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Anti-D
erythroblastosis fetalis
newborn

Rh_o (D)-negative infants
Rh_o immune globulin

The Detection of Anti-D in Rh_o (D)-negative Infants Born of Rh_o (D)-positive Mothers

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Extract

It has not been determined whether Rho_o (D)-negative infants born of Rh_o (D)-positive mothers are sensitized during gestation or during parturition. Sensitization before use precludes the efficacious use of human Rh_o immune globulin as a prophylactic. The purpose of the present study is to identify the time of sensitization. Cord blood was collected from the placentas of 68 Rh_o (D)-negative infants whose mothers were Rh_o (D)-positive. Sixty-three of the 68 infants had one blood sample obtained between 1 and 9 months later. The paired samples were analyzed for anti-D by standard Coombs test and by automated antibody detection techniques. With the technique of automated antibody detection, we have been unable to demonstrate antibody in cord blood of the Rh_o (D)-negative infants of whom at least 7 of 63 (11%) had detectable anti-D between 1 and 9 months of age. These data show that Rh_o (D)-negative infants do not have detectable antibody at birth but may develop detectable anti-D in the first months of life. This observation suggests that the sensitizing dose of Rh_o (D) antigen occurs at parturition rather than during gestation.

Speculation

The detection of anti-D in Rh_o (D)-negative infants born of Rh_o (D)-positive mothers confirms the neonate's ability to respond to antigen exposure. If Rh_o (D) exposure produces sensitization only at parturition as this study implies, then there may be an immunoprophylactic role for human Rh_o immune globulin in the neonate.

Rh_o (D) erythroblastosis fetalis continues to be a neonatal problem despite the introduction of human Rh_o immune globulin in 1968 (2). Before that time, it was estimated that there were 300,000 women of child-bearing age in the United States who had been sensitized by a prior Rh_o (D)-positive fetus (1). With the advent of human Rh_o immune globulin, this number was expected to decrease to almost zero. Yet there persist two groups of women who continue to bear Rh_o erythroblastotic infants. The first are Rh_o immune globulin failures estimated at approximately 1% of women who are candidates for prophylaxis, most of whom probably have had a larger than usual fetal-maternal hemorrhage (2). The second group is composed of Rh_o (D)-negative women who themselves have had Rh_o (D)-positive mothers (15, 17).

Approximately 40% of all Rh_o (D)-negative infants will have Rh_o (D)-positive mothers (3). Assuming that the incidence of Rh_o (D)-negative births in the United States is about 15% of all deliveries (2), then approximately 450,000 of 3,000,000 births/year are Rh_o (D) negative. Simple calculations reveal that about 90,000 will be Rh_o (D)-negative females whose mothers are Rh_o (D) positive. Levine and Waller (10) have shown that 28 of 700 (40/1,000) Rh_o (D)-negative women bore Rh_o erythroblastotic infants in their first pregnancies, 19 of whom had previous Rh_o (D) blood exposure. Thus, there remained an unexplained 9/700 (13/1,000). Ramos De Almeida and Rosado (15) report an approximate 20% (200/1,000) incidence of first pregnancy Rh_o erythroblastosis when the Rh_o (D)-negative mother's own mother is Rh_o (D) positive. Such a wide discrepancy in incidence probably relates to the selected population in the latter study (10).

It has not been determined whether these mothers are sensitized during their own fetal life or during parturition. Whether or not human Rh_o immune globulin would be an effective prophylactic depends on the timing of sensitization, inasmuch as once circulating anti-D is formed, passive immunization is probably not effective. The purpose of the present study is to identify the time of sensitization of Rh_o (D)-negative infants whose mothers are Rh_o (D) positive.

MATERIALS AND METHODS

Cord blood was collected from the placentas of 68 Rh_o (D)-negative, Du-negative, full term, appropriate for gestational age infants born at the George Washington University Hospital and the William Beaumont Army Medical Center between January 1973 and March 1974. All study infants' mothers were Rh_o (D) positive. ABO and Rh typing and Coombs tests of mother and infant blood were done independently by two experienced blood bank technicians using standard typing sera. The infants' cord sera were collected and frozen at −20°. The study infants were seen once again between 1 and 9 months of age when a 3.0-ml blood sample was obtained by venipuncture. Before venipuncture, the parents were informed completely of the research nature of this project and subsequently of its results. Five of 68 parents refused permission for venipuncture and those infants are not included in the data. The venipuncture sera were frozen at −20°.

After collection, the sera samples were coded and assayed for anti-D antibody by the standard Coombs test and by the automated method described by Lalezari (8). Since the automated method is said to be able to detect both complete (IgM) and incomplete (IgG) antibody at concentrations as low as 1 ng/ml, it will reveal antibody that the Coombs test would miss, (8). Precautions and controls as described by Lalezari (8) to eliminate false positives were taken. In addition, anti-D-positive samples were confirmed by using a 10-red cell panel of known antigenicity.

