Phenotypic Characterization of Gamma Interferon-Induced Human Monocyte Polykaryons

By J. Brice Weinberg, Marcia M. Hobbs, and Mary A. Misukonis

Multinucleated giant cells of mononuclear phagocyte origin (monocyte or macrophage polykaryons [MPs]) are seen in various normal and pathologic states including viral, bacterial, and fungal infections, foreign body reactions, cancer, and inflammatory conditions of unknown cause such as sarcoidosis and rheumatoid arthritis.1,2 Osteoclasts of bone, which function physiologically to resorb bone, are MPs derived from circulating uninucleate monocytes (UMs).3 These various MPs are thought to form by a process of UM fusion.1,4 Viral-induced cell fusion is thought to be caused by the action of viral envelope proteins that interact with cell membranes.5 Lymphokines have been shown to induce fusion of UMs.6-8 We have demonstrated that the lymphokine gamma interferon (IFN-γ) (highly purified natural or recombinant) causes MP formation from normal, freshly isolated human monocytes by a process of cell fusion.9 The functions of MPs formed in vivo in physiologic and pathologic settings, and in vitro in experimental circumstances, are not fully known. The purpose of this study was to characterize the IFN-γ-induced human MPs phenotypically, and to evaluate certain aspects of their function. The work shows that the MPs retain many characteristics of UMs, but that, when compared to UMs, they have decreased ability to reduce nitroblue tetrazolium (NBT); to phagocytose polystyrene spheres; and reduced expression of antigens recognized by monoclonal antibodies OKM1, DU-HL60-3, DU-HL60-4, TE5, and V1 were not expressed or were expressed poorly in MPs; they were expressed normally in control and treated UMs. However, HLA-DR expression was increased in treated UMs and MPs. The binding of the lectins RCA, Con A, WGA, DBA, UEA, and PNA was equal in all cells. Thus, MPs formed by fusion of UMs in vitro after culture with IFN-γ differ in several features from UMs.

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ensiformis or concanavalin A or Jack bean agglutinin (Con A; α-D-mannose), Dolichus biflorus or horse gram agglutinin (DBA; α-N-acetylgalactosamine), Ulex europaeus agglutinin I or gorse seed agglutinin (UEA; α-L-fucose), and Arachis hypogaea or peanut agglutinin (PNA; β-D-galactose).

Monocytes. Blood monocytes from normal male and female donors were isolated as described before using a combination of Ficoll-sodium diatrizoate and Percoll density gradient sedimentation. The donors gave written, informed consent according to a protocol approved by the VA and Duke University Clinical Investigations Committees. Cells isolated by this technique were composed of 80% to 95% monocytes, with the remainder being lymphocytes. In general, there were <4–6 platelets per monocyte. The monocytes (3 × 10⁷ per microtiter well) were incubated for one hour at 37 °C; the nonadherent cells were then removed by two washes with saline. This process resulted in monolayers with >95% monocytes. All cultures were in 10% autologous, unheated serum.

H₂O₂, acid phosphatase, plasminogen activator, and NBT reduction assays. H₂O₂ generation in response to 200 nmol/L of phorbol myristate acetate (PMA) was measured with monolayers of monocytes in microtiter plates as described previously, using phenol red as an oxidizable substrate in the presence of horseradish peroxidase and glucose. A Multiskan automatic eight-channel photometer (Flow Laboratories, Inc, Beltsville, Md) was used to measure the absorbances of the phenol red (600 nm) in microtiter plates. Protein was measured by the method of Lowry. NBT reduction by UMs and MPs in microtiter chambers was assessed by washing the cells twice with saline, incubating them with 0.2 mL of medium containing 200 nmol/L of PMA with 2 mg/mL NBT for 20 minutes, washing twice with saline, and counterstaining with 0.15% Safranin. The cells containing the reduced blue-black formazan were quantitated visually at 375 × in situ in the microtiter chambers.

Phagocytosis. Glutaraldehyde treatment and antibody opsonization of sheep erythrocytes was done as previously described. Cell monolayers containing UMs and/or MPs were incubated for two hours at 37 °C in medium with 1 × 10⁹ erythrocytes or 1 × 10⁷ polystyrene spheres. The chambers were then washed three times with saline, fixed in methanol, and stained with Wright’s stain. The number of particles phagocytized per cell or per nucleus was assessed by visual counting at 375 ×.

