

Brief Report

TRANSPLACENTAL TRANSMISSION OF NATURAL-KILLER-CELL LYMPHOMA

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THE placenta is an organ of embryonic origin that supports the growing fetus by facilitating the transfer of nutrients from the mother. It is not well understood how the allogeneic fetus thrives and avoids immune rejection in an environment where only trophoblastic and fetal capillary basement membranes separate the maternal and fetal circulations. Reports of vertical transmission of cancer are exceptionally rare, although maternal cells do reach the fetus^{1,2} and cancer occurs in nearly 1 in 1000 pregnant women.³⁻⁵ Malignant melanoma is the best known example of a cancer that can metastasize to the fetus. We report the transfer to the fetus of an aggressive natural-killer-cell lymphoma in the mother, with fatal consequences to the infant.

CASE REPORTS

Mother

The mother was a 15-year-old Thai girl (gravida 1, para 0) whose pregnancy was unremarkable until two weeks before delivery, when coughing, vomiting, and fever developed. Subsequently, swelling of the legs, dull abdominal pain, and vaginal discharge appeared, and the fever persisted. At 33 weeks' gestation she was hospitalized, appearing acutely ill. The laboratory findings included signs of hemolytic anemia, thrombocytopenia, and elevated levels of liver enzymes. The HELLP syndrome (hemolysis, elevated liver enzymes, and a low platelet count) and sepsis were diagnosed. Fetal distress became evident, requiring an urgent cesarean section. During the procedure, bilateral nodular masses in the mesosalpinx were noted and biopsies performed. The patient's condition deteriorated, and she was transported to Massachusetts General Hospital. Preliminary examination of the biopsy specimens suggested the presence of high-grade lymphoma. A bone marrow aspirate contained 30 percent large pleomorphic cells, a finding also consistent with the possibility of lymphoma (Fig. 1).

The patient's lungs were mechanically ventilated after dyspnea with progressive lethargy and acidosis developed. She was treated

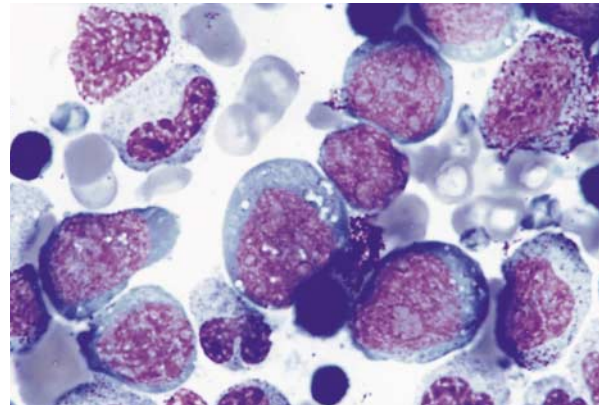


Figure 1. Bone Marrow Aspirate from the Mother.

Large neoplastic lymphoid cells with prominent nucleoli and blue-stained, occasionally vacuolated cytoplasm with a few azurophilic granules are present, along with residual hematopoietic elements (Wright's stain, $\times 800$).

with antibiotics and vasopressors and with cyclophosphamide, doxorubicin, vincristine, and prednisone for the lymphoma. Blood cultures were positive for *Candida tropicalis*. On postpartum day 17, the patient's condition deteriorated further, and another bone marrow aspirate again showed infiltration with lymphoma. After extensive family meetings, a decision was made to limit resuscitative efforts, and the patient died 24 hours later. Permission for an autopsy was denied.

Infant

The infant, a 1.6-kg boy born at 33 weeks' gestation, had poor muscle tone and apnea at birth. His initial course was consistent with the presence of transient tachypnea of the newborn. At 10 days of age, he was tolerating feedings and gaining weight and so was transferred to Massachusetts General Hospital to be with his mother.

When he was four weeks old, the infant's temperature rose to 38.6°C and he was lethargic and tachycardic. Blood analysis revealed a white-cell count of 8300 per cubic millimeter (19 percent polymorphonuclear leukocytes, 2 percent band forms, 66 percent lymphocytes, 2 percent atypical lymphocytes, and 11 percent monocytes). On cytologic examination, the cerebrospinal fluid appeared normal. A chest radiograph revealed a thymic shadow and was interpreted as normal. Administration of antibiotics was begun, but the fever persisted and distention of the abdomen and hepatosplenomegaly developed. Blood cultures were sterile. Nasal, conjunctival, and rectal specimens were obtained for viral cultures, and treatment with acyclovir was begun. The nurses wondered whether the infant might have acquired his mother's lymphoma. Two days later, his skin became mottled and he became increasingly lethargic and was transferred to the intensive-care nursery.

