CONCISE REPORT

Eosinophilic Cytoplasmic Inclusions in Fetal Leukocytes: Are Auer Bodies a Recapitulation of Fetal Morphology?

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Among the most striking morphological features of acute nonlymphoblastic leukemias (ANLL) is the occurrence of eosinophilic cytoplasmic inclusions known as Auer rods on Auer bodies. We examined immature myeloid cells from the peripheral blood of 9 human fetuses of 16–19 wk gestation for the presence of such structures. Five of these 9 samples contained cytoplasmic inclusions, which were identical to the Auer rods typically seen in blast cells from patients with ANLL. The incidence of positive cells was low (1–5 cells/10,000 cells surveyed). The inclusions were azurophilic with Wright-Giemsa staining and were cytochemically positive with peroxidase, acid phosphatase, and Sudan black staining. We observed no inclusions in identically prepared control myeloid cells from the bone marrow of 5 patients with acute lymphoblastic leukemia in remission and 3 patients with chronic myelogenous leukemia in stable phase. Nor were they present in peripheral blood myeloid cells of 10 normal adults. Myeloid precursors in long-term bone marrow culture from 2 normal adult donors did not develop the inclusions during 24 hr of incubation with prostaglandin F_2 (the abortifacient). These observations suggest that Auer rod formation is an occasional but normal phenomenon in fetal hematopoiesis.

HUMAN LEUKEMIAS are a heterogeneous group of diseases of leukocyte growth and differentiation.[1] Identification and classification of leukemic cells is based on a wide variety of morphological, cytochemical, immunologic, cytogenetic, and biochemical characteristics.[2] Among the most striking morphological features of acute nonlymphoblastic leukemias is the occurrence of cytoplasmic inclusions known as Auer rods or Auer bodies. First described by John Auer in 1906,[3] these eosinophilic, cytoplasmic structures vary from round to needlelike in shape, but classically assume a rodlike structure. They can be identified in blast cells of 50%–60% of patients with acute myeloid leukemias and may define the group most responsive to some currently used chemotherapy.[4] Although sometimes readily apparent, Auer bodies are usually found in only a small proportion of leukemic cells; their detection requires the scanning of a minimum of 500 cells and often many more.[4] They are sought with uncommon diligence because of the virtually universal view that Auer bodies are a leukemia-specific finding, pathognomonic of the diagnosis of acute nonlymphocytic leukemia.[5] To date, they have never been reported to be present in normal bone marrow. Since a variety of other markers of malignant cells, at first thought to be specific for neoplasia, have subsequently been shown to be expressed in normal fetal or embryonic tissues,[6-8] we examined human fetal peripheral blood, which normally contains many immature myeloid forms,[9] for the presence of Auer rods. We report the presence in these cells of cytoplasmic inclusions, which resemble by morphological and histochemical appearance the Auer bodies of myeloblastic leukemia cells.

MATERIALS AND METHODS

Dr. David Kurnit (Children's Hospital Medical Center, Boston, Mass.) and Dr. Paul Rosenthal (Sidney Farber Cancer Institute) provided peripheral blood from 9 human fetuses at 16–19 wk gestation (calculated from crown-rump lengths) drawn immediately following abortion induced by intraamniotic administration of prostaglandin $F_{2\alpha}$.[10] No fetus was known to be at risk for any congenital disorder.

Leukocytes were prepared by sedimentation in 1% Dextran T500 (Pharmacia Fine Chemicals, Piscataway, N.J.) and brief (2–5 sec) hypotonic lysis of erythrocytes,[11] washed with phosphate-buffered saline, pH 7.4, resuspended in 0.2 ml of 5% human serum albumin, and cytocentrifuged. Alternatively, buffy coat cells were directly cytocentrifuged. Cell yields were 5×10^3 to 4×10^4 per fetus, depending on the size of the original sample and its nucleated cell count. Slides were prepared by cytocentrifugation of 10^3–10^4 nucleated cells/glass slide, then fixed and stained by standard Wright-Giemsa, Sudan black B, acid phosphatase (azo dye method) and Graham-Knoll peroxidase techniques.[12] Slides were reviewed by three observers. All cells were surveyed and appropriate fields photographed through a Zeiss light microscope. Differential counts were in the range of those previously reported:[9] 1%–9% myeloid cells, 10%–70% of which were blasts or promyelocytes.

