The Transplacental Passage of Fetal Leukocytes into the Maternal Blood (36955)

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In 1948, Levine (1) proposed a theory for the pathogenesis of hemolytic disease of the newborn. He stated that the disease was caused by the entrance of fetal cells into the maternal circulation resulting in isoimmunization of the mother. Support for this theory has been provided by the detection of erythrocytes of fetal origin in the circulation of the pregnant female, and at the time of delivery (2). Overweg *et al.* (3) reported that cytotoxic antibodies against the husband's leukocytes were found during and after the first pregnancy in 15 of 116 women. Lewis *et al.* (4) have observed hyporeactivity of mother's lymphocytes to their husband's leukocytes; Ceppelini *et al.* (5) have made similar observations, when the mother's cells were cultured with either their newborn's or husband's cells in one-way mixed leukocyte reactions. Field and Caspary (6) showed by a macrophage electrophoretic method that incompatibility between father and child was greater than between mother and child. These results might be a result of passage of leukocytes from mother to fetus or vice versa. Although there is conflicting evidence from investigations in mice (7) as to whether maternal leukocytes find their way into the offspring, the preponderance of evidence is against its occurrence to any extent in man (8). To further understand the relationship, mechanism, and significance of these biological events, the sex chromosome was used as a marker to detect leakage of fetal cells through the placenta into the maternal circulation during the course of pregnancy.

Materials and Methods. A total of 83 healthy pregnant women, who attended the Obstetrics Clinic of George Washington University Hospital in the past two years, was used in this study. These women had no history of ever receiving transfusions of blood. Sixty-four of them were studied prior to delivery, as early as 8 weeks after conception and as late as one week prior to delivery. Thirty-five of them were primipara and 29 were multipara. Twelve of these multiparous women had previously delivered a male offspring ranging from 1 to 8 years before this pregnancy.

Six other women who delivered male infants had peripheral blood cultures performed for chromosome analysis twice. The first was performed during the last month of pregnancy, from 6 days to 27 days before term (median, 11 days). The second was performed from blood drawn 10 min to 54 hr after delivery (median, 14 hr).

An additional thirteen women were studied only post partum after they had delivered males. The cultures were performed on each individual on one occasion from one to six months post partum.

Heparinized venous blood (10 ml) was obtained one or more times from each individual as indicated. Three milliliter leukocyte cultures were prepared using 3×10^6 leukocytes, 20% autologous plasma, and NCTC 135 medium (Grand Island Biological). Phytohemagglutinin (PHA) was added to the culture at the beginning of incubation. After 72 hr incubation at 37° and 2 hr before harvest, colchicine was added; air-dried chromosome preparations were made as previously described (9). Two hundred metaphases were analyzed from each culture and cells with a male karyotype were scored. Pilot studies in which up to 1000 metaphases were analyzed

TABLE I. Incidence of Male Cells in Mother's Peripheral Blood with Time of Gestation and Post Partum.

Offspring/ mother	Trimester of study	Total no. studied	Incidence of cells/200 metaphases examined		
			2 or 3 ♂ cells	1 ♂ cell	No ♂ cells
♂/34	First	12	4	2	6
	Second	14	1	3	10
	Third	8	1	1	6
♀/30	First	14	0	0	14
	Second	9	0	2	7
	Third	7	0	0	7
♂/6	Last mo.	6	0	0	6
	<3 days post partum	6	4	1	1
♂/13	<6 mo. post partum	13	2	6	5

indicated that 200 cells were usually sufficient to detect any contaminating male karyotypes.

Results. Chromosome studies on 64 prenatal women are shown in Table I. Thirty-four of these women (18 were primipara, 16 were multipara) subsequently delivered male babies. Of those who delivered males, twelve women had been studied in their first trimester; four of these were found to have 2 or 3 male cells in a total of two hundred metaphases analyzed, and two were found to have 1 male cell each. Fourteen of the 34 were studied in the second trimester; one was found to have 2 male cells and three others had 1 male cell each. Eight were studied while in their third trimester. Of these, one had 2 male cells and one had 1 male cell. The remaining 30 women (17 were primipara, 13 were multipara) later delivered female babies. Two of these multiparous women were found to have 1 male cell each. The statistical significance of these findings will be valid only when the detection of male cells is considered. Based on this statement, the detection or nondetection of male cells from the patients who had male babies, the prediction of male made by detection of the male karyotype was highly significant ($p < 0.0008$) (10).

A total of six women were studied successfully both before and after delivery of a male child (Table I). None of these six had male cells found in their circulation before delivery, but five of the six had 1, 2, or 3 male cells in 200 metaphases analyzed following delivery.

Chromosomes from the blood of thirteen women were studied at various intervals after the delivery of a male infant (Table I). Male karyotypes were found in the leukocyte cultures from eight of them. Two mothers had male cells at two months, two at three months, two at four months, one at five months, and one still had circulating male cells at six months following delivery. The remainder of these women did not have male cells in their chromosome preparations.

Discussion. In our series, it appears that there are two time periods with the greatest possibility of detecting male cells in the maternal circulation. One occurred from eight weeks to the end of the first trimester. The second occurred immediately following delivery. Detection of male cells during the first period may be due to the fact that fetal blood leaks into the maternal circulation at the beginning of the gestation period, while the placental barrier is not well organized (11). The finding of male karyotypes following delivery may be a result of separation of the placenta from the uterine wall, allowing a large number of fetal blood cells to enter the maternal circulation.

