AN ANTENATAL EVALUATION OF INCREASED RH-ANTIBODY STIMULATION*

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Since the recent discovery that Rh-negative mothers can be protected against Rh immunization by the passive administration of anti-D gammaglobulin,[1,2] considerable attention has been focused on the incidence and effect of foeto-maternal bleeds occurring at delivery. Evidence is also available to show that foetal red cells often find their way into the maternal circulation during pregnancy, indicating that it is possible that antigenic stimuli for Rh-antibody formation can be expected before, as well as after, the delivery of an Rh-positive infant.[3-7]

Even though investigators employ different methods for the antenatal detection of foetal red cells, there has always been close agreement that the frequency of positive findings increases during the final months of pregnancy. While not every foeto-maternal bleed necessarily constitutes an active process of iso-immunization (because individual differences in the mechanism of antibody formation can be expected), it nevertheless raises the problem that antenatal haemorrhage from the foetus to the mother can initiate or further the intensity of antibody stimulation. It is therefore possible to postulate that recorded antenatal variations in antibody specificity, nature of immunoglobulin involved, or simple antibody titre differences can be considered as evidence that renewed foetal bleeds have taken place.

In this section of a three-part report, results of an investigation are presented to show that Rh-antibody follow-up studies performed throughout pregnancy can often clearly reveal the frequency of transplacental haemorrhage and its effect on foetal wastage. Although past observations have consistently suggested that the initial development of Rh immunization occurs as a result of the trauma of parturition, it is also shown that such events can occur as a consequence of foetal bleeds during pregnancy.

MATERIALS AND METHODS

All Rh-immunized mothers analysed in this study were examined from the first trimester of pregnancy onwards. In no instance were mothers included in whom the production of Rh antibodies might have been the result of previous Rh-incompatible blood transfusions, and only Rh-incompatible mother-infant combinations were examined.

For the evaluation of Rh-antibody titres the standardized method of indirect antiglobulin titration was used.[8] The importance of thorough washing of Rh-sensitized cells to avoid contamination by human serum before adding antihuman globulin reagent is emphasized. The necessity for this mode of testing, particularly for establishing consistent titration results, was recently confirmed by Gibbs and Camp.[9]

Since the frequency of antenatal episodes of Rh immunization is known to differ in Rh-negative mothers who become immunized by foetal Rh-positive red cells,[10] the question of when and how often these mothers do show increased episodes of antibody production is important. To determine these variations it is necessary that the investigator first resolves the accuracy of his indirect antiglobulin titration procedure. Table I shows that within the range of one dilution tube nearly 20% of inaccurate results can be recorded for the same samples of blood when tested by two investigators. For a 2-dilution-tube difference the percentage of error is narrowed to 5%. The absence of titration inaccuracies exceeding 3 dilution tubes is significant in that an observed increase of this magnitude can confidently be regarded as a renewed episode of immunization. Assuming that titre differences of 3 dilution tubes or more can also imply that transplacental passage of Rh-incompatible foetal red cells has taken place, then it should also be possible to assess how often such occurrences generally take place during pregnancy.

TABLE I. ACCURACY IN EVALUATION OF RH-ANTIBODY TITRES BY STANDARDIZED INDIRECT ANTIGLOBULIN METHOD WHEN DETERMINED BY 2 INVESTIGATORS (BLIND TEST)

	134 samples of Rh antibodies examined			
Titre differences	*First investigator % error*	*Second investigator % error*	*Average variation % error*	*Accuracy in predicting increased episodes of Rh immunization when variations exceed*
Three dilution tubes	0·0	0·0	0·0	100%
Two dilution tubes	6·7	5·9	6·3	93·7%
One dilution tube	15·6	17·9	16·7	83·3%

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RESULTS AND DISCUSSION

Table II records an analysis of 818 Rh-negative mothers who either were already immunized before the present pregnancy, or became immunized at some stage during the present pregnancy. The findings observed for different gestation periods clearly show that a significantly greater frequency of episodes of increased Rh-antibody production occurs after 32 weeks of gestation. Considering that this probably indicates increased foetal red cell leakage across the placental barrier, the results would appear to be in agreement with the findings of Betke,[11] who showed that a greater percentage of foetal red cells is generally observed in the maternal circulation towards the end of pregnancy.

