Acidic isoferritins have been previously found to be highly potent inhibitors of hematopoietic progenitors at concentrations of 10^{-11} to 10^{-16} mol/L, and it has been suggested that acidic isoferritin inhibitory activity plays a role in the regulation of normal hematopoiesis and also in the pathogenesis of leukemia. To characterize the ferritin species that affect the in vitro growth of human colony-forming unit–granulocyte-macrophage (CFU-GM), we tested different preparations of basic (L-subunit-rich) and acidic (H-subunit-rich) isoferritins. Three preparations of human liver (basic) ferritin did not show any effects on CFU-GM growth at concentrations up to 10^{-8} mol/L, irrespective of the degree of glycosylation. Acidic isoferritins were purified both from HeLa cells and human heart. HeLa cell ferritin did not affect in vitro colony formation. One of two preparations of human heart ferritin, containing 5% glycosylated ferritin, showed a mean inhibition of 26% ± 8% of the control at 10^{-8} mol/L (P < .02), whereas the other preparation, which contained no glycosylated ferritin, did not show any effect of CFU-GM growth. A preparation enriched for glycosylated acidic isoferritins from human heart was found to produce a mean inhibition of 32% ± 11% of the control at 10^{-8} mol/L (P < .01), whereas another one was ineffective. A significant part of the inhibitory activity was removed by preincubation with the monoclonal antibody 2A4 directed against human heart ferritin. The present findings indicate that basic isoferritins, ie, the predominant ferritin type in human blood, have no effect on the growth of human CFU-GM, and this is in keeping with indirect clinical evidence. Inhibition of colony formation may be obtained by some preparations of acidic isoferritins that are rich in H subunits and bind to concanavalin A. The mechanism(s) responsible for this are not clear, but the effective concentrations are higher than those found in human blood both under normal conditions and in leukemia. At present, the physiologic significance of the observed inhibitory activity is uncertain.

FERRITIN is ubiquitous in all cells, and its major functions are iron storage and detoxification. The structural and immunologic properties of this molecule vary from one tissue to another and are related not only to the iron status but also to other factors including cell differentiation and malignant transformation. Multiple forms of ferritin occur in most cells, with all molecules consisting of 24 subunits arranged as hollow protein shells having an approximate molecular weight of 500,000. These isoferritins are families of heteropolymers fashioned from different proportions of two distinct subunit classes, heavy (H) and light (L), with molecular weights of approximately 21,000 and 19,000, respectively. The L-subunit–rich isoferritins are basic (high pI) and predominate in iron storage organs (liver and spleen) and plasma, whereas the H-subunit–rich isoferritins are acidic (low pI) and occur in tissues of low nonheme iron content (heart, erythroid cells, monocytes, lymphocytes) and in fetal and some malignant tissues. This model for tissue ferritin phenotypes based on two distinct subunit species has been validated by the demonstration that the two subunits are encoded by distinct messenger RNAs (mRNAs) and recent evidence for the existence of at least two ferritin genes. However, the matter could be more complex because a glycosylated G subunit (molecular weight about 23,000) has been isolated from human serum. It is possible to evaluate basic and acidic isoferritins separately using immunoassays based on monospecific antibodies raised against L-subunit–rich and H-subunit–rich molecules.

Although ferritin function is inextricably linked to iron metabolism, there has been evidence of a role for ferritin in lymphocyte migration and T cell function as well as in the control of myelopoesis. In fact, a leukemia-associated inhibitory activity (LIA) directed against human granulocyte-monooyte progenitors identified by Broxmeyer and his associates as acidic isoferritins. Acidic isoferritin inhibitory activity (AIFIA) was later found in bone marrow and blood cells of normal donors, and a specific role for monocyte-macrophage–derived acidic isoferritins as feedback regulators of hematopoietic progenitor cells was suggested. However, this has been disputed because of certain inconsistencies with the present picture of human isoferritins.

In this work, we have studied the effects of different isoferritins on the growth of cultured granulocyte-macrophage precursor cells and correlated these effects with biochemical and immunologic characteristics of the ferritin tested.

