Non-Small Cell Lung Cancer Cells Induce Monocytes to Increase Expression of Angiogenic Activity

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Non-Small Cell Lung Cancer Cells Induce Monocytes to Increase Expression of Angiogenic Activity

Eric S. White, Scott R. B. Strom, Noel L. Wys, and Douglas A. Arenberg

Tumors are dependent on angiogenesis for survival and propagation. Accumulated evidence suggests that macrophages are a potentially important source of angiogenic factors in many disease states. However, the role(s) of macrophages in non-small cell lung cancer (NSCLC) have not been determined. We hypothesized that monocyte-derived macrophages are induced by NSCLC to increase expression of angiogenic factors. To define the role of macrophage-tumor cell interaction with respect to angiogenesis, human peripheral blood monocytes (PBM) were cocultured with A549 (human bronchoalveolar cell carcinoma) or Calu 6 (human anaplastic carcinoma) NSCLC cells. The resultant conditioned medium (CM) was evaluated for angiogenic potential and for expression of angiogenic factors. We found that endothelial cell chemotactic activity (as a measure of angiogenic potential) was significantly increased in response to CM from cocultures of PBM/NSCLC compared with PBM alone, NSCLC alone, or a combination of NSCLC and PBM CM generated separately. Subsequent analysis by ELISA revealed markedly increased CXC chemokine expression, with a lesser increase in vascular endothelial growth factor, in CM from PBM/NSCLC coculture. Neutralizing Ab to angiogenic CXC chemokines blocked the increase in endothelial cell chemotaxis. Furthermore, with separately generated CM as a stimulus, we found that macrophages are the predominant source of increased CXC chemokine expression. Finally, we found that NSCLC-derived macrophage migration-inhibitory factor is responsible for the increased expression of macrophage-derived angiogenic activity. These data suggest that the interaction between host macrophages and NSCLC cells synergistically increases angiogenic potential, and that this is due to an increased elaboration of angiogenic CXC chemokines. The Journal of Immunology, 2001, 166: 7549–7555.
clear cells (~30% monocytes and 70% lymphocytes) were washed twice with HBSS without Mg²⁺ or Ca²⁺, and resuspended in serum-free RPMI 1640 with 0.1% BSA at a concentration of 3 × 10⁶ cells/ml. To generate monocyte-conditioned medium (CM), 500 μl of the suspension was plated out in each well of a 24-well plate. Plates were incubated at 37°C with 5% CO₂ for 1 h, and nonadherent cells were removed by washing with RPMI 1640. Wells then were filled with 500 μl of serum-free RPMI 1640, and specimens were incubated for 48 h at 37°C with 5% CO₂. At 48 h, CM was harvested and centrifuged to remove any cell debris. All samples were stored at −20°C until used in assays.

**Monocyte/NSCLC coculture**

A549 or Calu 6 cells were grown to 80–90% confluence in six-well plates. Cells then were washed twice with serum-free medium, and PBM (isolated as above) were added into the upper chamber of a 25-mm transwell insert (Nunc, Naperville, IL) and allowed to adhere for 1 h. Cells were washed twice with RPMI 1640 and then transwell inserts were placed directly into A549-containing wells. The upper chamber then was filled with 1 ml of serum-free medium. Cocultures were incubated for 48 h at 37°C with 5% CO₂. At 48 h, CM was harvested and centrifuged at 10,000 × g for 5 min. All CM was stored at −20°C until used for the various assays. In some experiments, PBM were exposed to NSCLC CM, and NSCLC cells were exposed to PBM CM.

