Identification and Characterization of a Functional Receptor for Interferon-γ on a Megakaryocytic Cell Line

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We have previously shown that human interferon-γ (Hu-IFN-γ) induces platelets to become efficient effector cells, capable of killing young larvae of the parasite Schistosoma mansoni. Recently, binding sites for IFN-γ on platelets have been characterized. We show here the presence of high-affinity receptors for IFN-γ on the surface of the human megakaryocytic Dami cell line. Scatchard analysis indicated the presence of about 11,000 binding sites per cell, with a kd of 3 ± 0.5 × 10−10 mol/L; the apparent molecular weight of the receptor was 90 Kd. Receptor-bound 125I-Hu-recombinant IFN-γ was rapidly internalized and degraded when the temperature was increased from 4°C to 37°C. The half-life of this receptor was about 7 hours, and pretreatment of cells with IFN-γ or phorbol myristate acetate had very little effect on the surface receptor number and no detectable effect on IFN-γ receptor messenger RNA (mRNA) expression. The receptor was functional, because 24 hours of treatment with IFN-γ led to the increase of HLA class I mRNA expression and to the initiation of HLA class II mRNA expression. These effects were selective because platelet glycoprotein Ib, IIb, or IIa mRNA expression and cell proliferation were unaffected.

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INTERFERON-γ (IFN-γ) is a lymphokine produced by activated T lymphocytes that exerts immunomodulating, differentiating, antiproliferative, and antiviral effects on various cell populations. These pleiotropic activities require the interaction of this lymphokine with species-specific, high-affinity membrane receptors that are constitutively expressed by a variety of cell types. Recently, progress has been made in the molecular characterization of the human IFN-γ receptor (Hu-IFN-γ-R). It has been purified from Raji cells and placenta membranes as a glycoprotein (GP) with an apparent molecular weight of 90 Kd, and recently cloned from a Raji cell-derived cDNA library. The deduced amino acid sequence has indicated a molecular weight of 54 Kd, and the presence of several potential N- and O-linked glycosylation sites. The difference between the molecular weight of the native receptor and of the cloned protein has suggested a high degree of glycosylation of the native receptor. Indeed, the different molecular weights of the monoyctic receptor (103 Kd) and of the Raji cell receptor (90 Kd) seem indeed to be due to different glycosylation.

The study of immune effector mechanisms against helminth parasites has led to the identification of antibody-dependent cell cytotoxicity (ADCC) mechanisms involving inflammatory cells, including platelets, that can kill parasite larvae (Schistosoma mansoni) in the presence of anaphylactic antibodies (IgE). These effector functions of platelets can be regulated by mediators released by activated T cells. Thus, Hu-IFN-γ or tumor necrosis factors (TNF) are able to induce resting normal platelets into effector cells against parasite larvae in the absence of specific IgE antibodies. In line with these results, Molinas et al. have described a high-affinity specific receptor for IFN-γ on the human platelet surface membrane.

These data led us to search for the presence of receptors for this lymphokine on the platelet-precursor-hematopoietic cells, the megakaryocytes. Indeed, because even platelets express this receptor, the question remains whether it is synthesed and expressed already in megakaryocytes before their fragmentation into functional platelets. To study this question, we used throughout this work a human megakaryocytic cell line, the Dami line, that has been previously reported by Greenberg et al. to exhibit many of the morphologic and biochemical characteristics of bone marrow megakaryoblasts and megakaryocytes, including polyploidy, and expression of HLA class I, platelet GP Ib and IIb-IIIa complex, and von Willebrand factor (vWF). Moreover, these cells can be induced to differentiate further along the megakaryocyte/platelet lineage with the tumor-promoting phorbol ester, phorbol myristate acetate (PMA).

In the present work, we report the identification of a high-affinity receptor for IFN-γ on the Dami megakaryocytic cell line and show that this receptor is functional.

MATERIALS AND METHODS

Cells

Dami cells, originally isolated by Greenberg et al, were cultured in Iscove's modified Dulbecco's medium (IMDM; Gibco-BRL, Cergy-Pontoise, France) supplemented with 10% horse serum. Only exponentially growing cells were used for experiments.