Cell staining. Cells adherent to the plastic in the microtiter chambers were stained with either Wright’s stain, nonspecific esterase (NSE) with or without 27 mmol/L of sodium fluoride, or acid phosphatase with or without 40 mmol/L of sodium fluoride. The slides were counterstained with 0.1% Safranin. All the slides were observed with a Zeiss Universal fluorescent microscope. All incubations were done in 0.9% NaCl. Two hours at 37 °C in medium with 1 × 10⁶ latex (polystyrene) spheres and glutaraldehyde-fixed erythrocytes were phagocytized less avidly by MPs than by UMs when assessed as the phagocytic index.

Fluorescent and immunoperoxidase staining. Monocyte monolayers were established from 1 × 10⁶ monocytes per chamber on eight-chambered LabTek slides with chambers of 1-cm² surface area. The cells were incubated for three days without or with 100 U/mL IFN-γ. The UMs and MPs adherent to the slides were then washed twice with saline; incubated for three hours with 10% goat serum; washed once with saline; incubated for one hour with the control supernatant (P3) or the primary antibody (1:100 for anti-Leu M3; 1:500 for DU-HL60-3; 1:300 for DU-HL60-4, 1:100 for OKM1, 1:500 for TES and V1, and 1:200 for anti-HLA-DR); washed twice with saline; incubated for one hour with fluoresceinated goat anti-mouse IgG (1:40); washed twice with saline; and then observed with a Zeiss Universal fluorescent microscope (Carl Zeiss, Inc, Thornwood, NY). Cells stained with anti-lysozyme (1:200) were fixed with 100% methanol, processed as described above, and stained with fluoresceinated goat anti-rabbit IgG. All the incubations and washes were done at 23 °C. Immunoperoxidase staining was done on cells fixed in cold acetone using the labeled avidin–biotin method. The cells were treated sequentially at 23 °C for the indicated times with intervening saline washes: 5% horse serum in 2% bovine serum albumin (15 minutes); primary antibody or control supernatant (P3) (60 minutes); biotinylated anti-mouse IgG (1:200 for 30 minutes); avidin DH–biotinylated horseradish peroxidase H complex (30 minutes); and 0.5 mg diaminobenzidine in 0.05 mol/L of Tris buffer with 0.05 mol/L of imidazole (pH 7.6) with 0.018% H₂O₂ (1 to 2 minutes). The slides were then stained with Mayer’s hematoxylin, and coverslips were mounted with Permount for observation.

RESULTS

General characteristics. As noted before, normal, freshly isolated human blood monocytes cultured in 10% autologous unheated serum for three days at 37 °C developed only rare MPs, whereas those cultured under identical conditions except for the addition of 10 to 400 U/mL IFN-γ developed numerous MPs. The MPs appeared after 36 to 72 hours in culture. The largest numbers of MPs were found when 20 to 100 U/mL IFN-γ was used. The MPs were 28 to 100 μm in diameter with three to 168 nuclei per MP with a maximal fusion index (percentage of all nuclei within MP) of 50% to 70% (v 0% to 10% for control monocytes). The nuclei were usually positioned near the center of the MP. The MPs did not incorporate tritiated thymidine into their nuclei.

Phagocytosis. Latex (polystyrene) spheres and glutaraldehyde-fixed erythrocytes were phagocytized less avidly by MPs than by UMs when assessed as the phagocytic index (number of particles per nucleus in each cell). However, the more voluminous MPs did phagocytize more of these particles when considered on the per cell basis (Table 1). UMs from control and IFN-γ–treated cultures phagocytized the particles to a comparable degree. Phagocytosis of IgG-coated erythrocytes by MPs was less than that by IFN-γ–treated UMs or control UMs. This was even more dramatic when assessed on the basis of the phagocytic index. In general, there was no correlation between the degree of multinuclearity in the MPs and the phagocytic index for the opsonized erythrocytes (data not shown). Figure 1 shows the differences in phagocytosis by treated UMs and MPs.

Cytochemistry. In populations of monocytes cultured for three days in 100 U/mL IFN-γ, neither UMs nor MPs had peroxidase activity. All cells had NSE, but MPs stained more intensely than did UMs. Fluorochrome abolished NSE in all
cells. Resistance to fluoride is a characteristic of NSE of UM and MPs. Resistance to the inhibitory action of tartrate has been noted to be a characteristic of the osteoclast MP.6,7 However, all of these cells were only minimally inhibited by tartrate, the UM and MPs being approximately equivalent in their relative resistance to the inhibitory effect.

