Positive Modulation of Hemoglobin, Heme, and Transferrin Receptor Synthesis by Murine Interferon-α and -β in Differentiating Friend Cells

PIVOTAL ROLE OF HEME SYNTHESIS*

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Administration of highly purified preparations of murine interferon (IFN)-α, -α, -α, or -β to Friend leukemia cells induced to differentiate by dimethyl sulfoxide leads to a 100% increase of benzidine-positive (B+) cells. Different efficiencies for the two IFN species have been observed; a 10-fold higher dose of IFN-α is needed for stimulation of hemoglobin production and inhibition of cell growth as compared with IFN-β. Both species of IFN induce a substantial increase in heme, hemoglobin, and transferrin receptor levels.

In vitro run-on transcription assays indicate that IFN-β moderately stimulates transcription of the α-globin gene but not the transferrin receptor gene. It is postulated that IFN induces the enhancing effect on differentiation via a marked increase of heme synthesis and number of transferrin receptors, which in turn leads to an enhancement of globin chain synthesis. In this regard, the negative feedback reported in a variety of other cell types for the regulation of transferrin receptor expression in heme, hemoglobin, and transferrin receptor levels.

Interferons (IFN) are a protein family with a wide range of biological effects, which include inhibition of viral replication and cell growth as well as modulation of the immune response (reviewed in 1). Once IFN are bound to specific cell surface receptors (2, 3), they induce a variety of biochemical changes including the induction of several enzymes and alterations in the synthesis of other as yet unidentified proteins (4–7). At least two mechanisms, i.e. the 2-5A synthetase and the protein kinase pathways, are involved in the induction of the antiviral state, but little is known on the mechanism(s) responsible for the other effects elicited by IFN.

The effects of IFN on cell differentiation have been mainly studied in hematopoietic models (for a review, see 8), including Friend virus-induced erythroleukemia cells (9). Friend leukemia cells (FLC) are erythroid precursors blocked in their differentiation pathway at the proerythroblastic stage. These cells are induced to differentiate by treatment with dimethyl sulfoxide (Me2SO) and other inducers (for a review, see 10), thus providing a model for studies of erythroid differentiation at both the cellular and molecular level.

The present study reports the effects of IFN-α and -β on the pattern of hemoglobin (Hb) production, i.e. globin mRNA levels and chain synthesis, heme production, and transferrin receptor expression in differentiating FLC.

The stimulatory effects of IFN on Hb synthesis are associated with a substantial increase in transferrin binding to its receptor, heme content, and globin mRNA levels and chain synthesis. Transferrin is the major and perhaps the only source of iron for heme synthesis in erythroid cells (11). The molecular mechanisms leading erythroid cells to synthesize a high number of transferrin receptors are largely unknown.

The binding of dimorphic transferrin to its receptors on the cell surface is the first step in iron transport across the plasma membrane (11, 12). Transferrin receptors are generally expressed on rapidly dividing cells (13) and particularly in proliferating erythroblasts, which require a large amount of iron for the Hb production. The ability of FLC to bind transferrin and take up iron increases substantially during Me2SO-stimulated differentiation (14). Induction of FLC increases the number of transferrin receptors with respect to mouse reticulocytes (4.4 × 10⁵/cell versus 0.86 × 10⁵/cell) (15) thereby presenting an improved model for the study of transferrin receptor synthesis regulation.

In contrast to the negative feedback observed in other cell types, i.e. leukemic cell lines (16), fibroblasts (17), and mitogen-activated T lymphocytes (18), in which iron or hemin treatment induces a decline in transferrin receptor synthesis, differentiating FLC show a heme-induced up-regulation of transferrin receptor expression.

EXPERIMENTAL PROCEDURES

Cells—FLC 745A and L929 were grown in RPMI 1640 supplemented with 5% fetal calf serum and antibiotics. K562 cells were grown in RPMI 1640 supplemented with 10% fetal calf serum and antibiotics.

Virus—Stocks of vesicular stomatitis virus (Indiana strain), ob-
tained by infecting B10.D2 cell monolayers with low multiplicity of infection (0.1 plaque-forming unit/cell), were titrated by plaque assay on the same cells. Titers ranged between 10^4 and 10^6 plaque-forming units/ml.

