GM-CSF expression by human lung microvascular endothelial cells: in vitro and in vivo findings

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Burg, Jürgen, Vera Krump-Konvalinkova, Fernando Bittinger, and Charles James Kirkpatrick. GM-CSF expression by human lung microvascular endothelial cells: in vitro and in vivo findings. Am J Physiol Lung Cell Mol Physiol 283: L460–L467, 2002. First published March 22, 2002; 10.1152/ajplung.00249.2001.—Recently, many findings indicate that granulocyte-macrophage colony-stimulating factor (GM-CSF) plays an important role in the pathogenesis of acute and chronic lung diseases. In the present paper, the production of this cytokine in human pulmonary microvascular endothelial cells (HPMEC) is investigated. In an in vitro study, quiescent HPMEC did not express GM-CSF, either at the transcriptional or at the protein level. After activation for 4 h with tumor necrosis factor (TNF)-α (30/300 U/ml), lipopolysaccharide (LPS; 0.1/1 μg/ml), or interleukin (IL)-1β (100 U/ml), a significant release of GM-CSF was measured by enzyme-linked immunosorbent assay, with a time-dependent increase over 72 h. IL-8 (4, 16, or 64 ng/ml) or IL-1α at a concentration of 10 U/ml did not induce the release of GM-CSF. Human umbilical vein endothelial cells (HUVEC) and the angiosarcoma cell line HAEND served as reference cell lines. GM-CSF release in HPMEC was significantly (P < 0.025–0.05) less inducible by IL-1β than in HUVEC. A constitutive expression of GM-CSF by HAEND was observed. Additionally, GM-CSF expression in vivo by the lung microvasculature was confirmed by immunohistochemistry in lung tissue. To our knowledge, this is the first report of the ability of human pulmonary endothelial cells to synthesize and release GM-CSF. These results support the hypothesis that the lung microvasculature via the production of GM-CSF is a potential contributor to the cytokine network in lung diseases. This could be of particular importance in the pathogenesis of the acute respiratory distress syndrome in which endothelial dysfunction plays a central pathogenetic role.

granulocyte-macrophage colony-stimulating factor; human lung; microvasculature; endothelium

besides hemodynamic and hemostatic functions, the microvasculature of the lung is involved in the recruitment of inflammatory blood cells into the interalveolar space and is therefore crucial in both acute and chronic lung diseases (6, 35, 47, 48). Apart from these more permissive functions, little is known about the direct contribution of the microvascular endothelium to lung pathogenesis.

Granulocyte-macrophage colony-stimulating factor (GM-CSF), which was originally identified because of its effects on hematopoietic progenitors, has proved to exert varying effects on fully differentiated cells. The human GM-CSF gene is located on chromosome 5 in the same region as interleukin (IL)-3, IL-4, IL-5 and the macrophage colony-stimulating factor receptor. The human GM-CSF protein contains 144 amino acid residues, including a 17-amino-acid signal peptide, which is removed during secretion. Native GM-CSF is heavily glycosylated, resulting in a molecular mass of 14.5–34 kDa (for review, see Ref. 11). The GM-CSF receptor is a member of the hematopoietin receptor superfamily and is comprised of an α-chain (GM-CSF receptor-α) specific for GM-CSF and a β-chain shared with the IL-3 and IL-5 receptors (24, 30).

Recent findings reported from different laboratories suggest a pivotal role of GM-CSF in the pathogenesis of inflammatory lung diseases, acute respiratory distress syndrome (ARDS), and lung fibrosis. In asthma, enhanced GM-CSF production by lung inflammatory cells and bronchial epithelial cells has been well documented (5, 8, 10). There is evidence that GM-CSF is important in regulating pulmonary surfactant homeostasis and that reduced activity of GM-CSF and its receptor is associated with alveolar proteinosis (34, 36, 40, 50). In patients suffering from ARDS, it has recently been shown that GM-CSF is elevated in bronchoalveolar fluid and that GM-CSF has an influence on granulocyte viability (13, 28). Septic preterm infants had significantly higher plasma concentrations of GM-CSF than healthy preterm infants (18). In addition, after intravenous endotoxin challenge in humans, an increase in plasma GM-CSF was observed (23).

