Inhibition of Differentiation and Affinity Purification With a Monoclonal Antibody to a Myeloid Cell Differentiation-Inducing Protein

By Yosef Shabo and Leo Sachs

The normal myeloid hematopoietic regulatory proteins include four growth-inducing proteins called colony-stimulating factors (CSF), including interleukin-3 (IL-3), or macrophage and granulocyte inducers, type 1 (MGI-1), and another type of protein (MGI-2) with no myeloid cell growth-inducing activity that induces differentiation of normal myeloid precursor cells and certain clones of myeloid leukemic cells. An IgG2a monoclonal antibody was prepared and it neutralized two forms of MGI-2 (MGI-2A and MGI-2B) produced by mouse Krebs ascites tumor cells. This monoclonal antibody was used for affinity purification of MGI-2. This antibody also neutralized MGI-2 produced by normal mouse macrophages, normal myeloblasts incubated with IL-3, and MGI-2 produced by the lungs and found in the serum of mice injected with lipopolysaccharide (LPS). The anti-MGI-2 antibody did not inhibit the activity of any one of the four myeloid growth-inducing proteins (CSF or IL-3 = MGI-1), IL-1, tumor necrosis factor, or lymphotoxin. This antibody also inhibited induction of differentiation of myeloid leukemic cells by LPS, which is mediated by the endogenous production of MGI-2, but did not inhibit induction of differentiation in these leukemic cells by dexamethasone or corticosteroids, which is not mediated by MGI-2. Anti-MGI-2 antibody thus inhibited differentiation when MGI-2 was added externally to cells or when it was mediated by endogenously produced MGI-2.

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MATERIALS AND METHODS

Cells and cell culture. Two growth factor-independent clones of mouse myeloid leukemic cells were used in the present experiments. Clone 11 was derived from a spontaneous myeloid leukemia in an S1 mouse and clone 7-M12 from an X-ray-induced myeloid leukemia in an SJL/J mouse. Clone 11 but not clone 7-M12 can be induced to differentiate by MGI-2.

Two growth factor-independent clones of (MGI-1G) macrophages and granulocytes (GM-CSF = MGI-1GM), or macrophages, granulocytes, eosinophils, mast cells, megakaryocytes, or erythroid cells (IL-3). The genes for these four growth factors (murine and/or human) have been cloned and are unrelated in their nucleotide sequence. In addition to these growth factors, there is another type of myeloid regulatory protein that induces differentiation in normal myeloid precursor cells and some clones of myeloid leukemic cells but does not have myeloid cell growth factor activity. We have called this type of protein, which can be obtained from different sources, MGI-2. MGI-2 and myeloid cell differentiation-inducing activities from some other sources have been called differentiation-factor or differentiation-inducing factor. Normal myeloid precursor cells cultured with any one of the myeloid growth factors endogenously produce MGI-2, so these growth factors induce differentiation of normal precursor cells indirectly via production of a differentiation-inducing protein.

We have now produced a monoclonal antibody that neutralizes two forms of MGI-2 (MGI-2A and MGI-2B) produced by mouse Krebs ascites tumor cells. This monoclonal antibody also neutralized MGI-2 from other sources but did not neutralize CSF or IL-3 (MGI-1). We have used this antibody for affinity purification of MGI-2 and to show that this antibody can inhibit differentiation of myeloid leukemic cells that is mediated by endogenously produced MGI-2.

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bind to the heparin column, but MGI-2B did and was eluted with a linear gradient of 0 to 0.25 mol/L NaCl. Further purification of the separated MGI-2A and MGI-2B on phenyl-sepharose, hydroxyapatite, and reverse-phase high-performance liquid chromatography (HPLC) columns were performed as described. MGI-2 in serum and CM from lungs of normal mice injected with LPS and in CM from normal mouse resident peritoneal macrophages and M-CSF from L-929 cells were also used. MGI-2 induced by IL-3 was obtained by collecting CM four days after incubating 10^6 normal myeloid precursor cells/mL with 10 ng/mL pure recombinant mouse IL-3. Pure recombinant human GM-CSF from Drs Ken-Ichi Arai and Atsushi Miyajima, and pure recombinant mouse IL-3 from Dr Pierre Vassalli.

