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Prevention of Rhesus Immunization. A Controlled Clinical Trial with a Comparatively Low Dose of Anti-D Immunoglobulin

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Summary: A controlled clinical trial was carried out to test the effectiveness of a comparatively low dose of anti-D immunoglobulin (250 μg) in preventing rhesus immunization.

In the control group 17 out of 329 women (5%) formed rhesus antibodies, whereas in the treated group only 3 out of 333 women (0.9%) showed active immunization, all three of whom had an exceptionally large transplacental bleeding.

Introduction

Clinical trials carried out in Great Britain and in the United States have convincingly shown that rhesus immunization can in nearly all cases be prevented by the administration of anti-D immunoglobulin shortly after delivery of a rhesus-positive infant (Combined Study, 1966; Freda *et al.*, 1967).

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Progress is also being made in other countries to introduce this kind of prophylaxis. At present, however, the widespread application is severely limited by the supply of anti-D plasma from which the immunoglobulin is prepared. For this reason it is important to determine the minimum effective dose for the prevention of rhesus immunization.

In the New York and Liverpool trials doses of approximately 5,000 and 1,000 μg. of anti-D immunoglobulin respectively were used (Clarke, 1967), with practically 100% therapeutic effectiveness. Recently Ascari *et al.* (1968) published the results of a trial in which a dose of 300 μg. had been used, with similar success.

The present study records the effect of a 250 μg. dose.

Material and Methods

Patient Selection.—Ten obstetrical clinics participated in the trial over a period of 12 months. The patients comprised all non-immunized rhesus-negative women who were delivered of a rhesus-positive child, irrespective of parity or ABO compatibility. Women were randomized by treating those with odd-numbered birthdays. The immunoglobulin was administered by intramuscular injection within 24 hours after delivery.

Anti-D Immunoglobulin.—Anti-D immunoglobulin was prepared from 20 litres of pooled plasma donated by 18 naturally immunized women who had been selected for high-titre anti-D antibodies. Details regarding the preparation and assay have been given (Dudok de Wit and Borst-Eilers, 1968). The anti-D content of the preparation was 250 μg./ml.

Tests for Antibody.—Heparinized blood samples were collected on three occasions: at 32–34 weeks' pregnancy, immediately after delivery, and four to six months later. The plasma was tested for rhesus antibodies by standard saline, bromelin, and antiglobulin techniques, against a red cell suspension consisting of a 1:1 mixture of O CDe/cde and O cDE/cde cells. The same test cells were used throughout the study.

Estimation of Transplacental Haemorrhage.—All blood samples obtained at 32–34 weeks' pregnancy and immediately after delivery were examined for the presence of foetal red cells. Blood was diluted 1:3 with saline, and from 0.01 ml. of this suspension a blood film was prepared. This film was treated according to the method of Kleihauer *et al.*, (1957). Typical Hb F cells were counted in an area of 82.5 sq. mm. By mixing cord blood with adult blood in vitro it was found that a foetal cell score of 60 corresponded to the presence of about 1 ml. foetal blood in the maternal circulation.

Results

The trial comprised 740 rhesus-negative women with a rhesus-positive child. In eight cases rhesus antibodies (anti-D) which had not been present at 32–34 weeks' pregnancy were found in the post-delivery blood sample. For various reasons the sample at four to six months' post partum could not be obtained from 70 women. Of the remaining 662 women, 329 had previously been allotted to the control group and 333 to the treatment group. In the former there were 261 ABO compatible babies (79%) and 182 primiparae (55%), whereas in the latter these figures were 272 (82%) and 181 (54%), respectively.

In the control group 17 women (5%) formed anti-D, whereas among the treated women only 3 (0.9%) became immunized. The difference is significant ($P<0.01$). In Table I the incidence of immunization is correlated with the outcome of the Kleihauer test on the post-delivery blood samples. In all three women who formed rhesus antibodies despite treatment, exceptionally large transplacental haemorrhage (50, 140, and 180 ml.) had taken place. Details of two of these cases have been published (Dudok de Wit and Borst-Eilers, 1968, Cases 1 and 4). However, none of the six cases in the treated group with moderately large bleeds (of 5, 8, 10, 15, 19, and 25 ml. respectively) formed rhesus antibodies.