There is now a large body of evidence indicating that GM-CSF plays a particular role in the development of lung fibrosis, although reports are conflicting. Xing et al. (43, 44, 45) have shown that overexpression of GM-CSF in the rat lung causes accumulation of eosinophils and macrophages in the early stages, followed by fibrosis in later stages. Overexpression of GM-CSF...
in type II alveolar cells of transgenic mice increased lung size and caused type II cell hyperplasia, which is associated with acute and chronic lung diseases (16). In bleomycin-induced rat lung fibrosis, total mRNA of GM-CSF in the lung was already upregulated after 6 h and returned to basal levels after 24 h, followed by upregulation of transforming growth factor (TGF)-β (2), which suggests a particular role in the early phase of pulmonary fibrosis. Piguet et al. (33) observed that the application of a neutralizing antibody to GM-CSF markedly aggravated collagen deposition in bleomycin-induced lung damage in mice. On the other hand, results from Moore et al. (31) support the theory of an anti-fibrotic potential of GM-CSF, since bleomycin-induced lung fibrosis was more marked in GM-CSF knock-out mice. After bleomycin injury, they also observed a diminished expression of GM-CSF in isolated alveolar epithelial cells from rats (7).

Cultured human lung fibroblasts release GM-CSF constitutively, and this cytokine has been shown to exert a chemokinetic effect on monocytes (22). Moreover, there is evidence from in vitro experiments using human umbilical endothelial cells (HUVEC) and human monocytes that direct monocyte-endothelial interaction induces GM-CSF production in both cell types (39). Recently, coculture experiments using IL-1β-activated HPMEC and human eosinophils have shown that GM-CSF mRNA expression is upregulated in transmigrated eosinophils and that in vitro survival is longer (46).

In humans, inflammatory cells, lung macrophages, and bronchial epithelial cells are capable of producing GM-CSF (1, 3, 10, 21) so that in the complex microanatomy of the lung a variety of the cell types could contribute to GM-CSF levels in various pulmonary diseases.

Little is known about the role of microvascular endothelial cells of the lung in this context. Nevertheless, because of the essential pathogenetic role of the dysfunctional microcirculation in the development of ARDS and multiple organ dysfunction syndrome (19, 26), we investigated GM-CSF expression by primary isolated human pulmonary microvascular endothelial cells (HPMEC), both in the unstimulated state and after pretreatment with proinflammatory stimuli (tumor necrosis factor (TNF)-α, IL-1β, lipopolysaccharide (LPS), and IL-8). Macrophage-like cells from HUVEC and an angiofibroma cell line (HAEND) served as reference cell lines. To study the in vitro relevance of these data, the expression of GM-CSF in inflammed lung tissue was also investigated by immunohistochemistry.

MATERIALS AND METHODS

Cell Isolation, Culture, and Characterization

HUVEC were isolated according to the method described by Jaffe et al. (17). The cells were cultured in a 1:1 mixture of Ham’s F-12 medium and Iscove’s modified Dulbecco’s medium (GIBCO-BRL) supplemented with 20% FCS, penicillin-streptomycin solution (20 U/ml-20 µg/ml; GIBCO-BRL), and L-glutamine (2 mM; GIBCO-BRL) in a humidified atmosphere containing 5% CO2 and 10% O2.

