Identification of the Bone Marrow Colony Mononuclear Phagocyte as a Macrophage

By Martin J. Cline, Noel L. Warner, and Donald Metcalf

Bone marrow colony-derived mononuclear phagocytes are macrophages on the basis of morphology, glass adherence, the phagocytosis of a range of particles including erythrocytes, yeast, and bacteria, and the possession of receptors for IgG of predominantly IgG2a specificity.

Two cell lines develop in cultures of mammalian bone marrow in soft agar: granulocytic cells and mononuclear phagocytes.1,2 The latter have been tentatively identified as macrophages on the basis of morphology and ability to phagocytize particulate matter such as carbon.3 In these studies we have been able to identify conclusively these cells as macrophages on the basis of glass adherence, range of particles phagocytized, and the presence of surface receptors for IgG. These receptors were of subclass specificity identical to those of peritoneal macrophages.

MATERIALS AND METHODS

Bone marrow cells of C57BL mice were cultured in soft agar as previously described.1 Cultures were performed in 35-mm plastic Petri dishes (Falcon Plastics, Los Angeles, Calif.) and contained 25,000 or 75,000 bone marrow cells in 1 ml of agar medium. Colony formation was stimulated by inclusion in the medium of 0.1 ml of a 1:6 dilution of pooled serum from C57BL mice injected 3 hr previously with 5 μg S. typhimurium endotoxin. Cultures were incubated in 10% CO2 in air. At various intervals, colonies were removed from the agar and suspended in medium 199 (M199) containing 30% fetal calf serum. Suspension was achieved by aspirating colonies into a siliconized glass pipette with an internal bore of approximately 100 μ. Under visual control the cells were vigorously pipetted to and fro in M199, then collected by centrifugation (100 g for 7 min), and finally resuspended in M199 before being allowed to adhere to a glass cover slip for 3 hr at 37°C. Over 98% of the cells from colonies 8 days-old or younger were viable (trypan blue); in colonies older than 10 days an increasing proportion of cells were nonviable. Nonadherent cells were washed off with warm M199, unsensitized or antibody-coated sheep red cells (SRBC, 1 × 107/ml) were added, and the incubation continued for 30 min. SRBC not bound or ingested by macrophages were then washed from the cover slip with warm M199. There was no significant loss of macrophages at this point. The cover slips were then fixed in methanol, stained with Giemsa, and scored for the number of macrophages with adherent or phagocytized SRBC.4

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Another technique for measuring erythrocyte adherence involved the incubation of sensitized SRBC \( (5 \times 10^6/\text{ml}) \) with isolated marrow colony cells (approximately \( 5 \times 10^4/\text{ml} \)) in microtiter plates in suspension at \( 37^\circ\text{C} \) for 30 min. A drop was removed, placed under a cover slip, and the percentage of leukocytes with adherent SRBC was determined visually at magnification of 400 \( \times \). The suspension method generally gave values 20–40\% lower than the glass-adherence technique. The probable explanation for this difference is that macrophages in suspension are less efficient than glass-adherent cells in trapping and phagocytizing sensitized erythrocytes. This phenomenon has been noted previously with monocytes.\(^4\)

Peritoneal macrophages were obtained from C57BL mice and cultivated in vitro for 48 hr.\(^5\) and their interaction with sensitized SRBC was determined by the two methods just described.

Antisera to SRBC were prepared in CBA mice by single or repeated immunization with washed SRBC given intraperitoneally. A 5-day primary serum and a 7-day secondary serum were separated on Sephadex G-100/200 into IgM- and IgG-rich fractions, respectively.

For inhibition studies, purified mouse myeloma proteins were isolated from the sera or ascites fluids of mice bearing plasma cell tumors of defined type: IgA (HPC-1, -38); IgG\(_1\) (HPC-77, -9, -22, -32, -78, -81); IgG\(_2a\) (GPC-7, -8, HPC-3); IgG\(_2b\) (MPC-86, HPC-71, -84); IgM (MOPC-104, HPC-76); light chain (-MPC-76, HPC-14).

The effects of these purified proteins on the binding of sensitized erythrocytes to macrophages were tested by adding the proteins immediately before the SRBC.

**RESULTS**

Analysis of colonies in cultures stimulated by endotoxin serum was performed by sampling 50 sequential colonies at various intervals during the incubation period. Colonies were picked off individually and stained with 0.6\% orcein in 60\% acetic acid. As shown in Table 1, a progressive change occurred in the cellular composition of the colonies. Initially, all colonies were composed of granulocytes, but the majority of colonies in this population transformed to cells with the morphology of macrophages having a round excentric nucleus and bulky vacuolated cytoplasm.

When cells from pooled mature bone marrow colonies at 8–10 days of culture were examined, over 90\% adhered to glass and were morphologically indistinguishable from peritoneal macrophages by phase microscopy and in fixed stained preparations. As the peritoneal macrophage, they were peroxidase negative. Such cells efficiently phagocytized added *Candida albicans*\(^6\) and adventitious bacterial contaminants (Fig. 1), generally ingesting more than ten microorganisms per cell.