To control for the effect of prostaglandin exposure and sample processing on the development of Auer rods, several control samples were studied. Peripheral blood was drawn from 10 normal volunteers and bone marrow aspirates obtained for routine surveillance from 5 patients with acute lymphoblastic leukemia in remission and 3 with chronic myelogenous leukemia in stable phase. Slides (at least 5 for

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Supported in part by Grant AM31097 from the National Institutes of Health, by a Charles H. Hood Foundation Child Health Research Grant, and by Grant IN-129 from the American Cancer Society.

Submitted August 20, 1982; accepted October 1, 1982.

0006-4971/83/6103-0028$01.00/0

each subject) were prepared identically to those from fetal samples. Bone marrow for long-term cultures was harvested from femoral heads removed for total hip replacements then processed and grown in vitro by a previously described modification[13] of the Dexter[14] method. Cultures 6 wk after establishment were exposed for 24 hr to prostaglandin $F_{2\alpha}$ (tromethamine salt, the Upjohn Co., Kalamazoo, Mich.) 50 μg/ml, a maximal fetal level,[15] then harvested. Peripheral blood was obtained from normal adult laboratory personnel. Procedures and consents were approved by the Research Advisory or Human Subjects committees of either the Brigham and Women's Hospital, Children's Hospital Medical Center, Sidney Farber Cancer Institute, or New England Baptist Hospital, as appropriate for the location of the procedure.

RESULTS

Figure 1A illustrates typical Auer rods in a myeloblast from a patient with acute myeloid leukemia. Five of the nine fetal blood samples contained myeloid cells with similar cytoplasmic inclusions, such as that shown in Fig. 1B. The inclusions occurred in 1–5 cells/10^4 surveyed, usually in myeloblasts but also (very rarely) in more mature myeloid cells. Histochemical staining showed peroxidase activity (Fig. 1C), acid phosphatase (D), and Sudan black staining (E)—all characteristics of Auer bodies.[12] Neither the presence of the inclusions nor their frequency correlated with fetal gestation age.

Control samples were studied to test for the effect of prostaglandin exposure and in vitro manipulations. We observed no inclusions in identically prepared myeloid cells from the bone marrow of five patients with acute lymphoblastic leukemia in remission and three patients with chronic myelogenous leukemia in stable phase. Nor were they present in peripheral blood myeloid cells of ten normal adults. Myeloid precursors in long-term bone marrow culture[13,14] from five normal adult donors did not develop the inclusions during 24 hr of incubation with prostaglandin $F_{2\alpha}$.

DISCUSSION

The cytoplasmic inclusions we have observed in fetal myeloid precursors are identical morphologically and histochemically to Auer rods of acute myeloblastic leukemia. As in leukemia cells, these fetal inclusions assume a variety of shapes. Their rarity (1–5/10^4 cells) precluded electron microscopic examination of ultrastructure.

Our observations suggest that formation of Auer bodies is an occasional but normal part of fetal myelopoiesis, which may be reactivated in acute myeloblastic leukemia. Expression of fetal phenotypic characteristics by neoplastic cells occurs with some solid tumors. Various carcinoma cells produce carcinoembryonic antigen,[6] α-fetoprotein,[7] or fetal sulphoglycoprotein.[8] Other tumors such as embryonal rhabdomyosarcoma, hepatoblastoma, neuroblastoma, Wilm's tumor, and endodermal sinus tumor show fetal or embryonal morphological characteristics. The mechanisms of such phenotypic expression are unknown. The formation of Auer bodies could be a characteristic of a subset of myeloid precursors that are present in the fetus and diminish to an undetectable number later in gestation (so called F-myeloid cells analogous to F-red cells[16]), but clonogenically expand in some cases of nonlymphocytic leukemia. The reported,[4] but still controversial,[17] positive prognostic value of Auer bodies in acute myeloid leukemias could derive from a difference in the sensitivity to chemotherapy of this cell type relative to that from which Auer-body-negative acute myeloid leukemias derive. Alternatively, Auer body formation in leukemia cells could represent transformation-induced expression of a fetal gene.

