Papers and Originals

Incidence of Maternal Rh Immunization by ABO Compatible and Incompatible Pregnancies

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Summary: The incidence of maternal Rh immunization in Rh-negative women following a single ABO compatible Rh-positive pregnancy is about 17%. This incidence was determined by following Rh-negative women through two Rh-incompatible pregnancies and analysing their sera for anti-Rh at the time of delivery of their second observed pregnancy. Maternal Rh immunization occurs almost exclusively after delivery; however, antibodies may not be detectable in the absence of further antigenic stimulation.

The incidence of maternal Rh immunization when maternal-foetal ABO incompatibility is also present is 9–13% and 17% for group O and non-group O women respectively. This study emphasizes the need to offer Rh-immune prophylaxis to Rh-negative women having Rh-positive infants whether or not ABO incompatibility exists between the mother and infant.

Introduction

Levine *et al.* (1941) recognized and described the pathophysiology of Rh-haemolytic disease of the newborn and its relationship to maternal isoimmunization. It was not until the rediscovery of the anti-human globulin technique by Coombs *et al.* (1945), however, that laboratories had a sufficiently sensitive serological test to detect incomplete antibodies in the sera of immunized women. Since then, estimates of the overall incidence of maternal Rh immunization have varied from 2·5 to 10% (Clemens and Walsh, 1954; Allen and Diamond, 1958; Nevanlinna and Vainio, 1962; Worlledge *et al.*, 1968) of the women at risk. These studies, however, do not allow one to determine the risk of maternal Rh immunization after a single Rh-incompatible pregnancy.

In the prospective studies on the prevention of maternal Rh immunization with Rh-immune globulin reported from the United States (Ascari *et al.*, 1968; Pollack *et al.*, 1968a), Canada (Zipursky, 1968), Liverpool (Finn, 1968), and Freiberg (J. Schneider, personal communication, 1968) the incidence of Rh immunization in the unprotected control groups as determined by the presence of detectable anti-Rh in their sera six months or longer post partum varied from 4 to 11·2%. In these studies only ABO compatible mother–infant pairs were followed, and in the Liverpool study the parturient women were also required to manifest evidence of foetal-maternal haemorrhages in excess of 0·25 ml. as determined by the Kleihauer–Bethke technique.

In our prospective observations of over 2,800 Rh-negative women having one or more Rh-positive infants, we have become aware of the inadequacy of the serological evaluation of the maternal sera six months post partum as an accurate assessment of the incidence of maternal immunization. Furthermore, this study has enabled us to re-evaluate the time when maternal antibody was first detected after an immunizing pregnancy. This report presents evidence for a much higher incidence of maternal Rh immunization by pregnancy in ABO compatible matings and includes a review of the pertinent literature concerning the extent of protection afforded by maternal-foetal ABO incompatibility.

Materials and Methods

First Observed Pregnancies

From March 1964 to May 1968 clinical trials were conducted in 43 centres throughout the United States and elsewhere to demonstrate the efficacy of anti-Rh_0 (D) immune globulin (human) (RhoGAM§) in preventing maternal Rh isoimmunization. The patients selected for the study were Rh_0 (D) and D^u negative women, showing no serological evidence of immunization to the Rh antigen, who gave birth to ABO compatible, Rh-positive infants. The patients were randomly allocated to control and treated groups so that both groups would be comparable according to age, parity, gravidity, and racial and socio-economic background. From March 1964 to January 1966 the treated patients received 4·5 to 5 ml. of the $15 \pm 1·5$ g. of globulin solution per 100 ml. containing 4,000–6,000 μg. of anti-Rh. From January 1966 to May 1968 the treated patients received a globulin solution containing not less than 300 μg. of anti-Rh, usually in 1 ml. The Rh-immune globulin was administered intramuscularly to the treated patients within 72 hours after delivery. Control patients received either an equivalent intramuscular injection of gammaglobulin solution devoid of anti-Rh or no injection at all.