**Endothelial cell chemotaxis assay**

Chemotaxis membrane filters (5.0-µm pore size; Osmonics, Livermore, CA) were first prepared by bathing in 2.8% glacial acetic acid for 24 h, followed by two 1-h rinses in deionized, distilled water. The filters then were transferred to a 0.01% gelatin bath and allowed to soak for 24 h. Filters were dried under a laminar flow hood and stored at room temperature for up to 1 month. Endothelial cell chemotaxis assays were performed in 12-well, blind well chemotaxis chambers (Neuroprobe, Cabin John, MD). Briefly, human endothelial cells were suspended at a concentration of 10⁶ cells/ml in their respective serum-free medium (in the case of HMEC-1, MCD-131 medium was supplemented with 0.1% BSA). Endothelial cells were placed into each of the bottom wells (165 μl). Membranes were placed over the wells, the gasket applied, and the chambers sealed. Chambers were inverted and incubated at 37°C with 5% CO₂ for 1 h (HMEC-1) or 2 h (HMVEC) to allow for endothelial cell adherence to the membrane. Chambers then were reinserted and the CM to be studied was placed in the upper well (116 μl). Chambers were reincubated for 2 h. Membranes then were scraped to remove any adherent, un migrated endothelial cells from the lower chamber, fixed in methanol, stained with a modified Wright-Giemsa stain, and cells that had migrated through the membrane were counted in five high-power fields (hpf; ×200). Results were expressed as the mean number of endothelial cells that had migrated per hpf ± SEM. Each sample was assessed in triplicate. Experiments were performed at least three times. Results obtained were similar whether HMEC-1 or HMVEC cells were used. Each experiment was accompanied by a positive control (VEGF 100 ng/ml) and a negative control (nonconditioned medium) to account for variable chemotaxis from one assay to the next (data not shown).

**ELISA**

CXC chemokines (IL-8 (CXCL8), ENA-78 (CXCL5), GRO-α (CXCL1), MIG, (CXCL9); and IP-10, CXCL10), MIF, and VEGF were quantitated by a double-ligand method described previously (5). In brief, flat-bottom 96-well microtiter plates (Immuno Plate F96; Nunc) were coated with the appropriate purified Abs overnight at 4°C and then were washed three times with PBS with 0.05% Tween 20 (washing buffer). Non-specific binding sites were blocked with PBS and 2% BSA. After washing three times,

![Figure 1](https://www.jimmunol.org/article-pdf/7550/NSCLC%20INDUCES%20MACROPHAGE-DERIVED%20ANGIOGENIC%20ACTIVITY/17382948/220851/1/220851)

**FIGURE 1.** Endothelial cell chemotaxis to CM from A549 cells, Calu 6 cells, PBM, or coculture of A549 or Calu 6 with PBM. Data shown are representative examples of a minimum of three repeated experiments. Results were similar whether HMEC-1 or HMVEC cells were used. A, Endothelial cell chemotaxis to CM from A549 cells (63.1 ± 2.6 cells/hpf), PBM (49.1 ± 4.0), coculture CM (119.9 ± 4.7 cells/hpf), or separately generated A549 and PBM CM (91.4 ± 4.1). *p < 0.002 for the comparison of coculture medium to either A549 CM, PBM CM, or separately generated A549 and PBM CM. B, Endothelial cell chemotaxis to CM from Calu 6 cells (18.6 ± 2.1 cells/hpf), PBM (19.5 ± 2.6 cells/hpf), coculture CM (36.7 ± 1.6 cells/hpf), or separately generated Calu 6 and PBM CM (17.3 ± 0.9 cells/hpf). *p < 0.0005 for the comparison of coculture CM to either Calu 6 CM, PBM CM, or separately generated Calu 6 and PBM CM.

*(Methods)*

A549 or Calu 6 cells were grown to 80–90% confluence in six-well plates. Cells then were washed twice with serum-free medium, and PBM (isolated as above) were added into the upper chamber of a 25-mm transwell insert (Nunc, Naperville, IL) and allowed to adhere for 1 h. Cells were washed twice with RPMI 1640 and then transwell inserts were placed directly into A549-containing wells. The upper chamber then was filled with 1 ml of serum-free medium. Cocultures were incubated for 48 h at 37°C with 5% CO₂. At 48 h, CM was harvested and centrifuged at 10,000 × g for 5 min. All CM was stored at −20°C until used for the various assays. In some experiments, PBM were exposed to NSCLC CM, and NSCLC cells were exposed to PBM CM.