MATERIALS AND METHODS

Purification of human liver, heart, and HeLa cell ferritin. Eight ferritin preparations were made and coded as A, B, C, D, E, F, G, and H. The main characteristics of these isoferritins are reported in Table 1.

The procedure for purification of human liver ferritin (preparations A and B) was essentially the same as previously described in detail by Arosio et al. It consisted of heat extraction at 75°C, ammonium sulfate precipitation (60% saturation), precipitation at

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Table 1. Biochemical and Immunologic Characteristics of the Isoferritin Preparations

<table>
<thead>
<tr>
<th>Name</th>
<th>Origin</th>
<th>Relevant Purification Steps</th>
<th>Purity (%)</th>
<th>Immunologic Reactivity*</th>
<th>Binding to Con A (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>liver</td>
<td>crystallization</td>
<td>&gt;97</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>B</td>
<td>liver</td>
<td>crystallization</td>
<td>&gt;97</td>
<td>100</td>
<td>0</td>
</tr>
<tr>
<td>C</td>
<td>heart</td>
<td>passage through Con A-Sepharose</td>
<td>95</td>
<td>15</td>
<td>85</td>
</tr>
<tr>
<td>D</td>
<td>heart</td>
<td>preparative electrophoresis; no passage through Con A-Sepharose</td>
<td>&gt;97</td>
<td>14</td>
<td>86</td>
</tr>
<tr>
<td>E</td>
<td>HeLa cells</td>
<td>as in D</td>
<td>&gt;97</td>
<td>9</td>
<td>91</td>
</tr>
<tr>
<td>F</td>
<td>heart</td>
<td>no heat extraction; elution from Con A-Sepharose with 0.1 mol/L α-methyl mannoside</td>
<td>&lt;5</td>
<td>46</td>
<td>54</td>
</tr>
<tr>
<td>G</td>
<td>heart</td>
<td>tissue heated at 75 °C and then as in F; ultracentrifuged</td>
<td>&lt;5</td>
<td>49</td>
<td>51</td>
</tr>
<tr>
<td>H</td>
<td>liver</td>
<td>as in G</td>
<td>~10</td>
<td>98</td>
<td>2</td>
</tr>
</tbody>
</table>

Abbreviation: Con A, concanavalin A.

*Ferritin concentration was evaluated with immunoradiometric assays based on polyclonal antibodies against human liver ferritin and the monoclonal antibody 2A4 against human heart ferritin; each value was then expressed as a percentage of the total ferritin concentration.

300,000 g for 90 minutes, gel filtration on a Sepharose 4B column, and crystallization with 5% cadmium sulfate.

Human heart (preparation D) and HeLa (preparation E) ferritins were purified by similar procedures except that the crystallization step was omitted and replaced with diethyl aminoethyl chromatography and preparative gel electrophoresis on a polyacrylamide gradient (10% to 30%) gel slab.

Human heart preparation C was purified with a similar procedure except that the heated homogenate was passed through a column of Affi gel Blue (Bio-Rad Laboratories, Richmond, Calif); equilibrated in 20 mmol/L Tris-HCl, pH 7.4, and on a column of Con A-Sepharose (Pharmacia Fine Chemicals, Uppsala, Sweden); and the break-through peaks collected. For this preparation, the preparative gel electrophoresis step was omitted.

Isoferritin preparations were analyzed by means of isoelectrofocusing, sodium dodecyl sulfate (SDS) electrophoresis, and immunoblotting as previously described. Preparations A, B, D, and E appeared pure by native and SDS gel electrophoretic analyses, whereas preparation C contained 5% contaminants. The ferritin protein concentration was determined by the method of Lowry et al.20

Glycosylated ferritins were obtained by loading nonheated tissue homogenates (preparations F and H) or heated homogenates (preparation G) on a Con A-Sepharose column. After extensive washing, glycosylated ferritins were eluted with 0.1 mol/L α-methyl glucoside. These preparations were concentrated either on an Amicon M 300 membrane (preparation F and H) (Amicon Corp, Danvers, Mass) or by precipitation at 300,000 g for 90 minutes (preparation G). The ferritin concentration was detected by immunobassays. In ferritin preparations F and G, ferritin did not account for more than 5% of the total protein, whereas preparation H was somewhat more than 10% pure.