The infant's abdominal girth increased, mechanical ventilation became necessary, and melena and thrombocytopenia developed. The blood smear revealed large, abnormal lymphocytes with condensed chromatin and one to four nucleoli, many granules, and occasional vacuoles in the basophilic cytoplasm (Fig. 2). His condition continued to deteriorate, and an abdominal film revealed pneumatisis intestinalis. During laparotomy, clear ascitic fluid was removed and an area of pneumatisis was noted. The ascites recurred despite fluid restriction, diuretic therapy, and paracentesis.

Fever, hepatosplenomegaly, increased serum levels of lactate dehydrogenase, and negative microbial cultures prompted consider-

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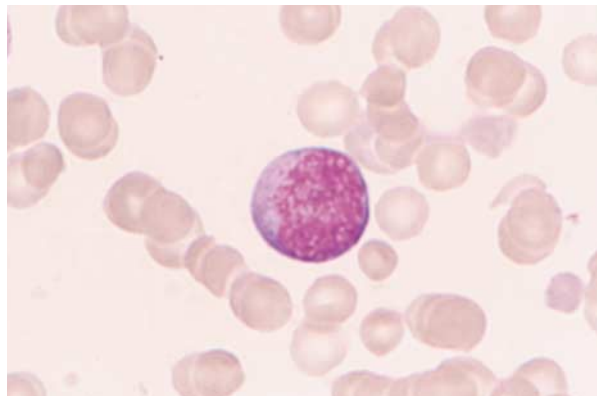


Figure 2. Peripheral-Blood Smear from the Infant. A large neoplastic lymphoid cell with an irregular nucleus, single nucleolus, and blue-stained cytoplasm is present. A few small azurophilic granules can also be seen (Wright's stain, $\times 800$).

ation of lymphoma or leukemia. A bone marrow aspirate contained 10 to 15 percent tumor cells. This finding, with the results of immunophenotypic analysis of peripheral blood, confirmed a diagnosis of lymphoma.

By the infant's fifth week of life, fewer tumor cells were noted in the peripheral blood, but the thrombocytopenia persisted. In an attempt to reduce the tumor burden, a trial of interferon alfa was started. The next day, the infant had hypotension, metabolic acidosis, increased intrapulmonary shunt, and rapid increases in the levels of peripheral white cells and serum levels of lactate dehydrogenase. The interferon alfa was discontinued. The possibility of adrenal insufficiency was considered, and glucocorticoids were administered. The baby died on the 59th day of life.

METHODS

Light Microscopy

Light microscopy was used to examine paraffin-embedded specimens from the bone marrow biopsy, the mother's mesosalpingeal mass, the placenta, the mother's and the infant's bone marrow aspirates, and the infant's peripheral blood.

Immunohistochemical Analysis

Paraffin-embedded tissue from the maternal mesosalpingeal mass was used for immunohistochemical analysis. Immunoperoxidase staining was performed with the avidin-biotin complex technique (Vector Laboratories, Burlingame, Calif.). Sections were stained with antibodies to CD45 (panleukocytes; Dako, Carpinteria, Calif.), CD45RO (UCHL-1; Dako), CD3 ϵ (T3 ϵ , T cells and natural killer cells; Dako), CD43 (T cells and a B-cell subgroup; Camfolio, Becton Dickinson, San Jose, Calif.), CD56 (123C3 and natural killer cells; Accurate Chemical and Scientific, Westbury, N.Y.), CD20 (L26, B cells; Dako), CD79a (B cells; Dako), CD1a (cortical thymocytes; Immunotech, Westbrook, Me.), CD5 (T cells; Novocastra, Newcastle-upon-Tyne, United Kingdom), CD8 (cytotoxic-suppressor T cells; Dako), CD30 (Ber-H2, activated lymphoid cells; Dako), CD57 (Leu-7; Becton Dickinson), terminal deoxynucleotidyl transferase (Supertech, Rockville, Md.), myeloperoxidase (Dako), and immunoglobulin kappa and lambda light chains (Dako).

