Hand-Mirror Cell Leukemia Associated with Mental Retardation: Immunologic, Chromosome, and Morphological Studies


Cytochemical, morphological, immunologic, and cytogenetic studies were carried out on hand-mirror cells (HMC) from a mentally retarded patient with a constitutional chromosome abnormality, 46,XX.r(21), and acute lymphoblastic leukemia. Scanning electron and differential interference contrast microscopy showed microspikes on the uropodia, but little evidence of cellular motility, despite formation and disappearance of individual uropodia in cell suspensions. The cells rosetted with sheep erythrocytes, suggesting T-cell origin. Cells derived from the bone marrow (80% HMC) showed a high degree of polyploidy (80%) and a bimodal chromosome number of 49 (49,XX,+10.−21.+3 rings) and 94 (6 no. 10, 3 no. 18, 2 no. 21 chromosomes, 3 ring chromosomes, plus 4 copies of each other chromosome).

Hand-mirror cells have been seen in patients with acute lymphocytic leukemia (ALL) and AML and have been associated with a prolonged clinical course, despite remarkable resistance to therapy. Ultrastructural studies have revealed the hand-mirror shape in the nuclear as well as the cytoplasmic portion of the cell; the presence of glycogen, polyribosomes, and increased numbers of mitochondria in the uropod; and microspikes on the surface of the uropod.

A detailed study of immunologic surface membrane markers, deoxyribonucleotidyl transferase, cytochemistry, scanning and electron microscopy, and cytogenetics of the HMC from a patient with a hand-mirror variant of ALL has recently been reported. It was concluded that the HMC is a lymphoblast with distinctive morphological, cytochemical, and immunologic features. The cells failed to grow in culture, and chromosome analysis showed a normal karyotype. We report a study of a mentally retarded girl with hand-mirror cell type ALL, a constitutionally abnormal karyotype, and acquired chromosome aberrations in the bone marrow.

From the Departments of Medicine and Pathology of the Dartmouth–Hitchcock Medical Center, and the Department of Biology, Dartmouth College, Hanover, N.H.

Supported in part by NCI Grants CA 23102, CA 55199, and CA 03426, the York Cross of Honour Research Foundation, and National Institute of General Medical Science Grant GM-22356.

Submitted February 8, 1979; accepted May 24, 1979.

Address reprint requests to Doris H. Wurster–Hill, Ph.D., Department of Pathology, Dartmouth Medical School, Hanover, N.H. 03755.

© 1979 by Grune & Stratton, Inc.
CASE REPORT

C. F. was a 12-yr-old mentally retarded white female, who was the product of a 38-wk pregnancy and uncomplicated delivery. The mother was 19 and the father 22 yr old at the time of her birth. Her birth weight and length were 2620 g and 48 cm, respectively. There were no viral infections or drug usage during the pregnancy and no family history of congenital defects, cancer, leukemia, or neurologic disease. There was one normal younger sister, and a normal half-sister from her father's previous marriage. No consanguinity could be ascertained.

Mental development was retarded. The patient sat at 8 mo, walked at 15 mo, talked at 3½ yr (single words), and at 9 yr still had poor urinary control. At the age of 12, her reading and mathematical abilities were at third and first grade levels, respectively. In March 1975, at age 11, she had a grand mal seizure with tonic-clonic movements, oculogyral movements, and incontinence. Meparal was substituted for seizure control following skin rash and fever associated with phenobarbital and dilantin.

Two months later, she was first noted to have neutropenia, $1.2 \times 10^9$/liter, which became progressively more severe and gradually evolved over 18 mo into acute leukemia. When first referred to this center in November 1976, the patient had multiple infections and a low-grade fever. Six months later, she was in the 40th percentile for height and weight. Physical examination showed a first branchial cleft defect with a short mandible, a deformed tube-like left auricle, microtia, stenosis of the ear canal, left hearing loss, and left scoliosis of the spine. There were a few small submandibular and anterior cervical lymph nodes. The spleen and liver were felt 2 and 4 cm below the left and right costal margins, respectively.