**Table I. ELISA analysis of A549, PBM, Calu 6, or coculture CM for CXC chemokines and VEGF**

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-8 (CXCL8)</th>
<th>ENA-78 (CXCL5)</th>
<th>GROα (CXCL1)</th>
<th>IP-10 (CXCL10)</th>
<th>MIG (CXCL9)</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549</td>
<td>0.3 ± 0.1</td>
<td>18.1 ± 1.1</td>
<td>0.3 ± 0.01</td>
<td>ND</td>
<td>0.4 ± 0.1</td>
<td>3.7 ± 0.8</td>
</tr>
<tr>
<td>Calu 6</td>
<td>0.5 ± 0.2</td>
<td>ND</td>
<td>1.2 ± 0.01</td>
<td>0.11 ± 0.01</td>
<td>0.5 ± 0.1</td>
<td>2.3 ± 0.1</td>
</tr>
<tr>
<td>PBM</td>
<td>26.4 ± 6.0</td>
<td>27.5 ± 7.5</td>
<td>8.4 ± 2.6</td>
<td>0.03 ± 0.01</td>
<td>0.6 ± 0.1</td>
<td>ND</td>
</tr>
<tr>
<td>A549/PBM</td>
<td>53.7 ± 7.1*</td>
<td>67.8 ± 9.4*</td>
<td>31.8 ± 10.1*</td>
<td>1.2 ± 0.5*</td>
<td>0.9 ± 0.2</td>
<td>2.5 ± 0.12</td>
</tr>
<tr>
<td>Calu 6/PBM</td>
<td>44.5 ± 2.9**</td>
<td>67.1 ± 15.9**</td>
<td>48.5 ± 9.6**</td>
<td>2.1 ± 0.5**</td>
<td>0.7 ± 0.1</td>
<td>0.8 ± 0.1</td>
</tr>
</tbody>
</table>

*Results are expressed as nanograms per milliliter ± SEM. ND, Levels below the lower limit of detection (<1 pg/ml). *p ≤ 0.01 for comparison with PBM alone or A549 alone. **p ≤ 0.05 for comparison with PBM alone or Calu 6 alone. Data are representative of three repeated experiments.*
samples (neat and 1:10 dilution) and standards were added as 50-μl aliquots and incubated at 37°C. Plates were washed three times and incubated with the respective biotinylated Ab at 37°C. Plates were washed three times and incubated with polyperoxidase-avidin substrate (Endogen, Woburn, MA) at room temperature to the desired extinction. The reaction was terminated with 0.5 M H₂SO₄. Plates were read at 490 nm in an ELISA reader. Standards were prepared as ½ log dilutions of purified recombinant Ab, from 100 ng/ml to 0.001 ng/ml per well. For VEGF and MIF, we developed an ELISA by performing multiple assays on standards of human recombinant protein from 1 pg/ml to 100 ng/ml using varying concentrations of coating and detecting Abs until optimal working concentrations were defined. VEGF ELISA used 20 ng/ml for coating and 75 ng/ml for detecting, and consistently and specifically detected quantities of VEGF₁₂₁ and VEGF₁₆₅ (the predominant secretory forms of human VEGF) at concentrations as low as 1 pg/ml. For MIF, 75 ng/ml was used for coating, and 20 ng/ml was used for detection. This ELISA had a similar sensitivity and specificity (data not shown).

Immunodepletion of MIF from NSCLC CM

A total of 250 mg of Protein A immobilized on Sepharose beads (Sigma, St. Louis, MO) was reconstituted in 15 ml of PBS (pH 8.0) overnight at 4°C, washed twice, then brought up in PBS to a final concentration of 150 mg/ml. A total of 1 μg of goat anti-human MIF or goat IgG (R&D Systems) was added to 100 μl of the protein A-Sepharose solution and brought up to a total volume of 500 μl in PBS, mixed thoroughly, and incubated for 2 h at 4°C. The beads then were washed and resuspended in 100 μl of PBS. Then 1 ml of A549 or Calu 6 CM was added to the beads and incubated on a rocker plate for 3 h at 4°C. The precipitate then was removed by centrifugation at 7500 × g for 5 min. Supernatants then were removed and used to stimulate adherence purified PBMs. One aliquot of each CM was subjected to specific ELISA to confirm that MIF had been depleted by the immunoprecipitation.

Statistical analyses

All generated data were compared by Student’s t test for unpaired observations, and were considered significant if p values were < 0.05. Results were presented as the mean number of migrated endothelial cells/hpf ± SEM. All data are representative of a minimum of three separate experiments. Data were analyzed on a Dell computer with the StatView 5.0.1 statistical software package (SAS Institute, Cary, NC).

Results

NSCLC cells and monocytes demonstrate constitutive expression of angiogenic activity that is synergistically increased in PBM/NSCLC coculture

We hypothesized that because monocyte-derived macrophages are found within tumors, these two cell types may interact to increase angiogenic activity. To test this hypothesis, we performed endothelial cell chemotaxis assays on medium generated from the coculture of PBMs and NSCLC cell lines. In comparison to CM from PBM alone, A549 CM alone, or a combination of the two separately generated CM, coculture CM demonstrated a marked increase in angiogenic activity as assessed by endothelial cell chemotaxis (p < 0.002; Fig. 1A). Similarly, PBM CM alone, Calu 6 CM alone, or separately generated Calu 6 and PBM CM demonstrated significantly less angiogenic activity than coculture CM (p < 0.0005; Fig. 1B).