**Interferons**—Amounts of mouse IFN are given in laboratory units, i.e. the amount of IFN causing a 50% decrease of plaque production by a murine tumor virus. This unit equals 4 research reference units of the NIH standard preparation, code G-002-904-511, whose titer was 12,000 IU/ml when reconstituted. Details are reported in research Reagents note 15 (World Health Organization standard, 1979).

IFN-α, IFN-β, and IFN-γ were produced constitutively by Chinese hamster ovary cells transfected with the corresponding murine IFN gene (19) (a generous gift of M. Van Heuvel and E. C. Zwarthoff, Erasmus University, Rotterdam, The Netherlands). Serum-free supernatants of 24-h confluent cultures were collected. Purification of IFN preparations was performed by (NH₄)₂SO₄ precipitation (45%). The supernatant, washed and concentrated on Amicon equipment YM-10 membrane, was purified by affinity chromatography, coupling monoclonal antibodies (generous gift of Dr. Zwarthoff, Erasmus University) anti-murine IFN-α, or -β on CNBr-activated Sepharose 4B. Specific activity of purified preparations ranged between 5 x 10^4 and 5 x 10^5 U/mg protein.

**Induction of FLC Differentiation**—FLC were seeded at 10^5/ml in the presence of the inducer MeSO₄ (Merck, stock solution undiluted, at room temperature) added to culture medium prior to cell seeding. Cells were counted daily using a Coulter Counter or hemocytometer. Cell suspensions containing 10^6 cells were centrifuged at 500 × g for 10 min. After removal of the supernatant, 2 ml of 2 M oxalic acid was added to the pellet. The mixture was vigorously shaken for 30 min at 120 °C in a preheated autoclave. A tissue blank containing cells with the oxalic acid was run without heating to check for the presence of endogenous porphyrins in the cell. Standards were made by adding 10 µl of hemin solution prepared in 1% (w/v) bovine serum albumin, 0.1% KOH, 50% (v/v) methanol to 2 µl oxalic acid solution and then heating as above.

After cooling, fluorescence was determined in a Perkin-Elmer LS-5B luminescence spectrophotometer. The exciting light was at 400 nm and the fluorescence emission at 622 nm.

**RLA and RBL Analysis**—FLC were harvested, centrifuged, and washed twice with phosphate-buffered saline. Poly(A) RNA was extracted and purified by the guanidine/cesium chloride method (22, 23). The RNA (5 µg) was run on denaturing (1.2%) agarose gels containing formaldehyde, transferred onto Hybond N+ nylon membrane (Amersham), and hybridized with nick-translated [32P]-labeled 1.3 × 10^6 cpm/ml cDNA probes. The hybridization was carried out with globin cDNA clones designated pα1G10 and pCR, βsG9 (24) (generous gifts of M. G. Farace, University of Catanzaro, Italy) and with pTR2, cDNA for transferrin receptor (25) (generous gift of J. Godding, The Walter and Eliza Hall Institute for Medical Research, Post Office Royal, Melbourne Hospital, Victoria, Australia).

The hybridization conditions were as follows: 50 mM sodium phosphate, pH 7.0, 7.5% formamide, 5 × SSC, 4 × Denhardt’s, 0.1% SDS, and 200 µg/ml sonicated salmon sperm DNA at 42 °C for 20 h. The filter was washed twice in 1 × SSC and 0.1% SDS for 30 min at room temperature and two times in 0.1 × SSC and 0.1% SDS for 10 min at 42 °C and then exposed to Fuji x-ray film using intensifying screens.