Assays for growth-inducing (CSF or IL-3) and differentiation-inducing (MGI-2) activity and some other proteins. Growth-inducing (CSF or IL-3 = MGI-1) activity was assayed in 35-mm Petri dishes by seeding 5 x 10^4 normal nucleated bone marrow cells taken from the femurs of 2- to 3-month-old SJL/J mice in 0.8 mL of 0.33% agar on top of a 2.5-mL harder agar base (0.5%) that contained different amounts of the material to be assayed. Both layers contained EM and 20% horse serum, and the medium was also supplemented with 1% normal rabbit serum to increase the cloning efficiency. Colonies of macrophages and/or granulocytes containing 50 or more cells were counted after seven days' incubation at 37°C. Differentiation-inducing (MGI-2) activity was assayed by seeding 7.5 x 10^4 differentiation-competent (D+) growth factor-independent, clone 11 myeloid leukemic cells per milliliter and measuring the amount of lysozyme produced after four days' incubation as described in the linear part of the concentration curve. One unit of MGI-2 activity is defined as the amount that induced the production of 1 μg equivalent of lysozyme per 5 x 10^4 cells after four days. The standard error in these assays was up to ±15% of the average values. Polymyxin B, 5 μg/mL completely blocks induction of differentiation in clone 11 leukemic cells even by high concentrations of LPS (200 ng/mL) but has no affect on induction of differentiation by the proteins used. This amount of polymyxin B was therefore added in the assays for induction of differentiation by these proteins. The same results were obtained with or without adding polymyxin B so that induction of differentiation was not due to the possible presence of some contaminating LPS in the protein preparations. Tumor necrosis factor (TNF) and lymphotoxin activity were assayed by incubation of L929 cells for 16 hours in the presence of 1 μg/mL actinomycin D and counting the number of viable cells. Interleukin-1 (IL-1) activity was assayed by using the cell line LBR M 33-1A5 as described.

Production of monoclonal antibody. MGI-2A from 35 mL of 100-fold–concentrated Krebs cell serum-free CM was partially purified as described earlier except for the step of reverse-phase HPLC. To concentrate the MGI-2A, it was loaded on a small DEAE-Sephadex column (5-mL bed volume) as described earlier and eluted in one step with 0.25 mol/L NaCl. The pooled MGI-2A was dialyzed against PBS, pH 7.4, containing 0.01% PEG and found to contain 2,000 U/mL of MGI-2. This partially purified MGI-2A was used for immunization. Four female SPD spleen cells were injected twice subcutaneously and in the footpads with 0.5 mL of the partially purified MGI-2A emulsified in Freund's complete adjuvant (Difco) at 1:1.5 vol/vol antigen/adjuvant) at 2-week intervals and then injected three times at 2-week intervals with 0.5 mL of 100-fold–concentrated crude Krebs CM (containing MGI-2A and MGI-2B) emulsified in Freund's complete adjuvant at 1:1.5 vol/vol ratio. The sera of the four rats were tested two and three days after the last injection for antibody to MGI-2 by the ability of the serum to inhibit MGI-2–induced differentiation in clone 11 myeloid leukemic cells. The sera of two rats were positive, and one rat was boosted with 0.5 mL of concentrated crude Krebs CM three days before fusion of spleen lymphocytes with mouse myeloma cells of the NSO line provided by Dr Z. Eshhar of this institute. This myeloma cell line is an HGPRT(-) variant of MOPC 21, which was obtained from Dr C. Milstein, MRC Laboratory of Molecular Biology, Cambridge, England, and does not produce immunoglobulin.

For fusion, the spleen lymphocytes were mixed at a ratio of 6:1 with NSO myeloma cells and the cells fused by adding PEG 1500 to a final concentration of 41% as described. The fused viable cells that appeared larger than the unfused cells were suspended at a concentration of 1.5 x 10^7 cells/mL in EM containing 10% horse serum, 10^-4 mol/L hypoxanthine, 4 x 10^-7 mol/L aminopterin, and 1.6 x 10^-2 mol/L thymidine (EM-HS-HAT), and 0.2 mL was distributed into each well of two microculture plates (2 x 96 wells). Seven days after fusion, the cultures were fed by aspirating half of the culture fluid and replacing it with prewarmed fresh EM-HS-HAT medium. Screening for anti-MGI-2 antibody was performed by adding hybridoma supernatants from the 192 individual wells to culture plates containing clone 11 cells and 4% Krebs CM containing MGI-2A and MGI-2B and testing for inhibition of differentiation. Two hybridomas that were found to secrete anti-MGI-2 activity were transferred to 24-well plates and then to 2-mL tissue culture Petri dishes. Two weeks after cell fusion, the HAT medium was replaced by HT medium and 2 weeks later by regular EM-HS medium. A monoclonal antibody–producing hybridoma was obtained by cloning one of these hybridomas three times at limiting dilution (one cell per well in the last step), and the single-cell origin was confirmed under the light microscope.