In Situ Hybridization

In situ hybridization was performed on paraffin-embedded sections of the mesosalpingeal mass and the placenta with the use of an oligonucleotide probe for Epstein-Barr virus (EBV)-encoded RNA transcript (EBER-1) (Novocastra). Fluorescence in situ hybridization was performed with commercial probes (Vysis, Downer's Grove, Ill.).

Flow Cytometry

Peripheral-blood lymphocytes from the infant and bone marrow specimens from both the mother and the infant were prepared

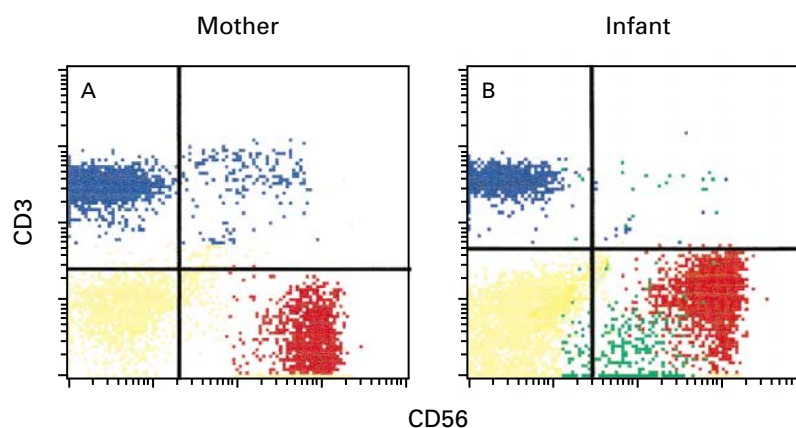
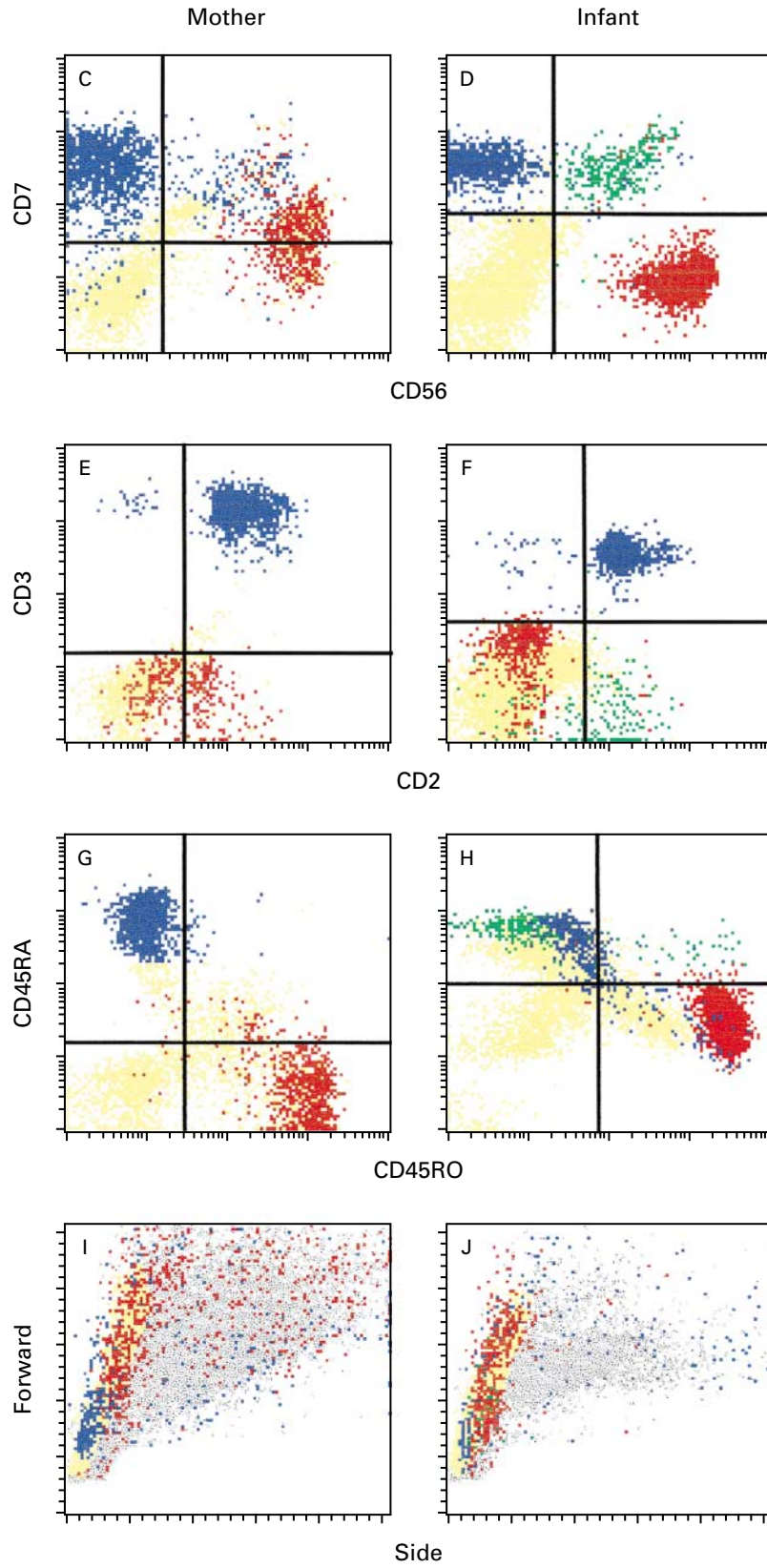