Cohen *et al.* (2), using the method described by Kleihauer, demonstrated fetal red cells circulating in the maternal blood at the end of the first trimester, and progressively increasing numbers of these cells with the

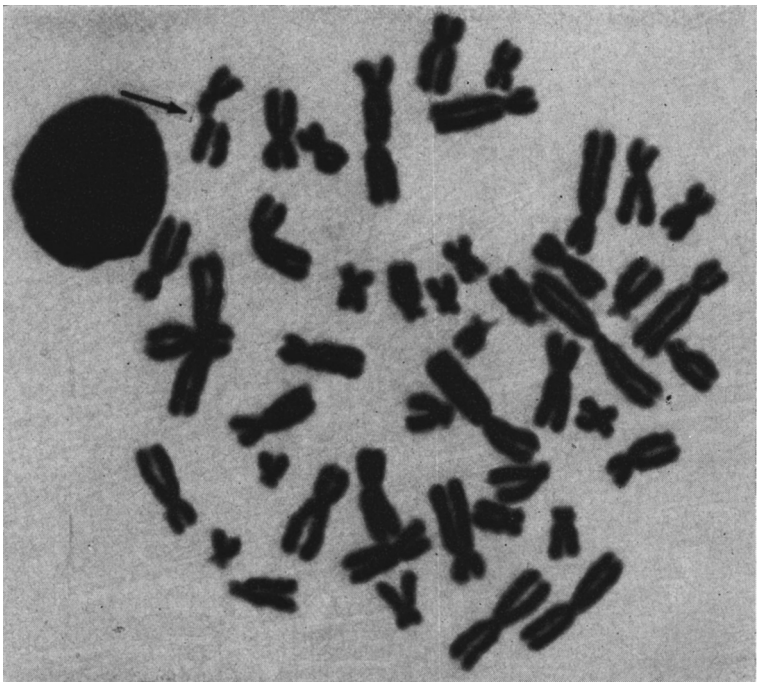

FIG. 1. A metaphase plate of peripheral blood preparation from a pregnant woman, arrow indicates breakage of the long arms near centromere of C group chromosome. If the fragment is lost, this could be mistaken for a Y chromosome and provide a false positive.

length of gestation. The greatest increase occurred during labor, at the time of the separation of the placenta from the uterine wall. This information along with our data demonstrates that not only red cells, but also leukocytes, pass the placental barrier into the maternal blood at the time of delivery. In our study, no progressive increase in the incidence of cells bearing the male karyotype in the maternal blood was observed after the first trimester until delivery.

Douglas *et al.* (12) demonstrated syncytiotrophoblasts in the circulating blood of pregnant women at various stages from 18 weeks of gestation to term. They suggested that the passage of these cells may be a means of achieving "tolerance" to fetal tissue as a form of homograft. Lewis *et al.* (4) using a mixed-leukocyte culture, demonstrated that in a normal pregnancy, there is a reduced maternal lymphocyte responsiveness, specifically to the father's leukocytes. There was less response in multiparous than in primiparous women, but an essentially normal response to the cells of unrelated males. In this study, in which we stimulated the peripheral blood leukocytes with PHA, there is an indication that some of these fetal cells are definitely lymphocytes. These findings may be a result of leakage of fetal cells bearing histocompatibility antigens which leads to partial immunological tolerance.

The question has been raised as to how long the fetal cells will survive in the maternal circulation. Thirteen post-partum women were followed for six months in an attempt to resolve this question. One mother still had male cells at 6 months after delivery. One mother in the prenatal series delivered a female baby but had one cell with a male karyotype; she had had a male child three years previously. De Grouchy *et al.* (13) found that 3 of their 21 pregnant women who had male cells had delivered a girl, and all had previously delivered a male child. These results strongly suggest that fetal cells can exist in the maternal circulation for as long as three years after delivery and may account for "false positives" in subsequent pregnancies. A false positive may be due to

a previously aborted pregnancy with a male child of which the mother was unaware.

The other mother, in our series who had one cell with a male karyotype in the 200 metaphases analyzed, had only female children and no other known pregnancies. Walknowska *et al.* (14) demonstrated false-positive results in one female with no history of a previous male pregnancy. Such findings may be a result of breakage of the long arms near the centromere of a C group chromosome (Fig. 1) or may possibly be a rearrangement or double nondisjunction as Jacobs has described (15).

The question has also been raised as to whether the detection of male cells will be pertinent to prenatal determination of the sex of the fetus, or detection of chromosomal abnormalities. According to the above results, the possibility of finding male cells is most promising during the first trimester. In addition, for fetal sex determination, this type of analysis will probably be reliable only if the mother has had no previous male pregnancies. As seen in Table I, there were no false positives in the peripheral blood of mothers containing two or more male cells in the two hundred metaphases analyzed. It would appear, therefore, that this determination of fetal sex could be used only when both of these latter conditions prevail.

The inconsistencies of false positive results may be further clarified by the use of new staining techniques such as the fluorescence stain or the special Giemsa stain. The use of these special stains should improve the identification of chromosome pairs.

Summary. The sex chromosome was used as a marker to detect leakage of fetal cells through the placenta into the maternal circulation during the course of, and after pregnancy. PHA-stimulated peripheral blood cultures from healthy pregnant and post-partum women were used for this study.

Twelve of 14 women, with male cells in their peripheral blood, gave birth to male babies. The cultures of six women, who bore male infants, were examined pre and post partum. None of them revealed male cells prior to delivery but five of them had male cells after delivery. Male karyotypes could be detected in the maternal blood for as long as six months after delivery. Our results indicate that the fetal leukocytes can traverse the placenta and enter the maternal circulation more readily at two time periods. One occurs during the first trimester; and the other, immediately following delivery. The passage of these cells may be important in the immunologic phenomena of parity related to paternally derived transplantation antigens.

Fetal sex determination will probably be reliable only if the mother has had no previous male pregnancies, and if two or more male cells per 200 metaphases are found.

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