RESULTS

Sixty-three cord blood specimens were obtained for which there was follow-up. The 63 infants were all 38–42 weeks gestational age and weighed between 2,730 and 3,360 g. There were 34 males and 29 females. Fifty-eight were delivered vaginally and five by cesarean section (three elective repeat, two for fetal distress). The pregnancies, labors, and deliveries were all unassociated with abnormal bleeding or evidence of placental pathology. Blood types of the mothers were all Rh_o (D) positive and all infants were Rh_o (D) negative, Du negative.

Of the total 63, 15 were a possible O-A or O-B incompatibility. Of that 15, 3 (all O-A) were direct Coombs test positive, the antibody being identified as anti-A. None of the 63 infants developed serum indirect bilirubins in excess of 14.5 mg/100 ml. None required either phototherapy or exchange transfusion. Furthermore, none of the ABO incompatible infants developed anti-D.

The results of positive anti-D levels at the various follow-up ages of the infants are presented in Table 1. It is interesting to note that the highest incidence of recovery occurred between 1 and 3 months of age, indicating that the antibody may decrease as the infant becomes older.

DISCUSSION

The results of the present study indicate that some Rh_o (D)-negative infants who have no evidence of Rh_o (D) isoimmunization at birth do develop anti-D antibody in the first months of life. There are currently two hypotheses to explain this observation.

The first is that the infant is exposed to his mother's Rh_o (D)-positive erythrocytes during fetal life. Taylor (17) and Owen *et al.* (12) have suggested that fetal exposure may result in tolerance rather than sensitization. The data of Pollack *et al.* (13, 14) showing that 20–30% of susceptible Rh_o (D)-negative adults will not form anti-D after antigenic exposure support the concept of tolerance. There is, however, no direct evidence to support or refute this hypothesis, and clearly such a phenomena requires further study. Furthermore, if sensitization rather than tolerance occurred, one should detect anti-D in the infant's cord blood.

The alternative hypothesis is that the infant is exposed to Rh_o (D) antigen during parturition. Levine and Waller (10) have shown clearly that Rh_o (D)-positive erythrocytes given to the female neonate can result in an Rh_o erythroblastotic infant from that exposed female's first pregnancy. In addition, if the neonate's sensitizing exposure occurred during parturition, one should not detect anti-D in cord blood but later in the infant's life.

With the technique of automated antibody detection, we have been unable to demonstrate antibody in cord blood of the Rh_o (D)-negative infants of whom at least 7 of 63 (11%) had detectable anti-D between 1 and 9 months of age. It is noted in our data that the 17.1% incidence of infant sensitization detected during the 1–3-month test period correlated with the incidence of maternal-fetal transfusion reported by Zarou *et al.* (20). Such a correlation, coupled with the negative cord blood anti-D levels, supports infant sensitization at birth rather than during gestation. This supposition is supported by Beer and Billingham's (3) data which revealed that only 1 of 40 similar infants studied between 3 and 20 months of age demonstrated anti-D antibody. Presumably the antibody response may only be of sufficient titer for detection for a short period of time.

In support of maternal-fetal sensitization at birth, Zarou *et al.* (20) have documented the occurrence of maternal-fetal tranfusion during labor in 17% of pregnancies studied using ^{51}Cr-tagged erythrocytes. The volume transfused in Zarou *et al.*'s (20) patients ranged from 1.0 to 13.0 cc. Although the sensitizing dose of erythrocytes for the infant is unknown, the dose of fetal Rh_o (D)-positive erythrocytes that can sensitize a susceptible Rh_o (D)-negative adult is as low as 0.1–0.4 cc (14).

The observation that Rh_o (D)-negative infants can be sensitized by Rh_o (D) antigen has now been documented by our data and by that of Ramos De Almeida and Rosado (15), Taylor (17), Hindemann (7), Beer and Billingham (3), and Levine and Walter (10). Further, Ramos De Almeida and Rosado (15), Beer and Billingham (3), and Taylor (17) have confirmed the strong correlation between rate of sensitization of the Rh_o (D)-negative mother by an Rh_o (D)-positive fetus and the Rh_o (D)-negative female infant of an Rh_o (D)-positive mother. Neither Beer and Billingham (3) nor Hindemann (7), however, present documented paired data on both cord blood and follow-up blood antibody levels to support either hypothesis.

