**γ-Interferon Modulates Human Monocyte/Macrophage Transferrin Receptor Expression**

By Raymond Taetle and J. Michael Honeysett

Although circulating human monocytes do not express transferrin (Tf) receptors, cultured adherent blood cells display high-affinity Tf binding sites. In the present studies, effects of various cytokines and biologically active proteins on human monocyte/macrophage Tf receptors were investigated. After culture, Tf receptor expression by adherent blood cells was time dependent and plateaued by 7 days. The addition of interleukin-1 (IL-1), α-interferon (α-IFN), granulocyte/macrophage-colony stimulating factor (GM-CSF), or human IgG to macrophages cultured for 4 days did not alter Tf receptor expression. Fe-saturated, human Tf caused a significant, dose-dependent decrease in receptor expression. At a dose of 100 U/mL, γ-interferon (γ-IFN) significantly increased Tf receptor expression by macrophages cultured for 4 (230% ± 51% of control) or 7 days (150% ± 22%). Scatchard analyses showed increased binding sites but no change in receptor affinity. Northern and slot blot analysis of cellular mRNA from macrophages cultured for 4 or 7 days and exposed to γ-IFN showed a two- to fivefold increase in Tf receptor mRNA, but ≤30% increase in β-actin mRNA. Ferritin content of γ-IFN-treated macrophages was 47% to 83% of control cells. Net uptake of $^{59}$Fe from Tf by γ-IFN–treated cells was 10% to 17% of control, but uptake of radiolabeled Tf was comparable. When macrophages were labeled with $^{59}$Fe and then exposed to γ-IFN, cell-associated Fe was reduced by 43%, indicating that γ-IFN caused macrophage Fe release. γ-IFN specifically modulates Tf receptor display by inducing Fe release and reducing cellular Fe content. Regulation of Tf receptor expression in macrophages is controlled by cellular Fe content and is thus similar to regulatory mechanisms in dividing cells.

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**MATERIALS AND METHODS**

*Cell culture techniques.* Peripheral blood was collected in heparin, sedimented with 6% Dextran/phosphate-buffered saline (PBS) and separated on Ficoll/Hypaque (Ficoll/Paque, Pharmacia Chemicals, Piscataway, NJ) as previously described. The interface mononuclear cells were aspirated and washed four times in RPMI 1640 (GIBCO, Grand Island, NY) supplemented with 10% fetal bovine serum (FBS; Hyclone, Logan, UT). For transferrin binding, 3 to 4 × 10⁶ cells in serum-free RPMI 1640 were added to individual wells of a 24-well plate (Costar, Van Nuys, CA) that had been pretreated with 0.25 mL concentrated H₂SO₄ and then washed five to seven times with sterile, distilled water. The cells were incubated for 2 to 3 hours at 37°C in 7.5% CO₂ and nonadherent cells were removed by four to five washes with media. Adherent cells were incubated in media with 5% fetal calf serum (FCS) for 1 to 14 days at 37°C in 7.5% CO₂.

For cellular characterization, cells were plated onto glass coverslips as previously described or into 75-cm² tissue culture flasks (Costar, Cambridge, MA) and incubated as described above. For RNA extraction, 1.2 to 1.5 × 10⁶ cells were added to acid-treated tissue culture flasks and incubated, and nonadherent cells were removed as described above.

Characterization of adherent blood cells. Adherent cells were characterized after incubation for 4 or 7 days. Coverslips containing adherent blood cells were washed, mounted on glass slides, and stained using Wright/Giemsa to assess morphology or for nonspec-
specific esterase as previously described.21 Phorbol ester-stimulated superoxide generation was assessed by nitroblue tetrazolium (NBT) dye reduction using cells mounted on coverslips using a modification of previously described techniques,7 or on >95% viable cells mechanically detached from tissue culture flasks. T-lymphocyte-specific antigens were detected by staining with a mixture of T101 (Hybritech, San Diego) and T11 (Coulter Diagnostics, Hialeah, FL) monoclonal antibodies. Granulocyte/monocyte-specific antigens were detected using a pool containing antibodies MY7, MY9 (Coulter) and OKM2 (Ortho Diagnostics, Raritan, NJ). Staining for Tf receptors was performed using B3/25 (provided by Dr Ian Trowbridge, Salk Institute for Biologic Studies, La Jolla, CA) or T9 (Coulter). A mixture of mouse immunoglobulins served as a control.