Coculture of PBM with NSCLC cell lines is associated with increased expression of angiogenic CXC chemokines

To determine the degree to which CXC chemokines or VEGF were responsible for the increased endothelial cell chemotaxis seen in NSCLC and PBM CM, we assessed levels of the angiogenic CXC chemokines IL-8 (CXCL8), ENA-78 (CXCL5), GRO-α (CXCL1), and angiostatic CXC chemokines IP-10 (CXCL10) and MIG (CXCL9) as well as VEGF by ELISA. Coculture of PBM with NSCLC resulted in a marked increase in angiogenic CXC chemokine expression with a much smaller change in VEGF levels. In contrast, expression of angiostatic CXC chemokines remained

![Image](http://www.jimmunol.org/)
very low (Table I). LPS contamination was excluded as a cause of increased CXC chemokine expression by adding polymyxin B to coculture, which had no effect on NSCLC-induced CXC chemokine expression from PBMs. LPS levels were below the limit of detection by Limulus lysate assay (data not shown).

**Increased endothelial cell chemotaxis in response to coculture CM is markedly reduced in the presence of neutralizing Ab to angiogenic CXC chemokines or their receptor, CXCR2**

We hypothesized that the increased endothelial cell chemotaxis to PBMs/NSCLC coculture was attributable to the observed increase in angiogenic CXC chemokines. To test this hypothesis, endothelial cell chemotaxis was performed with PBMs/NSCLC coculture CM in the presence of neutralizing Ab to IL-8 (CXCL8), ENA-78 (CXCL5), and GRO-α (CXCL1). We found that endothelial cell chemotaxis to A549/PBM coculture CM was significantly reduced in the presence of neutralizing Abs to either IL-8 (CXCL8), ENA-78 (CXCL5), or GRO-α (CXCL1) as compared with control IgG (p < 0.0007; Fig. 2A). Similarly, endothelial cell chemotaxis to Calu 6/PBM coculture CM in the presence of neutralizing Ab to IL-8 (CXCL8) or GRO-α (CXCL1) was significantly less than in the presence of control IgG (p < 0.002; Fig. 2B). Recently, CXCR2 was identified as the receptor mediating the angiogenic activity of CXC chemokines (23). Therefore, we performed endothelial cell chemotaxis to coculture CM using endothelial cells preincubated with neutralizing Ab to CXCR2 or control IgG. We found neutralizing Ab to CXCR2 markedly reduced the increased angiogenic response to coculture CM (Calu 6/PBM or A549/PBM; Fig. 2C; p < 0.0001 for both conditions).

**PBM are the primary source of increased angiogenic CXC chemokine expression in PBMs/NSCLC coculture CM**

We demonstrated that angiogenic CXC chemokine levels are markedly increased when PBM are cocultured with NSCLC. To determine the source of the increased chemokine levels, we first incubated PBMs in cell-free NSCLC CM and NSCLC in cell-free PBM CM to assess chemokine expression. We found that when PBMs are incubated with CM from NSCLC, there is a significant increase in expression of the angiogenic CXC chemokines similar to that seen when PBM are cocultured with NSCLC cells (Table II). Interestingly, NSCLC cells cultured in cell-free PBM CM also expressed increased levels of angiogenic CXC chemokines as compared with baseline expression (Tables I and II), albeit to a much lesser degree. This suggests that PBM are the predominant source of the increased chemokine expression in coculture. All CM were tested and found to be free of endotoxin by Limulus lysate assay (data not shown).