Isolation and culture of HPMEC were performed by a modification of the method of Hewett and Murray (14) and Kirkpatrick et al. (20). Normal human lung tissue was obtained from lobectomy specimens resected because of lung tumors. No adjuvant chemotherapy had been given. Briefly, isolation of HPMEC was performed as follows: subpleural lung tissue was cut into small fragments with scissors. After removal of debris and erythrocytes through a 40-µm nylon net, the tissue was treated with dispase (1.18 U/ml at 4°C for 18 h). After filtration through a 100-µm nylon net, the tissue was treated in a volume of 4 ml with elastase (40 units), trypsin (0.05%), and EDTA (1.8 mM) for 30 min at 37°C followed by a further 100-µm net filtration. The cell clumps were repeatedly resuspended in PBS-BSA and filtered through a 40-µm net, followed by centrifugation for 10 min and resuspension in medium-199 plus 20% pooled FCS. This mixed cell culture had a purity of ~30 ± 5% concerning the GM-CSF-positive endothelial cells. The culture was cultivated at 37°C in a gas mixture of 5% CO2 in air for 5–7 days, but not longer, to avoid overgrowth of contaminant cells. The developing monolayer was disrupted by treatment with 0.2% EDTA and 0.2% BSA for 30 min at 37°C followed by a mixture of 0.25% trypsin and 0.25% EDTA for 1 min. The positive selection of HPMEC was achieved by interacting the cell suspension with magnetic beads (1 µm diameter; Dia- anova) coated with a mouse monoclonal antibody against human platelet endothelial cell adhesion molecule-1 (PECAM-1; Immunotech). The subsequent pure cultures of PECAM-1-positive HPMEC were also shown to be positive for CD34, factor VIII-related antigen, CD38 (thrombospo- nadin receptor), Ulex europaeus agglutinin-1, prostacyclin, IL-1, IL-6, and plasminogen activator inhibitor-1. The cells also showed uptake of 1,1′-dioctadecyl-3,3′,3′-tetramethyl-indo- carbocyanine-acetylated low-density lipoprotein (Dil-Ac-LDL). Contaminant cells were detected by immunocytochemistry with antibodies against CD68, cytokeratins, or smooth muscle actin (SMA). Fibroblasts or SMA-negative myofibroblast-like cells were identified because of their characteristic morphology and rapid growth with nest formation. When no contaminant cells were detected, purity was determined at >99%. The viability, growth characteristics, and immunohistochemical phenotype were unchanged until passage 12.

HAEND cells were a gift of Dr. V. Vetvicka (University of Louisville, Kentucky). HAEND cells were originally derived from a human liver angiosarcoma (15). In our laboratory, we confirmed immunohistochemical positivity for von Willebrand factor and Ulex europeaus agglutinin-1; uptake of Dil-Ac-LDL was also demonstrated. The cells were cultured in RPMI 1640 supplemented with 10% FCS, penicillin-streptomycin solution (20 U/ml-20 µg/ml; GIBCO-BRL), and L-glutamine (2 mM; GIBCO-BRL).

Activation of Cells, Analysis by Enzyme-Linked Immunosorbent Assay, and RT-PCR

Cell activation. Before activation, the viability of the cells was tested by morphological inspection and Trypan blue exclusion. Cells were seeded in 24-well culture plates and allowed to adhere overnight (125,000 cells/well; 0.625 ml growth medium). Subconfluent cultures of HPMEC (passages 3–7) or HUVEC (passages 2–3) were stimulated by adding TNF-α (30/300 U/ml; Sigma), IL-1β (10/100 U/ml; Strathmann Biotech, Hannover, Germany), LPS (0.1/1 µg/ml; Sigma), or IL-8 (4, 16, or 64 ng/ml; Sigma) to the culture medium for 4, 24, or 72 h.
After the activation period, the supernatant was harvested and stored at −20°C. Supernatant (100 μl) correlated approximately with an original cell count of 20,000. For RNA isolation, cell lysates were stored in TRIzol (Life Technologies). The activation experiments with HUVEC and HPMEC were each repeated with four different donors.

HAEND cells were activated after 4 and 24 h with LPS (1 μg/ml), TNF-α (300 U/ml), and IL-1β (100 U/ml). These experiments were repeated three times.

Enzyme-linked immunosorbent assay analysis. The concentration of GM-CSF in the supernatant was measured at three different dilutions (1:1, 1:8, and 1:16) by a highly sensitive and specific sandwich enzyme-linked immunosorbent assay (ELISA) according to the manufacturer’s instructions (Quantikine; R&D Systems). Each dilution was measured in triplicate. Samples were quantitated from the linear portion of the standard curve, with detection limits between 3 and 5 pg/ml.