Figure 3. Flow-Cytometric Analysis of Bone Marrow Aspirate from the Mother and the Infant. CD56+CD3- neoplastic cells (red), normal CD3+ T cells (blue), and normal CD56+CD3- natural killer cells (green) are shown (Panels A through H), in addition to their forward and side light-scatter characteristics (Panels I and J). Normal natural killer cells are typically CD56+CD3-CD7+CD2+CD45RA+. Normal T cells are CD3+CD7+CD2+CD45RA+ or CD45RO+ and are demonstrated in both the mother's and the infant's bone marrow specimens. In contrast, neoplastic cells in the mother did not express or only weakly expressed CD7 (Panel C) and CD2 (Panel E) and strongly expressed CD45RO (Panel G). CD7 expression (Panel D) and CD2 expression (Panel F) were absent on the infant's CD56+CD3- cells, whereas CD45RO (Panel H) was strongly expressed. In both the mother and infant, the CD56+CD3- neoplastic cells had greater forward and side light-scatter characteristics than normal lymphocytes. Yellow areas represent unstained cells.



for staining with the use of standard techniques.⁶ Samples were stained with the following directly conjugated antibodies, in combinations of four antibodies per tube: CD2, CD3, CD4, CD5, CD7, CD8, CD10, CD14, CD16, CD19, CD20, CD23, CD33, CD34, CD45, CD45RO, CD71, and kappa and lambda light chains (Simultest Anti-Kappa/Anti-Lambda) (all Becton Dickinson); CD43 and CD45RO (Dako); CD1, CD45RA, CD13, and terminal deoxynucleotidyl transferase (Beckman Coulter, Fullerton, Calif.); and glycophorin (Caltag, Burlingame, Calif.). The samples were then incubated at 4°C for no more than 24 hours.

RESULTS

Mother

Biopsy of the right mesosalpingeal mass revealed a lymphoma composed predominantly of large, atypical lymphoid cells with scant cytoplasm. Mitotic figures were common. Many small areas of necrosis were interspersed among the neoplastic cells. Immunohistochemical analysis of paraffin-embedded sections showed that tumor cells expressed CD45, CD45RO, CD3 ϵ , CD43, and CD56 and did not express CD20, CD79a, CD1a, CD5, CD8, CD30, CD57, terminal deoxynucleotidyl transferase, immunoglobulin light chains, or myeloperoxidase. In situ hybridization revealed that most tumor cells were positive for EBER-1. These results were consistent with the presence of an EBV-positive lymphoma of natural-killer-cell lineage, because of the expression of CD56 and CD3 ϵ without the expression of CD3 or other T-cell- or B-cell-specific surface antigens.