To produce larger amounts of the antibody the hybridoma was grown as an ascites in immunosuppressed BALB/c x C57BL/6 F mice. Four-month-old female mice were injected intraperitoneally with 0.5 mL pristane (Aldrich Chemical Co, Milwaukee, WI). Ten days later the mice were irradiated (750 R) and after five to six hours injected with 5 x 10^6 hybridoma cells in 1 mL PBS. Nine days later the ascites fluid was removed, centrifuged, and stored frozen at -80°C. Purification of antibody from thawed ascites fluid was performed by caprylic acid precipitation followed by 45% ammonium sulfate precipitation as described. Antibodies were resuspended either in coupling buffer for the affinity matrix as described later or with PBS for the experiments on biologic activity.

The class and subclass of the monoclonal antibody were determined by the Ouchterlony immunodiffusion technique using 1.5% Noble agar (Difco) in PBS containing 0.05% NaN₃, and goat antiserum against 1gG2a, rabbit antiserum against 1gM, and sheep antiserum against 1gG1 (Miles, Naperville, IL).

Affinity chromatography of MGI-2 with the monoclonal antibody. Antibody affinity resin was prepared by covalent binding of purified antibody from ascites fluid to cyanogen bromide–activated Sepharose 4B (Pharmacia) according to the manufacturer's instruction manual. Following precipitation with ammonium sulfate, the antibody was resuspended in water and then dialyzed against 0.5 mol/L NaCl in 0.2 mol/L NaHCO₃/Na₂CO₃, pH 8.6 (coupling buffer). Immunoglobulin at 2 mg/mL (absorbance at 280 nm) was mixed with swollen activated Sepharose at a ratio of 1:2 buffer to gel. The slurry was rotated end over end for 48 hours at 4°C, and the residual active groups were blocked by 1 mol/L ethanolamine-HCl, pH 8.5, for 24 hours at 4°C. The resin was then washed three times alternately with coupling buffer and 0.5 mol/L NaCl in 0.1 mol/L sodium acetate, pH 4.0. The efficiency of the coupling was 98.5%, and 4 mg immunoglobulin was bound to 1 mL gel. Before use the antibody affinity resin was washed once under affinity chromatography conditions.

For chromatography, a column (1 x 5 cm) of anti-MGI-2 antibody coupled to Sepharose was equilibrated with 10 mmol/L Tris-HCl, pH 7.0, 0.01% PEG 6000, and 0.02% NaN₃ (azide).
MG1-2-containing differentiation medium was dialyzed against the same buffer and loaded at 4°C at a flow rate of 2.5 mL/h. The column was washed with equilibration buffer, followed by the addition of 1 mol/L NaCl in this buffer, washed again with equilibration buffer, and 1-mL fractions were collected. The strongly bound material was eluted with 0.2 mol/L glycine-HCl, pH 2.7, containing PEG and azide, and 0.7-mL fractions were collected into tubes containing 0.3 mL of 1 mol/L K2HPO4, PEG, and azide.

Polyacrylamide gel electrophoresis and immunoblotting. Sodium dodecyl sulfate (SDS) polyacrylamide gel electrophoresis was performed with a discontinuous buffer system containing 0.1% SDS as described, and the gels were then stained with Coomassie blue or with silver. For immunoblotting, samples were subjected to electrophoresis on SDS–polyacrylamide gels and transferred to nitrocellulose filters. The filters were first incubated overnight with 10% low-fat milk and 0.04% NaN3 in PBS (quenching buffer) and then incubated for two hours at room temperature with anti-MGI-2 antibody diluted in this buffer at 60 µg/mL immunoglobulin. After washing the same buffer, an 125I-labeled Fab fragment of rabbit antirat IgG, obtained from Dr Z. Eshhar, was added to a final concentration of 0.5 × 10⁶ cpm/mL and the filters incubated for one hour at room temperature. The filters were washed with quenching buffer and then with this buffer containing 0.1% Tween 20. The autoradiographs were exposed for six days at –80°C, Agfa x-ray film (Curix PR2) was used.