TABLE I.—*Prevention of Rhesus Immunization Following Pregnancy. Results of Controlled Clinical Trial with 250 μg. of Anti-D*

Approximate Amount of Foetal Blood in Maternal Circulation after Delivery	Not Treated		Treated	
	No.	Immunized	No.	Immunized
None found	171	3	179	0
<0·1 ml.	125	9	108	0
0·1–1 ml.	30	3	37	0
1–10 ml.	3	2	2	0
10–50 ml.	0	0	4	0
50–100 ml.	0	0	1	1
≥100 ml.	0	0	2	2*
Total	329	17 (5%)	333	3 (0·9%)

$\chi^2 = 8{\cdot}70$, 1 d.f., $P < 0{\cdot}01$.
* Previously reported (Dudok de Wit and Borst-Eilers, 1968).

In addition to the 20 women who formed anti-D there was one woman in the control group who formed anti-Kell. More detailed information on these 21 cases as well as on the eight cases where anti-D antibodies appeared towards term are given in Table II. It is apparent that neither primiparae nor ABO compatible cases showed a higher immunization rate.

TABLE II.—*Data on 29 Cases of Immunization by Pregnancy*

Parity	Blood Group		Estimated Volume of Foetal Blood in Maternal Circulation (ml.)		Titre of Immune Antibody 4–6 Months' Post-delivery			Anti-D Immunoglobulin Given
	Mother	Child	32–34 Weeks' Pregnant	Post-delivery	Saline	Bromelin	Antiglobulin	
Rhesus-antibodies Formed before Delivery								
II	A Rh⁻	A Rh⁺	0	0	0	4	2	—
I	O Rh⁻	O Rh⁺	0	0	0	64	32	—
II	A Rh⁻	O Rh⁺	0	—	0	16	32	—
III	O Rh⁻	O Rh⁺	0	—	0	4	8	—
III	A Rh⁻	A Rh⁺	0	<0·1	0	1	1	—
I	A Rh⁻	A Rh⁺	<0·1	0	256	512	256*	—
I	O Rh⁻	O Rh⁺	<0·1	—	8	32	16	—
I	A Rh⁻	A Rh⁺	3	0	1	8	2	—
Rhesus-antibodies Formed after Delivery								
I	O Rh⁻	O Rh⁺	0	0	0	4	0	—
I	O Rh⁻	A Rh⁺	0	0	0	2	2	—
III	B Rh⁻	AB Rh⁺	0	0	0	2	4	—
I	O Rh⁻	O Rh⁺	0	<0·1	0	4	8*	—
I	A Rh⁻	A Rh⁺	<0·1	<0·1	0	2	0	—
I	B Rh⁻	B Rh⁺	<0·1	<0·1	0	2	16	—
I	A Rh⁻	A Rh⁺	<0·1	<0·1	16	16	8	—
II	AB Rh⁻	AB Rh⁺	<0·1	<0·1	0	4	1	—
II	O Rh⁻	O Rh⁺	0	<0·1	0	2	2†	—
II	B Rh⁻	A Rh⁺	0	<0·1	0	4	2	—
II	O Rh⁻	A Rh⁺	<0·1	<0·1	0	2	4	—
II	A Rh⁻	A Rh⁺	0	<0·1	0	2	4	—
III	A Rh⁻	A Rh⁺	0	<0·1	0	±	1	—
I	A Rh⁻	A Rh⁺	<0·1	0·1	0	2	2	—
I	AB Rh⁻	B Rh⁺	<0·1	0·5	4	4	4	—
III	O Rh⁻	O Rh⁺	0	0·9	0	4	2	—
I	A Rh⁻	A Rh⁺	0	1·5	0	2	2	—
I	AB Rh⁻	B Rh⁺	0	7	1	4	32	—
I	O Rh⁻	O Rh⁺	<0·1	50	0	8	64	750 μg
II	A Rh⁻	A or O‡	<0·1	140	2	8	32*	250 μg
II	A Rh⁻	A Rh⁺	180	180	±	8	16*	500 μg

* Both anti-C and anti-D. † Only anti-Kell, no anti-D. ‡ Died.

So far only four women, all of whom belonged to the treatment group, have given birth to another rhesus-positive child. In none of these cases have rhesus antibodies been found.

Discussion

The results of this trial show that rhesus immunization could largely be prevented by the administration of 250 μg. of anti-D immunoglobulin within 24 hours of delivery. There were, however, circumstances in which prophylaxis failed.

