Regulation of Transferrin Receptors in Human Hematopoietic Cell Lines*

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Cells grown in the presence of ferric ammonium citrate or hemin exhibited a concentration and time-dependent decrease in $^{125}$I-transferrin (Trf) binding. In contrast, cells grown in the presence of protoporphyrin IX or picolinic acid (an iron chelator) exhibited a marked increase in Trf binding. The decrease or increase in binding activity observed under these different conditions of culture reflected, respectively, a reduction or increase in receptor number rather than an alteration in ligand receptor affinity. Growth of the cells in the presence of saturating concentrations of apotransferrin only induced a slight reduction in receptor number. Investigation of the Trf receptors' turnover and biosynthesis clearly showed that iron and hemin decreased the synthesis of Trf receptors without any modification of the receptor turnover; in contrast, protoporphyrin IX and picolinic acid markedly increased the synthesis of Trf receptors.

Our results suggest that hemin, iron, and protoporphyrin IX may represent the main molecules involved in the regulation of Trf receptors.

It was recently suggested that polypeptide receptors on mammalian plasma membranes can be classified into two different categories on the basis of function (Kaplan, 1981). Such categorization is based on whether the major function of the receptor is to transmit information (class I receptors) or internalize the ligand (class II receptors) (Kaplan, 1981). Ligand internalization by class II receptors provides the cell with a required factor, for example, covalin (Youngdahl et al., 1979) or cholesterol (Brown and Goldstein, 1975). Exposure to ligand, class I receptors may be down-regulated (the number of surface receptors is reduced); in contrast, binding of ligand by class II receptors does not lead to regulation of receptor number. The best-studied class II receptor is the human fibroblast low-density lipoprotein receptor which is involved in the transport of cholesterol to the cells. Incubation of the cells with low-density lipoproteins results in a decrease in receptor number. Conversely, incubation of cells in the absence of low-density lipoproteins leads to an increase in surface receptor number (Brown et al., 1975). The regulation of low-density lipoprotein receptors results from modulation of receptor biosynthesis which is controlled by the concentration of free cholesterol (Brown et al., 1975).

Transferrin receptors appear to be another candidate for class II receptors. The plasma glycoprotein transferrin transports iron from the plasma to the cells (Finch and Huebers, 1982). The first step in the uptake of iron by the cells requires binding of Trf to specific surface receptors. The interaction of Trf receptors in reticulocytes (Jandl and Katz, 1963; Kailis and Morgan, 1974), placenta (Wada et al., 1979; Galbraith et al., 1980a), activated lymphocytes (Galbraith et al., 1980b), hepatocytes (Young and Aisen, 1980), rat and human fibroblasts (Octave et al., 1981; Ward et al., 1982a), and a variety of neoplastic cell lines (Larrick and Cresswell, 1979; Hamilton et al., 1979; Sutherland et al., 1981; Testa et al., 1982) has been reported. There is compelling evidence that Trf is required for the growth of cells in vitro (Hutchings and Sato, 1978). Recent studies have shown that iron salts down-regulate the Trf receptors (Ward et al., 1982b) and that the intracellular iron concentration may represent one of the most important factors in the control of the number of Trf receptors (Ward et al., 1982b).

In the present paper, we define the role of iron salts, heme, and protoporphyrin IX in the control of the number of Trf receptors in human leukemic cell lines. The results showed the following. 1) Preincubation of the cells with ferric ammonium citrate markedly reduced the number of Trf receptors. 2) Addition of an iron chelator (such as picolinic acid) in the culture medium greatly increased the number of Trf receptors. 3) Hemin, as previously reported by us (Pelicci et al., 1982), exhibited an effect similar to that of iron salts. 4) Preincubation of the cells with protoporphyrin IX enhanced the number of Trf receptors. 5) Preincubation of the cells with apotransferrin only slightly modified the Trf-binding capacity. These studies clearly suggest that the biosynthesis of Trf receptors is modulated by iron salts, heme, and protoporphyrin IX, but not by previous exposure to the ligand.