IFN

Pure human recombinant IFN-γ (Hu-r IFN-γ) (specific activity, 2 to 5 × 10^6 U/mg of protein) was kindly provided by Roussel-UCLAF (Romainville, France). It was iodinated by the chloramine-T procedure as previously described without loss of biologic activity. The specific radioactivity ranged from 65 to 120 µCi/µg.

Binding Assay

Absorption isotherms were obtained by incubating 2 × 10^6 cells at 4°C for 2 hours with various concentrations of 125I-Hu-r IFN-γ, in U-bottomed microtitrater plates (Nunc, Roskilde, Denmark) at a cell density of 2 × 10^6 cells/mL in RPMI 1640 medium containing 10%...
fetal calf serum (FCS; Gibco-BRL). After incubation, the cells were washed four times by centrifugation in the same cold medium. Cell-associated radioactivity was measured in a gamma counter (Perkin-Elmer, Bois d'Arcy, France). Nonspecific binding was determined in parallel experiments in the presence of 100-fold excess of unlabelled Hu-r IFN-γ. Nonspecific binding, which never exceeded 15% of total counts at saturation, was subtracted from total counts to determine specific binding.

**Kinetic of ^125^I Hu-r IFN-γ Binding to Dami Cells**

Cells were incubated at 4°C with ^125^I Hu-r IFN-γ (0.325 nmol/L) in the presence or the absence of 100-fold excess of unlabelled Hu-r IFN-γ. Aliquots of cells (2 x 10^6) were spun down (13,000g for 2 minutes) through a sucrose cushion (25% in incubation medium) at the indicated times and the radioactivity of the pellet was determined.

**Dissociation of ^125^I Hu-r IFN-γ From Dami Cells**

Cells, preincubated with 2 nmol/L ^125^I Hu-r IFN-γ, were washed and resuspended in the same volume of medium containing 200 nmol/L Hu-r IFN-γ. At the indicated incubation times, cells were isolated as above and the pellet counted to determine cell-associated radioactivity.

**Internalization of Receptor-Bound Hu-r IFN-γ**

Dami cells were incubated with ^125^I Hu-r IFN-γ (2 nmol/L) for 2 hours at 4°C. After incubation and washing, cells were kept at 4°C or warmed to 37°C. At different incubation times, aliquots of cell suspensions were spun down at 4°C. The supernatants were mixed with one volume of 20% trichloroacetic acid (TCA) on ice. TCA soluble and insoluble fractions were separated by centrifugation at 4°C (2,000g; 20 minutes) and radioactivity measured. The cell pellets were eluted by 5 minutes of incubation on ice with 10 mmol/L glycine-HCl buffer (pH 2.5) to remove surface-bound ^125^I Hu-r IFN-γ. The cells were subsequently centrifuged to determine the radioactivity internalized by the cells.

**Cross-Linking of ^125^I Hu-r IFN-γ**

Cells, 2.5 x 10^7, were incubated with 2 nmol/L of ^125^I Hu-r IFN-γ. After incubation for 2 hours at 4°C, unbound radiolabeled IFN-γ was removed by four washes with cold phosphate-buffered saline (PBS) containing CaCl₂ and MgCl₂ (2 mmol/L, without serum). Cells were then incubated in the same medium with an uncleavable cross-linking reagent, disuccinimidyl suberate (DSS; Pierce, Oud-Beijerland, Netherlands) at a final concentration of 1 mmol/L for 30 minutes on ice. The reaction was stopped by the addition of glycine (10 mmol/L final concentration) and centrifuged. The samples were then treated with lysis buffer (100 mmol/L phosphate, 150 mmol/L NaCl, 2 mmol/L phenylmethylsulfonyl fluoride [PMSF], 1,000 U/mL aprotinin, 0.5% NP-40; pH 7.4) for 1 hour on ice and centrifuged for 20 minutes at 13,000g at 4°C. The supernatant was analyzed by sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) (8%) according to the method described by Laemmli.