TABLE II. ANALYSIS OF FREQUENCY OF ANTENATAL EPISODES OF RH-IMMUNIZATION OBSERVED AMONG 818 RH-IMMUNIZED MOTHERS EXAMINED AT VARIOUS STAGES OF PREGNANCY

Gestation range	*Total number of mothers examined*	*Episodes of Rh immunization observed**	
		No.	*%*
25 weeks or less	818	0	0·0
26–28 weeks	818	53	6·4
29–31 weeks	805	106	13·1
32–34 weeks	773	166	21·4
35–37 weeks	713	254	35·6
38–40 weeks	289	167	57·7

* Represents Rh-antibody titre increase of 3 dilution tubes or more.

Since none of the Rh-immunized mothers studied in this report was subjected to amniocentesis, it is intended to carry out comparative evaluations in future of the rate of increased antibody production observed between mothers not subjected to amniocentesis and those who were.

Table III records a similar investigation of 818 Rh-immunized mothers, comparing those who were immunized before the present pregnancy and those who were not. Of importance here is the observation that the frequency of raised episodes of immunization is significantly greater among those mothers who showed the presence of Rh antibodies for the first time. This increase appeared to be closely associated with the duration of pregnancy, showing that mothers not immunized before the present pregnancy recorded a significantly greater frequency of episodes of immunization before 35 weeks of pregnancy than the Rh-negative mothers who were already immunized.

Since it would be unrealistic to assume that such differences are directly influenced by significant variations in the incidence of transplacental bleeds occurring between mothers immunized and not immunized before the present pregnancy, it would appear that the most likely explanation for the decreased occurrence of episodes of immunization is that the mother who is already immunized has a remarkable ability to conceal the recognition of the foetal Rh-positive antigen as a potential stimulus. Such a hypothesis seems quite acceptable, particularly when we consider that these mothers can only be the recipients of foetal Rh-positive red cells which were already sensitized *in utero*, whereas mothers who were not immunized before the present pregnancy would be more often exposed to foetal red cells possessing the full expression of the Rh antigen. As such, the mechanism of Rh-antibody stimulation is unquestionably comparable with the studies of Stern *et al.*[12] These investigators were able to show that sensitized Rh-positive red cells are far less antigenic than unsensitized red cells, and their original work has led to the evaluation of the prevention of Rh immunization by the injection of gammaglobulin Rh antibodies.

If the present indication of apparent suppression of increased antibody production is an accurate deduction of the experimental findings of Stern *et al.*,[12] it can be seen that such a protective situation is not constant throughout pregnancy. In fact, the observations show that this apparent protection appears to decrease among the already immunized mothers as pregnancy progresses towards term. Since only small transplacental bleeds are anticipated before 30 weeks of pregnancy with more profuse bleeds generally occurring after this time,[13] it is possible to conclude that a breakdown in protection can be induced by the introduction of significant variations in the dosage of Rh-positive red cells crossing the placental barrier after 30 weeks' gestation. This situation bears a striking resemblance to the inability of anti-D gammaglobulin to secure complete protection against rhesus immunization when massive foeto-maternal bleeds are encountered.[13-15]

Such serological developments suggest that there are at least 2 ways in which the intensity of increased Rh-antibody production can be stimulated during pregnancy. One is the result of imperfectly understood defects of the placenta, which allows greater quantities of foetal red cells into the maternal circulation, while the other is undoubtedly closely associated with external version,[7,16] the repeated application of abdominal amniocentesis,[17,18] or certain modes of obstetric management during the third stage of labour.[19]

TABLE III. COMPARATIVE ANALYSIS OF FREQUENCY OF ANTENATAL EPISODES OF RH IMMUNIZATION OBSERVED AMONG MOTHERS NOT IMMUNIZED AND THOSE ALREADY IMMUNIZED BEFORE PRESENT PREGNANCY

Gestation range	*Mothers not immunized before present pregnancy*			*Mothers already immunized before present pregnancy*			*Chi-square values (Yates correction applied)*
	Total No. of mothers	*Episodes confirmed**		*Total No. of mothers*	*Episodes confirmed**		
		No.	*%*		*No.*	*%*	
25 weeks or less	208	0		610	0		
26–28 weeks	208	27	12·9	610	26	4·2	$x^2_{(1)}$ 16·449 P = 0·001
29–31 weeks	208	43	20·6	597	63	10·5	$x^2_{(1)}$ 9·454 P = 0·01
32–34 weeks	208	62	29·8	565	104	18·4	$x^2_{(1)}$ 6·771 P = 0·01
35–37 weeks	200	87	43·5	513	167	32·5	$x^2_{(1)}$ 3·160 n.s.
38–40 weeks	167	109	65·2	122	58	47·5	$x^2_{(1)}$ 2·178 n.s.
Average for all gestations	199	54	27·1	502	69	13·7	x^2 11·838 P = 0·001

* Represents Rh-antibody titre increase of 3 dilution tubes or more.

