in the lung reversed this deficit as shown by the enhanced activity of AM from SP-C-GM mice. To extend these in vitro observations to the intact host, mice of all three strains were inoculated intratracheally with FITC-labeled latex microspheres. After 1 h, AM were recovered by BAL. Binding and phagocytosis of microspheres by AM then were assessed by microscopic counting. In GM(−/−) mice, both the percentage of AM that contained latex microspheres (Fig. 7A) and the phagocytic index (Fig. 7B) were reduced significantly compared with those in wild-type mice and SP-C-GM mice. These data demonstrate that phagocytic activity of AM is impaired in GM-CSF(−/−) mice and that phagocytic function is in fact greater than normal in the setting of targeted overexpression of GM-CSF in the lung.

Impaired production of leukotrienes by AM from GM(−/−) mice. AM are an important source of leukotrienes in the context of host defense. Inhibition of endogenous leukotriene synthesis has resulted in diminished AM phagocytosis of bacterial pathogens. To determine whether the impaired macrophage inflammatory activity of GM(−/−) AM extended to leukotriene secretion, AM were collected from wild-type and GM(−/−) mice and stimulated in vitro with calcium ionophore. Total peptidoleukotrienes (leukotrienes C, D, and E4) were measured in the culture supernatants.
by EIA. As anticipated, wild-type AM released abundant leukotrienes (2,830 ± 528 pg/ml). However, leukotriene secretion was completely suppressed in the GM(−/−) AM (22.6 ± 4.5 pg/ml; P < 0.01 vs. wild type). Interestingly, leukotriene secretion in AM from SP-C-GM mice (496 ± 45.6) was greater than that from GM(−/−) AM but was not restored to the level of wild-type mice. Thus congenital absence of GM-CSF resulted in the loss of normal leukotriene synthesis by AM. This deficit was only partially corrected by exclusive expression of GM-CSF in the alveolar space.

**Diminished TNF-α expression by AM from GM(−/−) mice in response to LPS.** In addition to their important role as phagocytes, AM function as sentinel cells, amplifying the innate immune response through the initiation of a cascade of inflammatory mediators. TNF-α is a critical component of this inflammatory cascade. AM from GM(−/−) and wild-type mice were collected by BAL and placed in culture in DMEM with or without 10% FCS. LPS was added to the medium at increasing concentrations. After 24 h, TNF-α antigen was measured in the culture supernatants (Fig. 8). AM from wild-type mice released abundant TNF-α after LPS stimulation. As anticipated, the response to low-dose (1–10 ng/ml) LPS was greater in the presence of serum than under serum-free conditions, although, at higher doses of LPS, large amounts of TNF-α were released under either condition. In contrast, AM from GM(−/−) mice expressed no detectable TNF-α at baseline, with very little TNF-α induced after exposure to LPS in the presence or absence of serum. AM from SP-C-GM mice consistently secreted at least as much TNF-α in response to LPS as wild-type AM. Thus, in the absence of GM-CSF, AM secretion of TNF-α in response to LPS is greatly impaired, whereas expression of GM-CSF solely in the lung is sufficient to reverse this abnormality.

*Fig. 8.* In vitro tumor necrosis factor-α (TNF-α) secretion by stimulated AM in vitro. AM from wild-type mice (hatched bars) and GM(−/−) mice (open bars) were exposed to increasing doses of lipopolysaccharide (LPS) in the presence of 10% FCS or in serum-free medium. After 24 h, the culture supernatants were harvested, and TNF-α protein content was determined by ELISA. For GM(−/−) mice, each data point represents data from a separate animal (n = 4), whereas, for wild-type mice, each data point was determined from cells pooled from 2 or 3 mice (n = 3). Data are presented as means ± SE.

