ple of a cellular enhancer element adjacent to a known gene strengthens the hypothesis that enhancer elements may be important in regulation of cellular genes. In agreement with our results, Banerji *et al.* have also observed that portions of J_h-Cμ enhance transcription of the SV40 early promoter (*15*). It is difficult to extrapolate from viral systems to B cells where the J_h-Cμ sequences may not activate V_h promoters. However, cellular promoters from human, rabbit, and chicken can be enhanced with viral sequences (*5, 16*), and cellular DNA with enhancer activity has been detected previously (*17*). Furthermore, that V_h gene activation is mediated by sequences on the same chromosome located distant from the Vh promoter is entirely compatible with the properties of viral enhancers. Thus, the sequences identified in our study may act in situ as enhancers for immunoglobulin promoters and may explain how V_h gene transcription is activated on VDJ joining. This model is supported by the work of Gillies *et al.* (*18*) who have shown that specific deletions in the J_h-Cμ intron result in decreased transcription from a V_h promoter when the deleted gene is transfected into myeloma cells.

No transcriptional enhancer element was detectable by our assay between Cα and the alpha switch site—the region located 5′ to the translocated c-myc oncogene in many murine plasmacytomas. It is a formal possibility that a mouse lymphoid cell specific enhancer element does exist in this region but was not detected. However, this seems unlikely for two reasons. First, our system provides a very sensitive test for enhancers because small amounts of CAT enzyme are measurable and because COS cells amplify the number of templates. Second, we have used this system to identify enhancer activity in the J_h-Cμ region, where it has also been observed by others with different cells and promoters. The J_h-Cμ enhancer, like its viral counterparts, is able to act on various promoters in different cell types. Thus, some regulatory mechanism other than chromosome 12 enhancer elements is probably responsible for aberrant transcription of translocated c-myc genes. This would be consistent with the recent findings of Erikson *et al.* (*19*) who have shown that increased c-myc transcription occurs in all Burkitt's lymphoma lines regardless of the proximity of the translocation juncture to the c-myc gene or of the region in the heavy chain locus to which the c-myc gene is translocated. Possibly different chromatin structure or the removal of negative regulatory sequences is responsible for the altered transcription of the translocated c-myc.

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Chromosomal Mosaicism Confined to the Placenta in Human Conceptions

Abstract. *Placental and fetal tissues from 46 human pregnancies were cultured and cytogenetically analyzed in an attempt to document the existence of chromosomal mosaicism confined strictly to tissues of extraembryonic origin. In two gestations in which chromosomal mosaicism was found, it was expressed exclusively in placental chorionic cells and was not detected in cells derived from the embryo proper. This demonstration of confined chorionic mosaicism may have implications for the understanding of the fetoplacental unit and for prenatal diagnosis.*

The number and distribution of chromosomally abnormal cells in a mosaic conceptus has been thought to reflect the relative viability of the cells in the early cleavage embryo in which a nonlethal nondisjunctional event first occurred. Cell lineage now appears to be equally important (*1*), and therefore a single random nondisjunctional event occurring at a very early stage could produce mosaicism in the placenta or the fetus, but not necessarily in both, since only three or four cells in a mammalian blastocyst are selected as progenitors of the embryo proper (*2*). There should be many instances of mosaicism in which cells of only one line are selected for the embryo; since most of the remaining cells will become the placenta, the incidence of mosaic placentas should be higher than the incidence of mosaic fetuses. To test this prediction, we analyzed matched chorion, amnion, and cord blood samples from 46 human conceptuses. In the two instances in which chromosomal mosaicism was found, it was expressed exclusively in placental chorionic cells and was not detected in cells derived from the embryo proper.

Table 1. Chromosomal mosaicism in placental cultures from 46 gestations.

Category	Number of cultures		
	Total	One cell line	Two cell lines
Normal term male infants	9	9	0
Aneuploid infants and fetuses	6	6	0
Infants with IUGR	31	29	2

Chromosomal mosaicism confined to chorionic tissue has not been previously described. Although mosaicism has been

Table 2. Chromosomal analysis of two cases of confined placental mosaicism.

Mosaic conception	Cultured tissue	Number of analyzed metaphases			
		Main line	Second line	Random loss or gain	Total
		Case 1			
46,XY/47,XY,+22	Chorion	28	12	0	40
	Amnion	52	0	1	53
	Cord blood	53	0	1	100
	Skin	99	0	1	100
		Case 2			
46,XX/47,XXX	Chorion	58	10	3	71
	Amnion	74	0	0	74
	Cord blood	62	0	3	65

observed in placental tissue (chorion and amnion) from induced (*3, 4*) and spontaneous (*5*) abortions, it has been interpreted to represent mosaicism of the whole conceptus. In studies of abortuses it is frequently difficult to identify the precise origin of the tissues selected for culture, and it is rarely possible to compare embryonic and chorionic cultures.