**Isoelectric Focusing of Globin Chains**—Cells (15–20 × 10^6) were harvested on the third day of culture. Cells were centrifuged in 1 ml of medium without methionine and serum. After overnight starvation, 100 µCi of [35S]methionine was added for 1 h at 37 °C. Cells were then harvested by centrifugation, washed three times in phosphate-buffered saline, and lysed in 10 mM KCl, 1.5 mM magnesium acetate, 0.5% Nonidet P-40 for 20 min in ice. The samples were then centrifuged for 10 min in an Eppendorf centrifuge. DNA was precipitated by adding 1 ml of 50 mM citrate buffer (pH 5.5) containing 150 mM NaCl, 0.5 µg/ml bovine serum albumin, and 0.1% SDS. Samples were then digested with RNase and then with Pronase to release the globin chains in their functional state. The digests were precipitated by adding 1 ml of 50 mM citrate buffer (pH 5.5) containing 150 mM NaCl, 0.5 µg/ml bovine serum albumin, and 15% polyethylene glycol 6000. After further incubation for 15 min at 4 °C, the suspension was centrifuged at 10,000 × g for 15 min. Nonspecific binding was determined in the presence of a 1,000-fold excess of unlabeled iron-loaded transferrin. The amount of free 125I-transferrin was added to the lysate. The mixture was then incubated for 60 min at 4 °C. Three hundred µl of 100 mM citrate buffer (pH 5.5) containing 150 mM NaCl, 0.5 µg/ml bovine serum albumin, and 1% SDS was then added. After mixing and incubation for 10 min at 4 °C, the transferrin receptor complex was precipitated by adding 1 ml of 50 mM citrate buffer (pH 5.5) containing 150 mM NaCl, 0.5 µg/ml bovine serum albumin, and 15% polyethylene glycol 6000. After further incubation for 15 min at 4 °C, the suspension was centrifuged at 10,000 × g for 15 min. Nonspecific binding was determined in the presence of a 1,000-fold excess of unlabeled iron-loaded transferrin. The amount of free 125I-transferrin that was estimated was subtracted from the total binding. The amount of [125I]-transferrin bound to the incubation mixture and never exceeded 3% of the specific binding. As the case of binding to whole cells, results are the mean of two experiments and represent specific binding.

**Nuclear Run-on Transcription Assay**—Nuclei from 745 cells treated for 48 h with MeSO₄ and/or MeZSO were isolated. The newly synthesized RNAs were labeled with [3P]-UTP and purified according to (30).

Labeled RNA (2 × 10^6 cpm/ml) was hybridized to g-ribin, pTR2, glyceraldehyde-3-phosphate dehydrogenase, malathtionin II, β-microglobin, and 2-5′ A synthetase cdNA probes immobilized on nitrocellulose membrane. Hybridizations were performed in a solution containing 50% formamide, 5 × SSC, 5 × SDS, and 0.1% SDS. Samples were then hybridized to the membranes for 3 days at 42 °C. Filters were washed twice for 15 min in 0.1 × SSC and 0.1% SDS at room temperature and then for an additional 30 min at 65 °C.
RESULTS

Effects of Different Species of IFN on Hb Content in Me2SO-treated FLC—Mature IFN-α and -β have enhancing effects on erythroid differentiation of Me2SO-induced FLC as shown in Fig. 1, panel B. On the 3rd day of culture, FLC induced to differentiate by Me2SO exhibit increases of B\(^+\) values from 25% to \(\approx 60\)% upon addition of 500 units/ml IFN-β and from 25% to \(\approx 45\)% upon treatment with the same dose of IFN-α, or -α1.

The correspondent curves of cell growth (Fig. 1, panel A) indicate a pronounced inhibitory effect of 500 units/ml IFN-β compared with the slight effects observed for two subspecies of IFN-α at the same dose.

Dose-response studies (Fig. 2B) indicate that IFN-β has a greater stimulatory effect on Me2SO-induced FLC differentiation. Indeed, 500 units/ml IFN-β is sufficient to reach a plateau value of B\(^+\) cells whereas a 10-fold higher dose of IFN-α is necessary to achieve the same effect. Comparable results were obtained with IFN-α1 and -α4 (data not shown).

IFN-β produces a more inhibitory effect on FLC growth when compared with IFN-α or -α1 (Fig. 1A and Fig. 2A).

The specificity of the stimulation of differentiation was determined by neutralizing this effect by preincubation of IFN preparations with specific monoclonal antibodies. A representative experiment is shown in Fig. 3. The same result was obtained for IFN-β (not shown).