Examination of a smear of bone marrow aspirate showed that 30 percent of the cells were large, atypical lymphoid cells with a high ratio of nucleus to cytoplasm and scant basophilic cytoplasm (Fig. 1). Some cells had azurophilic granules or cytoplasmic vacuoles. The remaining cells were maturing hematopoietic precursors. Flow-cytometric analysis revealed an abnormal population of cells with light-scatter characteristics typical of large lymphoid cells and monocytes (Fig. 3). They accounted for 11 percent of the CD45+ population, expressed CD43, CD45RO, and CD56, and had reduced expression of CD7. Approximately half the cells had reduced expression of CD2; the remainder were CD2-. They lacked other T-cell antigens (CD1, CD3 [surface and cytoplasm], CD45RA, CD4, CD5, and CD8), B-cell antigens (CD10, CD19, CD20, and immunoglobulin light chains), myeloid antigens (CD13 and CD33), erythroid antigens (glycophorin and CD71), or natural-killer-cell-associated antigen CD16. The expression of CD56 and CD2 without surface expression of CD3 suggested an immunophenotype closely associated with natural killer cells. HLA typing of frozen bone marrow cells revealed the following haplotypes: A1, B57, Cw6, DR7, and DQ9; and A33, B58, Cw10, DR3, and DQ2.

Examination of the marrow aspirate from postpartum day 17 revealed cells with similar morphologic characteristics and the same results on flow cytome-

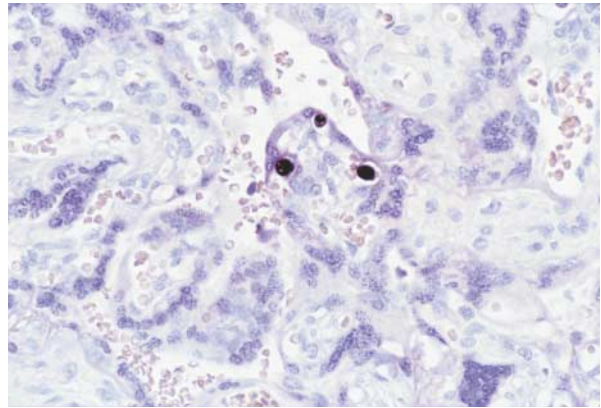


Figure 4. In Situ Hybridization for EBER-1 in the Placenta. Three EBER-1-positive cells are present within one chorionic villus (in the fetal circulation) ($\times 100$).

try. The findings in the bone marrow specimens, in conjunction with those in the mesosalpinx, were diagnostic of an aggressive natural-killer-cell lymphoma.

The placenta weighed 274 g, a value below the 10th percentile for 33 weeks' gestation. A 1-cm infarct was present. There were foci of necrotizing inflammation in the decidua, but no inflammation was present from the chorion to the amnion or in the villi or intervillous spaces. Stained sections were negative for microorganisms. No neoplastic lymphoid cells were recognizable on sections of placental tissue stained for light microscopy, but in situ hybridization revealed many cells within the decidua and a few cells within the blood vessels of the chorionic villi that had been transcribing EBV messenger RNA (Fig. 4).

Infant

The bone marrow and peripheral blood (Fig. 2) obtained from the infant revealed tumor cells with an immunophenotype similar to that of the mother's bone marrow and blood cells, although CD2 was not present and CD7 was detected only in the blood sample. These cells, constituting 10 percent of the cells in the lymphocyte light-scatter gate, had light-scatter characteristics (Fig. 3) similar to those of cells observed in the mother's bone marrow. In addition, CD4+ T cells, CD8+ T cells, natural killer cells (CD3-CD56+CD16+), and a polyclonal population of CD19+ B cells were identified in these and subsequent blood samples. During the infant's clinical course, the proportion of circulating tumor cells diminished to 4 to 5 percent of cells in the lymphocyte light-scatter gate. Cell-cycle analysis of samples of peripheral blood revealed a dramatically increased growth fraction (34 percent of cells in S and G2M phases) in CD3-CD56+ cells.

HLA typing of the infant's peripheral blood revealed the presence of A1, B57, and Cw6 (class I);

DR7 and DQ9 (class II) (the maternal haplotype); A24, B18, and Cw7 (class I); and DR12 and DQ7 (class II).

Cytogenetic Analysis

Cytogenetic analysis of the mother's bone marrow revealed the following karyotypes: 46,X,der(X)t(X1)(q22;q12)[2]/46,XX[28]; 30 cells in metaphase were analyzed. A clone of two cells showed an unbalanced translocation involving extra material from the long arm of chromosome 1 and an X chromosome (Fig. 5). Twenty-eight cells showed a normal female karyotype.