**Constitutively expressed, NSCLC-derived, macrophage MIF is responsible for increased expression of angiogenic activity by PBMs**

Recently, MIF has been associated with tumor angiogenesis (24–27), but it has not been shown to be directly angiogenic. We hypothesized that MIF may be the tumor-derived factor that induces angiogenic activity from PBMs. To test this hypothesis, we first measured levels of MIF in 48-h CM from unstimulated NSCLC cell lines. MIF was detected in both A549 (4.8 ± 0.2 ng/ml) and Calu 6 CM (6.9 ± 1.0 ng/ml). To determine whether MIF was responsible for increased PBMs-derived angiogenic activity, we performed endothelial cell chemotaxis assays on CM from PBMs that had been exposed to Calu 6 or A549 CM. Before stimulation of PBMs, NSCLC CM was subjected to immunodepletion with either IgG or goat anti-human MIF Ab. Depletion of MIF was confirmed by ELISA (data not shown). We found that NSCLC CM depleted of MIF was significantly less effective at inducing PBMs-derived angiogenic activity (p < 0.001; Fig. 3). Furthermore, PBMs stimulated with MIF-depleted A549 or Calu 6 CM resulted

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**Table II. ELISA analysis of CM generated from PBM cultured in NSCLC cell-free CM or NSCLC cells cultured in PBM cell-free CM for CXC chemokines and VEGF**

<table>
<thead>
<tr>
<th>Condition</th>
<th>IL-8 (CXCL8)</th>
<th>ENA-78 (CXCL5)</th>
<th>GRO-α (CXCL1)</th>
<th>IP-10 (CXCL10)</th>
<th>MIG (CXCL9)</th>
<th>VEGF</th>
</tr>
</thead>
<tbody>
<tr>
<td>A549/PBM</td>
<td>195.1 ± 27.9</td>
<td>25.1 ± 4.3</td>
<td>194.7 ± 21.1</td>
<td>1.4 ± 0.2</td>
<td>ND</td>
<td>2.5 ± 0.1</td>
</tr>
<tr>
<td>A549 in PBM CM</td>
<td>6.6 ± 0.6</td>
<td>3.3 ± 0.2</td>
<td>53.6 ± 3.5</td>
<td>ND</td>
<td>ND</td>
<td>5.9 ± 0.1</td>
</tr>
<tr>
<td>PBM in A549 CM</td>
<td>324.8 ± 2.0*</td>
<td>36.0 ± 3.8</td>
<td>294.8 ± 44.7</td>
<td>ND</td>
<td>ND</td>
<td>1.5 ± 0.2</td>
</tr>
<tr>
<td>Calu 6/PBM</td>
<td>323.5 ± 88.2</td>
<td>46.9 ± 5.7</td>
<td>103.2 ± 4.2</td>
<td>ND</td>
<td>ND</td>
<td>6.3 ± 0.6</td>
</tr>
<tr>
<td>Calu 6 in PBM CM</td>
<td>108.6 ± 2.3</td>
<td>1.6 ± 0.1</td>
<td>1.4 ± 0.4</td>
<td>ND</td>
<td>ND</td>
<td>0.8 ± 0.01</td>
</tr>
<tr>
<td>PBM in Calu 6 CM</td>
<td>406.7 ± 49.3</td>
<td>282.6 ± 42.5</td>
<td>124.4 ± 3.9</td>
<td>ND</td>
<td>ND</td>
<td>ND</td>
</tr>
</tbody>
</table>

*p Levels are expressed as ng/ml of CM ± SEM. Data are representative of three repeated experiments.

**FIGURE 3.** Angiogenic activity of monocyte CM after stimulation with NSCLC CM (A549 or Calu 6 cell line) that had been subjected to immunodepletion with either IgG or goat anti-human MIF Ab. Depletion of MIF was confirmed by ELISA. Data are representative examples of five experiments.
in significantly decreased CXC chemokine expression compared with control A549 or Calu 6 CM ($p < 0.05$ for each condition; Fig. 4).

**Addition of exogenous MIF is sufficient to increase PBM-derived angiogenic CXC chemokine expression**

The above data suggests that MIF is necessary for NSCLC cell line-induction of CXC chemokine-dependent angiogenic activity from PBMs. To determine whether MIF was sufficient to induce CXC chemokines, freshly isolated PBM were cultured in six-well plates in the presence of serum-free medium with or without the addition of 10 ng/ml MIF. CM was harvested at 24 h and assayed for IL-8, ENA-78, and GRO-α by specific ELISA. We found that PBM stimulated with MIF expressed significantly higher levels of IL-8 (252.7 ± 16.5 ng/ml vs 54.6 ± 2.0 ng/ml; $p < 0.01$), ENA-78 (264.8 ± 26.9 ng/ml vs 105.2 ± 6.7 ng/ml; $p < 0.01$), and GRO-α (7.9 ± 1.2 ng/ml vs 1.9 ± 0.4 ng/ml; $p < 0.01$) compared with unstimulated PBM (Fig. 5).