**EXPERIMENTAL PROCEDURES**

**Materials**—Human transferrin was purchased from Sigma, and it was electrophoretically homogeneous. Ferric ammonium citrate and ferric ammonium sulfate were obtained, respectively, from Sigma and Prolabo (Paris, France). Bovine or human serum albumin were from Sigma. Carrier-free $^{125}$I was bought from New England Nuclear. $^{60}$FeCl$_3$, 30 Ci/g of iron, was obtained from The Radiochemical Centre (Amersham, England).

Tissue culture flasks were from Costar (Cambridge, MA). Fetal and newborn calf serum were obtained from IBF or Eurobio (France). Hemin and protoporphyrin IX were from Sigma. These compounds were dissolved in 4 mM sodium hydroxide, the pH was neutralized with 0.1 M HCl, and they were stored at 4 °C under the form of stock solutions (2.5 mM). Hemin and protoporphyrin IX dissolved following

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† The abbreviations used are: Trf, transferrin; SDS, sodium dodecyl sulfate.

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this procedure were not toxic for cell culture.

Cells—K 562 (Lozzio and Lozzio, 1975), HEL (Martin and Papayannopoulos, 1982), HL-60 (Collins et al., 1977), and U937 (Sandstrom and Nilsson, 1976) cell lines were grown in plastic tissue culture flasks and Nillson, 1976) cell lines were grown in plastic tissue culture flasks. Annopoulou, 1982), HL-60 (Collins et al., 1977), and U937 (Sandstrom 59Fe followin a previously described method (Martinez-Medellin and Schulmen, 1972).

The cells were washed three times in serum-free RPMI 1640 medium and then incubated in the presence of 125 μg/ml 59Fe-Trf in RPMI 1640 medium. To measure "nonspecific" iron uptake, cells were incubated with 125 μg/ml 59Fe-Trf in the presence of 10 mg/ml unlabeled transferrin. The incubation temperature was 37 °C. At the end of the incubation period, the cells (1 × 10^6) were layered over a cushion of phthalate oil (1.02 density) and centrifuged 2 min at 10,000 x g to remove unbound 59Fe-Trf. 59Fe content of the cell pellet was measured in a γ counter. All data were averages of duplicate determinations (which were usually within 10% of each other) and were corrected for nonspecific binding (which did not exceed 5% of the total).

Transferrin Receptor Assay—Purified human transferrin was conju- gated with 125I by the solid-phase lactoperoxidase method (New England Nuclear, radiodiagnosis system) as previously described (Testa et al., 1982). The binding reaction was performed in polypropylene tubes (12 × 75 mm) in RPMI 1640 medium containing 0.1% bovine serum albumin (Sigma, Fraction V). Cell concentrations were 5 × 10^6 cells/ml, labeled Trf was 200 ng/ml, and unlabeled Trf was 1 mg/ml. Unbound ligand was removed by passage of cells through a density cushion, as previously described (Testa et al., 1982). After incubation, 200-μl aliquots of the cell suspension were layered over 150 μl of a mixture of dibutyl phthalate (Merc) and diethyl phthalate (Merc) to a final density of 1.025 in 400-μl plastic microfuge tubes and centrifuged at 20,000 x g for 5 min. At the end of centrifugation, the supernatant and the majority of phthalate cushion were aspirated. The tips of the vials containing cell pellets were then severed with a scalpel, transferred to plastic vials, and the radioactivity was measured in a γ counter. Total binding corresponded to the radioactivity in the cell pellet. Nonspecific bind- ing was represented by the radioactivity bound to the cells in the presence of cold Trf (1 mg/ml) and was less than 5% of the total radioactivity binding per 10^6 cells. "Specific binding" was the difference between total and nonspecific binding.

Before binding, the cells were washed four times in 40 ml of Hanks’ saline solution (Boehringer Mannheim, Germany). The number of washes did not modify the transferrin-binding capacity of the cells since they were grown in fetal calf serum, and bovine transferrin had a very low affinity for human transferrin receptors. All washes did not modify the transferrin-binding capacity of the cells since they were grown in fetal calf serum, and bovine transferrin had a very low affinity for human transferrin receptors, as previously reported by other investigators (Ward et al., 1982b) and by us (Titeux et al., 1984).

