Spontaneous *in Vitro* Transformation of Leukocytes from a Neonate[1] (37586)

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Some investigators consider infectious mononucleosis (IM) as a self-limiting lymphoproliferative disorder (1, 2). In an immunologically deficient host, such as a fetus, this lymphoproliferative process may result in acute leukemia (1). To organize a prospective study for the testing of this provocative hypothesis, a procedure capable of identifying neonates infected *in utero* by the agent of IM must first be developed.

Recent studies have strongly implicated the Epstein-Barr virus (EBV) and/or related agents as the causative agent of IM (3, 4). One consequence of prior infection by the EBV and/or related agents is the acquisition by the leukocyte of the infected person of the ability to transform spontaneously *in vitro* (5–7). Apparently, those leukocytes destined to transform *in vitro* are already present in the peripheral circulation of the infected person (8) but are prevented from rapid and persistent multiplication *in vivo* by specific serum inhibitor (9). If these observations on the relation between leukocyte transformation and the EBV and/or related agents are correct, the testing of umbilical cord blood for transformed lymphoid cells should be a convenient procedure for the identification of neonates who were infected *in utero* by the Epstein-Barr virus and/or the related leukocyte-transforming agent. The report describes the testing of 696 specimens of umbilical cord blood for transformed lymphoid cells.

Materials and Methods. Test for transformed cells. A previously described method was used (10). Briefly, 5 to 10 ml of blood was collected from the maternal end of the umbilical cord into a sterile tube with 1000 units of heparin. The leukocytes were separated partially from erythrocytes by sedimentation in dextran, washed in saline and suspended in culture medium to 5 million cells/ml. Two or more cultures, each consisting of 1 ml of the cell suspension in a 150 × 18 mm round bottom culture tube, were prepared from each sample of umbilical cord blood. The cultures were cared for and observed as described (10). Transformation was signaled by the appearance of enlarging aggregates of rapidly and persistently multiplying lymphoid cells. Once transformed, the cultures were divided 1 to 2 every 3 or 4 days. Cultures which failed to transform by week 12 were discarded as negative. The culture medium was 20% fetal calf serum in Eagle's minimal essential medium supplemented with the nonessential amino acids.

Test for EBV "capsid" antibody. The indirect immunofluorescent test on the EB3 cell was used (11). This test should detect specific EBV antibodies of IgM and IgG varieties.

Test for EBV antigen. A pool of convalescent mononucleosis sera was conjugated with fluorescein. This fluorescein-conjugated serum stained brilliantly cells positive for EBV antigens (EB3 and HRIK cells) but not cells negative for EBV antigens (Raji and SKLI cells).

Results. Six hundred and ninety-six specimens of umbilical cord blood collected at the Sacramento Medical Center from Jan. 1970 to Aug. 1972 were tested for transformed leukocytes. One was positive. Leukocyte

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cultures prepared from this blood sample developed enlarging aggregates of rapidly multiplying lymphoid cells on the tenth week of cultivation. The transformed culture was divided 1 to 2 serially every 3 to 4 days for 90 culture divisions without visible loss in growth vitality.

To rule out the possibility that transformed cells in this positive specimen might be due to inadvertent contamination of the blood or culture by transformed leukocytes from other established lines, the blood from this infant was retested on months 4, 6, 9, and 24 after birth; 14/20, 5/5, 7/7, and 2/10 cultures contained transformed leukocytes. In contrast, none of 88 leukocyte cultures prepared from the blood of 12 other infants contained transformed cells. Maternal blood collected 6 mo after delivery was also negative for transformed cells.

Sera collected when the infant was 4, 6, 9, 12, and 24 mo old were tested for EBV "capsid" antibody. All were negative at ¼ dilution. When tested without dilution, all gave weak positive reactions of questionable significance. Lymphoid cells established from the umbilical cord blood of this infant were examined for EBV antigen. No positive cell was seen among 40,000 examined.

Whiie this infant was under observation, data from a related project demonstrated the persistent oropharyngeal excretion by mononucleosis patients of a filtrable agent capable of transforming neonatal leukocytes (12, 13); oropharyngeal secretion was, therefore, collected from this infant (6 mo old) and tested for transforming activity by a previously described procedure (12). It was positive.

Physical examination and blood studies were performed on the infant at age 6, 9, 12, and 24 mo. No abnormalities other than mild anemia in the first 12 mo and, possibly, mild lymphocytosis were found. Results of blood studies are summarized in Table I.

TABLE I. Values of Complete Blood Count Done when the Infant Was 6, 9, 12, and 24 mo Old.

	6	9	12	24
WBC × 10^3	12.2	10.3	12.1	9.9
% lymphocyte	80	70	96	69
RBC × 10^6	4.55	4.36	4.27	4.4
Hb (g)	11.8	10.9	9.9	12.4
HCT	34.0	31.7	30.3	35.0
Mean cell vol (μm^3)	74	73	70	79
Mean cell hb (ng)	25.9	24.8	23.1	27.9

Discussion. This study shows that the blood from an occasional neonate does contain transformed lymphoid cells capable of prolonged and persistent multiplication *in vitro*. If this finding of transformed cells in the blood of neonates is accepted as evidence of *in utero* infection by the agent of IM (EBV and/or related agents), it should be a simple matter to initiate a prospective study to test the provocative and important hypothesis that infection of fetuses by the agent of IM may result in a lymphoproliferative disorder of a more serious nature than IM.

Of considerable interest is the failure to demonstrate unequivocally EBV-antibody of the IgG or IgM class in the serum of this infant whose blood contained transformed lymphoid cells. Intra uterine infection by other viruses such as rubella or cytomegalo virus results in a rising titer of specific IgM antibody after birth (14, 15). If the immune response of a fetus to infection by the EBV and/or related agents is similar to that by the rubella or cytomegalo virus, this finding may be interpreted as a possible antigenic difference between the responsible transforming agent and the EBV (16) or as a possible case of vertical transmission of the EBV (17, 18).

To the best of our knowledge, this is the first demonstration of transformed lymphoid cells in neonatal blood and of spontaneous transformation of leukocytes from a person whose serum EBV "capsid" antibody is $<¼$. This latter finding does not necessarily contradict the existing hypothesis (5–7) that prior infection by the EBV and/or related agents is a prerequisite for *in vitro* spontaneous transformation because the host was presumably infected at a time when its immune system was deficient.

Summary. Umbilical cord leukocytes were observed for spontaneous *in vitro* transformation. Leukocytes from one of 696 specimens transformed into rapidly and persistently

multiplying lymphoid cells. This finding was interpreted as *in utero* infection by the Epstein-Barr virus or other leukocyte-transforming agent(s). The neonate whose blood contained leukocytes that transformed spontaneously has been under observation for 24 mo; the only abnormalities were mild anemia and possibly a mild lymphocytosis.

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