TABLE IV. EVALUATION OF INCIDENCE OF FOETAL WASTAGE (STILLBIRTHS AND NEONATAL DEATHS) IN RELATION TO ANTENATAL EPISODES OF INCREASED RH-ANTIBODY PRODUCTION OBSERVED FOR 208 MOTHERS WHO WERE NOT IMMUNIZED BEFORE PRESENT PREGNANCY

Gestation range	Total No. of mothers observed	Antenatal episodes of immunization: Observed*				Not observed			
				Foetal wastage				Foetal wastage	
		No.	%	No.	%	No.	%	No.	%
25 weeks or less	208	0	0	0	0	208	100·0	0	0
26–28 weeks	208	27	12·9	0	0	181	87·1	0	0
29–31 weeks	208	43	20·6	0	0	165	73·4	0	0
32–34 weeks	208	62	29·8	0	0	146	70·2	0	0
35–37 weeks	200	87	43·5	2	2·3	113	56·5	0	0
38–40 weeks	167	109	65·2	5	4·6	58	34·8	1	1·7

* Represents Rh-antibody titre increase of 3 dilution tubes or more.

TABLE V. EVALUATION OF INCIDENCE OF FOETAL WASTAGE (STILLBIRTHS AND NEONATAL DEATHS) IN RELATION TO ANTENATAL EPISODES OF INCREASED RH-ANTIBODY PRODUCTION OBSERVED FOR 610 MOTHERS WHO WERE ALREADY IMMUNIZED BEFORE PRESENT PREGNANCY

Gestation range	Total No. of mothers observed	Antenatal episodes of immunization: Observed*				Not observed			
				Foetal wastage				Foetal wastage	
		No.	%	No.	%	No.	%	No.	%
25 weeks or less	610	0	0	0	0	610	100·0	0	0
26–28 weeks	610	26	4·2	10	38·4	584	95·8	3	0·5
29–31 weeks	597	63	10·5	27	42·7	534	89·5	5	1·0
32–34 weeks	565	104	18·4	26	25·0	461	81·6	8	1·7
35–37 weeks	513	167	32·5	49	29·3	346	67·5	21	6·0
38–40 weeks	122	58	47·5	8	13·7	64	52·5	3	4·6

* Represents Rh-antibody titre increase of 3 dilution tubes or more.

The results so far presented show that foetal red cells often cross the placental barrier to stimulate increased Rh-antibody production in the mother. Not firmly established, however, is the effect of these episodes of immunization on the clinical manifestation of Rh-haemolytic disease. Table IV details an analysis of 208 mothers who for the first time showed Rh antibodies during an Rh-incompatible pregnancy. It is interesting to note that foetal wastage (combined stillbirths and neonatal deaths) can be anticipated in the first immunizing pregnancy. Thus, the tendency to consider all primary immunization cases as mildly affected infants, who generally do not require further treatment, will involve a certain amount of risk unless constant follow-up studies are carried out to measure the intensity of the haemolytic process *in utero*.

Compared with the values of foetal wastage observed among the already immunized series of mothers as shown in Table V, it is significant that the foetal losses recorded among the primary immunization cases consistently took place after 35 weeks of pregnancy, when the incidence of Rh-antibody stimulation appeared to be most intense. This type of pattern also suggests that the time during which an Rh-positive foetus is exposed to Rh antibodies is of some importance in determining the severity of the haemolytic process *in utero*.

From the findings presented in Tables IV and V it is also evident that a very significant percentage of foetal wastage can be directly attributed to the occurrence of renewed episodes of Rh-antibody stimulation. This implies that the protective factor against increased Rh-antibody formation in the already immunized mother can be reversed through the reactivities of progressively large foetal bleeds, which in turn intensify the haemolytic process.

SUMMARY

In a study of 818 Rh-immunized mothers investigated throughout pregnancy it was shown that the frequency of raised Rh-antibody production, as determined by a 3-fold increase in the indirect antiglobulin titre, was significantly greater among mothers who were not immunized before the present pregnancy than among those who were already immunized. This type of variation appeared as evidence that increased Rh-antibody stimulation is not easy to induce when the already immunized mother is subjected to small transplacental bleeds of foetal Rh-positive red cells sensitized *in utero*. Foetal mortality studies also showed that the occurrence of increased Rh-antibody stimulation in the mother is directly responsible for a large percentage of foetal wastage observed in Rh-haemolytic disease of the newborn.

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