**Reactive oxygen species production.** We found, as various investigators have noted previously,22,23,32 that populations of IFN-γ-treated human monocytes produce more H2O2 in response to PMA than do control monocytes (Table 2). We wanted to harvest the adherent UM and MPs and study enriched populations of each, but could not obtain enough viable cells after enzyme, chelator, and/or mechanical treatments to do suitable studies (data not shown). However, we did examine the adherent cells individually for their ability to produce reactive oxygen species in response to PMA by visually studying NBT reduction. Using this technique, we found that the IFN-γ-treated UM were markedly positive (more so than the control UM), whereas the MPs in the same culture chambers had much reduced ability to reduce NBT (Table 2).

### Table 1. Phagocytosis by Control and IFN-γ-treated UM and MPs

<table>
<thead>
<tr>
<th>Phagocytosis</th>
<th>Control</th>
<th>IFN-γ-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td>Polystyrene spheres*</td>
<td>UM</td>
<td>MP</td>
</tr>
<tr>
<td></td>
<td>31.8 ± 3.0†</td>
<td>102.7 ± 8.8</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(16.9 ± 1.7‡)</td>
</tr>
<tr>
<td>Antibody-coated erythrocytes§</td>
<td>UM</td>
<td>MP</td>
</tr>
<tr>
<td></td>
<td>6.3 ± 1.2</td>
<td>2.3 ± 0.5</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(0.5 ± 0.1)</td>
</tr>
<tr>
<td>Glutaraldehyde-fixed erythrocytes‡</td>
<td>7.2 ± 1.1</td>
<td>14.6 ± 1.9</td>
</tr>
<tr>
<td></td>
<td></td>
<td>(3.3 ± 0.6)</td>
</tr>
</tbody>
</table>

- IFN-γ, gamma interferon; UM, uninuclear monocytes; MP, macrophage polykaryons.
- *Statistical comparisons: control UM vs treated UM (P < .04); control UM vs MP (P < .001 for particles per cell or particles per nucleus); treated UM vs MP (P < .001 for particles per cell or particles per nucleus); n = 12.
- †Mean number of particles or erythrocytes ± SEM per cell.
- ‡Mean number of particles or erythrocytes ± SEM per nucleus (phagocytic index). The number of nuclei per MP was 6.1 in the polystyrene sphere experiments, 4.6 in the antibody-coated erythrocyte experiments, and 4.4 in the glutaraldehyde-fixed erythrocyte experiments.
- §Statistical comparisons: control UM vs treated UM (P < .5); UM vs MP (P < .001 for erythrocytes per cell or erythrocytes per nucleus); treated UM vs MP (P < .001 for erythrocytes per cell or erythrocytes per nucleus); n = 8.
- ‡Statistical comparisons: control UM vs treated UM (P < .5); control UM vs MP (P < .03 for erythrocytes per cell or erythrocytes per nucleus); treated UM vs MP (P < .005 for erythrocytes per nucleus); n = 7.

**Antigen expression by MPs.** To determine if MP and UM differed in their expression of various antigens, the control and IFN-γ-treated monocytes were examined using different antibodies with immunofluorescence or immunoperoxidase techniques. Results using these two techniques were qualitatively similar. As seen in Table 3, neither UM nor MP stained positively with the control supernatant of the parent mouse myeloma (P3). The UM all reacted with the different antibodies studied. However, the MP did not react with the antibodies anti-Leu M3, DU-HL60-3, DU-HL60-4, TE5, V1, or OKM1 (anti-C3bi receptor). Figure 2 demonstrates the differential expression of anti-Leu M3 by IFN-γ-treated UM and MP. The IFN-γ-treated UM and MP had slightly enhanced staining for HLA-DR when compared with control monocytes, whereas staining for intracellular lysozyme was comparable in all cells.