**Cell Surface Radiolabeling**

Dami cells (5 x 10^4) were resuspended in 1.5 mL of PBS pH 7.4 and were incubated for 15 minutes on ice with 5 mCi ^125^I carrier-free (Amersham, Paris, France) and 500 μg of Iodogen (Pierce). After radiolabeling, the cells were washed three times with RPMI 1640 and resuspended in the appropriate medium.

**Immunoprecipitation**

Radiolabeled cells (2.5 x 10^7) were resuspended in lysis buffer. The supernatant was then incubated overnight at 4°C with a mouse monoclonal antibody (MoAb) directed against the Hu-IFN-γ-R of Raji cells (A6) or with a mouse MoAb directed against the platelet GPIIIa (Immunotech, Luminy, France) (5 μg/mL) as control. The complexes were then precipitated with protein-A sepharose (Pharmacia, Uppsala, Sweden) in the presence of a rabbit antinouse IgG serum (Nordic, Tilburg, Netherlands) (1:100). After washing, the immunoprecipitated molecules were analyzed by SDS-PAGE (7.5% to 15%).

**Northern Blot Analysis**

Total RNA was prepared from 5 x 10^6 cells by using RNezol (Cinna-Biotex, Houston, TX) and subjected to electrophoresis in 0.8% or 1.2% agarose denaturing gel. Equal amounts of total RNA (10 μg) from each sample were electrophoresed and were transferred to HYBOND-N filters (Amersham). The complementary DNA probes for Raji cell Hu-IFN-γ-R, for HLA-DP (HLA class II), for HLA-E (HLA class I; kindly provided by M.J. Truong, CIBP, Institut Pasteur, Lille, France), for platelet GP Ib (kindly provided by Dr K. Clemetson, Theodor Kocher Institut, Bern, Suisse), for platelet GPIb, and for platelet GPIIIa (kindly provided by Dr Frachet and Dr Marguerie, DRF/Laboratoire d'Hématologie, Grenoble, France) were radiolabeled using the method of random hexanucleotide priming and used for hybridization. Filters were washed with 0.1 SSC, 0.1% SDS at 55°C and were exposed to XAR Film (Kodak, Marne la Vallée, France) for autoradiography.

**RESULTS**

**Kinetics of ^125^I Hu-r IFN-γ Binding to Dami Cells**

Dami cells were exposed to ^125^I Hu-r IFN-γ (0.325 nmol/L), and the rate of binding was examined (Fig 1, representative of three experiments). During the first few minutes, the rate of binding was constant. The equilibrium binding was reached rapidly and the initial rate of association was directly proportional to the concentration of ligand added (data not shown). These results were consistent with a simple bimolecular reaction and allowed the determination of the forward kinetic constant kₐ, which was estimated to be 3.8 x 10⁻⁷ L/mol/min at 4°C.
Binding Studies

Figure 2 illustrates one representative experiment of 125I Hu-r IFN-γ binding to Dami cells at 4°C. The binding was dose-dependent and approached saturation at approximately 1 nmol/L of ligand. Scatchard analysis of the binding data (see insert) resulted in a linear plot compatible with noncooperative ligand binding to one homogeneous site. The apparent equilibrium constant $k_d$ was about 3.0 ± 0.5 × 10^-10 mol/L and the number of binding sites per cell was 11,000 ± 2,000.

Kinetics of Dissociation

Cells were incubated with 125I-r IFN-γ (2 nmol/L) for 2 hours at 4°C and then washed rapidly by diluting 50-fold in medium and centrifuged. The cells were resuspended in the original volume of assay with 200 nmol/L of cold IFN-γ. Zero time was taken immediately after resuspending cells and the reaction was stopped at each time by centrifugation through a sucrose gradient. The rate of dissociation ($k_{-1}$) calculated from the curve slope (Fig 3) was 7 × 10^-3/min. Using the association constant, $k_1$ of 3.8 × 10^7 L/mol/min (calculated above), the $k_d$ is 1.8 × 10^-10 mol/L.