Globin mRNA Steady-state Levels and Globin Chain Synthesis in FLC Induced to Differentiate and Treated with IFN-α or -β—Differentiation of Me2SO-treated FLC is associated with an increase of globin mRNAs, globin chains, and hence Hb accumulation (31, 32). The globin chains synthesized in response to Me2SO are identical to those synthesized in the erythroid lineage of mouse strain DBA/2, from which FLC (strain 745A) were originally derived (33). Two types of Hb are accumulated (Hb major and minor), which contain three species of globin polypeptides, namely α- and β-major and β-minor (10). After 96 h of culture in presence of 1.5% Me2SO, 80-90% of cells become B\(^+\), i.e. 25% of neosynthesized proteins are constituted by Hb, which represents 10% of all soluble proteins (34). Treatment with suboptimal doses of Me2SO (0.6-1%) induces only 40-60% B\(^+\) cells.

To investigate in more detail the stimulatory effect of IFN on Hb synthesis, globin mRNA steady-state levels and chains synthesis were analyzed.

Northern blot analysis of mRNA encoding α- and β-globin chains indicate that both IFN-β (500 units/ml) (Fig. 4) and IFN-α or -α4 (not shown) stimulate mRNA synthesis of both chains. Poly(A)\(^+\) RNA was extracted after 48 and 72 h of culture in the presence of Me2SO (0.6%) and 500 units/ml murine IFN-β. A higher level of both α- and β-globin mRNA was observed in FLC cells grown in the presence of Me2SO + IFN-β compared with cells treated with Me2SO alone. Analysis of globin chain synthesis provided comparable results, in that the level of production of all globin chains is 2-3-fold higher upon treatment with Me2SO + IFN-β than with Me2SO alone (Fig. 4, lowest panel).

The α-major:β-minor content ratio is 3:1 in FLC induced with Me2SO (35, 36); in contrast, the β-minor globin is more...
Effects of IFN on Transferrin Receptor Expression in Me2SO-treated FLC—\(^{125}\text{I}\)-Transferrin binding assays were performed to investigate whether the biological effects elicited by IFN on Hb synthesis correlate with a modulation of transferrin receptor number. The effect of heme content on the number of transferrin receptors was also analyzed.

Analysis of \(^{125}\text{I}\)-transferrin binding to uninduced FLC at different days of culture shows a marked induction of transferrin receptor due to the logarithmic growth of FLC in fresh medium, which peaks at 48 h and declines progressively until day 4 when the cultures reach saturation values. These results are in agreement with previous reports showing that transferrin receptor expression is correlated with cell proliferation.

### Table I

<table>
<thead>
<tr>
<th>Cells and treatment</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>B(^+)</td>
</tr>
<tr>
<td>745A</td>
<td>ND(^+)</td>
</tr>
<tr>
<td>745A + IFN-(\beta)</td>
<td>9</td>
</tr>
<tr>
<td>745A + Me2SO (0.6%)</td>
<td>26</td>
</tr>
<tr>
<td>745A + Me2SO (0.6%) + IFN-(\beta) (500 units/ml)</td>
<td>50</td>
</tr>
<tr>
<td>745A + Me2SO (0.6%) + IFN-(\alpha) or (\alpha) (2,000 units/ml)</td>
<td>46</td>
</tr>
</tbody>
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\(^+\) Not detected.

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abundant in nonstimulated FLC (2). Isoelectrophoretic separation of globin chains indicates that this ratio remains essentially unmodified during IFN-enhanced differentiation.

**Effect of IFN on Heme Concentration in Me2SO-treated FLC**—Since heme plays a critical regulatory role in various metabolic processes in erythroid cells and is also involved in the transferrin receptor regulation, it seemed of interest to assess whether the stimulation of Hb accumulation in FLC treated with Me2SO + IFN-\(\alpha\) or -\(\beta\) may be caused by an increased heme synthesis. Table I shows heme concentrations in FLC after 3 days of culture in the presence of Me2SO or Me2SO + IFN. The heme content in Me2SO + IFN-treated cells was markedly increased as compared with cells grown in the presence of Me2SO alone. The drastic increase of heme content could thus represent a major factor mediating the correspondent marked increase of Hb synthesis induced by IFN.