Investigation of the Binding of B3/25 Monoclonal Antibody to Transferrin Receptors on Whole Cells—In some experiments, the expression of transferrin receptors was evaluated by investigating the binding of B3/25 monoclonal antibody to transferrin receptors (Trow-bridge and Omary, 1981). The cells were washed four times with Hanks’ balanced saline solution containing 1 mg/ml bovine serum albumin (Sigma) and then 2 × 10^6 cells for each point were incubated 90 min at 4 °C in the presence of increasing amounts of purified B3/25 monoclonal antibody diluted in the same incubation buffer. After three washes at 4 °C, the cells were then incubated 90 min at 4 °C in the presence of 125I-goat IgG anti-mouse antibody (The Radiological Centre, Amersham, England; 10 pCi/μg). The cells were then layered on phthalate oil and processed as described above.

Dissolved Transferrin Receptor Assay—A simple assay was devised to determine solubilized and nonspecific transferrin receptor activity (Pelici et al., 1982). It is based on a difference in solubility of free and transferrin-bound receptors in polyethylene glycol. Cell samples were dissolved in phosphate-buffered saline solution containing 1% Triton X-100 (Sigma) and 1 mM phenylmethylsulfonyl fluoride (Sigma) and centrifuged at 10,000 x g for 10 min. Dissolved receptors were incubated with a total volume of 0.2 ml for 30 min at 37 °C in a 0.1 M citrate/Tris buffer solution (pH 5.0) containing 0.1% bovine serum albumin, 0.1% Triton X-100, and 200 ng of 125I-transferrin. The receptor-transferrin complex was precipitated with 0.5 ml of polyethylene glycol solution (12% w/v) in 0.1 M citrate/Tris buffer (pH 5.0) containing the carrier human γ-globulin (0.1%). The tubes were placed in a ice bath for 30 min and then centrifuged at 13,000 × g for 15 min at 4 °C. The supernatant and the precipitate were tested for radioactivity. Coprecipitation of free transferrin was measured by omitting the receptor from the tubes while nonspecific binding of transferrin was deter- mined by preincubating the samples with 1 mg of nonradioactive transferrin before adding the radioactive transferrin.

Investigation of the Biosynthesis of Transferrin Receptors—10 × 10^6 cells were washed three times in Dulbecco’s methionine-free medium and then incubated in the same medium containing 10% fetal calf serum (K 562 cells) at 37 °C under CO2/air (1:20). Cell concentrations were 5 × 10^6 cells/ml, and Nillson, 1976) cell lines were grown in plastic tissue culture flasks. 4 days after passage, at which point cell densities varied between 3 × 10^6 and 6 × 10^6 cells/ml.

Iron Uptake—Iron-free human transferrin was radiolabeled with 59Fe following a previously described method (Martinez-Medellin and Schulmen, 1972). The binding reaction was performed in polypropylene tubes (12 × 75 mm) in RPMI 1640 medium containing 0.1% bovine serum albumin (Sigma, Fraction V). Cell concentrations were 5 × 10^6 cells/ml, labeled Trf was 200 ng/ml, and unlabeled Trf was 1 mg/ml. Unbound ligand was removed by passage of cells through a density cushion, as previously described (Testa et al., 1982). After incubation, 200-μl aliquots of the cell suspension were layered over 150 μl of a mixture of dibutyl phthalate (Merc) and diethyl phthalate (Merc) to a final density of 1.025 in 400-μl plastic microfuge tubes and centrifuged at 20,000 x g for 5 min. At the end of centrifugation, the supernatant and the majority of phthalate cushion were aspirated. The tips of the vials containing cell pellets were then severed with a scalpel, transferred to plastic vials, and the radioactivity was measured in a γ counter. Total binding corresponded to the radioactivity in the cell pellet. Nonspecific bind- ing was represented by the radioactivity bound to the cells in the presence of cold Trf (1 mg/ml) and was less than 5% of the total radioactivity binding per 10^6 cells. "Specific binding" was the difference between total and nonspecific binding.