RT-PCR analysis. Total cellular RNA of unstimulated HPMEC and HUVEC or after 24 h of stimulation with LPS (1 μg/ml) was prepared using TRIzol reagent according to the instructions of the manufacturer (Life Technologies). First-strand cDNA synthesis was performed using the Superscript Preamplification System (Life Technologies) according to the manufacturer’s instructions. Two micrograms of total cellular RNA were reverse transcribed using random hexamers. The reaction products were diluted to 200 μl to be within the linear range of PCR amplification. Two-microliter aliquots of diluted cDNAs were used in PCR amplifications. Amplification reactions were run in a Perkin-Elmer 2400 thermal-cycler. PCR conditions for GM-CSF and platelet-derived growth factor (PDGF)-α were as follows: denaturation for 2 min at 94°C followed by 35 three-step amplification cycles of 94°C for 45 s, 55°C for 45 s, followed by a final extension step at 72°C for 8 min. PCR conditions for β-actin were denaturation for 2 min at 94°C followed by 35 three-step amplification cycles at 94°C for 45 s, 55°C for 45 s, 72°C for 45 s, and a final extension step at 72°C for 8 min. The PCR reaction mixtures (10 μl) were analyzed on a 2% agarose gel containing ethidium bromide. The oligonucleotide primer pairs were designed to preferentially amplify cDNA and to allow distinction between genomic and cDNA amplification products. The sequences of primers and length of PCR products were as follows: GM-CSF (190 bp): sense, 5'-CTG CTG AGA TGA ATG AGG-3' and antisense, 5'-GCA CAG GAA GTT TCC GGG GT-3'; PDGF-α (225 bp): sense, 5'-CAC GCC CAT TCG GAA GAG-3' and antisense, 5'-TTG GCC ACC TTG ACG CTG CG-3'; and β-actin (574 bp): sense, 5'-GAC CTG ACT GAC TAC CTC ATG A-3' and antisense, 5'-AGC ATT TGC GGT GGA CGA TGG AG-3'.

Tissue Preparation and Immunohistochemistry

Lung tissue was obtained from peritumoral pneumonia, from two cases of lung extirpation because of end-stage cystic fibrosis, and chronic candida pneumonia with sepsis. Uninflamed lung tissue served as a control and was obtained from a lung lobe unaffected by tumor. Small tissue fragments were fixed in an ethanol-based medium (Notox; Quartett) for 4 h taken through graded alcohols to 100% alcohol for 3 h and then routinely embedded in paraffin. Paraffin sections (4 μm thick) were stained using the avidin-biotin complex (ABC) method. After blocking unspecific avidin or biotin binding with a blocking kit (Avidin/Biotin Blocking Kit; Vector Laboratories, Burlingame, CA), nonspecific binding of immunoglobulins was blocked by preincubation with 4% nonfat dried milk/2% normal rabbit serum (Vector Laboratories) in Tris buffer (pH 7.6) at room temperature for 30 min. Primary polyclonal antibody against GM-CSF (sc-1321; Santa Cruz Biotechnology) was diluted 1:100 in a blocking reagent (Boehringer-Mannheim, Mannheim, Germany). Primary antibody and negative controls were incubated overnight at 4°C. Slides were then rinsed with Tris buffer (pH 7.6) and incubated for 30 min at room temperature with a secondary rabbit anti-goat antibody in a dilution of 1:200 (BA-5000; Vector Laboratories).

Incubation with the ABC complex conjugated with alkaline phosphatase (DAKO, Hamburg, Germany) for 30 min at room temperature was followed by the addition of substrate using the new fuchsin method. Endogenous alkaline phosphatase was blocked by adding levamisole (Sigma) to the substrate solution. Color development was stopped by immersing the slides in Tris buffer (pH 7.6). Counterstaining was performed with hematoxylin, and the slides were mounted with glycerin gelatine (Merck, Darmstadt, Germany).

