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# Transplacental Isoimmunization by Fetal Red Blood Cells

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Rh isoimmunization during pregnancy is thought to result from the transplacental passage of fetal erythrocytes. This theory has been supported by our own studies and those of others which have shown that at least 15% of pregnant women have fetal red cells in their circulation. During pregnancy these quantities seldom exceed 0.2 ml., however, at the time of delivery volumes greater than 1.0 ml. may enter the maternal circulation. These larger transplacental hemorrhages frequently result from manipulative procedures involving the placental site.

We have attempted to determine if the transplacental passage of fetal erythrocytes in the quantities noted above are of significance in Rh isoimmunization. Recently we have been able to show that repeated injections of 0.1 ml Rh (D) positive fetal red cells resulted in primary immunization in 4 of 15 Rh negative recipients. Two of the recipients had received a total of 0.2 ml., and two, 0.4 ml. The injection of 0.1 ml. of fetal cells is also able to produce a secondary antibody response. In addition it has been demonstrated that anti-D coated D-positive fetal cells are able to elicit a secondary immune response.

A theory of the pathogenesis of Rh isoimmunization based on the above findings is described. In addition a proposed plan for the prevention of Rh isoimmunization in mothers is discussed.

## Introduction

It was initially postulated by Levine *et al* that the stimulus for Rh antibody production during pregnancy resulted from the transplacental passage of fetal erythrocytes into the maternal circulation.<sup>1</sup> We know now that transplacental passage of fetal cells is a frequent occurrence and this knowledge has provided a foundation for a rational approach to the prevention of Rh immunization during pregnancy.

Direct evidence for the transplacental passage of fetal cells depends on the identification of these cells immunologically<sup>2</sup> or by their high hemoglobin F content.

The latter technique, originally described by Kleihauer, Braun and Betke in 1957<sup>3</sup> has been employed by us as a rapid, simple and reliable procedure to identify red cells of fetal origin.<sup>4</sup> This technique is sufficiently sensitive to detect 0.1 ml. of fetal cells in the maternal circulation. We have examined 1029 blood samples taken at various times during the pregnancy of 384 Rh (D) negative women (Table I). In 161 or 15.9% of the specimens fetal cells were found. The estimated volume of fetal cells in the maternal circulation was usually 0.1–0.2 ml., and only rarely exceeded 0.5 ml. The frequency with which fetal cells was found increased with the number of specimens examined in each pregnant woman and in those examined five times approached 50% (Table II). Since our technique has a lower limit of sensitivity of 0.1 ml. it seems likely that the majority of women *during* a normal pregnancy receive small quantities of fetal cells across the placenta.<sup>5</sup>

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TABLE I

## Incidence of fetal cells in 1029 maternal blood specimens.

Estimated volume of fetal cells in maternal circulation (ml.)	% positive
0.1-0.2	14
0.2-0.5	1.7
0.5	0.2

## Characteristics of Transplacental Passage of Fetal Red Cells

Any hypothesis equating the stimulus for Rh (D) immunization during pregnancy with the transplacental passage of fetal cells must be compatible with certain characteristic features of this process:

1. Rh (D) antibodies are rarely found during the first pregnancy.<sup>6</sup>
2. Rh (D) antibodies rarely appear after a pregnancy in which the fetal erythrocytes are ABO-incompatible with the mother's serum.<sup>7</sup>
3. Rh (D) antibodies are more common after abnormal (or complicated) pregnancies or deliveries.<sup>8</sup>

1. Although antibodies rarely appear during a first pregnancy, fetal cells are found as frequently in primipara as in multipara.<sup>5</sup> One explanation is that anti-Rh (D) usually is not demonstrable for at least two and often as long as five months following a primary antigenic stimulus.<sup>9</sup> Accordingly the antibody resulting from immunization during a first pregnancy may not be found until after delivery; usually when the blood is once again tested at the beginning of the next pregnancy. This appeared to be the case in 67 of 199 first-sensitized\* pregnancies that we have reviewed (Table III). These women were found to have antibodies early in pregnancy. In the other 132 women, however, antibodies only appeared after the 22nd week. Since antibodies are rarely found during a first pregnancy it is likely that their appearance in these 132 women resulted from a secondary antigenic stimulus in a previously immunized woman. This is consistent with the suggestion of Nevanlinna<sup>7</sup> who thought that the first or primary antigenic stimulus might result in a state of immunization without demonstrable antibodies. A second stimulus during a subsequent pregnancy would cause demonstrable antibodies to appear. Thus the need for multiple antigenic stimuli for the production of demonstrable antibodies is another explanation for the infrequency of antibodies in primipara.