Levels of pulmonary surfactant are significantly increased in BAL fluid of GM(−/−) mice compared with wild-type mice. Exposure of normal AM to exogenous surfactant protein A (SP-A) in vitro has been shown to impair AM TNF-α secretion (16). This SP-A-mediated inhibition was reversible upon removal of AM-associated SP-A. To determine whether SP-A bound to AM explained the reduced TNF-α secretion by GM(−/−) AM, AM from GM(−/−) were washed with EGTA (0.2 mM) before plating and exposure to LPS (16). Additional cells were sham washed in PBS. The EGTA wash did lead to increased TNF-α secretion in the GM(−/−) AM (308 ± 92 pg/ml with EGTA vs. 131 ± 19 pg/ml with sham wash). However, this change was modest, and TNF-α production remained significantly below that of wild-type AM (compare Fig. 8). Similarly, when BAL fluid cells from GM(−/−) and wild-type mice were subjected to differential centrifugation through albumin to remove surfactant before the TNF-α release assay, TNF-α production by GM(−/−) AM remained greatly diminished compared with that from wild-type AM (487 ± 17 vs. >2,500 pg/ml). These findings suggest that excess surfactant in the alveolar space in GM(−/−) mice is not the primary mechanism for impaired TNF-α secretion by GM(−/−) AM.

**Increased MCP-1 expression in lung lavage fluid and AM from GM(−/−) mice.** Our initial studies demonstrated increased numbers of AM recovered by lung lavage from GM(−/−) mice compared with wild-type controls. One possible explanation for this increased number of cells in the absence of GM-CSF would be that these cells are recruited to the lung by increased expression of a monocyte chemoattractant. Therefore, we determined the expression of MCP-1, a monocyte chemoattractant that is produced by a number of different cells in the alveolar space, in BAL fluid. MCP-1 protein was not detected in BAL fluid from normal wild-type mice (limit of detection 15.6 pg/ml). In contrast, there was abundant MCP-1 (654.5 ± 186 pg/ml, n = 3) in the BAL fluid from GM(−/−) mice. To determine whether AM themselves might be one source of increased MCP-1 expression in these mice, AM from GM(−/−) and wild-type mice were exposed to LPS (10 ng/ml) in the presence of 10% FCS. There was significant MCP-1 in the culture supernatants from GM(−/−) AM (189 ± 67 pg/ml), whereas MCP-1 was not detectable in undiluted supernatants from similarly treated wild-type AM (<16 pg/ml).

**DISCUSSION**

Previously, it has been recognized that GM-CSF is produced in abundance in lung tissue after inflammatory stimulation (6) and plays an important role in regulation of pulmonary surfactant (23). More recently, based on studies using mice congenitally deficient in GM-CSF, it has become clear that GM-CSF plays an important role in pulmonary host defense. GM(−/−) mice demonstrate enhanced susceptibility to pneumonia resulting from group B streptococci (13) or *P. carinii* (21). We now provide new information con-
cerning the specific consequences of the absence of GM-CSF for AM function. We found that GM(−/−) mice had increased numbers of AM in BAL fluid compared with wild-type mice. Associated with this increased number of AM was increased expression of MCP-1 in BAL fluid from GM(−/−) mice compared with wild-type mice. Although more abundant, AM from GM(−/−) mice demonstrated a diminished capacity to perform a number of classic functions of AM. In particular, they were less effective than AM from wild-type mice in binding and phagocytosis of latex microspheres both in vitro and in vivo. In contrast to normal AM, AM from GM(−/−) mice produced very little TNF-α or peptidoleukotrienes upon stimulation in vitro. AM from GM(−/−) mice also demonstrated an altered pattern of cell surface expression of β2-integrins and diminished BS-I lectin binding compared with wild-type AM. Finally, with the exception of leukotriene expression, these deficiencies could be corrected by expression of GM-CSF exclusively in the alveolar space.

