ACIDIC ISOFERRITINS AS SUPPRESSOR MOLECULES

To the Editor:

After reading the recent article by Sala et al we felt that several issues required clarification. We have reported a role for acidic isoferritins as suppressor molecules of myeloid colony formation.2-4 Sala et al cast doubt on this role because their own preparations of acidic isoferritins did not demonstrate reproducible inhibitory activity against CFU-GM colony formation. Furthermore, they state that the preparations which we sent them, although containing inhibitory activity, were not pure acidic isoferritins and these latter preparations were not inactivated by a monoclonal antibody (2A4) that recognizes acidic isoferritins.

What is not made clear is that our original studies were done with purified acidic isoferritins,2-4 isolated after isoelectric focusing and with H subunits isolated from pure apo-human liver ferritin provided by another investigator.8 Moreover, two other investigators using our purified preparation of chronic myelogenous leukemia (CML)-spleen acidic isoferritins reported suppressive activity.6,27 At his request, I sent Dr Jacobs preparations which contained acidic isoferritins, had inhibitory activity in our assays, and could be inactivated by preincubation with neutralizing monoclonal antibodies to acidic isoferritins. Two other groups12,13 using their own preparations of purified ferritins have confirmed our reports. Dezza et al11 have found the acidic isoferritin preparations correlated best with acidic isoferritin inhibitory activity. Furthermore, two other investigators using our own acidic isoferritin preparations have demonstrated that the inhibitory activity can be inactivated by neutralizing monoclonal antibodies that recognize acidic isoferritins. Moore et al15 have demonstrated that pure rat liver ferritin suppresses colony formation by an 1A antigen-positive population of monocyte-macrophage progenitor cells, substantiating our earlier report.1 Additionally, Cuervo et al have demonstrated that human placental acidic isoferritins inhibit the entry of normal granulocyte-macrophage progenitors into S phase of the cell cycle and that a similar effect of medium conditioned by the human myeloid leukemic cell line, ML-2, can be inactivated with antibodies to human placental ferritin. The cDNAs for human H- and L-ferritin subunits have been cloned16 and we now have evidence17 that only the purified recombinant acidic H-subunit isoferritins can suppress colony formation of human bone marrow hematopoietic progenitors. This suppressive effect is inactivated by several different neutralizing monoclonal antibodies which recognize acidic isoferritins, but is not inactivated by several monoclonal antibodies that precipitate purified basic ferritins.

With regard to the data generated by Sala et al1, the following comments are noted. While in Cardiff, Sala et al were not able to demonstrate inhibitory activity with our preparations (Table 1), although they were able to demonstrate inhibitory activity with prostaglandin E2 (PGE2), and with medium conditioned by K562 and U937 cells. The data in Table 1 from Sala et al contain very large standard deviations in percent change from control within an individual experiment which could mask the type of inhibition noted with acidic isoferritins. In this context it should be realized that it is much easier to detect an inhibition with PGE and extracts or conditioned medium from certain established cell lines because these materials decrease both the size and the number of the colonies and clusters. This is not the case with the acidic isoferritins, which exert their suppressive effects only on colony-forming cells in S phase (usually 25% to 50%) and which do not decrease the size of the colonies and clusters.24,25 The data shown in Table 1 from Sala et al reflect the relative insensitivity of the assay used in Cardiff, since PGE2 was active against human bone marrow cells at concentrations only as low as 10-8 mol/L. This is two to four log units below the activity of PGE noted against human bone marrow cells by us18 and by others.19,20 The same ferritin samples purported to be inactive in assays performed in Cardiff (Table 1, Sala et al) were found to be active when assayed by Sala et al in my laboratory (Table 5, Sala et al), even though substantial variability within an experiment is still apparent from the standard deviations. It is stated that the data in Table 6 from Sala et al demonstrate that the inhibitory activity of our samples is not inactivated after preincubation with monoclonal antibody 2A4. However, when compared to control values, the CML-spleen ferritin significantly suppressed colony formation, and after preincubation with monoclonal antibody 2A4, the effects of ferritin-containing preparation were not significantly different from control.

My comments to some of the statements made in the introduction to the article by Sala et al are as follows: First, our original studies suggested that the inhibitory activity of acidic isoferritins was due to a glycosylated molecule,2 yet Sala et al suggest that only a small percentage of acidic isoferritin molecules are glycosylated, although they do point out that the evidence about glycosylation of ferritin is contradictory. Of relevance here is that Dezza et al have found the activity of their acidic isoferritin preparations correlated best with glycosylated molecules. Our recent studies using purified recombinant acidic isoferritins suggest that glycosylation is not an absolute requirement for suppressive activity. Second, acidic isoferritin inhibitory activity could be detected by us only in extracts and conditioned medium of monocytes and macrophages,2-4 yet Sala et al point out correctly that T lymphocytes and neutrophilic granulocytes contain ferritin with a greater ratio of H-subunits than L-subunits. We have pointed out the difficulties of attempting to characterize acidic isoferritins only on the basis of the ratio of H- to L-subunits,21 and ferritin has not yet been purified from blood cells. Thus, there may be differences in the acidic isoferritin molecules obtained from cells of these different lineages.

Third, we detected acidic isoferritin inhibitory activity in extracts and conditioned medium of HL-60 cells22 but not in K562 cells.3 Sala et al point out that HL-60 ferritin has a low H-subunit content
while K562 ferritin has a high H-subunit content. It is important to point out that different subsets of noninduced HL-60 cells have ferritins differing in the ratio of H-subunits to L-subunits.\(^2\) We found that noninduced HL-60 cells containing ferritin with a high H-to-L-subunit ratio contained more acidic isoferritin inhibitory activity than noninduced HL-60 cells containing ferritin with a higher ratio of L- to H-subunits.\(^21\) Furthermore, K562 cells produced a potent inhibitory activity not inactivated by heteroantibodies to acidic isoferritins.\(^3\) Until this inhibitory activity is separated from ferritin, it is not possible to state whether or not K562 cells contain and release acidic isoferritin inhibitory activity.

Fourth, Sala et al.\(^1\) show in Table 7 of their paper that immunologically reactive ferritin can be found in medium conditioned for 18 hours by bone marrow cells. I assume they are trying to make the point that acidic isoferritins released endogenously should mask an effect of exogenously-added acidic isoferritins. Their studies are consistent with our own data demonstrating release of acidic isoferritin-inhibitory activity from monocytes into conditioned medium within 12 to 24 hours.\(^6,8\) However, pulse exposure of bone marrow cells for an hour or less with acidic isoferritins results in maximal inhibition and, within three to five hours of culturing cells at 37°C, the cells become insensitive to inhibition by acidic isoferritins\(^4,11\) unless they are reinduced into a state of responsiveness.\(^1\) Thus, ferritin released by 12 to 18 hours would not interfere with suppression due to acidic isoferritins added exogenously at an earlier time.

In summary, I believe that the evidence still supports a role for acidic isoferritins as suppressor molecules of in vitro colony formation by normal myeloid progenitor cells.

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REFERENCES

Acidic isoferritins as suppressor molecules [letter]

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