\*First-sensitized pregnancy is defined as the first pregnancy in which antibodies are demonstrable, no antibodies having been detected in the previous pregnancy.

TABLE II

## The incidence of fetal cells in mothers after repeated examinations.

No. of times examined	No. of women	% with fetal cells
1	384	17.2
2	215	30.7
3	102	35.6
4	62	40.4
5	31	43.6

2. It has been demonstrated that ABO incompatibility between the mother and fetus protects against primary Rh (D) immunization.<sup>7</sup> Our findings (Table IV) and those of Finn et al<sup>10</sup> indicate that fetal cells are rarely found post-partum in ABO incompatible pregnancies. This suggests that the ABO incompatible cells had been destroyed by the iso-agglutinins of the mother, and that this may constitute a mechanism which protects against Rh (D) immunization.

3. A third characteristic of maternal Rh (D) immunization is that antibodies frequently appear during a pregnancy immediately following a complicated pregnancy or delivery. This suggests that the complications cause larger or more frequent transplacental fetal cell passage and subsequent Rh (D) immunization, with antibodies appearing in the next pregnancy. We therefore studied a group of women with complicated pregnancies or deliveries. The results of these studies are shown in (Table V.) The incidence of fetal cells in the maternal circulation in patients in this group was not significantly increased, but large transplacental hemorrhages were more common than in normal pregnancies. In those pregnancies in which there had been manipulation or injury to the placenta, there were significantly more women who had received fetal cells in excess of 1.0 ml. Thus the frequent occurrence of Rh (D) immunization after complicated deliveries or pregnancies may be related to the frequency of large transplacental hemorrhages in such pregnancies.

## Antigenicity of Small Quantities of Fetal Red Cells

It has been suggested that abnormal pregnancies or deliveries may account for most, if not all, instances

TABLE III

## The time of appearance of anti-Rh (D) in Rh (D) negative women in a first-sensitized pregnancy.

Anti-Rh (D)	Number of women
Present in first 22 weeks	67
Absent in first 22 weeks	132



**TABLE IV**

**The effect of ABO compatibility on the incidence of fetal cells in post-partum blood samples.**

	With fetal cells	Without fetal cells
ABO Compatible	40	119
ABO Incompatible*	1	37

\*ABO incompatible refers to those pregnancies in which the mother's serum contains isoagglutinins against the ABO antigens of the fetal erythrocytes.

of immunization.<sup>11</sup> However, we have recently obtained evidence that the small quantities (0.1–0.2 ml.) of fetal cells entering the maternal circulation during normal pregnancy are capable of producing immunization. Similar findings have recently been reported by Cohen and Zuelzer.<sup>12</sup>

Evidence supporting this concept has been obtained from the immunization of fifteen Rh (D) negative women in the childbearing age. These women are all severely retarded permanent residents of a home for mentally defective persons, which precludes the risk of subsequent pregnancy. Each woman received repeated injections of 0.1 ml. of Group O Rh (D) positive fetal red cells (obtained from a placental vein at the time of delivery). The results of this study are shown in (Table VI). Following two injections two women developed antibodies. An additional two developed antibodies after four and five injections, respectively. These results suggest that the small quantities of fetal cells entering the

**TABLE V**

**The incidence of large (>1.0 ml.) transplacental hemorrhages after complicated pregnancies.**

Complication	Women tested	Women with more than 1.0 ml. fetal cells
Pre-eclampsia	39	1 (2.5%)
Mid-forceps delivery	53	1 (1.9%)
Ante-partum hemorrhage or placenta praevia (vaginal delivery)	9	0 (0%)
Caesarian Section	86	9 (10.5%)
Manual removal of placenta	17	3 (17.6%)
Amniocentesis	64	7 (10.9%)
Control	121	1 (0.8%)

maternal circulation during an uncomplicated pregnancy are sufficient to produce primary Rh (D) immunization.