**Fig. 3. Effect of neutralization of IFN-\(\alpha\) preparations on cell growth and on percent of B\(^+\) cells in Me2SO + IFN-\(\alpha\)-induced FLC.** Cells were treated with Me2SO and the indicated doses of IFN-\(\alpha\) (\(\Delta\)—\(\Delta\) or Me2SO + IFN-\(\alpha\) neutralized with anti-mouse IFN-\(\alpha\) monoclonal antibodies (\(\Delta\)—\(\Delta\)). Neutralization was performed by preincubation of IFN 30 min at 37 °C and 90 min at 4 °C with anti-mouse IFN-\(\alpha\) monoclonal antibodies (ratio between neutralizing units and IFN units, 2:1). After 3 days of culture, cell growth (lower panel) and differentiation (upper panel) were evaluated. Each condition was performed in duplicate.

**Fig. 4. Effect of IFN-\(\beta\) on globin chain synthesis and RNA levels in Me2SO-induced FLC.** After 2 (lanes 1–4) and 3 days (lanes 5–8) of culture, 1 × 10\(^6\) cells were collected and poly(A)\(^+\) RNA purified for Northern blot analysis. Hybridization with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) probe is shown for Northern blot analysis. Each experimental panel was performed in duplicate.

**Fig. 5. Effect of IFN-\(\alpha\)-\(\alpha\) and -\(\beta\) on \(^{125}\text{I}\)-transferrin (\(^{125}\text{I}\)) binding in Me2SO-induced FLC.** Binding of \(^{125}\text{I}\)-transferrin was evaluated on each day of culture as described under "Experimental Procedures." Panel A: O—O, control cells; \(\bullet\)—\(\bullet\), 0.6% Me2SO; \(\Delta\)—\(\Delta\), 0.6% Me2SO + 2,000 units/ml IFN-\(\alpha\); \(\Delta\)—\(\Delta\), 0.6% Me2SO + 2,000 units/ml IFN-\(\alpha\); Panel B: O—O, control cells; \(\bullet\)—\(\bullet\), 0.6% Me2SO; \(\Delta\)—\(\Delta\), 500 units/ml IFN-\(\beta\); \(\Delta\)—\(\Delta\), 0.6% Me2SO + 500 units/ml IFN-\(\beta\).
cultures, the amount of transferrin receptor RNA peaks at 48 h assed by Northern blot (Fig. 7 and Table 11). This pattern is in agreement with the transferrin RNA in Me2SO-induced FLC in the presence or absence of IFN-β. After 2 days (lanes 1–4) and 3 days (lanes 5–8) of culture, 10^6 cells were collected, and poly(A)^+ RNAs purified for Northern blot analysis. The filters were hybridized with the pTIR cDNA probe glyceraldehyde-3-phosphate dehydrogenase (GaPDH). Lanes 1 and 5, control cells; lanes 2 and 6, 500 units/ml IFN-β; lanes 3 and 7, 0.6% Me2SO; lanes 4 and 8, 0.6% Me2SO + 500 units/ml IFN-β. KB, kilobases.

**TABLE II**

<table>
<thead>
<tr>
<th>Cells and treatment</th>
<th>Day 2</th>
<th>Day 3</th>
</tr>
</thead>
<tbody>
<tr>
<td>745A</td>
<td>2.95 ± 0.5</td>
<td>2.05 ± 0.4</td>
</tr>
<tr>
<td>745A + IFN-β (500 units/ml)</td>
<td>4.55 ± 1.1</td>
<td>1.88 ± 0.8</td>
</tr>
<tr>
<td>745A + Me2SO (0.6%)</td>
<td>6.7 ± 0.8</td>
<td>4 ± 0.7</td>
</tr>
<tr>
<td>745A + Me2SO (0.6%) + IFN-β (500 units/ml)</td>
<td>12.1 ± 1.3</td>
<td>7.85 ± 1.2</td>
</tr>
</tbody>
</table>

In untreated FLC on day 2 of culture. In Me2SO-treated cells, a level of transferrin receptor RNA higher than in control FLC was observed at days 2 and 3 of culture, again in agreement with the data reported previously (38). A slight increase was observed after addition of 500 units/ml IFN-β to Me2SO-treated cells as compared with FLC treated with Me2SO alone.