Monoclonal antibody to heart ferritin. The monoclonal antibody 2A4 against human heart ferritin was developed by Cavanna et al. and its characteristics were reported by Arosio et al.18

Immunobasys for liver and heart ferritin. The two-site immunoradiometric assay for human liver ferritin was prepared as previously described. The two-site immunoradiometric assay for heart ferritin was prepared as described by Cavanna et al. using the monoclonal antibody 2A4.

Binding to Con A. This was determined as previously detailed.18

CFU-GM assay procedures. Normal bone marrow was obtained from normal volunteers or from patients undergoing chest surgery for nonmalignant diseases. Bone marrow cells were collected in preservative-free heparin (50 to 100 U/mL). A quantity of 1 x 10⁵ low-density bone marrow cells, separated as previously described, were plated in a 1-mL mixture containing Iscove’s modification of Dulbecco’s medium (1MDM), 15% fetal bovine serum, 0.3% agar (Difco Laboratories, Detroit, Mich), and 10% conditioned medium from the 5637 cell line as a source of colony-stimulating activity (CSA) in the presence or absence of the various isoferritins. Sodium azide was removed from the isoferritin preparations by dialysis prior to the cultures. Preliminary studies showed that ferritin concentrations in the 5637-conditioned medium were below the lower detectability limit of both the liver ferritin and 2A4 assays. Cultures were incubated at 37 °C in a humidified atmosphere of 5% CO₂ in air. Plates were scored for colonies after seven days of incubation, a time that has been shown previously to be optimal for the demonstration of inhibition of CFU-GM from normal donors by AIFIA. All aggregates containing more than 40 cells were counted as colonies.

To study the neutralization of a possible isoferritin-associated inhibitory activity by a specific antibody, active isoferritin preparations were preincubated at 10⁻⁴ mol/L with the monoclonal antibody 2A4 prior to the culture. The final dilution of the ammonium sulfate precipitate of the 2A4 ascitic fluid was calculated so as to have an excess of antibody as compared to ferritin molecules.

The bone marrow culture studies were carried out in a blind fashion. The various isoferritin preparations were made by two of us (S.L. and P.A.) and marked with different letters (A to H). People working with the CFU-GM assays did not know the content of such preparations. Two plates were scored for each sample by two independent investigators, and the mean value of the four scores was considered the result of that single experiment. Colony growth in the presence of different preparations was expressed as a percentage of the control, and the results of different experiments were expressed...
ISOFERRITINS AND GRANULOPOIESIS

Fig 1. Isoferritin analysis of the heart ferritin preparation G containing only glycosylated isoferitins eluted from a Con A-Sepharose column with 0.1 mol/L α-methyl mannoside. In this analysis, isoferitins were separated on polyacrylamide gel isoelectrofocusing.17 The bands containing isoferitins were cut and eluted. Then, on each sample, the pH was measured, and the ferritin concentration was evaluated by means of immunoradiometric assays based on polyclonal antibodies against human liver ferritin (HLF) and the monoclonal antibody 2A4 against human heart ferritin. Values for pH (----) and concentrations of liver-type (-----) and heart-type (-----) ferritin of different fractions are reported.

as means ± 1 SD. Differences between means were determined by using the t test for paired observations after logarithmic transformation of the original colony scores (number per plate).

RESULTS

Isoferritin preparations. Table 1 summarizes the biochemical and immunologic characteristics of the ferritin preparations used in the following experiments. Liver ferritin preparations A and B did not show any significant differences in their isoelectrofocusing profiles or subunit composition as judged by SDS electrophoresis. Biochemical analyses of heart preparations C and D showed that these isoferitins had about 85% H subunits; their isoelectrofocusing profiles covered a large spectrum of isoferitins and were not distinguishable. The only difference between preparations C and D was that a minor portion (5%) of ferritin D bound specifically to Con A whereas ferritin C was 100% nonglycosylated. HeLa cell ferritin (preparation E) had approximately the same proportion of H subunits as heart ferritin and did not bind to Con A.