Negative controls were incubated with an isotype control (normal goat IgG, sc 2028; Santa Cruz Biotechnology) and showed no reactivity.

Statistical Analyses

Data were statistically evaluated using the Mann-Whitney U-test. Statistical analyses were performed with the help of Microsoft Excel 97. P values <0.05 were taken as statistically significant.

RESULTS

Analysis of GM-CSF Expression in HPMEC by ELISA and RT-PCR

Quiescent HPMEC neither transcribe nor release GM-CSF. Analysis of supernatants of unstimulated HPMEC, cultivated in fresh growth medium for 4, 24, and 72 h, was performed. The data from the ELISA technique indicate that the cells did not release measurable amounts of GM-CSF. This observation was confirmed with HPMEC from different donors. Furthermore, RT-PCR of total cellular RNA from unstimulated HPMEC showed no constitutively expressed GM-CSF transcript, in contrast to β-actin (a housekeeping gene) and PDGF-α, which were both transcribed constitutively (Fig. 1).

Activation of HPMEC with TNF-α, IL-1β, and LPS led to the expression of GM-CSF. Primary cell isolates were activated with TNF-α (300 U/ml), LPS (1 μg/ml), and IL-1β (100 U/ml) for 4, 24, and 72 h (Fig. 2A). To study the dose-response relationship, 10 times lower concentrations of TNF-α, LPS, and IL-1β were applied (Fig. 2B).

In contrast to quiescent HPMEC, after 24 h of activation with LPS (1 μg/ml), analysis of total cellular RNA revealed a strong signal at 190 bp, corresponding to the transcript of GM-CSF (Fig. 1).

After 4 h of stimulation with TNF-α (300 U/ml), GM-CSF was released in the supernatant. After 24 h, a marked increase in the expression was observed followed by a further increase after 72 h (Fig. 2A). A 10 times lower concentration of TNF-α (30 U/ml) also induced the time-dependent increased expression of GM-CSF, with concentrations being proportionally
lower compared with the 10 times higher activation dose (Fig. 2).

The strongest stimulus for HPMEC to produce GM-CSF was LPS (1 μg/ml), with maximal concentrations measured after 3 days, corresponding to $1,305 \pm 104 \text{ pg}\cdot\text{ml}^{-1}\cdot2 \times 10^{-5}$ cells. Pretreatment with 0.1 μg/ml LPS led to the expression of GM-CSF in a concentration range only slightly lower than that induced by the 1 μg/ml concentration (Fig. 2B).

As was the case with TNF-α and LPS, IL-1β (100 U/ml) also caused a time-dependent release of GM-CSF over the time period from 4 to 72 h (Fig. 2). However, at a concentration of 10 U/ml, IL-1β did not induce a measurable GM-CSF production in HPMEC after 4, 24, and 72 h of stimulation (Fig. 2). Three different concentrations of IL-8 (4, 16, or 64 ng/ml) did not induce any release of GM-CSF by HPMEC after 4, 24, or 72 h.

**GM-CSF Expression in HUVEC and HAEND Compared With HPMEC**

GM-CSF expression in the unstimulated state and after activation with proinflammatory stimuli was different in various cells of endothelial origin. As was the case for HPMEC, HUVEC also showed no constitutive expression of GM-CSF at the protein level or the transcriptional level, as was demonstrated by RT-PCR.
Like HPMEC, a time-dependent increase in the production of the cytokine after 4, 24, and 72 h of HUVEC stimulation with TNF-α (300 U/ml), IL-1β (100 U/ml), and LPS (1 µg/ml) was measured in the supernatant (Fig. 2A). Additionally, with 10 times lower concentrations of TNF-α, IL-1β, and LPS a strong response was observed even after 4 h.

