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Acidic Isoferritins as Feedback Regulators in Normal and Leukemic Myelopoiesis*

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A. Background on Leukemia-Associated Inhibitory Activity

The absence of normal hematopoiesis during acute leukemia not in remission and the recovery of apparently normal blood cells during chemotherapy-induced remission suggest that suppressive cell interactions may be involved in the pathogenesis of acute leukemia. Others have shown inhibition of normal progenitor cell proliferation and differentiation by cells from patients with leukemia, but little or no information was provided regarding the actual characterization of the inhibitory cells or the mechanisms of action (Broxmeyer and Moore 1978). We have demonstrated the existence of an S-phase specific inhibitory activity [leukemia-associated inhibitory activity (LIA)] against normal granulocyte-macrophage progenitor cells (CFU-GM) which was produced by bone marrow, spleen, and blood cells from patients with acute and chronic myeloid and lymphoid leukemia and "preleukemia" (Broxmeyer et al. 1978a,b, 1979a,b). Greater concentrations of LIA were found during acute leukemia (newly diagnosed and untreated, or on therapy but not in remission) than during chronic leukemia (Broxmeyer et al. 1978a,b). Remission of acute leukemia was associated with low levels of LIA (Broxmeyer et al. 1978b, 1979a), and LIA at that time was not found in hemopoietic cells from normal donors (Broxmeyer et al. 1978a,b, 1979a,b). In contrast to its action on normal CFU-GM, LIA was not effective in suppressing the growth of normal CFU-GM from patients with acute leukemia who were not in remission and from many patients with acute leukemia during remission and with chronic leukemia (Broxmeyer et al. 1978b, 1979a,b). We postulated that LIA may thus confer a proliferative advantage to abnormally responsive cells (Broxmeyer et al. 1978b).

We have now documented these inhibitory interactions in neonatal and adult Balb/c mice infected with Abelson virus (Broxmeyer et al. 1980). Within 2–4 days after virus infection, the CFU-GM from bone marrow and spleen became insensitive to inhibition by human LIA and by mouse LIA-like material, even though colony morphology appeared normal. Shortly after or simultaneously with the detection of the colony forming cell resistance phenomenon LIA was found in bone marrow, spleen, and thymus cells. The abnormal interactions appeared to be related to induction of lymphoma in Balb/c neonates and to a lymphoproliferative disease in adult Balb/c mice. In contrast, normal cellular interactions were noted in adult C57B1/6 mice which were not susceptible to Abelson disease after virus inoculation and in untreated neonatal and adult Balb/c and adult C57B1/6 mice. Their CFU-GM were sensitive to inhibition by LIA and no LIA-like material was detected in their bone marrow, spleen, and thymus cells. We have also noted LIA interactions in mice given Friend virus (SFFV plus helper) (L. Lu and H. E. Broxmeyer, unpublished work) and others have developed a model for LIA based on induction of leukemia in mice with the RFV strain Friend virus, the spontaneous regression of the disease and its recurrence (Marcelletti and Furmanski 1980).

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B. Isolation, Characterization and Identification of LIA as Acidic-Isoferritins

After isolating and characterizing LIA (Bognacki et al. 1981) we noted that it was similar to a subclass of ferritins (Drysdale et al. 1977). The LIA to be purified was pooled from more than 5000 samples of extracts from bone marrow, spleen, and blood cells collected over a 5-year period from more than 1000 different patients with all types of acute and chronic leukemia and at all stages of disease progression. LIA was isolated by a combination of procedures including ultracentrifugation, Sephadex G-200, carboxymethyl cellulose, SDS-polyacrylamide gel electrophoresis, analytical and preparative isoelectric focusing, and Concanavalin A Sepharose (Bognacki et al. 1981). LIA had an apparent molecular weight of $\sim$550,000 and a pI of 4.7 and copurified with the acidic isoferritins. LIA was detected in all the ferritin preparations tested (Broxmeyer et al. 1981). Additionally, purified preparations of LIA were composed almost entirely (>90%) of acidic isoferritins as determined by radioimmunoassay and isoelectric focusing, and the inhibitory activity in the LIA and ferritin samples was inactivated by a battery of antisera specific for ferritins, including those prepared against acidic isoferritins from normal heart and spleen tissues from patients with Hodgkin's disease (Broxmeyer et al. 1981). LIA and the acidic isoferritin-inhibitory activity had similar physico-chemical characteristics as treatment with trypsin, chymotrypsin, pronase, and periodate, and breakdown of the ferritin into subunits by reduction inactivated the inhibitory activity. DNase, RNase, neuraminidase, lipase, phospholipase C, iron depletion, and heat treatment (75°C for 20 min) did not inactivate the activity (Broxmeyer et al. 1981). Inhibitory activity was detected at concentrations as low as 10^{-17} to 10^{-19}M and all samples were inactive against CFU-GM from patients with nonremission acute leukemia. A similar curve of inhibition was noted when mouse ferritin was assayed against mouse CFU-GM. The human and mouse acidic isoferritin inhibitory activity suppressed colony formation of cells giving rise to colonies containing purely granulocytes, macrophages, eosinophils, or mixtures of granulocytes and macrophages (L. Lu and H. E. Broxmeyer, unpublished work).

We were not able previously to detect LIA in bone marrow and blood cells from normal donors, but we have now found it in heart, spleen, placentae, and liver ferritin isolated from normal individuals (Broxmeyer et al. 1981) and mice. This was probably due to a combination of factors. Acidic isoferritins are elevated in leukemia and lymphoma but are in very low concentrations in bone marrow, blood cells, and serum from normal donors. Additionally, we now have evidence that medium conditioned by normal human bone marrow and blood monocytes, placental cells, mouse macrophages and WEHI-3 cells, which are used to stimulate colony formation, contain acidic isoferritins. Removal of the ferritins by preincubation with antisera to acidic isoferritins or by passing the material over Sepharose 6B columns or over columns to which ferritin antibodies have been fixed to Sephadex beads by CNBr enhances the stimulatory capacity of the conditioned medium by 50% to 100%. Not surprisingly, acidic isoferritins (LIA) demonstrate greater inhibition of colony and cluster formation (e.g., 60% inhibition vs 40%) when the preparations free of ferritin are used to stimulate CFU-GM. The relevance in vivo of acidic isoferritins as regulators of myelopoiesis is still to be determined, but the low concentrations needed for activity on the progenitor cells in vitro (10^{-17} to 10^{-19}M) suggest that they may be of importance as physiologic regulators, a role we have postulated previously for lactoferrin (Broxmeyer et al. 1979c), an iron-binding glycoprotein which acts on factor production rather than on the progenitor cells.

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