Internalization and Degradation of Receptor-Bound 125I Hu-r IFN-γ

We investigated the fate of Hu-r IFN-γ in Dami cells. For this purpose, cells were first incubated with 125I-Hu-r IFN-γ for 2 hours at 4°C to allow equilibrium binding to specific cell surface receptors. After washing, cells were kept at 4°C or warmed to 37°C and cell-internalized IFN-γ was determined after elimination of IFN-γ bound on the cell surface by using the acid elution method.25-27

As shown in Fig 4, radioactivity internalized by the cells (□) increased with time, was maximal at 20 minutes, and decreased thereafter, whereas TCA-soluble radioactivity (% bound) increased in the supernatant. This result indicated that IFN-γ was internalized after binding to its cell-surface receptor and was subsequently degraded.

Ligand Effect on IFN-γ-R Expression

In several cellular systems, IFN receptors have been shown to be regulated by the ligand itself.23,32 The following experiments were designed to investigate the effect of IFN-γ treatment on the expression of its cell surface receptor. Cells were treated (for 2.5 or 5 hours at 37°C) with a saturating amount of cold Hu-r IFN-γ in the presence or absence of cycloheximide, or with cycloheximide alone. Then, cell-surface bound IFN-γ was eliminated by the acid-elution procedure and the number of surface receptors available determined by measurement of binding of 125I-r IFN-γ to the cell surface. As shown in Fig 5, the level of surface bound 125I-IFN-γ was not reduced during the incubation period with lymphokine alone (□) despite internalization of IFN-γ/IFN-γ-R complex (Fig 4). This result indicated that the receptor level was maintained at a constant level either through receptor recycling, by de novo synthesis, or by use of a cryptic receptor pool. Incubation of cells with cycloheximide alone (to inhibit protein synthesis), followed by saturation-binding experiments at 4°C, allowed the Hu-IFN-γ-R half-life determination (Fig 5 (▲)). This half-life was calculated as approximately 7 hours. Finally, preincubation of the cells with a saturating concentration of Hu-r IFN-γ together with cycloheximide did not decrease the level of surface binding below the reduction with cycloheximide alone (Fig 5 (△)), indicating that de novo receptor synthesis was not responsible for the constant level of receptors on the cell surface. Therefore, the constant level of cell surface receptor expression must be due to either receptor recycling or to a cryptic receptor pool. In addition, the receptor was acid resistant because binding capacity of cells was not modified by acid elution (pH 2.5; 10 minutes).
Characterization of Hu-IFN-γ Receptor of Dami Cells

Cross-linking of 125I-Hu-r IFN-γ to Dami cell surface component. Cross-linking of 125I-Hu-r IFN-γ to Dami cells, using DSS (Fig 6) as the cross-linking reagent, yielded a radiolabeled complex with an apparent molecular weight of 105 Kd on SDS-PAGE electrophoresis and autoradiography (lane 4). Excess unlabeled IFN-γ (lane 3) or the absence of DSS (lane 1) prevented the formation of this complex. In Fig 6, A and B indicate noncross-linked dimer (A) and monomer (B) of IFN-γ. Assuming a complex consisting of one molecule of ligand and one molecule of receptor, the molecular weight of the receptor or its binding subunit would be approximately 90 Kd.

Detection of Hu-IFN-γ-R messenger RNA (mRNA). As shown in Fig 7, Northern blot analysis of total RNA from Dami cells treated with 0.02, 0.1, 0.2, 1, or 2 nmol/L of IFN-γ, PMA (10 ng/mL), or medium for 24 hours permitted detection of the IFN-γ-R mRNA (2.3 kb). Total RNA of the monocytic cell line U937 was used as a positive control (data not shown). There was no apparent change in the 2.3-kb mRNA level on treatment with IFN-γ. These results were consistent with the absence of downregulation of this receptor after lymphokine binding. PMA slightly decreased the number of surface receptors (10%; data not shown) but had no effect on the mRNA level.