HPMEC responded in a much weaker fashion to IL-1β (100 U/ml) than did HUVEC, as was confirmed with endothelial cells from four different lung donors (Fig. 2A). The difference was statistically significant, as was evaluated by the U-test ($P < 0.05$ after 4 h, $P < 0.025$ after 24 or 72 h). To compensate for different levels of response because of the primary nature of the endothelial cells, concentrations of GM-CSF released by IL-1β (100 U/ml) were related to the concentration of GM-CSF induced by LPS (1 µg/ml) or TNF-α (300 U/ml) in each experiment. Again, the difference was found to be significant ($P < 0.025$–0.05). Moreover, HPMEC in contrast to HUVEC did not respond to IL-1β at a concentration of 10 U/ml (Fig. 2B).

After activation with TNF-α (30 and 300 U/ml), the mean concentration of GM-CSF released by HUVEC was higher than that by HPMEC, especially with the lower concentration, although differences were not statistically significant (Fig. 2). After activation with LPS (0.1 and 1 µg/ml) again no significant difference between the amount of GM-CSF released by HPMEC or HUVEC was observed.

In contrast to HPMEC or HUVEC, the angiosarcoma cell line HAEND exhibited a constitutive expression of GM-CSF (Fig. 3). The expression of GM-CSF was also upregulated by TNF-α (300 U/ml), LPS (1 µg/ml), or IL-1β (100 U/ml), with a marked time-dependent increase (Fig. 3).

**GM-CSF Production by the Human Lung Microvasculature Was Confirmed by Immunohistochemistry**

Lung tissue excised from inflamed peritumoral areas (Fig. 4A), from a candida pneumonia with sepsis...
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(Fig. 4B), and from a lung with end-stage cystic fibrosis demonstrated strong focal positivity for GM-CSF in the microvasculature in areas with florid inflammation (Fig. 4).

In addition to the endothelial cells, GM-CSF was expressed in bronchial epithelial cells, intra-alveolar macrophages, and granulocytes (Fig. 4).

By contrast, in uninfammed lung tissue, expression of GM-CSF in the microvasculature was absent, but bronchial epithelial cells exhibited a positive signal both in the uninfammed bronchi and in acute bronchitis (Fig. 4, C and D). No expression in lung fibroblasts was seen.

DISCUSSION

In the pathogenesis of acute and chronic lung diseases, many different cell types act together (resident cells as well as cells recruited from the circulation). Recent findings suggest a pivotal role of GM-CSF in the development of various lung diseases (2, 5, 28, 40), although a picture of the pathogenetic role of this important cytokine is still far from complete. In contrast to many previous investigations, which have concentrated on inflammatory cells, especially lung macrophages as a source of this cytokine, we focused in the present paper on the lung microvascular endothelium. Because it is located in immediate anatomical and physiological relationship to intravascular, interstitial, and alveolar cells, cytokines released by dysfunctional microvascular endothelial cells could exert local effects in a paracrine fashion. This is even more feasible when taking into account that, in the dysfunctional state of the endothelium, transudation of blood plasma components is seen. Moreover, it represents in its entirety 30–40% of the total cells in the alveolar functional unit and thereby could be a potentially important contributor to systemic production of GM-CSF.

Our results show that primary isolated human microvascular endothelial cells from the lung, simulating an inflammatory state by activation with TNF-α (30 or 300 U/ml), IL-1β (100 U/ml), and LPS (0.1 μg/ml), are able to produce considerable amounts of GM-CSF. However, quiescent HPMEC showed neither transcriptional activity nor release of the GM-CSF protein over a period of 72 h. After activation for 24 h with LPS, a strong signal for the GM-CSF transcript was observed, demonstrating that de novo synthesis of GM-CSF is taking place. In accordance with Lenhoff and Olofsson (27), GM-CSF production in HUVEC is inducible with LPS, TNF-α, or IL-1β. We confirmed the observation of Takahashi et al. (39) by RT-PCR using different PCR primers that unstimulated HUVEC do not show any transcriptional activity for GM-CSF.