RESULTS

Properties of the monoclonal antibody to MG1-2 and its use in affinity purification. The hybridoma obtained after immunization with MG1-2 was grown in an ascites form in BALB/c × C57BL/6 F1 mice as described in Materials and Methods. The ascites fluid from these mice neutralized MG1-2 activity so that induction of differentiation in clone 11 myeloid leukemic cells by MG1-2 was almost completely neutralized at a dilution of 1:10 and 50% inhibition was obtained at a dilution of 1:4 × 10⁵ (Fig 1). Inhibition of MG1-2–induced production of lysozyme by the ascites fluid (Fig 1) was also accompanied by inhibition of MG1-2–induced cell adherence and morphologic maturation (data not shown). In addition, the cells continued to multiply in cultures containing MG1-2 and ascites fluid as compared with MG1-2–induced cultures containing only MG1-2 in which cells stop multiplying as they become mature. This ascites fluid did not affect the growth rate of the cells in the absence of MG1-2 (data not shown).

To determine whether the differentiation-inhibitory material in the ascites fluid was an immunoglobulin, the IgG fraction from the ascites fluid was isolated by the caprylic acid/ ammonium sulfate precipitation procedure. After SDS–gel electrophoresis, the IgG fraction showed a 150-Kd major band typical of IgG under nonreducing conditions. Two major bands at 50 Kd and 25 Kd representing the heavy and light chains, respectively, were observed under reducing conditions. This purified IgG, like the ascites fluid, inhibited MG1-2–induced cell differentiation in clone 11 cells, and complete inhibition was obtained with 2.5 µg/mL. The subclass of immunoglobulin was characterized by Ouchterlony immunodiffusion. A precipitin line was obtained only with antirat IgG2a, but not with anti-IgG2b, anti-IgG1, anti-IgA, or anti-IgM. This indicates that the antibody was IgG2a.

An immunoabsorbent column (4-mL bed volume) was prepared by conjugating the anti–MG1-2 antibody to Sepharose (4 mg immunoglobulin/1 mL gel) for the purification of MG1-2. Crude Krebs CM that contained MG1-2A,23 MG1-2B,23 and GM-CSF26 or partially purified MG1-2A from Krebs cells were loaded on the affinity column. The results show that the MG1-2A and MG1-2B in crude CM and the partially purified MG1-2A bound to the affinity column and could be eluted with an acidic buffer (pH 2.7) (Fig 2, Table 1). This low-pH–eluted MG1-2 gave a 230-fold purification in a single step with crude CM and a 577-fold purification of MG1-2A with partially purified MG1-2A (Table 1). All the input GM-CSF in crude Krebs CM was found in the unbound fractions, and the affinity-purified MG1-2A did not contain any CSF or IL-3 activity. There was some nonspecific binding of non-MG1-2 proteins to the immunoaffinity column, especially when crude CM was used, and these proteins could be removed with 1 mol/L NaCl (Fig 2). This 1 mol/L NaCl did not remove MG1-2- bound to the column.

The affinity-purified MG1-2A showed a few bands on a silver-stained SDS–polyacrylamide gel (Fig 3) that have
MGI-2 activity. The several bands at 23 to 27 Kd even after reverse-phase HPLC as described were all detected by immunoblotting with the anti-MGI-2 antibody (Fig 3E). There was no signal with material from the column that did not contain MGI-2 (Fig 3D) or in the absence of antibody (Fig 3F).

Specificity of the anti-MGI-2 antibody. MGI-2A and MGI-2B from Krebs cells have different characteristics as indicated by separation on DEAE-cellulose, DEAE-Sephacel, heparin-agarose, phenyl-sepharose, and reverse-phase HPLC columns (see Materials and Methods). Both MGI-2A and MGI-2B from these cells were neutralized by the anti-MGI-2 monoclonal antibody (Table 2). In addition, MGI-2A produced by normal mouse macrophages, normal mouse myeloblasts incubated with IL-3 and induced by LPS in mouse myeloid leukemic cells, the MGI-2 produced by normal mouse macrophages, normal mouse myeloblasts by rIL-3, and M-CSF (Table 3). It also did not neutralize the cytotoxic effect on L929 cells of mouse rTNF (10 U/mL) or human recombinant lymphotoxin (100 U/mL) or the activity of IL-1 (10 U/mL). Antibody to mouse rTNF, human recombinant lymphotoxin, and a mouse monoclonal IgG2a antibody to dinitrophenol did not neutralize MGI-2 (data not shown).