Chemicals and cytokines. Purified, Fe-saturated Tf was obtained from Miles Pharmaceuticals, (Naperville, IL). Human IgG and F(ab')2 IgG fraction were obtained from Sigma Chemicals (St Louis). a-Interferon (a-IFN) (>5 x 10^4 U/mg protein) and -IFN (1.6 x 10^9 U/mg protein) were obtained from Amgen (Thousand Oaks, CA). Ultrapure human IL-1 (25% IL-1 a, 75% IL-1 b, 8 x 10^6 U/µg protein) was obtained from Genzyme Corporation, Boston. Placenta-conditioned medium (placenta CM) containing high titers of granulocyte/macrophage (gm-) and granulocyte (g-) colony-stimulating factors (CSF) was prepared, and activity was assessed as described elsewhere.22

Treatment with additives and cytokines. Human monocyte/macrophages cultured for 3 or 6 days were washed twice in media and overlaid with media containing 1% FCS. For 24-well plates, 1 mL was added to each well, or proportionate amounts were added to other culture vessels. Additives (human IgG, F(ab')2, IgG, or transferrin) or cytokines (a-IFN, -IFN, placenta CM CSF, IL-1) were added in a final volume of 10 µL/mL media, and incubation was continued for 14 to 16 hours at 37°C in 7.5% CO₂.

Transferrin binding and uptake. Iron-saturated human Tf was labeled to a specific activity of ~5 x 10^7 cpm/mg using I125 and chloramine T, and specific binding was determined at 4°C as previously described.12,23 Non-specific binding was determined by incubation with 200x unlabeled Tf and subtracted from total bound counts. For cell counts, control cells or cells treated with cytokines were detached with trypsin/EDTA from quadruplicate wells on each plate and complete removal of all cells was confirmed by microscopy. Cell counts on plates treated with additives or cytokines did not differ from controls.

To determine whether a-IFN inhibited Tf uptake through Tf receptors, day 7 macrophages were incubated at 4°C for 30 minutes with 125I-labeled, Se-saturated Tf as previously described.23 The cells were then incubated at 37°C for 30 minutes, washed, and harvested to determine total cell-associated radioactivity. To determine Tf uptake, duplicate cell aliquots were treated with 0.5 mol/L NaCl/0.2 mol/L acetic acid to remove residual surface-bound Tf.13 Percentage of Tf uptake was determined by acid-resistant counts divided by total cell counts x 100.

Extraction of cellular RNA and Northern hybridization. To isolate total cellular RNA, monocytes cultured for 4 or 7 days were mechanically detached from tissue culture flasks and RNA was extracted by the guanidine isothiocyanate method.11 For Northern blot analysis, cellular RNA (10 µg) was electrophoresed in 1% agarose gels containing 18% formaldehyde and transferred onto nylon membranes (Biotrans; ICN Biomedicals, Irvine, CA) as previously described.13 The probes used were a 2.96-kilobase (kb) Hind III fragment from the human Tf receptor cDNA clone, pCD-TR14 and a 0.9-kb chicken b-actin probe that detects human actin RNA (provided by Dr Carol MacLeod, UCSD Cancer Center).15 Nick translation was performed according to manufacturer's instructions (Nick Translation Kit, Bethesda Research Laboratories, MD). Northern and slot blot hybridization were also performed as previously described.13 All RNA specimens analyzed by slot blotting showed single discrete bands when probed using Northern blots. For some studies, Northern or slot blots were placed in 10 mmol/L Na phosphate, pH 6.5, with 50% formamide to remove radioactive DNA and then were rehybridized to a second probe. Relative amounts of RNA were determined by scanning slot blots using computerized, digital-matrix photometry through the courtesy of Dr William Neely, San Diego Veterans Administration Medical Center.