To study the antigenicity of small quantities of fetal erythrocytes during pregnancy a group of Rh (D) negative women were examined repeatedly during pregnancy for the appearance of fetal cells and antibody. The findings in two women are shown in (Figs. 1 and 2). The first woman, Mrs. C. was followed during three pregnancies; fetal cells (estimated volume = 0.1–0.2 ml.) were noted at the 26th week of the second pregnancy and persisted until the 35th week. Antibodies were suspected four weeks following delivery and were clearly demonstrable at the 29th week of her third pregnancy. The infants were all Rh (D) positive and ABO compatible with the mother. The appearance of antibodies four weeks after the second pregnancy suggests that the primary antigenic stimulus was the transplacental passage of 0.1–0.2 ml. of fetal cells during the second pregnancy. A second patient, Mrs. F. was followed through her first and second pregnancy (Fig. 2). Both infants were Rh (D) positive. No fetal cells were found during or immediately after either pregnancy, yet antibodies appeared during the second pregnancy. In this woman Rh immunization was not associated with a large transplacental hemorrhage at the time of delivery but resulted from a smaller quantity of fetal cells than is detectable by our technique (less than 0.1 ml.).

**Mechanisms of Transplacental Immunization**

These findings suggest that there may be two mechanisms by which mothers can be immunized during pregnancy (Fig. 3):

**TABLE VI**

**Immunization of four Rh (D) negative women by repeated injections of 0.1 ml. fetal cells, given at six-week intervals.**

Number of injections	Antibody titers* in subjects 6 weeks after injection			
	K.	T.	A.	F.
0	—	—	—	—
1	—	—	—	—
2	32/32	1/1	—	—
3	4/4	1/1	—	—
4	1/8	1/8	0/4	—
5	8/32	2/64		+
6	16/32			0/64

\* Antibody titers are expressed as saline titer/albumin titer.  
 — No antibodies demonstrable.  
 + Antibodies suspected.



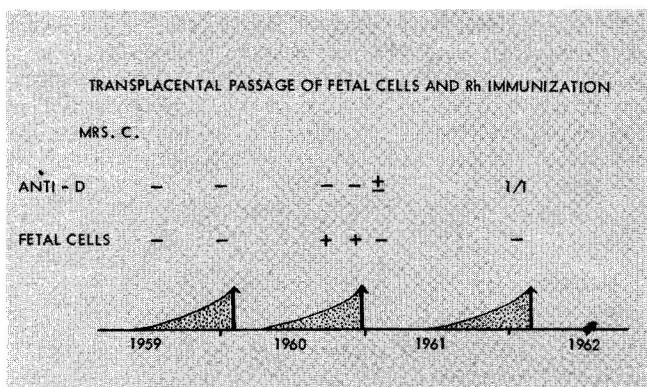


Fig. 1. The appearance of fetal cells and anti-D in Mrs. C. Anti-D: - = negative; ± = trace; 1/1 = saline/albumin titer. Fetal cells: - = negative; + = 0.1 - 0.2 ml. fetal cells in maternal circulation. The stippled areas represent pregnancies.

a. Small quantities of fetal erythrocytes (0.1 ml. or less) enter the maternal circulation during pregnancy. Because of the time required for antibody to appear as well as the possible need for a further stimulus, immunization may not be demonstrable for some time after delivery. This immune state may be recognized by the presence of anti-Rh (D) at the beginning of the next pregnancy or more commonly by the development of antibodies during the subsequent pregnancy as the result of a further antigenic stimulus by fetal red cells.

b. The transplacental passage of a large number of cells as a result of an abnormal pregnancy or delivery. At present there is not sufficient evidence available to determine the relative importance of these mechanisms as a cause of Rh (D) immunization in pregnancy.