Inhibition of differentiation by endogenously produced MGI-2 by antibody to MGI-2. Clone 11 and similar clones

**Table 1. Binding of MGI-2 to a Column of Anti-MGI-2 Antibody Coupled to Sepharose**

<table>
<thead>
<tr>
<th>Sample</th>
<th>Total Protein (mg)</th>
<th>Specific Activity (U/mg)</th>
<th>Amount Loaded</th>
<th>Total Protein (mg)</th>
<th>Specific Activity (U/mg)</th>
<th>Amount Bound</th>
</tr>
</thead>
<tbody>
<tr>
<td>Crude CM from Krebs cells</td>
<td>44</td>
<td>164 ± 13</td>
<td>7,200 ± 576</td>
<td>0.17</td>
<td>6,400 ± 350</td>
<td>37,647 ± 2.058</td>
</tr>
<tr>
<td>Partially purified MGI-2A from</td>
<td>11</td>
<td>1,964 ± 98</td>
<td>21,600 ± 1,080</td>
<td>0.21</td>
<td>19,872 ± 1,589</td>
<td>94,628 ± 7,566</td>
</tr>
</tbody>
</table>

*The amount of protein was determined with a fluorescamine analyzer as described after dialysis against 10 mmol/L sodium phosphate, pH 7.4, containing 0.01% PEG and 0.02% azide.
†Fold purified as compared with crude CM from Krebs cells.
‡Partially purified after hydroxyapatite as described in Materials and Methods.

Fig 3. SDS-polyacrylamide gel electrophoresis and immunoblotting of MGI-2 eluted from an immunoaffinity column containing anti-MGI-2 antibody. Proteins were subjected to electrophoresis on a 12.5% polyacrylamide gel with 0.1% SDS and silver stained in lanes A, B, and C. Lane A, molecular weight (mol wt) markers; lane B, partially purified MGI-2A before immunoaffinity purification; lane C, MGI-2 eluted from the immunoaffinity column after loading partially purified MGI-2A. MGI-2 activity is associated with mol wts that range from 23 to 27 Kd. The 80-Kd band in lane C is an artifact of the silver staining. Immunoblotting (lanes D, E, and F). Lane D, proteins that did not bind to the immunoaffinity column; lane E, MGI-2A eluted from the affinity column and then further purified by HPLC; lane F, the same as lane E but incubated without anti-MGI-2 antibody.

To measure induction of differentiation, clone 11 myeloid leukemic cells seeded at 7.5 x 10⁶ cells/mL were incubated for four days with or without 25 μg/mL anti-MGI-2 antibody. MGI-2A and MGI-2B from Krebs cells were purified by the steps described in Materials and Methods.

**Table 2. Neutralization of MGI-2 From Different Sources by Anti-MGI-2 Antibody**

<table>
<thead>
<tr>
<th>Source of MGI-2</th>
<th>Units of MGI-2 Added/mL</th>
<th>Differentiation Induction (µg Lysozyme Equivalent/5 x 10⁶ Cells) With or Without Anti-MGI-2 Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td></td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>MGI-2A from Krebs cells</td>
<td>2</td>
<td>10.6 ± 1.4</td>
</tr>
<tr>
<td>MGI-2B from Krebs cells</td>
<td>2</td>
<td>9.6 ± 1.2</td>
</tr>
<tr>
<td>Normal mouse macrophages</td>
<td>3</td>
<td>16.0 ± 0.8</td>
</tr>
<tr>
<td>Serum from mice injected with LPS</td>
<td>5</td>
<td>25.9 ± 1.5</td>
</tr>
<tr>
<td>Lungs from mice injected with LPS</td>
<td>5</td>
<td>25.1 ± 1.6</td>
</tr>
<tr>
<td>Induced in mouse myeloid leukemic cells by LPS</td>
<td>2</td>
<td>10.8 ± 0.9</td>
</tr>
<tr>
<td>Induced in normal mouse myeloblasts by rIL-3</td>
<td>1</td>
<td>4.5 ± 0.6</td>
</tr>
</tbody>
</table>