Auer bodies probably form by fusion of primary (azurophilic) granules;[5] the signal or mechanism for this process is not known. We considered the possibility

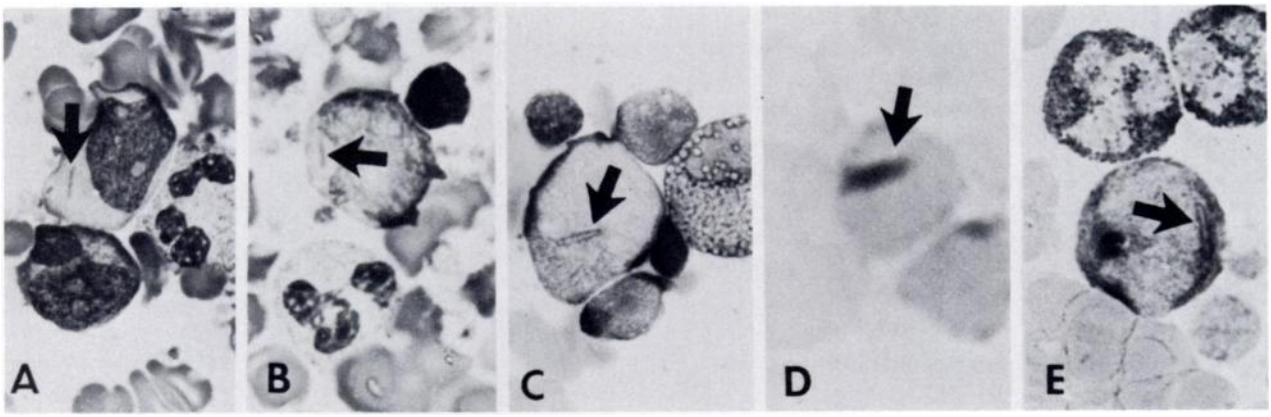

Fig. 1. Photomicrographs of myeloblasts from acute myeloid leukemia and from fetal peripheral blood. (A) Acute myeloid leukemia, Wright-Giemsa stain. (B) Fetal blood, Wright-Giemsa stain. (C) Fetal blood, peroxidase reaction. (D) Fetal blood, acid phosphatase. (E) Fetal blood, Sudan black stain. Arrows indicate cytoplasmic inclusions. Magnification ×1000.

that the abortion procedure could artifactually cause Auer body formation in fetal cells that would normally not contain them. The most likely culprit would be the inducing agent, prostaglandin $F_{2\alpha}$. Fusion of primary and secondary granules in Chediak-Higashi's syndrome leukocytes is associated with high intracellular cyclic AMP levels.[18] However, F-type prostaglandins, unlike E-type, usually do not interact with adenyl cyclase, and in fact, tend to counteract cyclic AMP effects on degranulation and cell motility.[19] We therefore deliberately attempted to induce Auer rods with $PGF_{2\alpha}$. We did not observe granule fusion products or other inclusions in the myeloid cells of long-term human bone marrow cultures incubated with prostaglandin $F_{2\alpha}$ at the expected concentration and duration of the fetal exposure. The possibility of artifactual inclusion formation during hypotonic lysis[20] of contaminating erythrocytes or during fixation and staining was ruled out by our findings in identically prepared myeloid cells from normal peripheral blood and from bone marrow of patients with acute lymphoblastic leukemia in remission and chronic myelogenous leukemia in stable phase. In none of these were inclusions induced by vitro manipulations.

We suggest that the presence of Auer bodies in fetal leukocytes may indicate that these structures, previously considered leukemia-specific, may actually be only leukemia-associated. A recent report of Auer bodies in lymphoid malignancies[21] suggests that the association may not even be strictly exclusive to myeloid leukemias. Like many other biochemical and histochemical signs of neoplasia,[6-8] they may represent a recapitulation of a stage of fetal development that is undetectable postnatally except during neoplastic cellular proliferation.