Table 1. *Rate of positive recovery of anti-D by age*

	Birth	1–3 mo	3–6 mo	6–9 mo	Total
n	63	35	20	8	63
No. positive	0	6	1	0	7
% positive	0	17.1	5	0	11.1

To additionally refute placental transfusion during gestation as the sensitizing event, Freda (6) showed that minute fetal-maternal erythrocyte exchange during gestation is more important in the amnestic response of the already sensitized Rh_o (D)-negative mother than in the initiation of anti-D formation in the nonsensitized individual. Furthermore, the occurrence of an amnestic response during the first pregnancy of an Rh_o-negative mother who previously was exposed to Rh_o-positive erythrocytes is well documented (4, 10, 16, 18, 19). These cited studies all include females who had their first Rh_o (D)-positive erythrocyte exposure many years (some as infants) before their first pregnancy and subsequently bore Rh_o (D) erythroblastotic infants, confirming that an earlier primary immunization is necessary for the amnestic response that occurred during these females' first Rh_o (D)-positive fetus pregnancy.

ABO incompatibility is a factor that further complicates the understanding of Rh_o (D) sensitization since the relationship of ABO incompatibility to Rh_o (D) sensitization and the amnestic response of pregnancy is incompletely understood. Although ABO incompatibility tends to protect the Rh_o (D)-negative mother from sensitization by her Rh_o (D)-positive fetus (9), there is no alteration of the severity of Rh_o erythroblastosis once sensitization has occurred (9). It is interesting that none of the 15 infants in this study who had maternal-fetal ABO incompatibility and the 6 who had fetomaternal ABO incompatibility developed anti-D. This observation is especially interesting since the O infant at birth rarely has his own A or B antibody. Perhaps a placental factor is involved in the maternal-fetal ABO incompatibility. Further evaluation of this finding is necessary to understand whether ABO incompatibility is related to Rh sensitization of the susceptible infant.

SUMMARY

These data show that infants can respond to Rh_o (D)-positive erythrocytes with anti-D antibody formation. Furthermore, the data suggest that the infant's primary exposure to Rh_o-positive erythrocytes occurs during parturition rather than during gestation. This assumption is based on the inability to detect antibody from cord blood by the most sensitive technique currently available for measuring circulating antibody in infants who a few months later have easily detectable anti-D.

Although these data are preliminary, it is tempting to speculate that Rh_o human immune globulin would have a place in the immunoprophylaxis of Rh_o (D)-negative infants whose mothers are Rh_o (D) positive. Whether or not the efficacy of Rh_o immune globulin would be the same in the neonate as in the adult is currently under investigation. If the data confirm our hypothesis, then another cause of primary Rh_o (D) immunization can be eliminated.

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Folic acid
formiminoglutamic aciduria
hydantoin-5-propionic aciduria

Hydantoin-5-Propionic Aciduria in Folic Acid Nondependent Formiminoglutamic Aciduria Observed in Two Siblings

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Extract

Two previously described sisters with folic acid nondependent formiminoglutamic aciduria also excrete abnormally high amounts of hydantoin-5-propionic acid (HPA). HPA was identified by gas chromatography-mass spectrometry as the *N,N'*-bis-trimethylsilyl methyl ester- and *N,O*-permethyl derivatives. About 600 and 1,100 mmol HPA/mol creatinine, respectively, were found in 24-hr urine samples before and after ingestion of free histidine (three equal doses of 66 mg/kg each, with 4-hr intervals). The corresponding values in normal adults were 4.5 ± 2.2 (n = 24) and 46.0 ± 16.4 mmol HPA/mol creatinine (n = 17) when measured by a new, highly specific mass fragmentographic method using deuterated HPA as the internal standard.

Speculation

Formation of hydantoin-5-propionic acid from imidazolone propionic acid (ImOPA) by aldehyde oxidase represents a bypass at elevated concentrations of ImOPA caused, *e.g.*, by formimino-L-glutamate:tetrahydrofolate-5-formiminotransferase (EC. 2.1.2.5) deficiency (as postulated for our cases), general or functional folate deficiency, or imidazolone propionic acid hydrolase deficiency (not yet detected). HPA excretion in urine measured by mass fragmentography may be a sensitive tool to detect such deficiencies and may complement or even replace the enzymatic measurement of formiminoglutamic acid excretion.

In 1974, we described two sisters with normal serum folate concentration who excreted formiminoglutamic acid (FIGlu) in excessive amounts (15), both about 600 mmol FIGlu/mol creatinine. We believe that they represent the first cases where a practically complete deficiency of formimino-L-glutamate:tetrahydrofolate-5-formiminotransferase can be postulated. Our cases did not respond to pharmacologic doses of folic acid. Recently Perry *et al.* (17) described two other cases with apparently the same metabolic error. They excreted markedly lower amounts of FIGlu under pharmacologic doses of folic acid. From the known catabolic pathway of histidine we also suspected an increase in the excretion of HPA (15). For the precise measurement of this compound in urine we developed a relatively simple mass fragmentographic method (16). In this report, the results of these measurements are presented.

MATERIALS AND METHODS

HPA was synthesized from L-glutamic acid according to the method of Dakin (10). Similarly, trideutero-HPA was synthesized from 2,4,4-[$^2H_3$]-DL-glutamic acid (19). HPA was analyzed quantitatively as the *N,O*-permethyl derivative with trideutero-