The hemoglobin was 6.6 g/dl, the white blood cell count $2.2 \times 10^9$/liter with 2% neutrophils, 2% eosinophils, 88% lymphocytes (of which 15% were lymphoblasts), and 8% monocytes. The platelet count was $8.5 \times 10^9$/liter. The bone marrow was hypercellular and showed a predominance (80%) of lymphoblasts with single prominent cytoplasmic uropodia resembling hand mirrors. The other normal hematopoietic elements were decreased. Silver staining showed increased reticulum. The serum muramidase was normal. Examination of cerebrospinal fluid did not reveal any white cells. She was transfused with packed cells and was started on vincristine, 2 mg i.v., once a week, and prednisone, 54 mg p.o. daily, in accordance with Cancer and Leukemia Group B (CALGB) protocol no. 7611 for acute lymphoblastic leukemia. Despite 4 weekly courses of vincristine, prednisone, and intrathecal methotrexate, followed by t-asparaginase (1000 U/kg i.v. daily for 3 days), her bone marrow did not improve, and therapy was discontinued. Despite generalized seizures, her clinical condition remained stable for 7 mo; she then developed leukemic meningitis, requiring therapy with dexamethasone, intermittent intrathecal ara-C, and whole-brain irradiation. She failed to respond and died October 8, 1977 with Escherichia coli septicemia 4 mo later. Post-mortem examination showed leukemic infiltration of the pericerebellar region and acute subarachnoid hemorrhage.

MATERIALS AND METHODS

Comparison of Fresh and Frozen Cells

Preliminary comparative studies of differential interference contrast studies, scanning electron microscopy, histochemistry, and cell surface markers were carried out on fresh cells and cells stored in liquid nitrogen. The freezing process did not influence the results of these studies on peripheral blood lymphocytes, lymphoblasts, or hairy cells. Consequently, except where noted otherwise, all studies described below were performed on cells stored in liquid nitrogen.

Morphological Studies and Histochemical Stains

In addition to the usual examination of the bone marrow, cell suspensions from the marrow and peripheral smears were stained for myeloperoxidase,7 with periodic acid Shiff stain,8 acid phosphatase, alpha naphthyl acetate esterase, and naphthol AS-D chloracetate esterase.9,10

Cell Surface Marker Studies

Suspensions of cells from peripheral blood were examined for sheep erythrocyte receptors by rosette formation with unsensitized sheep erythrocytes (E), for surface immunoglobulins (SIg) by immunofluorescence with monospecific antisera against heavy chains, light chains, and polyvalent immunoglobulin, and for complement receptors by the EAC rosette assay.11,12
Differential Interference Contrast Studies

One drop of a cell suspension of peripheral blood cells, containing lymphocytes and lymphoblasts suspended in fetal calf serum, was placed on a 24 × 60 mm glass slide, pre-treated with 0.2 ml of 1% polylysine solution and attached with tape to a stainless steel holder. The cells were covered with a 22 × 22 mm cover glass and sealed with valap (1:1:1 mixture of vaseline, lanolin, and paraffin). The cells were then allowed to settle for 20–30 min at 37°C. The preparation was then viewed with a Zeiss inverted Axiomat microscope with high extinction differential interference attachments. Photomicrographs were recorded on Kodak S.O. 115 film.

Scanning Electron Microscopy

Peripheral blood lymphocytes prepared and attached to slides, as described above, were rinsed in phosphate-buffered saline at 37°C, fixed in 1.5% buffered glutaraldehyde pH 7.2, and postfixed in 2% osmium tetroxide for 30 min. The cells were again rinsed in buffer and dehydrated stepwise in increasing concentrations of ethanol. The slides were transferred to the chamber of a Bomar carbon dioxide critical point drying apparatus and processed. Afterward, they were mounted on aluminum stubs and vacuum sputter coated with gold-palladium. The specimens were examined with a Coates & Welter Model 106A Cwikscan scanning electron microscope using an accelerating voltage between 10 and 15 kV, and tilt angle of 45°–50°. The micrographs were recorded on polaroid type 55 P/N film.

Cytogenetics

Direct chromosome preparations were made in June 1977 from a fresh bone marrow aspirate, showing 58% lymphoblasts, 22% lymphocytes, 8% myeloid, and 9% erythroid, according to a technique described previously. The patient had not had any therapy during the previous 5 mo. White cells from peripheral blood, containing 80% lymphoblasts, which had been stored in liquid nitrogen for 14 mo, were thawed, and examination by light microscopy showed that there had been no significant change in cellular morphology as a result of the freezing and storing procedures. These cells were cultured in Eagle's basal medium with calf serum (20%), antibiotics, and phytohemagglutinin (approximately 10⁶ cells/5 ml medium) for 72 hr, and subsequently harvested in the same manner as whole blood cultures following a 2-hr incubation period with colchicine. G-banding of the chromosomes was accomplished with the trypsin technique of Seabright, using Giemsa instead of Leischmann's stain. Chromosomes of the parents and siblings were studied.

RESULTS

Morphological and Histochemical Studies

Light microscopy. Hand-mirror cells aspirated from the bone marrow had an average length of 18 μm (range 14–22) and a width of 8–10 μm. The nucleus was spheroid or ovoid and occupied 75%–85% of the cell volume. Preparations stained with Wright's stain showed a fine nuclear chromatin pattern with 2–3 nucleoli (Fig. 1). The cytoplasm in the uropod was hyaline and light blue in color. The cells had negative staining characteristics for the following histochemical studies: myeloperoxidase, periodic acid Schiff stain, acid phosphatase, alpha naphthyl acetate esterase, and naphthol AS-D chloroacetate esterase.