ACKNOWLEDGMENT

We thank Drs. Howard J. Weinstein, David G. Nathan, and James B. Hanshaw for advice and manuscript review; Nancy Boardman and Angela Nardella for technical assistance; and Denise Maclachlan for manuscript preparation.

REFERENCES

1. Berard CW: Current concepts of leukemia and lymphoma: Etiology, pathogenesis, and therapy. Ann Intern Med 85:351, 1976

2. Gralnick HR: Classification of acute leukemia. Ann Intern Med 87:740, 1977

3. Auer J: Some hitherto undescribed structures found in the large lymphocytes of a case of acute leukemia. Am J Med Sci 131:1002, 1906

4. Mertelsman R, Thaler HT, To L, Geets, MacKenzie S, Schaur P, Friedman A, Arlin Z, Cirrincione C, Clarkson B: Morphological classification, response to therapy, and survival in 263 adult patients with acute nonlymphoblastic leukemia. Blood 56:773, 1980

5. Freeman JA: Origin of Auer bodies. Blood 27:499, 1966

6. Abelev GI: α-Fetoprotein as a marker of embryo-specific differentiations in normal and tumor tissues. Transplant Rev 20:3, 1974

7. Gold P, Shuster J, Freedman SO: Carcinoembryonic antigen (CEA) in clinical medicine. Cancer 42:1399, 1978

8. Hakkinen I, Viikari S: Occurrence of fetal sulphoglycoprotein antigen in the gastric juice of patients with gastric diseases. Ann Surg 169: 277, 1969

9. Playfair JHL, Wofendale MR, Kay HEM: The leukocytes of peripheral blood in the human fetus. Br J Haematol 9:336, 1963

10. Stubblefield PG, Naftolin F, Frigoletto F, Ryan KJ: Laminaria augmentation of intra-amniotic $PGF_{2\alpha}$ for midtrimester pregnancy termination. Prostaglandins 10:413, 1975

11. Stossel TP: Evaluation of opsonic and leukocyte function with a spectrophotometric test in patients with infection and with phagocytic disorders. Blood 42:121, 1973

12. Hayhoe FGJ, Quaglino D: Haematological Cytochemistry. Edinburgh, Churchill Livingstone, 1980, p 68

13. Greenberg HM, Newburger PE, Parker LM, Greenberger JS: Human granulocytes generated in continuous bone marrow culture are physiologically normal. Blood 58:724, 1981

14. Dexter TM, Testa NG: Differentiation and proliferation of hemopoietic cells in culture, in Prescott D (ed): Methods in cell Biology, vol 14. New York, Academic, 1976, p 387

15. Green K, Granstrom E, Bygdeman M, Wiqvist N: Kinetic and metabolic studies of 15-methyl-prostaglandin $F_{2\alpha}$ administered intra-amniotically for induction of abortion. Prostaglandins 11:699, 1976

16. Boyer SH, Belding TK, Margolet L, Noyes AN: Fetal hemoglobin restriction to a few erythrocytes (F cells) in normal human adults. Science 188:361, 1975

17. Sultan C, Deregnaucourt J, Ko YW, Imbert M, Ricard D'Agay MF, Gouault-Heilman M, Brun B: Distribution of 250 cases of acute myeloid leukemia (AML) according to the FAB classification and response to therapy. Br J Haematol 47:545, 1981

18. Rausch PG, Pryzwansky KB, Spitznagel JK: Immunohistochemical identification of azurophilic and specific granule markers in the giant granules of Chediak-Higashi neutrophils. N Engl J Med 298:693, 1978

19. Zurier RB, Weissman G, Hoffstein S, Kammerman S, Tai HH: Mechanisms of lysosomal enzyme release from human leukocytes. II. Effects of cAMP and cGMP, autonomic agonists, and agents which affect microtubule function. J Clin Invest 53:297, 1974

20. Archer GT, Blackwood A: Formation of Charcot-Leyden crystals in human eosinophils and basophils and study of the composition of isolated crystals. J Exp Med 122:173, 1965

21. Jehn U, Thiel E: Auer bodies in acute lymphocytic leukemia and B-cell lymphoma: Evidence for a common progenitor of myeloid and lymphoid cells. Blut 43:7, 1981