Macrophage ferritin content. Cellular ferritin was determined as previously described16 using lysed control or cells exposed to 100 U/mL a-IFN for 14 to 16 hours. Fe uptake and release. Human Tf was fully saturated with 59Fe as described by Bates and Schlabach.28 Labeled Tf was mixed with unlabeled Se-saturated Tf to give a final concentration of 50 µg/mL. Day 4 or day 7 adherent blood cells were incubated for 14 to 16 hours as described above with 59Fe labeled Tf or labeled Tf and 100 U/mL a-IFN. The cells were then harvested and counted. In other experiments, day 4 or day 7 macrophages were incubated with 59Fe-labeled Tf for 6 hours, washed, and then cultured under control conditions or with a-IFN. Cells were then harvested and counted.

Results

Adherent blood cells were characterized for morphology, surface markers, histochemistry, and phorbol ester-stimulated NBT dye reduction using modifications of previously described techniques.12,21 With the exception of Tf receptor display, results were identical for cells cultured for 4 or 7 days. By morphology, 90% ± 2% (x ± SE, six studies) of the cells were macrophages. The majority expressed myeloid surface markers (71% ± 13%), nonspecific esterase (85% ± 2%), and generated superoxide after phorbol stimulation (79% ± 7%). When assessed by immunofluorescence staining, peripheral blood monocytes and adherent blood cells cultured for 1 day did not express Tf receptors. Similar to previous studies,21 after 4 days, 40% ± 12% of cells expressed Tf receptors detected by immunofluorescence staining with monoclonal anti-Tf receptor antibody and, by 7 days, 85% ± 8% of cells were positive.

By means of radioligand binding, no Tf binding was detected on freshly isolated adherent cells, but low levels of specific binding were observed after culture for 1 day (Fig 1). After 4 days, a single class of Tf binding sites was detected (eg, Fig 2). Estimated affinities were 6 x 10^4 mol/L (Kd, mean of 3 studies), and maximum specific binding was 74 ± 9 fmol/10^6 cells (mean ± SE, seven studies). Because only ~40% of cells expressed Tf receptors detected by immunofluorescence, this indicated an average of ~10^7 binding sites/cell.

The effects of various biologically active proteins on Tf receptor display are shown in Table 1. In these studies, monocytes were cultured for 3 days, washed, exposed to cytokines (IL-1, Placenta CM CSF, a-IFN, -IFN), transferrin, or IgG for 14 to 16 hours in medium with 1% FBS, and Tf binding was assessed. Proteins chosen for study were those shown to bind to macrophage surface receptors (transferrin, IgG) or alter macrophage function. Placenta CM was
used to screen for effects of g-CSF and gm-CSF. The F(ab')₂
immunoglobulin preparation was used as a control for pro-
teins unable to bind to the macrophage surface, but related to
a protein for which these cells have surface receptors (ie,
IgG). Although several caused minor perturbations in Tf
binding, only γ-IFN significantly increased Tf receptor
expression. At the highest dose used, Fe saturated Tf caused
a significant decrease in receptor expression, consistent with
Fe loading.13,21,27

When adherent blood cells were cultured for 7 days, Tf
binding increased in a time-dependent manner (Fig 1).
Consistent with other recent studies,3 binding did not
increase further with incubation up to 14 days (data not
shown). Scatchard analyses again showed a single class of
high-affinity binding sites (Fig 2) (Kₐ = 3.0 × 10⁻⁹ mol/L,
mean of three studies) and an average of 1.8 ± 0.5 × 10⁵
sites/cell (mean ± SE, five studies). Overnight exposure of
adherent blood cells previously cultured for 6 days to 100
U/mL γ-IFN also increased Tf receptor display (150% ±
22% of control, five studies, P < .05), but ligand affinity
remained unchanged (Fig 2).