RESULTS

Effects on Cell Growth—The addition of hemin (from 1 to 100 μM) or protoporphyrin IX (from 0.1 to 10 μM) to the culture medium did not significantly affect the rate of cell growth. In contrast, ferric ammonium citrate or sulfate increased the rate of growth of the cells, especially the K 562 cells (Fig. 1). Picolinic acid at concentrations between 1–2 mM inhibited cell growth slightly; at higher concentrations (3
TABLE I

Effect of iron (ferric ammonium citrate), heme, protoporphyrin IX, picolinic acid, apotransferrin, and iron-saturated transferrin on Trf receptor number and affinity

K 562 cells were grown with ferric ammonium citrate (10 μg/ml), heme (0.1 mM), picolinic acid (2 mM), apotransferrin (50 μg/ml), iron-saturated transferrin (50 μg/ml), or protoporphyrin IX (10 μM) for 3 days before assay of Trf-binding activity. The results represent the range of values obtained in five different experiments.

| Effect                        | 125I-Trf bound (ng/l × 10⁶ cells) | Kₘ (× 10⁻⁹ M) |
|-------------------------------|-----------------------------------|---------------|
| Control                       | 47-64                             | 2             |
| Ferric ammonium citrate       | 11-31                             | 1.75          |
| Hemin                         | 19-35                             | 1.6           |
| Protoporphyrin IX             | 93-110                            | 3             |
| Picolinic acid                | 150-220                           | 2.4           |
| Apotransferrin                | 41-60                             | 1.8           |
| Iron-saturated transferrin    | 15-35                             | 2             |

Effects of Iron, Heme, Protoporphyrin IX, Picolinic Acid, Apotransferrin, and Iron-saturated Transferrin on the Expression of Trf Receptors—The addition of iron (ferric ammonium citrate or sulfate) and heme to the culture medium markedly decreased Trf-binding activity (Table I); in contrast, cells grown in the presence of protoporphyrin IX or picolinic acid (an iron chelator) exhibited a marked increase in their Trf-binding capacity (Table I). The addition of apotransferrin to the culture medium induced only a slight decrease in the number of Trf-binding sites; in contrast, iron-saturated transferrin elicited an effect similar to that of iron (Table I). Scatchard analysis revealed that the decrease or increase in binding activity observed under these different conditions were due, respectively, to a decrease or increase in receptor number rather than an alteration in ligand-receptor affinity (Fig. 2). As shown in Fig. 3, incubation of cells with ferric ammonium citrate, heme, or protoporphyrin IX led to a concentration-dependent decrease or increase in Trf-binding activity.

Comparable results were obtained when the Trf-binding capacity was estimated by investigating the binding of B3/25 or 42/6 monoclonal antibodies which specifically recognize Trf receptors (Trowbridge and Omary, 1981; Trowbridge and Lopez, 1982) (Fig. 4). Thus, cells grown in the presence of ferric ammonium citrate bound about two times less B3/25 antibody with respect to control cells, while cells grown in the presence of iron-saturated transferrin and heme bound about two times more B3/25 antibody with respect to control cells. These results also demonstrate the specificity of the binding of B3/25 and 42/6 antibodies to Trf receptors.
Effect of ferric ammonium citrate (iron), hemin, and protoporphyrin IX on the uptake of iron by K 562 cells. K 562 cells were grown for 4 days under standard conditions of culture (control) and in the presence of ferric ammonium citrate (25 µg/ml), hemin (100 µM), or protoporphyrin IX (10 µM). Iron uptake was measured as described under "Experimental Procedures."

The presence of protoporphyrin IX bound 2.5 times more B3/25 antibody than control cells. Experiments with Trf-²²⁵Fr showed that a decrease or increase in iron uptake occurred, respectively, proportional to the decrease or increase in receptor number (Fig. 5). However, the changes in iron uptake were less marked than those observed for Trf binding. The modulation of Trf receptors observed in the cells grown in the presence of these different compounds was not dependent upon (a) a change in the kinetics of association of ²²⁵Fr to the cells at 20 or 37 °C, or (b) a change in the dissociation of Trf from the cells (data not shown).