Preparations F and H could be characterized only immunologically because of the low level of ferritin purification, and the results of these studies are reported in Table 1. One major question addressed in this work was the relationship among subunit composition (immunologic reactivity), glycosylation, and pH. Figure 1 shows the results of isoelectrofocusing studies with the heart preparation G. A large number of isoferitin bands were seen, ranging in pH from 5.8 to 4.2. Immunoassays revealed two different isoferitin populations: (1) those detected by the 2A4 immunoradiometric assay and therefore containing more than 60% H subunits had a peak value of 4.6 for pH; (2) those detected by the immunoradiometric assay based on polyclonal antibodies against human liver ferritin and therefore rich in L subunits had a peak value of 5.2 for pH.

Effect of different isoferitins on CFU-GM growth. Results of these studies are reported in Table 2. Irrespective of the degree of glycosylation, the three preparations of human liver ferritin (A, B, H) did not show any effect on CFU-GM growth at concentrations equal to 10^-8 and 10^-12 mol/L. HeLa cell ferritin (preparation E) also did not affect colony formation. Of the two preparations of heart ferritin, preparation C, which had been passed through a Con A-Sepharose column to remove glycosylated proteins, did not show any effect on CFU-GM growth, whereas preparation D, containing 5% glycosylated ferritin, showed a significant inhibitory effect, mainly at 10^-8 mol/L. Of the two heart preparations containing glycosylated isoferitins (F and G), G was found to produce a mean inhibition of 32% ± 11% of the control at

Table 2. Effects of Different Isoferritin Preparations on In Vitro CFU-GM Growth

<table>
<thead>
<tr>
<th>Preparation (No. of Experiments)</th>
<th>Molarity</th>
<th>Origin</th>
<th>Colony Formation as Percentage of Control Medium (Mean ± 1 SD)</th>
<th>Comparison With Control Medium*</th>
</tr>
</thead>
<tbody>
<tr>
<td>A(4)</td>
<td>10^-12</td>
<td>liver</td>
<td>100 ± 8</td>
<td>0.01 NS</td>
</tr>
<tr>
<td>A(4)</td>
<td>10^-9</td>
<td>liver</td>
<td>98 ± 9</td>
<td>0.44 NS</td>
</tr>
<tr>
<td>B(4)</td>
<td>10^-12</td>
<td>liver</td>
<td>99 ± 4</td>
<td>0.31 NS</td>
</tr>
<tr>
<td>B(4)</td>
<td>10^-9</td>
<td>liver</td>
<td>93 ± 8</td>
<td>1.82 NS</td>
</tr>
<tr>
<td>C(4)</td>
<td>10^-12</td>
<td>heart</td>
<td>98 ± 10</td>
<td>0.43 NS</td>
</tr>
<tr>
<td>C(4)</td>
<td>10^-9</td>
<td>heart</td>
<td>87 ± 18</td>
<td>1.47 NS</td>
</tr>
<tr>
<td>D(4)</td>
<td>10^-12</td>
<td>heart</td>
<td>88 ± 6</td>
<td>3.99 &lt;.05</td>
</tr>
<tr>
<td>D(4)</td>
<td>10^-9</td>
<td>heart</td>
<td>74 ± 8</td>
<td>5.52 &lt;.02</td>
</tr>
<tr>
<td>E(4)</td>
<td>10^-12</td>
<td>HeLa cells</td>
<td>102 ± 12</td>
<td>0.32 NS</td>
</tr>
<tr>
<td>E(4)</td>
<td>10^-9</td>
<td>HeLa cells</td>
<td>96 ± 17</td>
<td>0.95 NS</td>
</tr>
<tr>
<td>F(4)</td>
<td>10^-12</td>
<td>heart</td>
<td>99 ± 8</td>
<td>0.27 NS</td>
</tr>
<tr>
<td>F(4)</td>
<td>10^-9</td>
<td>heart</td>
<td>95 ± 14</td>
<td>0.79 NS</td>
</tr>
<tr>
<td>G(4)</td>
<td>10^-12</td>
<td>heart</td>
<td>91 ± 11</td>
<td>1.54 NS</td>
</tr>
<tr>
<td>G(4)</td>
<td>10^-9</td>
<td>heart</td>
<td>68 ± 11</td>
<td>5.01 &lt;.01</td>
</tr>
<tr>
<td>H(4)</td>
<td>10^-12</td>
<td>liver</td>
<td>101 ± 15</td>
<td>0.12 NS</td>
</tr>
<tr>
<td>H(4)</td>
<td>10^-9</td>
<td>liver</td>
<td>96 ± 8</td>
<td>1.06 NS</td>
</tr>
</tbody>
</table>

Abbreviation: NS, not significant.