The candidacy of all patients was determined within 72 hours post partum and an attempt was made to follow patients by examining their sera for atypical antibodies at 4 to 10 days post partum as well as at one, three, and six months post partum by the indirect anti-human globulin technique with the use of commercially available screening cells and anti-human globulin reagents. The presence of anti-Rh in the patients' sera *six months post partum* was regarded as evidence of active immunization, except for a small number of treated patients who, having received 4,000–6,000 μg. of anti-Rh following their first observed pregnancies, had declining titres of passively acquired detectable anti-Rh six months post partum. These patients were followed for an additional six months to determine whether or not active immunization had occurred.

Subsequent Pregnancies

Patients having more than one Rh-positive pregnancy during the period of observation were examined serologically at regular

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intervals throughout their subsequent pregnancies. Previously treated and control patients were required only to give birth to another Rh-positive infant to be included in the subsequent pregnancy tabulation. The demonstration of anti-Rh in the sera of the women at the *time of delivery* of the subsequent Rh-positive infants was regarded as evidence of active immunization.

Results

A total of 2,876 mother–infant pairs were admitted to the Rh-immune globulin trials for their first observed pregnancies and followed for a minimum of six months post partum. Of the 1,834 treated patients, 300 received the large dose (4,000–6,000 μg.) of Rh-immune globulin and 1,534 received the smaller dose (not less than 300 μg.). The incidence of maternal Rh immunization as determined by the presence of anti-Rh in the maternal sera six months post partum is shown in Table I. Two of the 1,834 (0·1%) treated patients were actively immunized despite passive immunization within 72 hours post partum; whereas in the control series 73 of 1,042 (7·0%) were found to have circulating Rh antibodies.

TABLE I.—*Results of Serological Analysis Performed Six Months after the First Observed Pregnancies*

Group	Immunized	Not Immunized	Total
Treated	2	1,832	1,834
Control	73	969	1,042
Total ..	75	2,801	2,876

2/1,834 = 0·1%. 73/1,042 = 7·0%. P > 0·001.

Up to September 1968 a total of 252 Rh-negative women have had subsequent Rh-positive infants, 150 from the treated group and 102 from the control group. The incidence of immunization determined by the presence of anti-Rh antibodies in the maternal sera at the time of delivery is shown in Table II. Only 1 of the 150 patients treated after their previous pregnancies had evidence of Rh immunization. By contrast, 17 out of 102 (16·8%) control patients had detectable anti-Rh antibodies in their sera and gave birth to infants with Rh-haemolytic disease of varying severity.

TABLE II.—*Results of Serological Analysis Performed at Delivery of Patients Having Subsequent Rh-positive Infants*

Group	Immunized	Not Immunized	Total
Treated	1	149	150
Control	17*	85	102
Total ..	18	234	252

17/102 = 16·8%. 1/149 = 0·7%. P > 0·001.
* 10 out of 17 had detectable antibodies six months after their first observed pregnancies.

It is noteworthy that 7 of the 17 control patients who were found to be immunized at the time of delivery of the subsequent Rh-positive infants were not found to have detectable anti-Rh in their sera six months after their first observed pregnancies. The antibodies were first detected in their circulation during the fourth to the ninth months of their subsequent gestations.

Discussion

It is now widely accepted that active maternal Rh immunization by pregnancy can be prevented by the passive administration of 300 μg. or more of anti-Rh administered within 72 hours after the delivery of an Rh-positive infant. The single failure in the treated group followed through a subsequent pregnancy has been reported elsewhere (Walsh and Hewitt, 1967). She was found to have received 5 ml. of gammaglobulin for measles prophylaxis during the second month of her previous pregnancy and at the time of delivery of her subsequent infant was noted to have: (1) an IgM anti-Rh_0 (D), (2) an IgM anti-rh′ (C), (3) an IgG anti-Jk^a, and (4) an unidentified antibody.

It is tempting to speculate that the occurrence of an IgM anti-Rh and other antibodies of various blood group specificities in this case is due to antibody-mediated immune enhancement, since it resembles the serological findings described by Pollack *et al.* (1968b) in the male volunteers treated with 10 μg. of anti-Rh and 5 ml. of Rh-positive blood. While samples of the identical lot of measles immune globulin used to treat this woman were not available for examination, samples of two lots from the same manufacturer were analysed and found to have critical levels of blood group antibodies, including anti-Rh_0. This evidence strongly suggests that passively acquired anti-Rh in the measles gammaglobulin injection may have resulted in an enhanced immunological responsiveness to the various blood group factors.