To determine if the changed expressions of these different antigens was accompanied by changes in surface membrane glycoprotein or glycolipid, we evaluated the binding of various lectins. These agglutinins bind to specific terminal carbohydrates of surface glycoconjugates and have proven useful in the study of hematopoietic cells.8,31 Data in Table 4 demonstrate that there was essentially no difference between control UM and IFN-γ-treated UM and MP with respect to lectin binding. All the cells bound RCA, Con A, and WGA, in decreasing order of intensity. The binding of these

### Table 2. H2O2 Production and NBT Reduction by Control and IFN-γ-treated Monocytes

<table>
<thead>
<tr>
<th>H2O2 (nmol/h/mg) *</th>
<th>Control</th>
<th>IFN-γ-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UM</td>
<td>MP</td>
</tr>
<tr>
<td></td>
<td>33.9 ± 7.3</td>
<td>198.8 ± 28.5</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>NBT reduction (percentage positive) †</th>
<th>Control</th>
<th>IFN-γ-treated</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>UM</td>
<td>MP</td>
</tr>
<tr>
<td></td>
<td>67.2 ± 2.8</td>
<td>91.2 ± 2.5</td>
</tr>
<tr>
<td></td>
<td>17.9 ± 5.3</td>
<td></td>
</tr>
</tbody>
</table>

- IFN-γ, gamma interferon; UM, uninuclear monocytes; MP, macrophage polykaryons.
- *Mean ± SEM from seven determinations. Statistical comparison: control UM vs treated UM (P < .001).
- †Mean ± SEM from seven determinations. Statistical comparisons: control UM vs MP (P < .001); control UM vs treated UM (P < .001); treated UM vs MP (P < .001).
A granuloma, as defined by Adams is "... a compact (organized) collection of mature mononuclear phagocytes (macrophages and/or epithelioid cells), which may or may not be accompanied by accessory features such as necrosis or the infiltration of other inflammatory leukocytes." Granulomas are characteristic of host responses to many different living and nonliving agents. They form, in general, as reactions to particulate or indigestible agents that persist in tissues for long periods of time. Cells of granulomas serve both protective and destructive functions—protecting the host against microbial agents and possibly tumor cells, while also causing destruction and fibrosis of adjacent tissues. They form, in general, as a consequence of membrane fusion, and the pivotal change must involve an alteration of the mononuclear phagocyte's plasma membrane leading to macrophage polykaryons.

Much work has been expended in trying to dissect and understand the mechanisms by which MPs form (reviewed in ref. 2). It is clear that the MPs form by a process of monocyte fusion, and the pivotal change must involve an alteration of the mononuclear phagocyte's plasma membrane leading to the fusion. Chambers has suggested, based on in vitro studies, that the MPs form as a consequence of membrane fusion during simultaneous phagocytosis or endocytosis of a particle or ligand by two or more cells. There is evidence that lymphokines play a role in the development and maintenance of granulomas in vivo. Yoshida and others have demonstrated that lymphokines including chemotactic factors, macrophage migration inhibitory factors, and macrophage fusion factors are produced in vivo within granulomas. Various investigators have shown that different soluble lymphocyte factors alter the function of macrophages and cause fusion of mononuclear phagocytes to MP in vitro. We have shown that IFN-γ, in addition to serving as a "macrophage activating factor," also causes the fusion of human monocytes to form MP.

In this study, we show that the MPs, while maintaining many features of UMs, display several differences other than simple morphology. As noted by others, MPs have a decreased ability to phagocytize particles. In our system, IgG-coated erythrocyte phagocytosis was markedly depressed in MPs, whereas the nonspecific phagocytosis of polystyrene spheres and glutaraldehyde-fixed erythrocytes was only moderately depressed (when assessed by the phagocytic index). The cause of the decreased phagocytosis is unknown. Chambers has shown that the phagocytic ability of rodent MPs is dependent on the mechanism by which they are formed. For example, MPs formed by simultaneous phagocytosis of glutaraldehyde-fixed erythrocytes display a selective decrease in phagocytosis for this type of erythrocyte, whereas MPs formed by simultaneous phagocytosis of antibody-coated erythrocytes display a selective decrease in phagocytosis for this type. It is difficult to extrapolate this reasoning to experiments using IFN-γ-induced MPs.

We discovered that MP expression of certain cell surface lectins was blocked by the appropriate monosaccharide ligands.

