ACID ISOFERRITIN INHIBITORY ACTIVITY

To the Editor:

The paper by Lu et al. appearing recently in Blood adds to the already considerable contributions made by this group supporting the hypothesis that acidic isoferritins play an important regulating role for hemopoietic progenitor cells in vitro. While the evidence in favor of this hypothesis has now become substantial, a number of inconsistencies remain and the term "acidic isoferritin" has never been clearly defined in this context. The acidic isoferritins found in nonhemopoietic tissues, such as heart or HeLa cells, contain a high proportion of H subunits, react to an antibody raised against heart or HeLa ferritin, and do not bind to concanavalin-A. Similar isoferritins occur in most hemopoietic cells, including lymphocytes, monocytes, neutrophil polymorphs, erythrocytes, and erythroid blasts. In addition to these, acidic isoferritins, again defined by their isoelectric point, occur in plasma, but these do not normally react with an antibody to heart or HeLa ferritin; they contain a glycosylated subunit, and bind to concanavalin-A-Sepharose. They can be converted to more basic isoferritins by treatment with neuraminidase.

The properties described for the leukemia-associated inhibitory activity (LIA), which appears to have been identified as an isoferritin, are a composite of characteristics of different isoferritins and do not correspond to any species characterized up to now.

If, as Lu et al. suggest, the acidic isoferritin inhibitory activity derives only from normal bone marrow and blood cells of the mononuclear phagocytic lineage, then we have to explain how these specific isoferritins differ from the apparently similar molecules produced by other hemopoietic cells. It is particularly intriguing to speculate on the mode of action of acidic isoferritins on erythroid progenitor cells when recent studies indicate that erythroblasts may contain between 10 and 100 fg of acidic ferritin per cell.

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REFERENCES


To the Editor:

The letter from Professor Allan Jacobs addresses an important question that I and my colleagues have thought about. The question is how the acidic isoferritin inhibitory activity, which suppresses the growth in vitro of a subpopulation of Ia-antigen-positive cycling multipotential (CFU-GEMM), erythroid (BFU-E), and granulo-cyte-macrophage (CFU-GM) progenitor cells, fits in with the known physicochemical and immunologic characteristics of other isoferritins. A leukemia-associated inhibitory activity (LIA), derived from nonadherent, nonphagocytic, low density, Ia-antigen-negative populations of non-T, non-B cells with Fc receptors from patients with leukemia and subsequently found to be present in and released from Ia-antigen-positive monocytes and macrophages of normal donors, was identified by us as acidic isoferritins. This identification was based on the following criteria: LIA was detected in all of the preparations of purified ferritin tested and the activity corresponded best with those samples of ferritin known to be enriched for the acidic isoferritins. The ferritin preparations migrated as single high molecular weight bands (~550,000) on sodium dodecyl sulfate-polyacrylamide gel electrophoresis [SDS-PAGE, without 2-mercaptoethanol (2-ME)], and this was the only region on the gels demonstrating inhibitory activity. Under reducing conditions (plus 2-ME), the high molecular weight band broke down into the characteristic ferritin subunits of 21,000 (H subunit) and 19,000 (L subunit), but we could not detect inhibitory activity in the regions of these lower molecular weight bands elsewhere on these reducing gels. Upon isoelectric focusing (IEF), the inhibitory activity localized to a peak pl value of 4.7, which corresponded to a single band of protein. This band, upon SDS-PAGE (plus 2ME), demonstrated both H and L subunits with a greater proportion of H subunits. The inhibitory activity in the samples was inactivated with antisera specific for ferritin, including those prepared against acidic isoferritins and absorbed with basic isoferritins (pl > 5.6). Antiserum absorbed with acidic isoferritins (pl < 4.9) did not inactivate the inhibitory activity. The inhibitory activity was inactivated with trypsin, chymotrypsin, and pronase, but not with neuraminidase, DNase, RNase, lipase, or phospholipase-C. The glycoprotein nature of the inhibitory activity was established, as it was inactivated by periodate treatment and it bound to concanavalin-A-Sepharose and could be eluted off with a-methyl mannose. Thus, as mentioned by Professor Jacobs, the LIA appears to be a composite of different isoferritins. It should be pointed out, however, that we were measuring a functional activity that could be detected at very low molarities (e.g., <10^-16 M) and studies by others in characterizing ferritins have used as their assay systems physicochemical and/or immunoreactivity with antibodies. We are very interested in defining further the characteristics, both physicochemically and immunologically, of the ferritin molecules that have the capacity to act as a suppressor molecule in vitro, as we have described. This activity may reside in the relative spatial distributions of the H to L subunits and/or the presence or absence of specific sugar moieties on these subunits. It should be emphasized that the ratio or relative concentrations of H to L subunits detected by gel analysis and the relative immunoreactivities of ferritin to heart-type antibodies, which are believed to be detecting mainly H-subunits, and spleen-type antibodies, believed to be recognizing mainly L-subunits, gives little or no information about how the subunits are arranged sterically with regard to each
Acid isoferritin inhibitory activity [letter]

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