Immunoprecipitation of 125I-labeled cell surface components. After cell surface iodination, the Dami cells were lysed in the presence of NP-40 and the lysate was immunoprecipitated. As shown in Fig 8, the mouse MoAb A6 directed against the Hu-IFN-γ-R of Raji cells precipitated a molecule of approximately 90 Kd (lane 1). This result was in agreement with the molecular weight of Hu-IFN-γ receptor immunoprecipitated from other cells. As a positive control, we used a mouse MoAb directed against platelet GPIIIa. This antibody precipitated two molecules of approximately 105 Kd and 147 Kd. The 105-Kd molecule was probably the GPIIIa (molecular weight, 95 to 110 Kd) that is in cell membranes associated with GPIIb as the complex GPIIb-IIIa. The 145-Kd molecule corresponded to the molecular weight of GPIIb (153 Kd) and it detection likely due to the precipitation of nondissociated GPIIb-IIIa complex. The others molecules present in the two cases were nonspecifically absorbed by the reagents (protein-A, rabbit antiserum, or mouse IgG).

Biologic Effects of Hu-IFN-γ

To correlate IFN binding and biologic effects, we examined the action of this lymphokine on Dami cells. Dami cell proliferation or ploidy were not affected by IFN-γ concentrations up to 20 nmol/L (data not shown). Because Dami cells exhibited high-affinity receptors for IFN-γ without expressing the usual biologic effect (inhibition of proliferation), we examined the expression of genes known to be modulated by IFN-γ in sensitive cells, such as HLA genes. Figure 7 shows a Northern blot analysis of the mRNA extracted from Dami cells treated or not treated with IFN-γ. IFN-γ did not modify expression of platelet GPIb (2.4 kb), GPIIb (3.5 kb), or GPIIIa (6.1 kb), whereas PMA enhanced their expression. In contrast, whereas HLA class II gene expression was not detected in cells treated with buffer or PMA, mRNA (1.3 kb) expression appeared in a dose dependent manner on IFN action. Moreover, the HLA class I mRNA (1.7 kb) level was increased by IFN-γ or PMA. As a control, the same blot was hybridized with a β-actin cDNA probe. No significant modifications of its mRNA level were observed.

DISCUSSION

We present here evidence indicating that IFN-γ binds to a specific receptor on the human megakaryocytic, Dami cell
The existence of two molecular forms of Hu-IFN-γ-R has been proposed by Fischer et al, the first one being present on hematopoietic cells (monocytes) and the other one on nonhematopoietic cells (HeLa, Wish). These investigators reported that monocyte and HeLa receptors notably differed by the response to an excess of ligand and by their resistance or lability to acid treatment. Surprisingly, Dami cells present a receptor with the characteristics of the receptor described in cells of nonhematopoietic origin. It seems that the receptor for IFN-γ on monocytes has particular characteristics that could be due to different pattern of glycosylation or regulation, and not necessary to its hematopoietic origin. So, the presence of a receptor for IFN-γ on blood platelets and on a megakaryocytic cell line, which is similar to the receptor previously described on HeLa or Raji cells, suggests that this receptor is borne by bone marrow megakaryocytes and on their progenitors. In this context, it has been reported that r IFN-γ, as well as r IFN-α, markedly suppress the in vitro

![Image](1234567)

**Fig 6.** Cross-linking of 125I-r IFN-γ bound to Dami cells and analysis by SDS-PAGE. For each experiment, 2 x 10⁷ cells were incubated with labeled IFN (2 nmol/L) alone (lane 2 and 4) or in the presence of an excess of cold IFN-γ (lane 1 and 3) for 2 hours at 4°C. After washing, the cells were resuspended in cold PBS containing CaCl₂ and MgCl₂ (2 mmol/L) and treated (lane 3 and 4) or not treated (lane 1 and 2) with the cross-linking reagent DSS. Their lysates were prepared and analyzed by SDS-PAGE. This figure shows an autoradiograph of a dried gel. Not-cross-linked dimer (A) and monomer (B) of radiolabeled lymphokine.