AM are critically involved in pulmonary host defense, both as effector cells that bind and engulf pathogens and as sentinel cells that secrete inflammatory mediators to recruit and activate inflammatory cells. Studies in which AM have been specifically depleted by tracheal instillation of liposomes containing dichloromethylene diphosphonate have confirmed the importance of AM for host defense against gram-negative pathogens (5) and against P. carinii (14, 15). AM from GM(−/−) mice evidenced defects in binding and phagocytosis of latex beads that were apparent not only during in vitro studies but also when the beads had entered the alveolar space in vivo. The defect in binding/phagocytosis observed in the GM(−/−) mice would significantly impair the ability of these AM to engulf and kill pathogens that enter the lung either by aspiration of oropharyngeal contents or by aerosol deposition, rendering the animals susceptible to pneumonia in response to small numbers of organisms.

Transgenic mice lacking biological activity of GM-CSF can be generated by targeted deletion either of GM-CSF or of its receptor. Transgenic mice with a targeted deletion of the β-chain of this receptor develop pulmonary pathology resembling that found in GM(−/−) mice (19, 25). In vitro studies using macrophages from these receptor-deficient mice demonstrate diminished adherence and variable defects in phagocytosis with impaired phagocytosis of colloidal carbon. However, AM from these receptor-deficient mice demonstrate preserved phagocytosis of latex beads (28). The reasons for discrepancies between the GM(−/−) mice and β-chain-deficient mice are not yet clear (27).

AM are an abundant source of inflammatory mediators. The defect in binding and phagocytosis identified in AM from GM(−/−) mice would be less critical if these AM were still able to vigorously recruit and activate neutrophils and other inflammatory cells in response to invading pathogens. However, we identified profound abnormalities in expression of both TNF-α and leukotrienes by GM(−/−) AM in response to standard stimuli. Among the many AM secretory products, we chose to investigate TNF-α because it is a classic early-response cytokine that is produced in response to a broad array of microbial products. In turn, TNF-α stimulates macrophage production of C-X-C chemokines to recruit neutrophils (31). Other cellular sources may provide TNF-α in the lung in GM(−/−) mice (13; Paine, unpublished observations). However, the profound decrease in TNF-α expression by GM(−/−) AM greatly impairs the ability of these resident cells to initiate a vigorous inflammatory response. The effect of GM-CSF on leukotriene expression is quite striking. In the absence of GM-CSF, the production of all of the peptidoleukotrienes is greatly reduced. This defect appears to be the result of a diminished content of two proteins that are critical for leukotriene production. Expression of 5-lipoxygenase, the rate-limiting enzyme for leukotriene synthesis, and of 5-lipoxygenase-activating protein are greatly reduced in AM from GM(−/−) mice compared with AM from wild-type mice (Coffey, unpublished observation). Stimulated leukotriene secretion by AM from SP-C-GM mice remains significantly reduced compared with that of AM from wild-type mice, demonstrating that these AM are not equivalent to those from wild-type mice. This is in contrast to the normalization of other functional capabilities in AM from mice expressing GM-CSF exclusively in alveolar epithelial cells. The absence of GM-CSF during monocyte development in the bone marrow may contribute to this failure of SP-C-GM AM to secrete normal levels of leukotrienes. However, AM themselves are normally a source of GM-CSF. It is possible that normal leukotriene expression by these cells requires autocrine expression of this growth factor by AM.

The mechanisms that lead to impaired AM function in the GM(−/−) mice have not been defined. It is possible that the aberrant phenotype of the AM from GM(−/−) mice is a consequence of loss of expression of a series of GM-CSF-responsive genes such as TNF-α (26). It is also possible that a number of the functional capabilities of the mature AM are interdependent. For instance, expression and binding of β2-integrins can be critical for phagocyte adherence, phagocytosis, and respiratory burst (29). Thus significantly reduced expression of CD11a/CD18 and CD11c/CD18 by the GM(−/−) AM is likely to contribute to the functional deficiencies of these cells.