The decrease in Trf receptor number produced by ferric ammonium citrate or hemin was time-dependent and was maximal by 24 h (Fig. 6). The decrease in receptor number occurred regardless of growth state. Similarly, the increase in Trf-binding capacity produced by protoporphyrin IX was time-dependent and was maximal by 48 h. When cells grown in media supplemented with hemin, ferric ammonium citrate, or protoporphyrin IX were removed to nonsupplemented media, the Trf-binding capacity returned to control levels by 24–48 h (Fig. 7).
The binding experiments on whole cells at 20 °C permit the evaluation of surface receptors. Since several recent studies have afforded evidence that most of transferrin receptors are on intracellular membranes, we evaluated 125I-Trf binding also on cell samples dissolved in 1% Triton X-100. This technique allows the investigation of both surface-bound and intracellular localized transferrin receptors. Using this technique, we confirmed the results obtained by quantifying the transferrin-binding capacity on intact cells. Thus, we showed that cells grown in the presence of ferric ammonium citrate and hemin, respectively, exhibited a marked decrease in their Trf-binding capacity (Table II). In contrast, cells grown in the presence of picolinic acid and protoporphyrin IX, respectively, exhibited a marked enhancement of their transferrin-binding capacity (Table II).

**TABLE II**

Investigation of transferrin-binding capacity on cell samples dissolved in 1% Triton X-100

| Cell sample                  | Transferrin-binding capacity* (ng/mg protein) |
|-----------------------------|-----------------------------------------------|
| Control                     | 690 ± 45                                      |
| Ferric ammonium citrate     | 360 ± 52                                      |
| Hemin                       | 445 ± 30                                      |
| Protoporphyrin IX           | 880 ± 85                                      |
| Picolinic acid              | 1320 ± 78                                     |

*Transferrin-binding capacity was evaluated by Scatchard plots, and the results were expressed as nanograms of transferrin bound/mg of protein.

Fig. 8. Effect of actinomycin D on 125I-Trf-binding activity of cells grown with hemin, protoporphyrin IX, or ferric ammonium citrate. K 562 cells were grown in standard media or media supplemented with ferric ammonium citrate (25 μg/ml), hemin (100 μM), or protoporphyrin IX (10 μM). After 72 h of incubation, actinomycin D (1 μg/ml) was added. At indicated times, 125I-Trf-binding activity was determined under the conditions mentioned above. Actinomycin D completely inhibited cell growth, but until 24 h of incubation, did not modify the cell viability as estimated by the trypan blue exclusion test.

Fig. 9. Reappearance of Trf receptors after trypsin treatment of K 562 cells grown in media with and without iron or hemin supplementation. K 562 cells were grown in standard media supplemented with Fe3+ (25 μg/ml) in the form of ferric ammonium citrate, or with hemin (100 μM). Cells were exposed to trypsin (0.1 mg/ml) in serum-free RPMI 1640 medium for 30 min at 37 °C. The trypsin was neutralized by addition of fetal calf serum (20%); the cells were washed three times and then grown in media with or without iron or hemin supplementation. At specific times, cells were removed, and the Trf-binding capacity was measured under the conditions mentioned above. Cells in standard (○), in iron-supplemented (▲), or hemin-supplemented (□) media.
cells labeled with \([\text{\textsuperscript{\textit{35}}}S]\)methionine for 2 h. Affinity chromatography on Sepharose-transferrin from pg/ml; \(c\), 562 cells grown 72 h in the presence of ferric ammonium citrate (25 mM).

The figure shows the polyacrylamide gel electrophoresis of the transferrin receptor purified by affinity chromatography on Sepharose-transferrin from K 562 cells labeled with \([\text{\textsuperscript{35}}}S]\)methionine for 2 h. A, Coomassie Blue staining. B, fluorograph: \(a\), K 562 cells grown under standard conditions; \(b\), K 562 cells grown 72 h in the presence of ferric ammonium citrate (25 \(\mu\text{g/ml}\)); \(c\), K 562 cells grown 72 h in the presence of picolinic acid (2 mM). The arrows indicate the position on the gel of molecular weight standard markers (obtained from Bio-Rad).