**DISCUSSION**

Table 3. Antigen Expression by Control and IFN-γ-treated UMs and MPs

<table>
<thead>
<tr>
<th></th>
<th>Control UMs</th>
<th>Control UMγ</th>
<th>IFN-γ-treated UMs</th>
<th>IFN-γ-treated UMγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Anti-Leu M3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DU-HL60-3</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DU-HL60-4</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>TE5</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>V1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>OKM1</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Anti-HLA-DR</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
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</table>

Table 4. Lectin Binding by Control and IFN-γ-treated UMs and MPs

<table>
<thead>
<tr>
<th></th>
<th>Control UMs</th>
<th>Control UMγ</th>
<th>IFN-γ-treated UMs</th>
<th>IFN-γ-treated UMγ</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>RCA</td>
<td>+ (0*)</td>
<td>+ (0*)</td>
<td>+ (0*)</td>
<td>+ (0*)</td>
</tr>
<tr>
<td>ConA</td>
<td>+ (0†)</td>
<td>+ (0†)</td>
<td>+ (0†)</td>
<td>+ (0†)</td>
</tr>
<tr>
<td>WGA</td>
<td>+</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>DBA</td>
<td>0</td>
<td>0</td>
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<td>0</td>
</tr>
<tr>
<td>UEA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>PNA</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
</tbody>
</table>

No fluorescence above control, 0; fluorescence greater than control, +. IFN-γ, gamma interferon; UMs, uninuclear monocytes; MPs, macrophage polykaryons.

Reactivity in the presence of 0.1 mol/L of α-D-mannose.

†Reactivity in the presence of 0.1 mol/L of α-D-galactose.

Fig 2. Phase contrast (A) and immunofluorescent (B) photographs of the same field containing IFN-γ-treated uninuclear monocytes (UMs) and macrophage polykaryons (MPs). The cells had been incubated with mouse monoclonal anti-Leu M3 antibody and fluoresceinated goat anti-mouse immunoglobulin as described in the Materials and Methods section. The arrows indicate the MPs. Original magnification ×400; current magnification ×300.
MONOCYTE POLYKARYONS

antigens recognized by monoclonal antibodies was markedly depressed as compared with control or IFN-γ-treated UMs. These included antigens recognized by the antibodies DU-HL60-3, DU-HL60-4, TES, V1, anti-Leu M3, and OKM1. The cause of this loss of surface expression of these antigens is not known. It is not universal, since HLA-DR was expressed to an increased degree. This general enhanced expression of HLA-DR in IFN-γ-treated human monocytes corroborates the findings of others.42,43 We hypothesized that the decreased expression of certain antigens was related to changes in surface glycoproteins. However, the MPs and UM s showed comparable binding of various lectins with different monosaccharide-binding specificities.

The functions of all the antigens noted to be depressed in the MPs are unknown except for the OKM1 antigen. It has been shown that this antigen is, or is very closely related to, the cellular receptor for the complement component C3bi.44 Certain patients with recurrent bacterial infection have been described whose monocytes, granulocytes, and null cells lack OKM1 and the related antigens M01, LFA-1, and Mac 1.45-47 Cells from these patients have defective phagocyte adherence, phagocytosis of C3bi-coated particles, and depressed production of reactive oxygen species with phagocytosis of zymosan.

Populations of IFN-γ-treated human monocytes, as compared with control monocytes, have been shown to produce more H2O2 in response to PMA than do control monocytes.12,31,32 However, in this study, we demonstrate that, when examined on an individual cell basis using an NBT reduction assay, the MPs actually have diminished ability when compared with control monocytes, have been shown to produce more H2O2 in response to PMA than do control monocytes.12,31,32 However, in this study, we demonstrate that, when examined on an individual cell basis using an NBT reduction assay, the MPs actually have diminished ability to produce reactive oxygen species, whereas the IFN-γ-treated UMs have qualitatively enhanced NBT reduction. Although it is remotely possible that this decreased NBT reduction by MP was caused simply by diminished endocytosis of the indicator NBT, we think that this is unlikely. This finding suggests that the increased H2O2 production by populations of IFN-γ–treated monocytes is caused by increased production by the UMs that overshadows the decreased production by the MPs. The mechanism of this decreased ability is unknown. Others have noted that MPs formed by long-term culture in high concentrations of serum have normal NBT reduction in response to PMA.41

Human MPs will form spontaneously from normal monocytes in long-term cultures or in cultures done in high concentrations of serum.41,48-50 IFN-γ dramatically enhances this MP formation, causing an earlier appearance of MPs and larger MPs.11 The system using IFN-γ is an in vitro model that results in MPs that are very similar to those seen in histologic sections of MPs formed in vivo. Comparable to mononuclear phagocytes seen in experimental granulomas formed in vivo,51 the human monocytes treated in vitro with IFN-γ display a heterogeneity with respect to various parameters.

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Phenotypic characterization of gamma interferon-induced human monocyte polykaryons

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