Cell surface markers. The results of the surface marker studies of peripheral blood containing 80% HMC lymphoblasts were as follows: E's 77%, polyvalent immunoglobulin 17.5%, IgG 4.5%, IgM 12.5%, IgA 0.5%.

Differential interference contrast. This method demonstrated the hand-mirror shape well and showed 1–3 uropodia on individual cells (Fig. 2). During a 4-hr observation period, the cells were generally stationary, although individual uropodia were sometimes observed to appear and disappear.

Scanning electron microscopy. The surface of the HMC contained irregular
Fig. 1. Bone marrow smear demonstrating the hand-mirror cell configuration of two lymphoblasts. Note the microspikes on the uropod and the hyaline cytoplasm in the uropod (original magnification ×1600).

Fig. 2. Cell suspended in serum in the process of uropod formation (original magnification ×1600).
Fig. 3. Scanning electron micrograph. Notice the irregular short projections (p) on the surface (original magnification ×7160).

Fig. 4. Scanning electron micrograph. Notice the irregular microspikes (original magnification ×7760).
short projections (Fig. 3). The uropodia of several cells had microspikes attached to the substratum (Fig. 4).

**Cytogenetics.** Sixty percent of dividing cells in the bone marrow aspirate were polyploid. A count of 31 cells revealed a bimodal distribution with modes of 49 and 94 chromosomes. The modal karyotype with 49 chromosomes had trisomy 10, monosomy 21, and 3 ring chromosomes of undetermined origin (49,XX,+10,-21,+3 rings) (Fig. 5). The rest of the complement appeared normal. The polyploid modal karyotype with 94 chromosomes had 6 no. 10, 3 no. 18, and 2 no. 21 chromosomes in contrast to 4 chromosomes in each of the other positions. In addition, there were 3 ring chromosomes, larger than the ring in the constitutional karyotype, of undetermined origin (Fig. 6). All other chromosomes in the complement appeared structurally normal. A few nonmodal cells appeared to have some random, structurally abnormal (marker) chromosomes, but these cells also consistently had the changes shown in the modal karyotypes. No normal cells were seen. A Philadelphia chromosome was not present.

The frozen lymphocytes from the peripheral blood showed a normal modal number of 46 chromosomes with a consistent abnormality of the no. 21 interpreted as a ring 21 [r(21)] (Fig. 7). A lesser mode of 45 (25%) consisted of cells with random loss and variable unidentifiable marker chromosomes. No cells were seen to
Fig. 6. Modal karyotype (n = 94) from bone marrow cell. Note more than 4 copies of no. 10, less than 4 of nos. 18 and 21, and 3 ring chromosomes (arrows).

Fig. 7. Karyotype (n = 46) from frozen lymphocyte of peripheral blood. Note abnormal no. 21 [t(21)] (arrows). Chromosomes 1, 3, 7, and 8 show overlaps.
have 49 chromosomes, trisomy 10, monosomy 21, or the rings seen in the bone marrow karyotype.

Chromosomes of the parents and siblings were normal.

DISCUSSION

A small number of human lymphocytes with the hand-mirror configuration during the process of locomotion has been established previously as a normal finding. Large numbers of such cells are not normally present, but do occur as a rare manifestation of some hemopathies. In contrast to the observations of Stass, formation of new uropodia in our cells was not accompanied by subsequent locomotion.

As suggested by Sjögren's study of untreated patients with AML, there is positive correlation between an elevated percentage of cells with HMC and a longer survival time. This correlation is also true for nonresponders to therapy, and especially for patients with acquired karyotypic abnormalities. Similar observations have been made in individuals with AML as well as ALL.

Over 70% of patients with ALL have blasts lacking surface markers (null cells), whereas approximately 20%–30% have cells demonstrating T-cell characteristics; these data are summarized by Vogler et al. B-cell ALL is very uncommon. The HMC leukemia patient reported by Stass was classified as null cell in type, although the cells were acid phosphatase and β-glucuronidase positive, suggesting T-cell origin. A case of HMC ALL with surface markers suggesting B-cell origin has also been reported. In our patient, 77% of the peripheral blood cells (approximately 80% lymphoblasts) formed “E” rosettes, while 17.5% were positive for surface membrane-bound immunoglobulin. We suggest, therefore, that these malignant cells were of T-cell origin, even though they did not have histocytical characteristics (acid phosphatase, alpha naphthyl acetate esterase) of the T-cell type.