If Rh (D) immunization during pregnancy is to be prevented the RH (D) positive fetal erythrocytes which enter the maternal circulation must be destroyed or their antigenicity must be reduced. It has been suggested<sup>11</sup> that the major cause of Rh (D) immunization is transplacental hemorrhage at the time of delivery. Theoretically these women, when identified, could be given anti-Rh (D) antibodies which should destroy the fetal erythrocytes. Recently Clarke et al<sup>13</sup> and Freda et al<sup>11</sup> have reported studies which indicate that passive immunization with anti-Rh (D) antibodies does reduce the antigenicity of Rh (D) positive cells. Thus there would now appear to be available a method whereby Rh immunization can be prevented in Rh (D) negative mothers who have received Rh (D) positive fetal erythrocytes at the *time of delivery*.

Immunization resulting from transplacental hemorrhage *during pregnancy*, however, cannot be prevented by the administration of intact anti-Rh (D) antibodies

\*Prepared for us by Dr. A. Nisonoff, Department of Microbiology, University of Illinois.

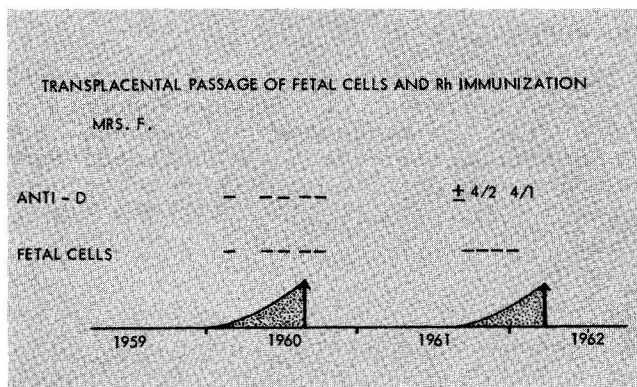


Fig. 2. The appearance of antibody during pregnancy of Mrs. F. Symbols: same as for (Fig. 1).

because such antibodies are able to cross the placenta, attach to and possibly destroy Rh (D) positive erythrocytes of the infant. Recently we have studied a pepsin-digested anti-Rh (D) antibody which may effectively destroy Rh (D) positive cells, yet will not cross the placenta\*. This product has a molecular weight of approximately 106,000, compared with intact gamma globulin which has a molecular weight of 150,000. The portion lost during pepsin digestion is thought to contain the "placenta-crossing" site<sup>14</sup> and it is likely that the remainder of the antibody will not cross the placenta. The pepsin-digested anti-Rh (D) that we have studied has antibody activity *in vitro* comparable to that of the intact gamma globulin. It may be possible therefore to treat Rh negative women *during pregnancy* with such an antibody to destroy and/or reduce the antigenicity of Rh positive fetal cells reaching her circulation.

It should also be noted, however, that, if Rh (D) immunization during pregnancy is to be prevented, prophylactic treatment may be necessary in all Rh (D) negative women (with Rh (D) positive husbands), since it is evident that most women receive fetal cells at some time during pregnancy. Furthermore our findings (Figs. 1, 2) suggest that with present techniques we cannot always predict which woman is at risk.

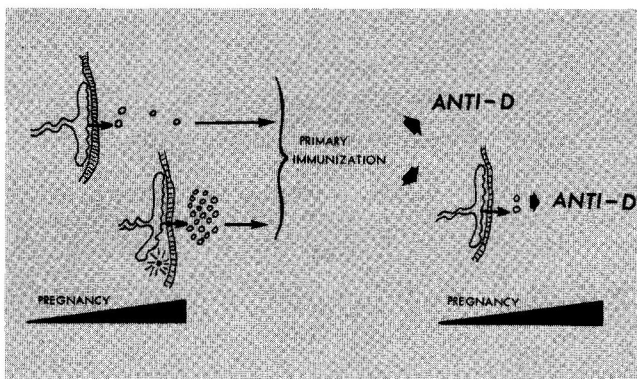


Fig. 3. A theoretical scheme of the mechanism of Rh (D) immunization during pregnancy.

**Summary**

Fetal erythrocytes can be found in the maternal circulation during pregnancy and after delivery. It is likely that Rh (D) immunization can result either from a large transplacental hemorrhage at the time of delivery or from repeated smaller hemorrhages occurring during

pregnancy. The prevention of Rh immunization based on these considerations is at present under study using passive immunization with anti-Rh (D). The prospects are bright that in the near future a practical method of prevention of Rh immunization will be developed which will significantly reduce the incidence of this unfortunate complication of pregnancy.

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