*Comparison was carried out by applying the t test for paired observations on colony scores after logarithmic transformation. Colony scores of the control medium ranged from 59 to 499 per plate.
10−9 mol/L, whereas F was ineffective. The major difference between these two preparations was that the step of heat extraction had been omitted with preparation F.

As reported in Table 3, a significant part of the inhibitory activity of preparations D and G on in vitro colony formation was removed by preincubation with the monoclonal antibody 2A4 directed against human heart ferritin.

Overall, nine experiments were carried out with heart ferritin preparations D and G at 10−9 mol/L. With ferritin D, colony formation was 70% ± 10% of the control medium, the difference being significant (t = 5.14, P < .001). With preparation G, colony formation was 68% ± 10% of the control medium, and the difference was again significant (t = 7.40, P < .001). In an attempt to investigate the reproducibility of our studies, we remade two preparations of heart ferritin according to the procedures followed with preparations D and G. The new preparation D1, produced at 10−9 mol/L an average inhibition of 24% ± 9%, which was significant (n = 4, t = 4.80, P < .02). Only two experiments were carried out with the new preparation G1, at 10−9 mol/L, and colony formation was 79% and 68% of the control medium, respectively.

**DISCUSSION**

There is controversy surrounding the role of acidic isoferritins in the control of hematopoiesis. According to Broxmeyer and his associates, the main characteristics of the acidic isoferritins that manifest suppressive activity upon the multipotential, granulocyte-macrophage, and erythroid progenitor cells in vitro are the following: (1) they have a molecular weight of about 550,000 and a pI ranging from 4.6 to 4.9; (2) they bind to Con A and are specifically eluted by α-methyl mannoside; (3) they are inactivated by monoclonal antibodies prepared against human heart ferritin and H subunits from heart ferritin, but not by antibodies against liver ferritin; and (4) they are present in normal monocytes and macrophages and, in greater amounts, in leukemic cells.

Jacobs and his associates have noted a number of inconsistencies in the data of the Broxmeyer group and have raised the following criticisms: (1) tissue ferritins, including those in monocytes and leukemic cells, are nonglycosylated or at least do not bind to Con A; (2) studies on human leukemic cell lines have shown no correlation between the ability of their extracts to inhibit human CFU-GM growth and their content in acidic, H-subunit–rich isoferritins; (3) biologic fluids, including serum, do not normally contain ferritins that react with antibodies against H-subunit–rich isoferritins; (4) AIFIA is reported to be active at concentrations as low as 10−18 mol/L, and this would mean approximately six molecules interacting with one cell within the target system; and (5) more important, the Jacobs group has not been able to demonstrate inhibition of granulocyte-macrophage progenitors by either heart, spleen, or serum ferritin. Although inconsistencies do exist in the data of Broxmeyer and his associates, not all the aforementioned criticisms are entirely valid.

1. Experimental evidence suggests that some 20% of ferritin subunits are made in the polyribosomes attached to the membranes of the endoplasmic reticulum. This is the intracellular site at which export proteins secreted from cells are made and glycosylation takes place. It has also been suggested that some ferritin made by membrane-bound polyribosomes is assembled within vesicles and that this ferritin could be more acidic than the ferritin in the surrounding cytoplasm. Shinjyo et al. reported fucose, mannose, galactose, and hexosamine in crystalline spleen ferritin. Previous studies in our laboratories and some data of the present work show that in human liver, spleen, and heart a minor component of ferritin exists that binds to Con A and is specifically eluted with α-methyl mannoside. This fraction accounts for 2% to 5% of the total tissue ferritin and is generally richer in H subunits than is the total ferritin of the tissue extract.