Differences in the activation profiles of HUVEC and HPMEC were seen. The microvascular endothelial cells from the lung responded significantly weaker (P < 0.05 after 4 h; P < 0.025 after 24 or 72 h) to IL-1β (100 U/ml) than did HUVEC. In addition, over a period of 72 h, no release of GM-CSF by HPMEC was observed after stimulation with IL-1β at a 10 times lower concentration. No significant difference was observed between HUVEC and HPMEC after stimulation with LPS and TNF-α. Previous studies concerning the secretion of urokinase-type plasminogen activator have already indicated differences between the human lung microvascular endothelium and HUVEC (38). In the bovine lung, TNF-α caused different effects on microvascular vs. macrovascular endothelial cell monolayers of the lung (29). These results support the fact that endothelial cells of different topography exhibit distinct properties and that therefore human microvascular endothelial cells from the lung are required when investigating interstitial lung pathology. The various endothelial cell models are not necessarily interchangeable.

Interestingly, HAEND, an angiosarcoma cell line, exhibited in contrast to HPMEC or HUVEC a constitutive expression of GM-CSF. It seems that, during sarcoma development, the control of GM-CSF gene transcription is lost in cultured angiosarcoma cells. Nevertheless, a marked upregulation of GM-CSF expression was observed after HAEND activation with TNF-α, IL-1β, and LPS.

Our in vitro findings that HPMEC have the capacity to express GM-CSF were confirmed in lung tissue by immunohistochemistry. Inflammed lung tissue from peritumoral pneumonia, candida pneumonia with sepsis, and from end-stage cystic fibrosis and un inflammed lung tissue were investigated by immunohistochemistry for the expression of GM-CSF. In areas of acute inflammation, a definitive positivity of the microvasculature for GM-CSF was observed. Additionally, intra-alveolar macrophages, granulocytes, and bronchial epithelial cells exhibited a strong positivity, with the latter cells being positive in inflammed and uninflammed areas. The potential of these cells to express GM-CSF is already known from in vitro experiments (8, 21, 39). However, expression of GM-CSF by fibroblasts could not be seen. This is in marked contrast to in vitro results, which have shown a constitutive expression of GM-CSF by lung fibroblasts (22).

We were able to demonstrate that the lung microvasculature is a potential contributor to the cytokine network in lung disease. The mechanism by which GM-CSF might initiate or accelerate pathological lung processes is poorly understood. In early phase inflammation, recruitment of inflammatory cells is of particular importance. In vitro and in vivo findings suggest that GM-CSF promotes inflammatory cell adhesion to the endothelium and could potentially assist in transmigration of inflammatory cells (4, 11, 37, 44, 49).

An increase in the number of inflammatory cells induced by GM-CSF could also be because of either an enhanced lifespan or possibly proliferative effects. HPMEC are in close vicinity both to intravascular and interstitial inflammatory cells and could, via the expression of GM-CSF, modulate the activity of inflammatory cells. In mice as well as in vitro, GM-CSF has been shown to upregulate neutrophil and eosinophil activity by increasing their survival and function (9, 12, 32, 41, 46). GM-CSF has also been described as
exerting proliferative effects on alveolar macrophages, which are increased in acute and chronic lung diseases (25, 42). It would appear that GM-CSF is particularly upregulated in early stage lung diseases and that the development of a fibrotic lung reaction after overexpression of GM-CSF in the rat lung is mainly attributable to an increase in TGF-β (2).

Besides the above-mentioned local effects, GM-CSF could exert systemic effects, e.g., on hematopoiesis, because in the circulation it has a biphasic half-life (t1/2) of 10 min followed by a second t1/2 of 85 min (11).

To our knowledge, this is the first report that activated human lung microvascular endothelial cells produce GM-CSF, which has recently been shown to be pathogenetically important in various lung diseases.

In conclusion, we postulate that the microvasculature, which holds a central position anatomically and physiologically, via its production of GM-CSF, could be a crucial contributor to the pulmonary production of this important cytokine. Although much work still has to be done to elucidate the sequential pathomechanisms, our observation supports the hypothesis that the dysfunctional pulmonary microvascular endothelium, that is, activated by pro-inflammatory stimuli, could contribute to the induction of the interstitial fibrosis characteristic of late-stage ARDS.

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