Results from studies directly comparing γ-IFN effects on
adherent blood cells cultured for 1 to 7 days are shown in Fig
1. Cells were cultured for varying periods, treated overnight
with control medium or 100 U/mL γ-IFN, and Tf binding
was assessed. Augmentation of Tf binding was most pro-
nounced after 4 days in culture, but was also detected after 1
and 7 days.

Although a previous study showed that Tf receptor tran-
scripts appeared in monocytes after culture,3 under some
conditions, macrophages alter surface Tf receptor display by
changing distributions of surface and intracellular recep-
tors.29 To determine whether γ-IFN increased synthesis of
new Tf receptors, steady-state Tf receptor transcripts were
assessed after γ-IFN treatment. As shown in Fig 3, exposure
of adherent blood cells to γ-IFN resulted in a two- to fivefold
increase in Tf receptor mRNA. In contrast β-actin mRNA
increased slightly (<30%) on day 4 (Fig 3B) or day 7 (not
shown). Thus, exposure of adherent blood cells to γ-IFN
increased steady-state Tf receptor mRNA, but transcripts for
a structural gene increased to a much lesser extent.

In proliferating cells, Tf receptor display increases when
cellular Fe content falls and decreases when cellular Fe
increases.13,21,30,31 To determine whether γ-IFN-induced
changes in macrophage Tf receptor display were due to
changes in Fe balance, intracellular ferritin levels were
determined in control and γ-IFN-treated macrophages. As
shown in Table 2, intracellular ferritin levels were 47% to
63% of control levels in γ-IFN-treated cells. The ability of
control and γ-IFN-treated macrophages to take up ⁵⁹Fe
from Tf was also compared. When cells were simultaneously
exposed to ⁵⁹Fe-Tf and γ-IFN, net Fe uptake by treated cells

<table>
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<th>Addition to Culture</th>
<th>Concentration</th>
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<td>γ-IFN</td>
<td>10 U/mL</td>
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<td></td>
<td>100 U/mL</td>
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<td>230 ± 5††</td>
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<td></td>
<td>1,000 U/mL</td>
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<td>188 ± 16*</td>
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<td>110 ± 13</td>
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<tr>
<td></td>
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<td>85 ± 7</td>
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<td>93 ± 9</td>
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Table 1. Effects of Various Biologically Active Proteins on
Macrophage Transferrin Binding

Human macrophages grown for 4 days from blood monocytes were
incubated for 14 to 16 hours with 1% FCS or various concentrations of
protein additives.

*Significantly different from control, P < .05.
†Percentage of control, mean ± SE.
‡Significantly different from control, P < .01.
was only 10% to 17% of controls (Table 2). This finding could result from decreased Tf-Fe uptake or from release of Fe already taken up by γ-IFN–treated cells. To examine Tf uptake, control and γ-IFN–treated macrophages were incubated with radiolabeled Tf and then warmed to 37°C for 30 minutes. Residual surface-bound Tf was then eluted by acid salt treatment. As shown in Table 1, control and γ-IFN–treated macrophages took up similar relative amounts of labeled Tf, indicating that IFN did not inhibit Tf receptor endocytosis. Thus, γ-IFN–treated macrophages contained less Fe and showed decreased net Fe uptake from Tf when compared with untreated cells.

These observations suggested that macrophage Fe release was increased in the presence of γ-IFN. To confirm this hypothesis, macrophages from peripheral blood cells cultured for 4 or 7 days were incubated with 59Fe-Tf for 6 hours and then cultured for 14 to 16 hours under control conditions or with γ-IFN. After this culture period, IFN-treated cells contained 43% to 45% of the Fe of controls, demonstrating decreased retention of intracellular Fe (Table 2).