2. A variety of products from leukemic cells can affect in vitro granulopoiesis. Consequently, there can be no simple relationship between immunologic characteristics of cell ferritin and the ability of cell extracts to inhibit colony formation.

**Table 3. Effects of the Monoclonal Antibody 2A4 Against Human Heart Ferritin on the Inhibitory Activity of Acidic Isoferritins Against Human Granulocyte-Macrophage Progenitor Cells**

<table>
<thead>
<tr>
<th>Ferritin*</th>
<th>Monoclonal</th>
<th>No. of Experiments</th>
<th>Colony Formation as Percentage of Control Medium (Mean ± 1 SD)</th>
<th>Comparison (t Test for Pared Observations)</th>
</tr>
</thead>
<tbody>
<tr>
<td>D</td>
<td>none</td>
<td>5</td>
<td>68 ± 11</td>
<td>t = 5.47, P &lt; .01 when compared with control medium</td>
</tr>
<tr>
<td>D</td>
<td>2A4</td>
<td>5</td>
<td>93 ± 12</td>
<td>t = 4.37, P &lt; .02 when compared with D alone</td>
</tr>
<tr>
<td>none</td>
<td>2A4</td>
<td>5</td>
<td>100 ± 5</td>
<td>t = 1.70, P &gt; .05 when compared with D + 2A4</td>
</tr>
<tr>
<td>G</td>
<td>none</td>
<td>5</td>
<td>67 ± 11</td>
<td>t = 4.96, P &lt; .01 when compared with control medium</td>
</tr>
<tr>
<td>G</td>
<td>2A4</td>
<td>5</td>
<td>85 ± 13</td>
<td>t = 6.91, P &lt; .01 when compared with G alone</td>
</tr>
<tr>
<td>none</td>
<td>2A4</td>
<td>5</td>
<td>100 ± 5</td>
<td>t = 2.35, P &lt; .05 when compared with G + 2A4</td>
</tr>
</tbody>
</table>

*In all experiments, the ferritin concentration was 10−9 mol/L.
3. Normal serum ferritin reacts with antibodies to liver or spleen ferritin, has a very low iron content, and is for the most part glycosylated. Its concentration reflects the amount of storage iron, and it is believed that this protein is synthesized by the rough endoplasmic reticulum of reticuloendothelial cells and hepatocytes. Acidic ferritins are present in many tissues, cells, and some biologic fluids, but cannot be detected in most sera or are present only in low concentrations. A possible explanation for this has been recently advanced by Covell and her colleagues and us. It has been shown in fact that human serum contains binding factors for acidic ferritins, probably related to complement proteins. The interaction of H-subunit–rich ferritin with these binding factors may be responsible for the rapid clearance of tissue ferritin from circulation and the low levels of acidic isoferritins in the plasma of normal subjects. However, it should be noted that this does not rule out the possibility of a role of acidic isoferritins in local cell-to-cell interactions within the bone marrow. In fact, if acidic isoferritins had a local function, there would therefore be mechanisms to make sure that they do not accumulate in plasma and other body fluids. Alternatively, as will be discussed, ferritin-binding proteins may play a role in the interaction of acidic isoferritin with cells.

4. The extremely low effective concentrations of AIFIA (10\textsuperscript{-16} to 10\textsuperscript{-18} mol/L) should not be considered biologic nonsense per se. It has indeed been found that the hematopoietic cell growth factors act at very low concentrations, and in addition, the receptor numbers per cell are extremely low. There is also intriguing evidence that very few receptors need to be occupied to elicit a biologic effect, for example, as few as ten molecules of erythropoietin need to be bound to erythroid need to be bound to erythroid progenitors. Within inhibitors of granulopoiesis, lactoferrin has been found to be effective in inhibiting colony stimulatory activity at concentrations as low as 10\textsuperscript{-11} mol/L by two different groups.