The increased phospholipid and protein content in the alveolar space of GM(−/−) mice could contribute to some of the abnormalities in AM function. Excess cell surface SP-A has been shown to impair AM secretion of TNF-α (4, 16). However, we found that washing AM from GM(−/−) mice with EGTA to remove surface-bound SP-A did not restore TNF-α secretion to that found in wild-type cells. Similarly, sedimentation of AM through albumin to remove excess surfactant phospholipid did not correct the defect in TNF-α expression. Importantly, there were characteristics that were increased in the GM(−/−) mice compared with controls. First, cell surface expression of CD11b was increased.

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in AM from GM(−/−) mice compared with wild-type AM, perhaps suggesting that in the absence of GM-CSF, alveolar mononuclear cells have a less mature phenotype. Second, MCP-1 secretion in response to LPS in vitro was increased in GM(−/−) AM compared with wild-type AM. This clearly indicates that the loss of TNF-α production is not simply a manifestation of a global defect in cytokine production. Thus, although we cannot exclude some effect of excess surfactant components on macrophage function, it is likely that the major mechanism accounting for impaired AM function in the GM(−/−) mice is the absence of GM-CSF.

Expression of GM-CSF exclusively in the lung in the absence of GM-CSF during monocyte development in the bone marrow is sufficient to induce expression of most of the characteristics of the mature AM phenotype in SP-C-GM mice. AM are differentiated cells (3) that differ from blood monocytes or tissue macrophages at other sites. Our data now indicate that GM-CSF plays a complex role in coordinating the maturation of AM in the alveolar space. In the absence of GM-CSF, both signaling and effector functions of AM are severely disrupted, whereas pulmonary expression of GM-CSF promotes expression of features of the fully differentiated AM phenotype. These data support the hypothesis that pulmonary GM-CSF acts as a local regulator of AM maturation.

The manner in which the number of AM in the normal lung is sensed and the means by which this number is controlled are poorly understood. Increased numbers of AM were recovered from both SP-C-GM and GM(−/−) mice compared with wild-type mice. In the SP-C-GM mice, this increase may be attributable to the mitogenic effect of GM-CSF for macrophages (32) or to the chemotactic activity of GM-CSF for AM (20). Our finding that MCP-1 was expressed in lung lavage fluid from GM(−/−) mice but not in normal controls is consistent with the hypothesis that monocyte chemotactants are induced in the absence of GM-CSF in an attempt to regulate AM activity in the alveolar space by increasing the recruitment of monocytes to the lung. Our studies broaden the concept of pulmonary GM-CSF as a regulator of alveolar homeostasis. Previous studies have defined a role for GM-CSF on AM in pulmonary surfactant homeostasis and have demonstrated that GM(−/−) mice are more susceptible to pneumonia because of two specific pathogens, group B streptococcus (13) and P. carinii (21). Our findings indicate that GM(−/−) AM have a global defect affecting both phagocytosis and expression of pivotal inflammatory signals. Based on these functional deficits, one would predict that GM(−/−) mice would be susceptible to pulmonary infections with a wide range of pathogens, from routine bacteria to eukaryotes. Thus one may think of pulmonary GM-CSF as playing a “homeostatic” role in host defense in the lung by providing an environment in which AM are equipped to provide an optimal innate immune response to invading pathogens.

In conclusion, we have found that GM(−/−) mice have increased numbers of AM but that these AM demonstrate a broad-based abnormality in host defense function compared with AM from wild-type mice. In particular, AM adherence and expression of β2-integrins are diminished, phagocytosis of latex microbeads in vivo and in vitro is impaired, and secretion of TNF-α and leukotrienes is greatly diminished. Interestingly, targeted expression of GM-CSF exclusively in the alveolar space is sufficient to restore most aspects of AM function, with the exception of leukotriene production. These studies provide further evidence that GM-CSF in the alveolar space is a critical component of the pulmonary innate immune response and can determine the adequacy of the innate immune response to the initial entry into the lung of noxious infectious agents.

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