<table>
<thead>
<tr>
<th>Day of Incubation</th>
<th>Granulocytic (%)</th>
<th>Mixed (%)</th>
<th>Macrophage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>100</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>7</td>
<td>50</td>
<td>20</td>
<td>30</td>
</tr>
<tr>
<td>9</td>
<td>20</td>
<td>15</td>
<td>65</td>
</tr>
<tr>
<td>11</td>
<td>5</td>
<td>12</td>
<td>83</td>
</tr>
<tr>
<td>14</td>
<td>0</td>
<td>4</td>
<td>96</td>
</tr>
</tbody>
</table>

*Classified from 50 sequential colonies sampled at each time point. Colonies scored as "pure" granulocytic or macrophage contained fewer than 5\% of other cell types.
Between 85 and 100% of the glass-adherent mature (8–10 day) bone marrow phagocytes were able to bind and ingest antibody-coated SRBC. Red cell binding was dependent upon the presence of IgG antibody. IgM-containing fractions of anti-SRBC sera were not effective (Table 2).

The presence on bone marrow macrophages of surface receptors for certain IgG subclasses was deduced from studies in which free immunoglobulins competed with IgG-coated SRBC for binding. In the presence of an excess of IgG₂a (and to a lesser extent of IgG₁) immunoglobulins, binding of sensitized SRBC by macrophages was inhibited (Table 2).

**Table 2. Binding and Phagocytosis of Red Cells by Surface-Adherent Bone Marrow Colony and Peritoneal Macrophages**

<table>
<thead>
<tr>
<th>RBC-Sensitizing Agent</th>
<th>Competing Protein (100 μg/ml)</th>
<th>% Phagocytes With Bound or Ingested Red Cells</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Bone Marrow Colony Macrophage</td>
</tr>
<tr>
<td>Albumin</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>IgM antibody</td>
<td>2 (0–4)</td>
<td>85 (80–92)</td>
</tr>
<tr>
<td>IgG antibody</td>
<td>94 (85–100)</td>
<td>66 (29–100)</td>
</tr>
<tr>
<td>IgG antibody IgG₂a</td>
<td>50 (28–69)</td>
<td>38 (9–61)</td>
</tr>
<tr>
<td>IgG antibody IgG₂b</td>
<td>96 (88–100)</td>
<td>73 (68–85)</td>
</tr>
<tr>
<td>IgG antibody IgA</td>
<td>97 (92–100)</td>
<td>78 (65–86)</td>
</tr>
<tr>
<td>IgG antibody IgM</td>
<td>96 (95–100)</td>
<td>80 (70–85)</td>
</tr>
<tr>
<td>IgG antibody Light chains</td>
<td>96 (86–99)</td>
<td>86 (79–93)</td>
</tr>
</tbody>
</table>
Fig. 2. Time course of appearance of cells with surface receptors for IgG-coated SRBC in colonies grown from mouse bone marrow in soft agar. Colonies were removed from agar at various intervals after initiation of culture and cell suspensions prepared in M199. Fraction of cells forming rosettes with IgG-coated SRBC was determined.

Inhibition of binding of IgG-coated SRBC by bone marrow macrophages was dependent upon the concentration of free immunoglobulin and was detected at concentrations below 50μg/ml in the case of IgG2a proteins. The wide range of values obtained with IgG1 proteins reflected the fact that some of the proteins tested were moderately inhibitory of rosette formation (HPC-9 and HPC-32), whereas others were noninhibitory (HPC-22, -78, -77, and HPC-81). For each series of proteins, between six and nine experiments were performed.

The immunoglobulin receptors on peritoneal macrophages were of identical specificity to those of bone marrow macrophages (Table 2). IgG2a proteins produced the greatest inhibition of rosette formation between peritoneal macrophages and IgG-coated SRBC. The same pattern of response to individual IgG1 proteins was seen with peritoneal macrophages as with bone marrow colony macrophages; HPC-9 and -32 were inhibitory.

The time course of acquisition of the IgG receptor property by bone marrow cells in culture is shown in Fig. 2. Small numbers of cells with IgG receptors were detectable by the third to fifth day of culture; however, large numbers of such cells were not detectable until after the sixth day of culture, when morphologically identifiable macrophages were abundant in the colonies. Macrophages in free suspension are less efficient than glass-adherent cells in forming red cell rosettes and in erythrophagocytosis. Consequently, sensitized SRBC were bound or ingested by 85–100% of mature glass-adherent macrophages but only by 60% of the same cells in suspension. Red cell rosettes were only seen around peroxidase-negative cells. The granulocytic cells of less mature colonies (3–7 days) only rarely had a single adherent red cell.

DISCUSSION

Among the phagocytic leukocytes, the possession of IgG receptors is a property of the macrophage-monocyte cell line. This observation was first made by Lo Buglio et al., and subsequently confirmed and extended. The macrophage receptors that bind IgG and those binding cytophilic antibody are probably similar if not identical. Therefore, the presence of IgG re-
ceptors is a useful means of identifying macrophages. By this means we have been able to demonstrate that the mononuclear phagocytes arising in bone marrow colonies in vitro are indeed macrophages and that they possess receptors of specificity identical with those of the peritoneal macrophages. That macrophage receptors have subgroup specificity was already known from studies of cytophilic antibody in the guinea pig.12

REFERENCES

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