*Clone 11 mouse myeloid leukemic cells seeded at 5 x 10⁶ cells/mL were incubated with 1 µg LPS/mL for one day, the cells collected and washed three times with PBS to remove LPS, and the serum-free CM containing MGI-2 collected after seeding 1 x 10⁶ cells/mL and incubation for another two days. The same results were obtained in the presence or absence of 5 µg/mL polymyxin B. The presence of polymyxin B completely blocks the induction of differentiation due to the possible presence of some contaminating LPS.
of myeloid leukemic cells can be induced to differentiate by compounds such as LPS,42 cytosine arabinoside,53 and some steroid hormones including dexamethasone.55 The induction of differentiation by LPS is mediated by the endogenous production of MGI-2,52,53 in the differentiating cells, whereas induction of differentiation by cytosine arabinoside53 and dexamethasone54 is not mediated by the production of detectable MGI-2. Incubation of clone 11 cells in the presence of the anti–MGI-2 monoclonal antibody has shown that LPS-induced differentiation was completely inhibited by this antibody at a low concentration of LPS and partially inhibited at higher concentrations of LPS. But this antibody did not inhibit induction of differentiation by cytosine arabinoside or dexamethasone at concentrations of these compounds that induced the same amount of lysozyme as did LPS (Table 4). The anti–MGI-2 antibody (25 μg/mL) did not inhibit the activity of LPS (0.5 μg/mL) as measured by the ability of LPS to induce IL-1 production in normal mouse spleen cells (5 x 10⁵ cells/mL), and a mouse IgG2a monoclonal antibody against dinitrophenol did not affect differentiation of clone 11 cells that was induced by LPS. The results indicate that the anti–MGI-2 antibody cannot inhibit differentiation induced by externally added or endogenously produced MGI-2.

Clone 7-M12 myeloid leukemic cells can be induced to differentiate to mature cells by GM-CSF,55,56 or IL-3,57 and clone 11 myeloid leukemic cells can be induced to partially differentiate by G-CSF.58 The anti–MGI-2 antibody did not inhibit induction of differentiation by 5 ng/mL of rGM-CSF or rIL-3 in clone 7-M12 or 50 ng/mL of rG-CSF in clone 11, so this differentiation may possibly also not be mediated by the production of MGI-2 in these cells (data not shown).

**Table 3. Lack of Neutralization by Anti–MGI-2 Antibody of IL-3, GM-CSF, G-CSF, and M-CSF**

<table>
<thead>
<tr>
<th>Growth-Inducing Protein</th>
<th>No. of Macrophage and/or Granulocyte Colonies With or Without Anti–MGI-2 Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>0</td>
</tr>
<tr>
<td>rL-3</td>
<td>102 ± 14</td>
</tr>
<tr>
<td>rGM-CSF</td>
<td>57 ± 8</td>
</tr>
<tr>
<td>rG-CSF</td>
<td>17 ± 4</td>
</tr>
<tr>
<td>M-CSF</td>
<td>47 ± 6</td>
</tr>
</tbody>
</table>

The different growth-inducing proteins, 2 ng/mL rL-3, 2 ng/mL rGM-CSF, 50 ng/mL rG-CSF, and 1% of 10-fold-concentrated serum-free CM from L-929 cells,57 were assayed for colony formation with 5 x 10⁴ normal mouse bone marrow cells as described in Materials and Methods with or without 25 μg/mL anti–MGI-2 antibody. The same results were obtained when these proteins were incubated for 30 minutes at 37°C with antibody before the assay for colony formation. There was a similar distribution of types of colonies in these cultures with or without this antibody.

**Table 4. Inhibition of Induction of Differentiation by LPS by Anti–MGI-2 Antibody**

<table>
<thead>
<tr>
<th>Inducer of Differentiation</th>
<th>Concentration of Inducer (μg/mL)</th>
<th>Differentiation Induction (μg Lysozyme Equivalent/5 x 10⁶ Cells) With or Without Anti–MGI-2 Antibody</th>
</tr>
</thead>
<tbody>
<tr>
<td>None</td>
<td>---</td>
<td>0.2 ± 0.1</td>
</tr>
<tr>
<td>LPS</td>
<td>0.05</td>
<td>0.9 ± 0.1</td>
</tr>
<tr>
<td></td>
<td>0.2</td>
<td>1.8 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.5</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td></td>
<td>0.04</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Dexamethasone</td>
<td>0.04</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.2 ± 0.3</td>
</tr>
<tr>
<td>Cytosine arabinoside</td>
<td>0.04</td>
<td>2.0 ± 0.2</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>3.2 ± 0.3</td>
</tr>
</tbody>
</table>