**Discussion**

Tumors require angiogenesis to grow >2–3 mm$^3$ (1, 9). Many factors are important in tumor angiogenesis, including VEGF (28–30) and CXC chemokines (5, 6, 10, 11). Evidence suggests that a balance between proangiogenic and antiangiogenic factors contributes to net tumor angiogenesis (9, 11, 31). The source(s) of angiogenic factor expression within NSCLC have not been completely elucidated. Malignant cells can and do produce angiogenic factors (22, 32), but recent evidence by Fukumura et al. (14) demonstrates that murine mammary tumors induce VEGF promoter activity in stromal cells. In addition, Anderson and coworkers (32) demonstrated induction of IL-8 (an angiogenic CXC chemokine) in human fibroblasts by malignant cells.

A number of different cell types contribute to angiogenesis, including cancer cells (12, 34), endothelial cells (35–37), fibroblasts (13, 15), eosinophils (38), platelets (39), and monocyte-derived macrophages (40–42). Each cell type displays a certain repertoire of angiogenic factors, depending on the stimulus. Because of the overwhelming redundancy within this system, it is important to determine the cellular source(s) of angiogenic activity in pathologic conditions. In this study, we found that PBM are induced by NSCLC cells to increase expression of angiogenic activity. In addition, we observed some increase in expression of angiogenic factors by NSCLC cells exposed to PBM CM.

Many authors have cited similarities between tumor growth and wound healing (43–45). Macrophages are critically involved in wound repair (46–48). In wounds, local tissue injury results in leakage of plasma and blood from damaged blood vessels resulting in a fibrin clot rich in fibronectin, fibrin breakdown products, and
platelets. The fibrin clot becomes a provisional matrix or stroma into which monocytes migrate to ingest wound debris (43). Monocyte-derived macrophages are essential in this role, as evidenced by defective wound repair in macrophage-depleted animals (49). In addition to their role in removing debris, macrophages in inflammatory conditions promote tissue repair by producing cytokines such as TNF-α (50), platelet-derived growth factor (51), and VEGF (52). Analogous events are thought to occur in the process of tumor angiogenesis (43–46).

Polverini and Leibovich (52) demonstrated that tumor-associated macrophages or their CM isolated from experimental fibrosarcoma display angiogenic activity, suggesting the role of macrophage-derived soluble factors in tumor angiogenesis. Similarly, Hildenbrand et al. (53) showed that TGF-β induced macrophages to produce urokinase-plasminogen activator, a known factor in the degradation of tumor matrix and tumor progression. Interestingly, this group also demonstrated that tumor-associated macrophages were induced to produce significantly more urokinase-plasminogen activator than either PBM or elicited tissue macrophages (53), suggesting that the tumor environment can alter macrophage phenotype.

We recently have demonstrated infiltration of monocytes in human NSCLC, which is mediated by tumor expression of CC chemokines (20). Because PBM are a potentially rich source of angiogenic factors, including angiogenic CXC chemokines (41, 55, 56), we hypothesized that NSCLC may augment the expression of macrophage-derived angiogenic factors. Our results show that NSCLC cells promote angiogenesis by inducing host PBM to up-regulate CXC chemokine-dependent angiogenic activity. This is important in the context of our previous findings demonstrating the role of the CXC chemokine family in NSCLC-mediated angiogenesis (5, 10, 31). Although it is likely that other factors such as VEGF play an important role in NSCLC angiogenesis, the results of our study do not support a predominant role for VEGF in the monocytic-derived angiogenic activity induced by NSCLC.

Recently, Addison et al. have demonstrated that CXCR2 is the putative receptor for the angiogenic activity mediated by angiogenic CXC chemokines (23). In our study, we found that the increased angiogenic activity in coculture CM could be neutralized by Abs directed against CXCR2. It is interesting to note that the degree of inhibition of angiogenic activity by anti-CXCR2 was comparable to, or only slightly greater than, the inhibition seen with Abs directed at the individual ligands. To account for this observation, one can speculate that Ab binding to an individual CXC chemokine ligand may prevent access to the receptor by other ligands, thereby effectively neutralizing the receptor as well.

We found that MIF, expressed constitutively by the A549 and Calu 6 cell lines, is responsible in large part for the induction of PBM-derived angiogenic activity. MIF was initially discovered as a proangiogenic factor in non-small cell lung cancer (NSCLC) tumorigenesis and spontaneous metastases. J. Exp. Med. 184:981.