In the first place antibodies had appeared before delivery in eight women. This could be due to primary immunization during pregnancy or it could be a booster response following earlier sensitization by pregnancy or blood transfusion. Four of the women were multiparae, for whom the latter explanation is the more likely. The remaining four primiparae all had a negative history of pregnancies or transfusion; nevertheless, the high anti-D titre in three of these cases suggests a booster reaction rather than a primary immunization (see Table II). From our results it would appear, therefore, that primary immunization during pregnancy is probably a rare occurrence. This is in agreement with the observations of Nevanlinna and Vainio (1962), who found only four examples of rhesus immunization during pregnancy in a study of more than 4,000 primiparae. A different opinion is held by Chown (1968), who estimated the incidence at 2%. In order to prevent antibody formation in these cases the Winnipeg group has started the administration of anti-D during the last trimester of pregnancy in a limited number of women (Zipursky and Israels, 1967).

In the second place immunoprophylaxis failed in all three cases in which a very large transplacental haemorrhage had occurred. So far only seven cases of attempted prophylaxis after massive transplacental haemorrhage have been reported, the dose of anti-D varying from 250 to 1,000 μg. (Dudok de Wit and Borst-Eilers, 1968, Hughes-Jones and Mollison, 1968; Woodrow *et al.*, 1968). In five of these cases active immunization occurred, suggesting that if immunosuppression can be achieved at all under these circumstances much higher doses

of anti-D will be required. Transplacental bleeding of 50 ml. or more occurred in 3 out of 740 cases in our series; the frequency, however, of such large foetomaternal transfusions is generally supposed to be lower. Thus Schneider and Schoof (1964), in a series of 1,300 hospital deliveries, observed no transplacental bleeds of more than 12 ml. We feel, therefore, that there is no justification in trying to adjust the standard dose of anti-D to the requirement met with in the rare instances of massive transplacental bleeding. In view of the limited supply of anti-D immunoglobulin the standard dose should be the smallest dose that is effective in cases with small and moderately large bleeds. In this context it is encouraging to note that in our study none of the six women in the treated group with bleeds of 5 to 25 ml. developed rhesus antibodies.

It therefore seems reasonable to propose that further trials be undertaken to determine whether, in fact, anti-D immunoprophylaxis may be achieved with doses even smaller than 250 μg.

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The immunoglobulin preparation was produced by Dr. H. W. Krijnen, Central Laboratory of the Blood Transfusion Service of the Netherlands Red Cross, Amsterdam.

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Significance of Rh-sensitization during Pregnancy: Its Relation to a Preventive Programme

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Summary: A study of the incidence of Rh-sensitization showed a 6.5% incidence of anti-D appearing for the first time during the last trimester of pregnancy. In 24 of the 29 subjects these antibodies were weak, and were found only when a sensitive technique using enzyme-treated Rh-deletion test cells was employed. The remaining five, however, had high-titre antibodies, which were associated with a positive Coombs test on cord blood.

Rh-immune globulin administered at delivery resulted in disappearance of the anti-D in all but one of the subjects with weak antibody to whom it was given, suggesting that this treatment can reverse early sensitization. There was no effect when Rh-immune globulin was given to one subject with a high anti-D titre.

Since sensitization has been found to occur frequently during the last trimester of pregnancy, an antenatal schedule of prophylaxis is advocated.

Introduction

It is now generally accepted that the administration of Rh-immune globulin to susceptible mothers at delivery may result in almost complete suppression of antibody formation in the postpartum period (*Brit. med. J.*, 1966; Freda, Gorman, and Pollack, 1966; Zipursky and Israels, 1967; Mollison, 1968).

Investigation into the prevention of Rh sensitization has been carried on in Edmonton since January 1967 as part of a co-operative study involving the western Canadian provinces (*Canad. med. Ass. J.*, 1967; Chown, 1967). Our part in the study was designed primarily to compare the effectiveness of two different doses of Rh-immune globulin and to try to establish a minimum effective dose. However, we were also interested in trying to establish the incidence of sensitization during the last trimester of pregnancy. Our interest was stimulated by the fact that in northern Alberta from 1965 to 1967 3.5% of women with anti-D agglutinins found in the last trimester of pregnancy were primigravidae.

Methods and Materials

Selection of Subjects

Criteria for entry into the study were: (*a*) Rh-negative mother of any parity with an Rh-positive husband; (*b*) no anti-D when first tested during pregnancy; and (*c*) delivery of an Rh-positive ABO-compatible infant.

The following tests were done on all of the possible candidates for the study: ABO and Rh phenotype of the mother, ABO

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