There is a striking discrepancy in the incidence of immunization in the control patients between those determined by the presence of detectable anti-Rh six months post partum (7%) and those determined by the presence of antibodies at the time of delivery of their subsequent Rh-positive infants (17%). This discrepancy can be explained by one of two possibilities: (1) primary Rh immunization may have occurred during the subsequent Rh-positive pregnancies in those patients who were apparently not immunized, as well as after the delivery of the first observed pregnancies; and (2) the women showing antibodies for the first time in their subsequent pregnancies may have been manifesting a secondary immune response. The latter possibility assumes that the primary sensitization occurred after the first pregnancy, but was insufficient to cause a humoral antibody response above the threshold of detectability.

Immunization During Pregnancy

In considering the first possibility, that immunization can occur during the course of pregnancy, it must do so very infrequently and may result from the extraordinary circumstances where large foetal-maternal haemorrhages occur intra partum. Nevanlinna and Vainio (1962) found only four examples of maternal Rh immunization in 4,153 Rh-negative primiparae in whom previous exposure to the Rh antigen could not be found. A relative state of maternal anergy exists during pregnancy, the reason for which is largely unexplained (Billingham, 1964). This immunological inertia pertains only to primary immune response, whereas the secondary response appears to be unaltered. If immunization occurred intra partum, it should have been observed in a higher incidence of immunization in the treated patients having subsequent Rh-positive pregnancies, instead of the single example of doubtful interpretation. The absence of intrapartum antibody formation in the treated group cannot be explained by the persistence of passively administered anti-Rh given after a previous pregnancy. The passive immunization with Rh-immune globulin decays exponentially with a half-life of 22–25 days, and therefore would be incapable of exerting any protective influence during a subsequent gestation.

Since antibody determinations were performed at the time of the subsequent deliveries, there was insufficient time for these patients to make detectable primary antibody responses. Judging from the events following the first observed pregnancies, Rh antibodies rarely appeared before the fourth week post partum. Therefore in the 7 of the 17 control patients who developed detectable anti-Rh for the first time during their subsequent pregnancies it is reasonable to conclude that their previous pregnancy provided the immunizing stimulus.

Secondary Immune Response

By exclusion, therefore, we are led to the second possibility, that active maternal immunization occurs primarily in the early

post-partum period, and that Rh antibodies may escape detection until a subsequent stimulation by another Rh-positive pregnancy.

It would appear therefore that the incidence of maternal Rh immunization after a single Rh-positive ABO compatible pregnancy is about 17%. Those examples of Rh immunization which have been reported to occur intra partum in all probability result from previous Rh-positive pregnancies or other exposure to the Rh antigen. Even in a group of Rh-negative primiparae one must be circumspect in ruling out the possibility of previous exposure to the Rh antigen because of: (1) a previously unsuspected Rh-positive pregnancy terminating in abortion; (2) previous transfusion of Rh-positive whole blood; (3) the injection of Rh-negative female infants with whole blood for the prevention of haemorrhagic disease of the newborn before the discovery of vitamin K; and (4) the intramuscular injection of whole blood for measles prophylaxis. While all of these practices have been abandoned as dangerous, a legacy of iatrogenically immunized Rh-negative women may still become apparent after subimmunizing doses of Rh antigen during pregnancy many years later.