In order to determine whether the effect of IFN on accumulation of globin and transferrin receptor mRNA is exerted at transcriptional or post-transcriptional level, run-on experiments were performed to monitor the transcription rate of these two genes in FLC. Endogenously labeled RNAs, extracted from nuclei of cells treated for 48 h with control medium, Me2SO, Me2SO + IFN-β, or IFN-β without Me2SO, were hybridized to a panel of cDNA probes immobilized on nitrocellulose filters and subjected to autoradiography. The intensity of the bands corresponding to the α-globin chain and transferrin receptor was measured by densitometry of the x-ray film and normalized to that of glyceraldehyde-3-phosphate dehydrogenase used as an internal control.

Fig. 8 shows the run-on experiment (panel A) and the average data evaluated by laser densitometry of the autoradiogram (panels B and C).

Fig. 8, panel B, shows transcription data for the α-globin gene. An increase in the transcription of the α-globin gene is observed in Me2SO-treated cells as compared with controls, in agreement with data reported previously (38). A slight increase was observed after addition of 500 units/ml IFN-β to Me2SO-treated cells as compared with FLC treated with Me2SO alone.

Panel C of the same figure shows transcription data for the transferrin receptor gene. In contrast to the α-globin gene,
transferrin receptor does not seem to be regulated at the transcriptional level, indicating a post-transcriptional and/or translational level of regulation. After treatment with 1.5% Me2SO, a slight increase in the transcription of the transferrin receptor gene is observed as compared with controls. Other cDNA probes, i.e. 2'-5'A synthetase, β₂-microglobulin, and α2-antiplasmin, were used as controls of genes induced or not induced by IFN-β.

**Effect of Heme Addition and Heme Synthesis Inhibition on Transferrin Receptor Expression in Me2SO-induced FLC**—These results support existing data that indicate that IFN stimulates Hb production and transferrin receptor expression in Me2SO-induced FLC. The present studies suggest further that these effects may be related to a stimulation of heme synthesis and/or synthesis of the transferrin receptor. In agreement with the present results, other studies have also shown that treatment of FLC with IFN and ribonucleoside precursors results in an increase in heme synthesis and transferrin receptor expression (532).

Since the regulation of transferrin receptor expression by heme observed in FLC is at variance with results reported previously in other cell types (16-18), control experiments in one of these cell types were performed. Thus, human K562 erythroleukemic cells were grown in vitro in the absence or presence of Me2SO and in the absence or presence of hemin. In the cells grown either in the absence or in the presence of Me2SO, hemin elicited a significant inhibition of their transferrin binding capacity (Table IV). Furthermore, the inhibition of transferrin receptor expression elicited by hemin in K562 cells is observed at a hemin concentration even lower than that used in FLC (Table IV).

**Discussion**

IFN is comprised of a family of proteins that act in a species-specific manner, eliciting a wide range of biological effects, including those on cell differentiation, which, at present, are poorly understood at the molecular level.

Several experimental models of cell differentiation exist which have facilitated the analysis of the effects of IFN (for review see 8, 39, 40, 42). Studies available thus far have produced conflicting data most likely due to the use of partially purified IFN preparations often containing a mixture of IFN types and/or IFN and other cytokines. Existing data on the effects of IFN on differentiation in the hematopoietic system support a possible in vivo regulatory action in the microenvironments of the spleen and bone marrow, in which low amounts of IFN are detectable. Studies performed in vitro on hematopoietic progenitors have demonstrated the inhibitory action of IFN-α, -β, and -γ on the development of myelomonocytic and erythroid colonies in clonogenic assays (8, 43).

The present results indicate that IFN-β and -α increase Hb production in FLC induced to differentiate by treatment with Me2SO. This phenomenon seems to be mediated by a cor-
decrease of transferrin receptor may be, at least in part, heme seems to be operative. The expression of transferrin receptor expression in the present studies, IFN exerted a marked inhibitory effect on transferrin receptor expression, which are physiologically involved in T lymphocytes (18), in which iron treatment induces a decline in cellular regulator of a variety of other metabolic pathways for systems that utilize oxygen.