line. To our knowledge, this is the first characterization of a receptor for IFN-γ on cells from the megakaryocytic lineage other than platelets. Its molecular weight was 90 Kd, as estimated by cross-linking or immunoprecipitation experiments. Our data are consistent with the existence of a single, high-affinity, noncooperative site. At 4°C the apparent equilibrium dissociation constant was 3 x 10⁻¹⁰ mol/L and there were approximately 11,000 binding sites per cell. At 37°C, surface-bound ligand was internalized and intracellular IFN-γ was rapidly degraded into TCA-soluble material. The cell surface Hu-IFN-γ-R was not downregulated after binding by its ligand, because a preincubation of cells with an excess of IFN-γ did not modify the number of cell surface receptors nor the expression of the receptor mRNA, even when protein synthesis was blocked with cycloheximide. This finding strongly suggests that the steady level of receptor obtained in the presence of an excess of ligand is not due to an increase in the amount of receptor synthesis but is probably related to the existence of preformed-receptor cryptic pool, or to a recycling of the receptor following IFN-γ/Hu-IFN-γ-R complex internalization and dissociation. The acid resistance (pH 2.5; 10 minutes) of the receptor was in agreement with its recycling. PMA had no effect on the receptor mRNA level, a result in agreement with recent observations obtained with Raji cells. Taken together, our data suggest that the Hu-IFN-γ-R on the Dami megakaryocytic cell line is very similar with Hu-IFN-γ-R, previously described on other human cells (Wish, HeLa, Raji). The existence of two molecular forms of Hu-IFN-γ-R has been proposed by Fischer et al, the first one being present on hematopoietic cells (monocytes) and the other one on nonhematopoietic cells (HeLa, Wish). These investigators reported that monocyte and HeLa receptors notably differed by the response to an excess of ligand and by their resistance or lability to acid treatment. Surprisingly, Dami cells present a receptor with the characteristics of the receptor described in cells of nonhematopoietic origin. It seems that the receptor for IFN-γ on monocytes has particular characteristics that could be due to different pattern of glycosylation or regulation, and not necessary to its hematopoietic origin. So, the presence of a receptor for IFN-γ on blood platelets and on a megakaryocytic cell line, which is similar to the receptor previously described on HeLa or Raji cells, suggests that this receptor is borne by bone marrow megakaryocytes and on their progenitors. In this context, it has been reported that r IFN-γ, as well as r IFN-α, markedly suppress the in vitro

![Image](8463428)

**Fig 7.** Identification of β-actin, Hu-IFN-γ-R, HLA class I, HLA class II, GPIb-α, GPIIb, and GPIIia transcripts in mRNA from Dami cells treated with IFN-γ or PMA. Total RNA (10 μg) from untreated cells (lane 1), from cells treated with 0.02 nmol/L (lane 2), 0.1 nmol/L (lane 3), 0.2 nmol/L (lane 4), 1 nmol/L (lane 5), 2 nmol/L (lane 6) of IFN-γ, or from cells treated with PMA (10 ng/mL) (lane 7) for 24 hours was fractionated on 1% agarose gel. The same blots were then hybridized with a 32P-labeled β-actin cDNA probe, Hu-IFN-γ-R cDNA probe, HLA-E cDNA probe, HLA-DRβ1 cDNA probe, GPIb-α cDNA probe, GPIIib cDNA probe, or GPIIia cDNA probe.
Fig 8. Analysis by SDS-PAGE gel electrophoresis of surface \textsuperscript{125}I-labeled extracts of Dami cells immunoprecipitated by anti-Raji cell IFN-\(\gamma\)-R (lane 1) or by antiplatelet GPI\(\alpha\) antibodies (lane 2).