The infant's bone marrow cells showed chimerism with the following karyotypes: chi 46,X,der(X)t(X;1)(q22;q12)[3]/46,XY[16]. Twenty cells in metaphase were analyzed. A clone of three cells showed the same t(X;1) translocation that was found in the mother's specimen. One nonclonal cell showed an apparently balanced t(6;22)(q27;q13) translocation in addition to t(X;1) (Fig. 6). Sixteen of 20 cells analyzed showed a normal male karyotype. Fluorescence in situ hybridization of interphase nuclei with an alpha satellite centromeric probe for the X chromosome and a probe for the classic satellite region of the Y chromosome showed 33 of 363 nuclei to be XX and the other 330 nuclei to be XY.

DISCUSSION

The clinical and laboratory data are consistent with the transplacental passage of maternal natural-killer-cell lymphoma and subsequent engraftment of these cells in the fetus. The karyotype of this boy's lymphoma was female and carried the same translocation as his mother's tumor cells. The immunophenotypes of their lymphomas were nearly identical.

During pregnancy, cells of various types are transferred between the mother and the fetus. Several studies have reported that small numbers of maternal erythrocytes, platelets, granulocytes, and lymphocytes can cross the placenta.⁷ When labeled maternal cells were transfused into mothers before delivery, they were identified in umbilical-cord blood.¹ Several infants with immunodeficiency have been found to have engrafted maternal cells.² Cytogenetic studies have detected maternal lymphocytes one to five years after birth in boys who had received in utero transfusions,⁸ and small numbers of male cells have been found in progenitor-cell populations prepared from the blood of women who gave birth to sons up to 27 years previously.⁹

The passage of maternal cells across the placenta occurs in species with hemochorial placentae, but the quantity of cells transferred and the timing of embryonic exposure to such cells are not known.^{10,11}

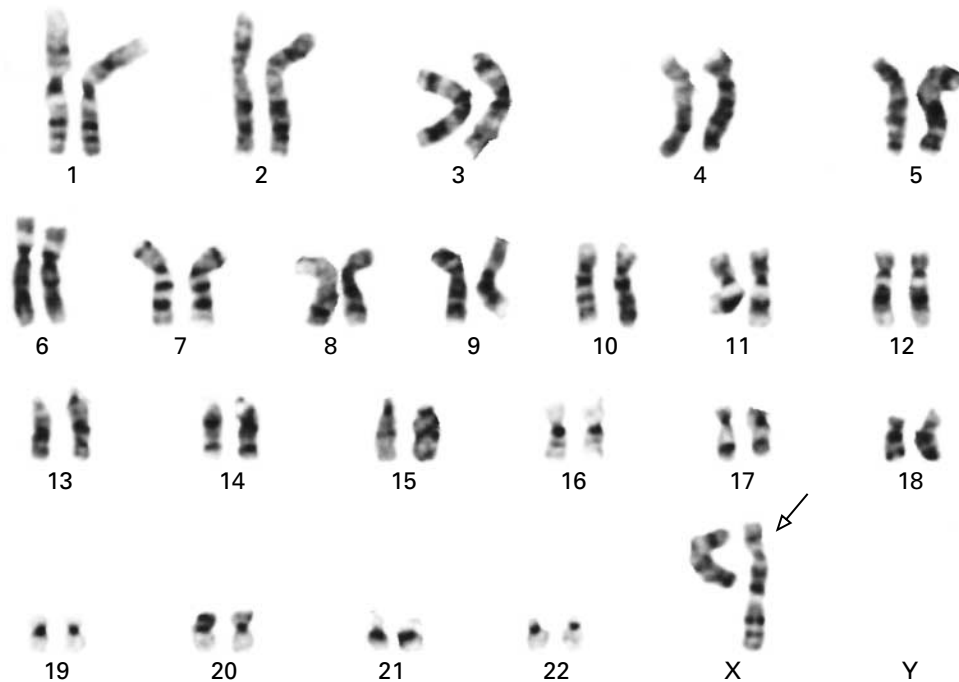


Figure 5. Karyotype of a Bone Marrow Cell from the Mother.

The karyotype reveals an unbalanced t(X;1) translocation (arrow) involving most of the long arm of chromosome 1 and an X chromosome.