These cells, as well as those described by others in ALL and infectious mononucleosis, had uropodia with microspikes on the surface. In addition, normal cultured phytohemagglutinin-stimulated lymphocytes have been observed to have uropodia with microspikes that attach to other cells and to the culture vessel. It is believed that these spikes play a role in exploration by the lymphocyte of solid structures surrounding it. Vesicles of lysosomal origin have also been observed in the uropodia. It is thus conceivable that hand-mirror type cells might represent immunologically stimulated lymphocytes, and that the uropod and microspikes are structures by which they not only attach but also secrete cytotoxic substances.

About one-half of patients with ALL have acquired chromosome abnormalities, mostly represented by diploid or hyperdiploid modes; the most common change is a gain of one or two no. 21 chromosomes followed by gains of an X, or nos. 8, 13, 14, or 4 in order of decreasing frequency, although the changes may be more random in juvenile than adult ALL. Our patient had both hyperdiploid and polyplloid modes, but in contrast to the most common change seen in ALL, i.e., gain of no. 21 material, our patient had a deficiency of no. 21 material. Involvement of the no. 10 in either marker formation or aneuploidy, such as the trisomy 10 in our patient, is uncommon in any of the hematologic disorders, although it has been reported previously in ALL. Ring chromosomes are often associated with therapy, particu-
larly radiotherapy. The rings in the karyotype of our patient are unusual, however, since she had not received any chemotherapy for 5 mo prior to chromosome analysis, nor had she had any radiation therapy prior to that time. The rings did not participate in the reduplication process that resulted in the polyploid mode.

Another report of chromosome studies in a patient with hand-mirror cell ALL described a normal karyotype from the direct bone marrow preparation. The HMC from that patient failed to grow in culture, and it is possible that the metaphase spreads studied in the direct preparation did not actually represent the HMC. In our case, we are assuming that the cells analyzed represent the leukemic population because of the acquired abnormalities present, but it is noteworthy that no normal metaphases were seen. Total aneuploidy and a very high rate of polyploidy (60%), as compared with a polyploid rate of 0%-2.5% in normal marrow, may be related to the subsequent rapid downhill course of our patient. Total absence of demonstrable normal metaphases in the marrow of patients with AML and ALL has been shown to be associated with a very unfavorable prognosis.

The 46,XX,r(21) karyotype seen in the frozen peripheral blood lymphocytes is presumed to represent the patient’s constitutional karyotype, and the chromosome abnormality involving the no. 21 may have been related to the patient’s mental retardation. An antimongoloid phenotype, resulting from partial deletion of a G chromosome, was first described by Lejeune. More recent cases of 21-deletion syndrome studied with banding confirm Lejeune’s finding to some extent, but the phenotypic findings are not entirely consistent and vary in severity. The characteristics of the 21-deletion phenotype in 25 cases have been summarized and discussed by Gericke. Our patient did have a number of these features, e.g., mental retardation, abnormal ears, micrognathia, genitourinary abnormalities, repeated infections, and microcephaly, many of which, however, are common to other chromosomal syndromes as well. She did not have an antimongoloid slant of the eyes.

It is interesting that the constitutionally abnormal no. 21 was lost, and new aberrations were acquired by the leukemic cells that were monosomic for no. 21. Excess no. 21 material (trisomy 21) is known to be associated with increased incidence of leukemia, but a similar association of deficiency of no. 21 material with leukemia has not been recognized. Relatively few cases with 21 deficiency have been described, however, and study of more of these rare cases may eventually reveal that any disturbance in the sum total of no. 21 material may be associated with increased incidence of leukemia.

ACKNOWLEDGMENT

The authors wish to thank Dr. O. R. McIntyre and Dr. E. Jaffe for critical review of this manuscript, Dr. R. Beck and Dr. L. Zaitlin for special assistance, M. Larson and J. White for technical help, and K. Hourihan for typing the manuscript.

REFERENCES

2. Norberg B, Rydgren L, Stenstam M: Amoeboid movement configuration. A cell configura-
tion observed in tumour cells from 3 cases of bone marrow neoplasia. Scand J Haematol 13:294-304, 1974
10. Sigma Chemical Company: Technical Bulletin 386, St. Louis, Mo, September 1978
Hand-mirror cell leukemia associated with mental retardation: immunologic, chromosome, and morphological studies

R Stern, ST Widirstky, DH Wurster-Hill, RD Allen, KA Smith, GG 3d Cornwell and CJ Jr Cornell
Hand-mirror cell leukemia associated with mental retardation: immunologic, chromosome, and morphological studies

R Stern, ST Widirstky, DH Wurster-Hill, RD Allen, KA Smith, GG Cornwell 3d and CJ Cornell Jr