We studied chorion, amnion, and cord blood specimens from a total of 117 placentas. Three different categories of fetuses and infants were included in the study: (i) term male infants of normal weight from uncomplicated pregnancies (to control for possible contamination of placental cultures with maternal cells); (ii) aneuploid infants, diagnosed by their appearance at the time of delivery, and aneuploid fetuses aborted after prenatal diagnosis; and (iii) term and premature infants with a clinical diagnosis of intrauterine growth retardation (IUGR). The last group, which comprised most of our study material, was included because trisomic placentas show abnormal morphological development and function (*6, 7*), and we therefore anticipated that mosaic placentas might also show some functional insufficiency.

Cultures of peripheral blood lymphocytes were established and harvested according to standard cytogenetic techniques (*8*). Several explants from different areas of the chorion and amnion of each placenta were seeded on plastic petri dishes containing nutrient media and incubated at 37°C. After 2 to 3 weeks, cells were harvested in situ (*9*), and chromosomes were studied after G-banding. The cases included in the study were those in which at least 100 metaphases suitable for analysis were obtained from the three types of cultures initiated (chorion, amnion, and blood) and in which these 100 metaphases were more or less equally divided between cells originating from the ectotrophoblast (chorion) and the embryo proper (amnion and cord blood). These criteria led to the exclusion of many cases in which amnion and cord blood grew well, but the yield of metaphases from chorion cell cultures was low. A culture was considered to exhibit true mosaicism only if three or more explants contained the minor cell line in more than one dish from a given tissue.

Forty-six of 117 placentas yielded metaphase data that met the established criteria for cytogenetic evaluation (Table 1). No maternal contamination was detected in cultures of chorion cells from control placentas (the normal male newborn group). The frequency of random loss or gain of individual chromosomes was similar for the three types of cultures we compared, namely, long-term chorionic cultures, long-term amniotic cultures, and short-term cord blood cultures. Cytogenetic findings were concordant in all cases except in the two with mosaicism. Both of these were from the IUGR group and showed mosaicism in the chorion only (Table 2). The first infant, showing trisomy 22 mosaicism in his chorion, was the third child of a 37-year-old woman and was born at 40 weeks of gestation. The diagnosis of IUGR was made before delivery. His birth weight was 2140 g. The infant showed no significant developmental abnormalities. The placenta weighed 610 g, and a diffuse increase in subchorial fibrin was apparent both on gross and histological examination. A skin biopsy for chromosomal analysis obtained at the age of 1 year showed only one cell line: 46,XY (Table 2). The second infant, showing triple X mosaicism in her chorion, was the first child of a 26-year-old woman and was born at 38 weeks of gestation. Her birth weight was 2540 g. The placenta weighed 325 g and showed no remarkable features. Permission for a skin biopsy was not granted.

Our findings provide conclusive demonstration of chromosomal mosaicism confined to placental chorionic tissue in humans. Similar findings in mouse conceptuses have been reported (*10*). The use of an in situ technique for harvesting cultured chorionic cells and the adoption of strict criteria for the definition of abnormal sublines make it unlikely that any of the trisomic cells observed arose in vitro. The present data clearly do not exclude the possibility that trisomic cell lines may have been present in some tissues of these two infants with mosaic placentas. However, since the amnion is a derivative of ectoderm (*11, 12*) the total number of analyzed cells originating from the embryo proper was 253 for the first infant and 139 for the second infant (Table 2). We can therefore exclude with 95 percent confidence a level of mosaicism of 2 percent or higher in the first infant and of 3 percent or higher in the second (*13*). In addition to these two cases, we have recently studied a case of pregnancy termination for prenatally detected mosaicism 46,XY/47,XY,+14 in which numerous fetal cultures from ectoderm, mesoderm, and endoderm yielded only a normal male karyotype, whereas cultures of the chorion revealed both the normal cell line and a prenatally detected trisomic cell line. This provides further support for the existence of mosaicism confined to the chorion.

Several investigators have reported significant chromosomal mosaicism in cultures of amniotic fluid obtained at 16 weeks of gestation (*14–16*) that could not be confirmed in cultures initiated from the subsequently aborted fetuses or liveborn infants. However, confirmatory cultures in these studies did not include chorionic tissue, with one exception (*14*) in which the trisomic clone was subsequently found in chorionic tissue. Accidental removal of chorionic fragments during amniocentesis is a recognized complication of this procedure (*17*), and contamination of aspirated amniotic fluid by chorionic cells is likely the explanation for detection of mosaicism in cultures of amniotic fluid.

Our finding of two cases of mosaicism confined to the chorion among conceptuses with IUGR suggests that some, if not all, conceptuses with chorionic mosaicism can successfully complete intrauterine development. Intrauterine growth retardation may represent a significant clinical manifestation of abnormal placental function in such conceptions. Among the 31 gestations with IUGR described in this report, 19 were associated with significant maternal smoking, which alone could explain the fetal growth retardation. Three originated from pregnancies associated with severe maternal hypertension. In nine, the IUGR was unexplained; both detected cases of confined chorionic mosaicism came from this group. Further clarifica-

tion of the frequency of confined chromosomal mosaicism, its association with IUGR, and its possible association with preterm unexplained intrauterine death is needed.