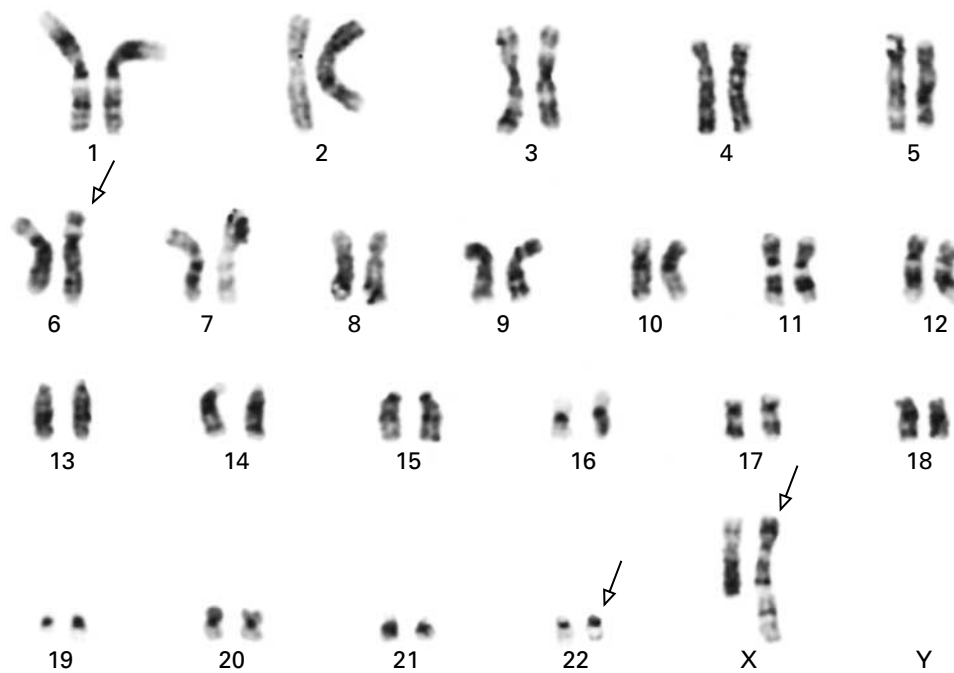


Figure 6. Karyotype of a Bone Marrow Cell from the Infant.

The same unbalanced t(X;1) translocation as that found in the mother's cells is present. In addition, there is a t(6;22) translocation in this cell. The arrows indicate the abnormal chromosomes.

In one study in which *lacZ*⁻, *scid/scid*, or wild-type mouse blastocysts were transferred into pseudopregnant *lacZ*⁺ transgenic mice, all fetuses examined late in gestation contained maternal cells. In the *scid/scid* (immunodeficient) fetuses, maternal cells crossed the placenta about midway through gestation and were found in lymphoid and nonlymphoid organs. In the wild-type (immunocompetent) fetuses, maternal cells were found exclusively in bone marrow.¹² Apoptosis of maternal cells that cross the placenta has been observed and may limit the number of such cells that enter the fetus.¹³

Why was the maternal lymphoma not rejected by the infant in this case? One possibility is that the mother was homozygous for one of the fetus's HLA haplotypes. In this situation, the fetus would not have recognized as foreign the major-histocompatibility-complex antigens on the mother's lymphoma cells. HLA homozygosity was found in one of two reported cases of infantile leukemia thought to be caused by vertical transmission of leukemic cells.^{14,15} However, in our case, the mother was not homozygous for one of her son's HLA haplotypes. It is also possible that the baby had a congenital immunodeficiency, although this is unlikely because the infant had relatively normal numbers of CD4⁺ and CD8⁺ T cells in peripheral blood and because cultured T cells re-

sponded in vitro to interleukin-2 (data not shown). Another consideration is that maternal lymphoma cells might have passed to the fetus early in pregnancy. Early in gestation, the fetus is immunologically immature and space is available in developing bone marrow for engraftment of hematopoietic cells. In this situation, donor cells can successfully compete with host cells for growth in the hematopoietic environment. We were unable to ascertain the points during gestation when maternal lymphoma cells reached the fetus. Finally, neoplasms of natural killer cells are among the most aggressive of the non-Hodgkin's lymphomas. They are more prevalent among Asians, are strongly associated with EBV, and may pursue a fulminant course,¹⁶⁻¹⁸ with rapid development of multiorgan failure, as was seen in both our patients. Since normal decidua exhibits an infiltrate of large granular lymphocytes with a natural-killer-cell phenotype,¹⁹ it is possible that the lymphoma in the mother had an affinity for binding to vascular endothelium in decidua. This affinity, coupled with the high proliferative capacity of the lymphoma, resulted in the localization of large numbers of tumor cells in the placenta and consequently their availability for entry into the fetal circulation. However, the paucity of tumor cells in the decidua does not support this idea.

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