**TABLE III**

**Effect of iron (ferric ammonium citrate), heme, protoporphyrin IX, and picolinic acid on the intracellular concentration of ferritin**

| Ferritin concentration (ng/mg protein) | Control | Ferric ammonium citrate | Hemin | Protoporphyrin IX | Picolinic acid |
|--------------------------------------|---------|-------------------------|-------|------------------|---------------|
| Control                              | 245-300 | 555-608                 | 300-355 | 115-137         | 1-12          |
| Ferric ammonium citrate              |         |                         |       |                  |               |
| Hemin                               |         |                         |       |                  |               |
| Protoporphyrin IX                    |         |                         |       |                  |               |
| Picolinic acid                       |         |                         |       |                  |               |

The reappearance of Trf binding is plotted against time, the reappearance of receptors reached half of the maximal level at 6–8 h for control cells, at 6–8 h for iron-supplemented cells, at 7–9 h for hemin-treated cells, and at 8–10 h for protoporphyrin IX-treated cells. These results indicate similar half-lives for Trf receptors under these different conditions of culture. Thus, our data suggest that incubation of the cells with iron, heme, or protoporphyrin IX affects receptor biosynthesis without affecting receptor degradation. To directly investigate this point, we studied the biosynthesis of Trf receptors by incubation of the cells in the presence of \([\text{\textsuperscript{35}}}S]\)methionine followed by purification of Trf receptors on Sepharose-transferrin (Fig. 10). In control cells, the synthesis of Trf receptors corresponded to 0.2% of the total protein synthesis of K 562 cells. In contrast, the synthesis of Trf receptors was lower in cells grown in the presence of ferric ammonium citrate (0.05–0.1% of total protein synthesis). Picolinic acid strongly increased the synthesis of Trf receptors to levels 3–5 times higher than in control cells (0.5–1% of total protein synthesis).

**Effect of Ferric Ammonium Citrate, Hemin, Protoporphyrin IX, and Picolinic Acid on the Intracellular Content of Ferritin**—In order to investigate the effect of ferric ammonium citrate, heme, protoporphyrin IX, and picolinic acid on the level of iron stored into the cells, we evaluated the intracellular concentration of ferritin. In fact, it was well established that the level of ferritin present in the cell was dependent upon the intracellular concentration of iron (Aisen andリストowski, 1980). These experiments showed that (a) ferric ammonium citrate induced a marked increase in the level of ferritin; (b) heme elicited moderate enhancement in the concentration of ferritin; and (c) both protoporphyrin IX and picolinic acid elicited a very marked decrease in the concentration of ferritin (see Table III).

**DISCUSSION**

Our results clearly show that several compounds related to iron or heme metabolism can modulate the transferrin-binding capacity of human leukemic cell lines. More particularly, we showed that incubation with iron salts or Trf-Fe\(_2\) resulted in time and concentration-dependent reduction in Trf-binding capacity of the cells. Analysis of the binding data by the method of Scatchard (Scatchard, 1949) revealed that such reduction was the result of decreased receptor number rather than alteration in ligand-receptor affinity. Incubation of cells with apo-Trf did not alter receptor number. These results indicate that an increased accumulation of iron in the cells leads to a decrease in Trf binding. Similar results were previously reported in Hela cells (Ward et al., 1982b).

This conclusion is further supported by the observation that an iron chelator, picolinic acid, in the culture medium greatly increased Trf-binding capacity. As previously reported (Fernandez-Pol, 1977; Fernandez-Pol, 1978; Gusley and Jett, 1981), picolinic acid at low concentrations (0.1 to 2 mM) reduces iron uptake; at higher concentrations (3 mM or higher), this compound completely inhibits iron uptake.

Furthermore, this interpretation is further supported by the experiments of ferritin quantification. These experiments clearly showed that cells exhibiting a higher level of intracellular ferritin (cells grown in the presence of ferric ammonium citrate and heme) have the lower number of Trf receptors; in contrast, cells possessing the lower level of ferritin exhibit the higher level of Trf-binding capacity.

Thus, our data clearly indicate that the expression of Trf receptors depends on the amount of iron accumulated into the cells. This system exhibits a simple physiological principle. When cells accumulate large amounts of iron, they reduce the number of Trf receptors in order to prevent further accumulation of iron; in contrast, when the intracellular concentration of iron is low and the cells need more iron, they induce an increase in Trf receptors to permit rapid accumulation of iron. This mechanism may be especially relevant for cellular physiology since iron is required for both cell growth and for synthesis of heme-containing molecules.