**DISCUSSION**

The present studies confirm previous observations by ourselves and other researchers demonstrating time-dependent Tf receptor expression by cultured human monocytes.5,7 In the present studies, monocyte/macrophage binding sites per cell were two to four times higher than in a previously published study.5 In this previous study, human macrophages obtained from cultured monocytes were detached using EDTA prior to binding, whereas in the present studies, attached cells were studied in situ. Either the EDTA treatment or detachment itself might alter intracellular or surface-accessible Tf receptor pools. Although monocyte/macrophages studied previously were cultured in 10% FBS,5 in the present studies cells were cultured for 14 to 16 hours in 1% serum. By reducing the FBS concentration, bovine Tf concentrations were reduced. Because reducing Tf/Fe concentrations increases hematopoietic cell Tf receptor display13,27 and synthesis,13 differing serum Tf concentrations may have altered macrophage Tf receptor expression. In proliferating cells that constitutively express Tf receptors,12,33 Fe balance appears to be the major determinant of Tf receptor display.31,27,30,31 Cellular ferritin follows an inverse pattern, increasing with Fe exposure and decreasing during Fe deprivation.13,21,30,31 Previous studies also demonstrated a close relationship between Tf receptor display, Tf receptor protein synthesis, and Tf receptor transcript levels in hematopoietic cells.13 The present studies indicate that
γ-IFN induces new Tf receptor synthesis. Because in other cells increased Tf receptor synthesis is accompanied by decreased cell ferritin and Fe content, Fe balance in γ-IFN-treated macrophages was examined. Results were consistent with decreased net Fe uptake from Tf due to increased Fe release (Table 2). Tf uptake was similar in γ-IFN-treated and control macrophages. Thus, although γ-IFN inhibits uptake of macrophase CSF-1 receptors, it did not affect Tf receptor uptake.

Several recent studies indicate that macrophase activation alters Tf receptor display and Fe balance. Hamilton et al. showed that murine macrophages from mice injected intraperitoneally with sterile stimuli had higher Tf receptors than cells harvested after injection of BCG or *Propionobacterium acnes.* Alvarez-Hernandez et al. showed a reduced rate of Fe release from thiglycollate-treated macrophages. Finally, McGowan et al. reported decreased Fe release and increased Fe content in alveolar macrophages from smokers. The present studies suggest that γ-IFN may mediate some of these effects on macrophase Tf receptors and Fe balance.

In vitro, γ-IFN is a potent activator of macrophase functions, such as tumor cytotoxicity, and increases class II HLA antigen expression. The complex responses elicited by γ-IFN enhance immune effector function and are accompanied by elaboration of cytokines and biologically active molecules, such as gm-CSF. Fe sequestration and Tf receptors may play a role in macrophase tumor cytotoxicity and fungistasis. Because Tf may also enhance CSF release from macrophages and CSF activates macrophase functions, multiple interactions between immune modulators (γ-IFN, CSF), mediators of Fe availability (Tf, Tf receptors) and macrophase effector function may exist.

Enhancement of Tf receptor expression by γ-IFN appears to be a specific response. Cytokines such as gm-CSF, IL-1, and α-IFN failed to increase surface Tf receptors. Similarly, proteins that bind to or are processed by macrophages, such as IgG, also failed to alter Tf receptor expression. Only Fe-saturated Tf lowered receptor expression, an effect consistent with known effects of increased cellular Fe on Tf receptor expression and synthesis.

The present studies differ from studies using murine peritoneal macrophages. When exposed to similar concentrations of γ-IFN, murine macrophages decreased Tf receptor display within 24 hours. The reasons for this apparent disparity are unclear but could relate to varying stages of macrophase activation or heterogeneity within macrophase populations. Because bone marrow macrophase Fe content is increased in patients with anemia due to inflammatory diseases, local differences in γ-IFN concentrations or complex interactions with other proteins affecting macrophase Fe balance may determine macrophase Fe content and Tf receptor display in vivo.

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