As a prosthetic group, heme regulates the structure and activity of hemoproteins. Heme is also involved in the regulation of the biosynthesis of many of these proteins by exerting its effect at different steps such as transcription, translation, transport, assembly, and protein degradation (44).

In FLC, experiments have revealed that heme plays a crucial role in the differentiation program triggered by Me2SO. In addition, hemin added exogenously increased the accumulation of globin mRNA and protein in FLC without blocking cell proliferation (45).

In the FLC system the increase in heme content is correlated with an increase in transferrin receptor number. This observation is in contrast to the negative feedback observed in other cell types, i.e. fibroblasts (17) and mitogen-activated T lymphocytes (18), in which iron treatment induces a decline in transferrin receptor synthesis. In differentiating FLC an up-regulation of transferrin receptor expression induced by heme seems to be operative. The expression of transferrin receptor is regulated by complex mechanisms. A variety of factors, e.g. hemin, protoporphyrin IX, and iron salts, modulate the expression of transferrin receptor (46). It is generally conceded that the expression of transferrin receptor is correlated directly with the rate of cell growth (47, 48) and inversely related to the amount of iron accumulated in the cells (49). The iron modulation has been demonstrated clearly by experiments of iron load or deprivation in a variety of cell types (18, 47, 49-52). This mechanism may be especially relevant for cellular physiology; when cells accumulate large amounts of iron, they reduce the number of transferrin receptors in order to prevent further accumulation of this mineral. In contrast, when the intracellular concentration of iron is low, cells induce an increase in transferrin receptor to permit rapid iron accumulation. However, it has been shown recently (53) that in macrophages, which are physiologically involved in iron storage, iron induces an up-regulation of transferrin receptor expression.

It seems, therefore, that in particular cell types, which are involved in iron storage and in erythroid differentiation, a positive feedback modulation of transferrin receptor expression is triggered by iron or heme, respectively.

Transferrin binding experiments indicate that in Me2SO + IFN-treated FLC the number of transferrin receptors is increased markedly. At the same time cell growth is inhibited significantly, and hemoglobinization is increased. A previous report (54) showed that human IFN-α inhibits the transferrin receptor expression and cell proliferation in a dose-dependent manner; it was therefore suggested that the decrease of transferrin receptor may be, at least in part, responsible for the antiproliferative action of IFN. In the present studies, IFN exerted a marked inhibitory effect on cell growth of Me2SO-treated FLC but also a marked stimulation of 125I-transferrin binding coupled with an enhancing effect on heme synthesis. Thus, the discrepancy between these data and those reported previously on the effect of IFN on transferrin receptor expression may be largely related to the different cell types used in the two studies.

It has been reported that heme, in addition to being a structural component of Hb, is also able to stimulate the transcription rate of globin mRNA (55) and is a necessary factor for optimized mRNA translation in reticulocytes (41, 55–60). Northern blot and run-on experiments (Figs. 7 and 8) suggest that globin chain stimulation is probably related to a transcriptional activation of the gene. In addition, an increasing level of intracellular heme is required to sustain optimal transferrin receptor synthesis in FLC induced to differentiate terminally. This conclusion is confirmed directly by the experiments performed by adding exogenous heme or an inhibitor of heme synthesis (Table III). These studies showed that in Me2SO-induced FLC an inhibition of heme synthesis by addition of succinyl acetone leads to a decrease of transferrin receptor expression; an opposite phenomenon is observed when heme content is increased by the addition of exogenous heme.

In conclusion, the present report provides evidence that in differentiating FLC, IFN-α and -β positively modulate heme, Hb, and transferrin receptor synthesis. These results further suggest that stimulation of heme synthesis may play a pivotal role in this complex process.

Acknowledgments—We gratefully acknowledge gifts of two Chinese ovary cell lines, transfected with the murine IFN-α and -β genes (M. Van Heuvel and E. C. Zwarthoff), pCR, αG10 and pCR, βG9 (M. G. Farace), and ptFR-2 plasmids (J. Goding).

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Positive Modulation of Friend Cell Differentiation by IFN-α and -β