In this work, we have tried to clarify the aforementioned controversy. Our findings clearly show that neither the pure form nor the glycosylated fraction of liver ferritin is able to consistently affect the in vitro growth of human CFU-GM. This is at variance with the findings of Broxmeyer et al, who found that normal human liver and spleen ferritins had inhibitory activity at 10\textsuperscript{-10} to 10\textsuperscript{-15} mol/L. Our data, however, are in keeping with indirect clinical evidence. Patients with thalassemia major who are not regularly treated with desferoxamine may have serum ferritin concentrations as high as 20,000 µg/L (4 × 10\textsuperscript{-8} mol/L), about 50% of the circulating protein binding to Con A (M. Cazzola, unpublished observation). Their total white cell count and differential is either normal or may be slightly elevated, and leukopenia may be observed only as a part of the picture of severe hypersplenism.

The results obtained with heart and HeLa cell ferritins are more intriguing. Only one of the two purified heart ferritin preparations and one of the two glycosylated fractions had a suppressive effect, whereas HeLa cell ferritin did not have any effect. The biochemical and immunologic analyses did not show major differences between active and inactive preparations. Only the differences in purification procedures and degree of glycosylation (Table 1) may explain, at least in part, the different effects on colony formation in vitro. The active purified heart ferritin preparation contained a minor portion (5%) of glycosylated ferritin, whereas the inactive one was fully nonglycosylated, as was the HeLa ferritin preparation. As regards the two glycosylated heart ferritin preparations (F and G), the active one (G) was heated and ultracentrifuged, whereas the inactive one (F) was not. The nonheated preparation, which is known to contain active proteases, could have been partially damaged upon storage, even if phenylmethylsulfonyl fluoride had been added as a protease inhibitor. Inactivation of the suppressive activity of acidic isoferritin preparations D and G by preincubation with the monoclonal antibody 2A4 (Table 3) is one of the strongest indications that the inhibitory activity was associated with the acidic isoferritin molecules.

Although a high proportion of H subunits and glycosylation appeared to be prerequisites for the observed inhibitory effect, this activity appears to be much less potent than the previously reported AIFIA, and its physiologic significance is uncertain. The inhibitory activity was detected by us at concentrations of 10\textsuperscript{-9} to 10\textsuperscript{-12} mol/L, and this means a range from 0.5 to 500 µg/L, assuming a molecular weight of 500,000. We have recently found that most normal subjects have serum concentrations of acidic ferritin lower than 1.5 µg/L (lower limit of detection) and values are generally below 5 µg/L in patients with leukemia. As previously pointed out, however, the low serum concentrations could be in keeping with the hypothesis of a local function.

The mechanism(s) by which acidic isoferritins exert their inhibitory activity are not clear at present. The active isoferritin preparations did not block in vitro growth of CFU-GM completely, the maximum inhibition being about 50%. This could mean that acidic isoferritins act on a subpopulation of human granulocyte-monocyte progenitors having specific receptors. For example, AIFIA has been found to suppress colony formation in vitro by an Ia-like (HLA-DR) antigen–positive subpopulation of cycling human CFU-GM. Alternatively, the possibility exists that ferritin does not have a direct effect on myeloid progenitors but interacts with other active molecules. There has been evidence that complement proteins may be ferritin binders in serum. More recently, we have observed an in vitro interaction between H-subunit–rich isoferritins and the third component of human complement, C3 (V. Bellotti, P. Arosio, and M. Cazzola, unpublished observations). C3 is synthesized not only by the liver but also by macrophages and monocytes, as is the C3 proactivator, factor B. Whether the interaction of acidic isoferritins and C3 can result in a reduced in vitro growth of myeloid progenitors remains to be established. Furthermore, it has been recently shown that lactoferrin can bind to ferritin, the ferritin-lactoferrin complex retaining its capacity of binding to lactoferrin receptors. Since lactoferrin is able to inhibit colony stimulatory activity by binding to specific receptors on monocytes and macrophages, the inhibitory activity of acidic isoferritins in the in vitro system could be explained by their capacity of interacting with lactoferrin.

It is hoped that a deeper insight into the effects of acidic isoferritins on in vitro growth of human myeloid progenitors will be obtained by using recombinant human homopolymers and investigating the aforementioned mechanisms.
REFERENCES


Effect of acidic and basic isoferritins on in vitro growth of human granulocyte-monocyte progenitors

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