However, compounds possessing a tetrapyrrolic ring can also modulate the Trf-binding capacity of the cells. Thus, hemin, as previously reported by us (Pelicci et al., 1982), significantly reduces the number of Trf-binding sites in a manner similar to iron. In contrast, protoporphyrin IX which is identical to heme but is devoid of iron markedly increases the number of Trf receptors. The effect of heme could be related to the presence of iron in its molecule; in fact, accumulation of heme by the cells corresponds equally to an accumulation of iron. In contrast, it is difficult to understand the mechanism by which protoporphyrin IX increases the number of Trf receptors. This effect does not derive from reduced iron accumulation since protoporphyrin IX does not inhibit iron uptake by the cells.

However, experiments of ferritin quantification showed that cells grown in the presence of protoporphyrin IX exhibited a marked reduction of their ferritin concentration. It is interesting that in other biological systems, hemin and protoporphyrin IX exhibit antagonistic effects; thus, protoporphyrin IX is a potent stimulator of guanylate cyclase which
is inhibited by heme (Ignarro et al., 1982); heme acts as a mitogen for lymphocytes, whereas protoporphyrin IX is unable to stimulate the proliferation of the cells (Stenzel et al., 1981). A possible role for cyclic nucleotides in the regulation of Trf receptors is now under investigation in our laboratory.

The modulation of Trf receptors by all the compounds mentioned above implicates a modification of the number of receptors without change in the affinity of the receptor for the ligand. Modifications in the kinetics of association or dissociation were not observed. Furthermore, differences in the proportion of membrane-bound and intracellular receptors were not found, as suggested by experiments of [125I]-Trf binding on cell samples dissolved in 1% Triton X-100.

The modulation by these compounds of the number of Trf receptors became apparent only several hours after their addition to the cells. Such changes in receptor number could have represented a modification of the degradation of Trf receptors and/or a modulation of the synthesis of receptors. When new receptor synthesis was blocked with actinomycin D or cycloheximide, both control cells and cells exposed to iron, heme, or protoporphyrin IX demonstrated the same apparent rate of receptor degradation. However, investigation of the half-life of a receptor by using protein or mRNA synthesis inhibitors may cause artifactual changes in the rate of receptor biosynthesis. Therefore, we investigated receptor half-life by measuring the reappearance of Trf-binding activity following trypsinization. The time required for both trypticized control cells and cells incubated with ferric ammonium citrate, heme, or protoporphyrin IX to resynthesize half of their maximal level of receptors was similar. This result strongly suggests that hemin, protoporphyrin IX, and ferric ammonium citrate did not alter the rate of receptor degradation.

To more directly investigate the mechanism of regulation of Trf receptors, we studied the effect of heme, ferric ammonium citrate, protoporphyrin IX, and picolinic acid on the biosynthesis of Trf receptors. Under standard conditions of culture, the biosynthesis of Trf receptors in K 562 cells represents about 0.1-0.2% of the total protein synthesis. Cells grown 3 days in the presence of ferric ammonium citrate or heme exhibited a marked reduction of Trf receptor biosynthesis. In contrast, cells grown in the presence of protoporphyrin IX or picolinic acid exhibited a strong increase in the synthesis of Trf receptors. It is interesting that cells grown in the presence of picolinic acid synthesize on the average five times more Trf receptors than do control cells. Thus, picolinic acid-treated cells may represent an interesting source of mRNA specific for Trf receptors with respect to gene cloning.

Short incubation (2 h) of the cells in the presence of ferric ammonium citrate, heme, protoporphyrin IX, or picolinic acid did not modify the rate of receptor biosynthesis with respect to the control. These results suggest that Trf receptors are probably modulated by a transcriptional mechanism.

This study affords evidence that Trf receptors can be modulated. The pattern of regulation appears to be analogous to that previously described for lipoprotein receptors (Brown et al., 1975). Receptor number is regulated not by binding of low-density lipoproteins, but by the intracellular concentration of cholesterol metabolites. Receptor number is also regulated by an alteration in the rate of receptor biosynthesis (Brown and Goldstein, 1975). Thus, great analogies exist between Trf receptors and lipoprotein receptors.

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