In the Rh-immune globulin studies reported here, only ABO compatible mother–infant pairs were examined. Whereas there is wide agreement that the coexistence of ABO and Rh incompatibility between mother and child decreases the incidence of maternal Rh immunization, the extent of this decrease is disputed. Numerous investigators (Van Loghem and Spaander, 1948; Wiener *et al.*, 1949; Donohue *et al.*, 1954; Reepmaker, 1955; Heistö, 1955) have confirmed Levine's (1943) early observation that fewer infants with Rh-haemolytic disease were born to mothers who were ABO incompatibly mated than expected. These observations, while valid, cannot be used to assign the degree of protection against maternal Rh immunization, since (1) the incidence of Rh-haemolytic disease is not equal to the incidence of maternal Rh immunization, and (2) cases of Rh-haemolytic disease tend to be less severe where dual (ABO and Rh) incompatibility exists between mother and infant. Hence, some of the latter cases of Rh-haemolytic disease may escape the attention of specialty clinics where severe cases are referred.

Expected and Observed Incidence

In three large independent studies Reepmaker (1955), Cohen (1960), and Donohue and Wake (1964) have reported the ABO blood group of Rh-immunized women and their husbands. In all three studies summarized in Table III, it may be seen that there was no statistical difference between the expected and observed incidence of Rh-immunized women mated A × B, B × A, AB × A or B, except for the minimal significance seen in the A × B matings in Reepmaker's study. There was, however, a 31–49% decrease from the expected incidence of Rh immunization in the group O women in the matings A × O and B × O.

TABLE III.—*Comparison Between Expected* and Observed Frequencies of Maternal Rh Immunization in ABO Incompatible Matings*

Investigators	No. of Patients	A × O		B × O		A × B		B × A		AB × A or B	
		Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.	Exp.	Obs.
Donohue and Wake (1964)	438	81·5	44	19	12	16	14	16	13	8·7	11
		P < 0·001 (46% reduction)		P < 0·001 (39% reduction)		N.S.		N.S.		N.S.	
Cohen (1960)	2,657	238	182	56	37	39	33	45	39	21·5	16
		P < 0·001 (31% reduction)		P < 0·001 (34% reduction)		N.S.		N.S.		N.S.	
Reepmaker (1955)	1,742	337	172	69	40	66	44	66	48	29	18
		P < 0·001 (49% reduction)		P < 0·01 (42% reduction)		P < 0·05 (33% reduction)		N.S.		N.S.	

* Determined by the Hardy–Weinberg equilibrium based on ABO gene frequency for the respective populations examined.
N.S. = Not significant.

The decrease in incidence of Rh immunization in the group O mothers and the absence of protection in the non-group O mothers may be due to the qualitative difference in the anti-A and anti-B isoagglutinins of the two groups, as has been shown by Rawson and Abelson (1960). The predominantly 7S anti-A and anti-B of group O women may be more effective in preventing ABO and Rh-incompatible foetal erythrocytes from gaining access to the maternal immune system.

If the incidence of Rh immunization after a single ABO compatible, Rh-incompatible pregnancy is about 17%, as is suggested by the present study, then the incidence of maternal Rh immunization for group O and non-group O women with ABO incompatibility is 9–12% and 17% respectively.

The upward revision of probability of maternal Rh immunization is important to establish the risk of an unprotected Rh-incompatible pregnancy, since specific immunoprophylaxis is now widely available. These studies indicate that the overall incidence is more than twice that previously reported for ABO compatible and incompatible matings. The recognition of the time of immunization is significant inasmuch as it justifies the rationale for using passive immunization as a single post-partum injection rather than as an intrapartum measure, as has been proposed by Zipursky and Israels (1967). In addition to the wasteful nature of treating Rh-negative women with a foetus of undetermined Rh type (12 of the 47 women treated intrapartum gave birth to Rh-negative infants), this practice suffers from one additional disadvantage. The Rh-positive infants born to Rh-negative women treated during the last trimester of pregnancy may have a positive direct Coombs reaction, which, if not properly evaluated, could mask the coexistence of haemolytic disease due to blood group antibodies other than anti-Rh. The prognostic value of this useful procedure on the cord blood specimen is unnecessarily surrendered.

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ADDENDUM.—Since submitting this manuscript for publication, J. C. Woodrow and W. T. A. Donohoe (*Brit. med. J.*, 1968, **4**, 139) have reported a 17% incidence of maternal Rh immunization in unprotected Rh-negative women having second Rh-positive pregnancies. The close corroboration of the present report provides excellent verification of this surprisingly high incidence.