Clone 11 myeloid leukemic cells seeded at 7.5 x 10⁴ cells/mL with LPS or dexamethasone and seeded at 1 x 10⁶ cells/mL with cytosine arabinoside were incubated with these differentiation inducers for four days with and without 25 μg/mL anti–MGI-2 antibody. A similar partial inhibition of differentiation by the higher concentrations of LPS was obtained when new antibody was added each day, and similar results were obtained in all the experiments when the antibody concentration was increased five-fold. Polymyxin B, 5 μg/mL, completely inhibited the differentiation induced by LPS but did not affect the differentiation induced by dexamethasone or cytosine arabinoside.

**DISCUSSION**

We have prepared an IgG2a monoclonal antibody to differentiation-inducing protein MGI-2, which induces differentiation in normal myeloid precursor cells and in certain clones of myeloid leukemic cells and does not have myeloid cell growth–inducing activity.7,4 The monoclonal antibody was then used for affinity purification of MGI-2. The affinity-purified MGI-2A showed several bands of 23 to 27 Kd on SDS–polyacrylamide gel electrophoresis that specifically bound the anti–MGI-2 antibody as shown by immunoblotting. Bands with these mol wts all have the biologic activity of MGI-2.46 The MGI-2 used for immunization was produced by Krebs mouse ascites carcinoma cells and contained two forms of MGI-2 (MGI-2A and MGI-2B),7 the monoclonal antibody neutralized both of these forms. MGI-2A and MGI-2B may represent different glycosylated forms of MGI-2. This antibody also neutralized the MGI-2 produced by normal mouse macrophages,46 normal myeloblasts incubated with IL-379 and induced in mouse myeloid leukemic cells with LPS,55 and the MGI-2 produced by the lungs57 and found in the serum of normal mice injected with LPS.58 This indicates that MGI-2 from these different sources have a common antigenic site(s). The anti–MGI-2 monoclonal antibody did not neutralize IL-3, GM-CSF, G-CSF, M-CSF, TNF, or lymphotoxin, all of which bind to cell surface receptors that are different from the receptors that bind MGI-2.57 This antibody also did not neutralize IL-1.

The clone of myeloid leukemic cells used in these experiments (clone 1130 from the myeloid leukemia M153) can be induced to differentiate by externally added MGI-2 and can also be induced to differentiate by some nonprotein inducers including LPS,42 dexamethasone,52 and cytosine arabinoside.53 Induction of differentiation by LPS is mediated by the endogenous production of MGI-2 in the differentiating cells,52,53 and induction of differentiation by dexamethasone54 and cytosine arabinoside55 is not mediated by the production of detectable MGI-2. The use of the anti–MGI-2 monoclonal antibody has shown that this antibody inhibited differentiation induced by LPS but did not inhibit differentiation induced by dexamethasone or cytosine arabinoside. It also...
did not inhibit induction of differentiation in clone 11 cells by G-CSF29 or induction of differentiation in another clone of myeloid leukemia (clone 7-M12) from another myeloid leukemia32 that is induced to differentiate by IL-329 and GM-CSF29,33,35 but not by MGI-2.

These results suggest that the anti-MGI-2 antibody can be used to analyze different pathways of myeloid cell differentiation induced by different proteins or other compounds1,5 so as to distinguish differentiation induced by endogenously produced MGI-2 from differentiation induced by other differentiation-inducing proteins that may be produced in these other pathways. However, the incomplete inhibition by the anti-MGI-2 antibody of differentiation induced by high concentrations of LPS also suggests that the antibody may not be able to neutralize nonsecreted cellular MGI-2 or all of the secreted MGI-2 that is rapidly bound to its surface receptors and then internalized.59 The use of this antibody should, however, be able to further clarify differences in both the structure and function of MGI-2 and other myeloid cell regulatory proteins. Studies on amino-acid sequence, neutralization by this monoclonal antibody, and myeloid cell differentiation-inducing activity of recombinant protein have now shown that MGI-2A is interleukin-6 (IL-6).58 The anti-mouse MGI-2 antibody is thus a monoclonal antibody for mouse IL-6.

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Inhibition of differentiation and affinity purification with a monoclonal antibody to a myeloid cell differentiation-inducing protein

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