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Hepatitis B Virus Infection in Cultured Human Lymphoblastoid Cells

Abstract. *Since it has been postulated that liver hepatocytes may become infected by hepatitis B virus (HBV)* in vivo *through direct contact with infected macrophages, the possibility that a circulating cell of hematopoietic origin might be susceptible to infection with HBV was investigated. Cells positive for HBV surface antigen were identified in aspirates of bone marrow cells from people infected with HBV. These cells were used to prepare a lymphoblastoid suspension culture that contains HBV-infected cells.*

Research progress with hepatitis B virus (HBV) has been hampered by the lack of a cell culture system for growing the agent. It has been postulated that liver hepatocytes may become infected by HBV in vivo through direct contact with infected macrophages (*1*). This raises the possibility that liver macrophages or a circulating cell of hematopoietic origin might be susceptible to infection with HBV. To address this issue we examined aspirates of bone marrow cells from people who were infected with HBV. After identifying HBV surface antigen (HBsAg)-positive cells in such biopsies we established a lymphoblastoid suspension culture that contains HBV-infected cells.

At present, the only available cultures that remain persistently infected with HBV are liver tumor cell lines derived from HBV-infected people (*1–5*) and cells transfected with HBV DNA (*6*). Neither type of culture appears to make whole virus or to release HBV into the culture supernatant. Most produce only HBsAg, although some transfected cells appear to make virion core antigen (HBcAg), at least for limited periods.

Bone marrow aspirates from four HBsAg-positive and five HBsAg-negative patients with various hematologic diseases were tested under code for HBsAg. Smears of acetone-fixed cells were tested by direct immunofluorescence with a 1:20 dilution of rabbit antiserum to HBsAg conjugated to fluorescein isothiocyanate (FITC) (Behring). Samples from the four HBsAg-positive patients revealed that 1 to 2 percent of the cells were positive for cytoplasmic fluorescence, while the other five were negative. A subsequent bone marrow aspirate was available from one of the four patients, and this was used to initiate a cell culture.

The donor was a 55-year-old anemic white male (RAC) who was hospitalized for acute hepatitis. The patient had been on dialysis for 1 year and had received multiple transfusions. He was serologically positive for HBsAg, HBV e antigen, and had antibodies to HBc. Three months after diagnosis the signs and symptoms of hepatitis were partially resolved and a bone marrow aspirate was checked for anemia. At this time a portion of bone marrow containing approximately 10^7 mononuclear cells was seeded in RPMI 1640 medium with 25 percent heat inactivated fetal calf serum and, per milliliter, 10,000 units of penicillin, 10,000 μg of streptomycin, and 25 μg of Fungizone (Gibco). This culture, designated RAC/BM and incubated at 37°C in 5 percent CO_2, has been maintained in suspension form for 10 months. It is subcultivated at 3- to 4-day intervals by disruption of the cell clusters and reseeding at a density of 5×10^5 cells per milliliter.

By examination with the light microscope the cells are round but pleomorphic, and some have small cytoplasmic projections. When examined with the May Grünwald Giemsa stain, most resemble lymphoblasts (Fig. 1). Karyotypic analysis indicated that the RAC/BM cells contained 46 chromosomes. Smears of RAC/BM were also stained with the periodic acid–Schiff (PAS) reagent. Forty to 50 percent of the cells were PAS-positive, whereas all were negative for chloroacetate esterase peroxidase and

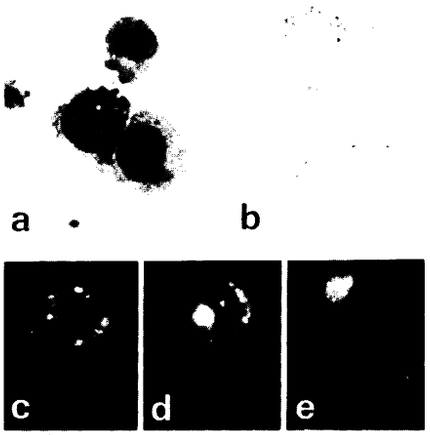

Fig. 1. Characteristics of RAC/BM cells at passage 31. (a and b) Smears of cells stained with May Grünwald Giemsa and PAS. (c) Living cells (10^6) were washed three times with phosphate-buffered saline (PBS), incubated with 50 μl of rabbit antibody to HBsAg conjugated with fluorescein (Behring) for 1 hour at 4°C, and washed again three times before examination. Whereas 20 percent of the RAC/BM cells were positive with either this technique or an indirect technique in which a human antiserum to HBsAg was used together with a conjugated rabbit antibody to human immunoglobulin G (Behring), less than 1 percent of the cells of a negative control line (CCRF-SB) (*10*) were positive and 10 to 15 percent of the PLC/PRF/5 cells were positive. (d and e) In air-dried smears of RAC/BM fixed in acetone at −20°C for 10 minutes, 15 percent of the cells were positive for cytoplasmic fluorescence when tested with human antiserum to HBsAg (d) and 12 percent were positive with human antiserum to HBcAg (e). The F(Ab)′ fragment of goat immunoglobulin directed to human immunoglobulin G labeled with fluorescein (Cappel) was used and each incubation step was for 40 minutes at 37°C. Less than 1 percent of the control cells were positive with either antiserum.