proliferation of megakaryocyte progenitors (colony-forming unit-megakaryocyte [CFU-Mk]), of pluripotent hematopoietic progenitor cells (CFU-granulocyte, erythroid, monocyte, megakaryocyte [CFU-GEMM]), and of other cells of restricted lineage (burst-forming unit-erythroid [BFU-E] and CFU-granulocyte-macrophage [CFU-GM]), whether cells are isolated from normal subjects,\textsuperscript{36-38} from patients with chronic myeloid leukemia,\textsuperscript{39} or from patients with myelofibrosis in the context of myeloid metaplasia.\textsuperscript{40} In contrast, Dami cell proliferation was not inhibited by IFN-\(\gamma\). It has been shown, however, that IFN-\(\gamma\) is unable to induce any effect on megakaryocytic progenitors when mononuclear light density bone marrow cells (LDBMC) are depleted of accessory cells, whereas IFN-\(\alpha\) remains active in these conditions.\textsuperscript{36,40} Various hypotheses could account for this observation. Treatment of accessory cells with IFN-\(\gamma\) induces cytotoxic functions that could be responsible for the inhibition of progenitor cell growth.\textsuperscript{41,42} IFN-\(\gamma\), but not IFN-\(\alpha\), also induces the production of TNF\textsuperscript{43,44} that directly inhibits progenitor cell proliferation.\textsuperscript{45} IFN-\(\gamma\) also increases TNF receptor expression,\textsuperscript{46} and induces cell responsiveness to inhibitory mediators such as transferrin.\textsuperscript{47}

In this study, modulation of HLA antigen expression provided a very useful marker, showing that IFN-\(\gamma\)-R was functional. As previously described on lymphoblastoid Na-malva cells,\textsuperscript{4} IFN-\(\gamma\) was able to induce HLA class II mRNA expression and to increase HLA class I mRNA expression in Dami cells. This effect was selective, because the expression of GPIb, IIb, and IIIa mRNA was not modified. HLA class II antigens are constitutively expressed only on a restricted number of cellular types (monocytes, B cells, follicular dendritic cells).\textsuperscript{48} These antigens are not expressed on platelets and normal megakaryocytes, but are present on early progenitors of this lineage (CFU-Mk).\textsuperscript{49,50} So, it seems that these antigens appear early in the differentiation process and are subsequently lost. In contrast, HLA class I antigen are present on all nucleated cells as well as on platelets and red blood cells.\textsuperscript{52} IFN-\(\gamma\) have been described to induce and to enhance HLA class I and HLA class II expression on numerous cells.\textsuperscript{53} Whereas, the intervention of HLA class II products in antigen presentation is well documented, we do not yet known their role on megakaryocytic progenitors as well as on IFN-\(\gamma\)-treated Dami cells.

The effect of IFN-\(\gamma\) on megakaryocytic progenitors could be due to modified synthesis of certain mRNAs that are important in modulating cellular functions during megakaryocytogenesis. The presence of a similar IFN-\(\gamma\)-R on human platelets\textsuperscript{54,55} raises the question of its role in a cell without a nucleus that contains only traces of rough endoplasmic reticulum and mRNA and synthesizes little protein.\textsuperscript{56-58} This platelet receptor appears functional, because platelets incubated with IFN-\(\gamma\) were able to kill \textit{S mansoni} parasite larvae in vitro\textsuperscript{59} and to reduce, after passive transfer, the parasite burden in vivo.\textsuperscript{60} One might speculate that the IFN-\(\gamma\)-R synthetized by megakaryocytes remains active in platelets and that IFN-\(\gamma\)-R mRNA remain present in the platelet cytosol after megakaryocyte segmentation.

In conclusion, the results presented here raise the question of the role of IFN-\(\gamma\) on the cells of the megakaryocytic lineage. Numerous cytokines do not directly exhibit in vitro hematopoietic colony activity but are able to modulate response to other cytokines in normally nonresponsive immature hematopoietic cells.\textsuperscript{57,58} To date, the possible relationships between IFN-\(\gamma\) and other factors influencing megakaryocytogenesis and megakaryocyte functions have not been explored. It would be of a great interest to study the modulatory effect of IFN-\(\gamma\) in the presence of other cytokines, such as, interleukin-3 (IL-3), IL-4, IL-6, granulocyte-macrophage colony-stimulating factor, or TNF.

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