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Prevention of Primary Rh Immunization:

First Report of the Western Canadian Trial, 1966-1968

AT a meeting held in Winnipeg in March 1966 of representatives from the Universities of Manitoba, Saskatchewan, Alberta, Calgary, British Columbia and Western Ontario, the Department of National Health and Welfare and the Connaught Medical Research Laboratories, the following program was proposed:

1. All centres taking part in the study (Winnipeg, Saskatoon, Edmonton, Calgary, Vancouver, London) undertook to gather, by means of plasmapheresis, anti-Rh plasma from women who had been immunized by pregnancy and who had had at least one baby with an extreme form of hemolytic disease. The Connaught Medical Research Laboratories undertook to extract Cohn fraction II from 200-litre lots of such plasma and prepare it in a 16% protein solution.

2. In all cases Rh-negative women were to be admitted to the study if:

(a) they had no Rh antibodies at delivery;

(b) the baby was Rh-positive, ABO-compatible and Coombs negative.

Women in the experimental group were to be treated by a single intramuscular injection of the Rh immune globulin within 72 hours after delivery, while "controls" would receive no such injection. The allotment to treated and control groups was to be random.

3. Parity and fetal cell counts (Kleihauer) would be recorded but not used in selection for or against treatment.

4. The sera of all women would be examined for antibodies at delivery, towards the end of their stay in hospital, and again six weeks and six months after delivery.

5. Patients would be admitted to the study over a period of one year, with the final follow-up six months later.

By April 1967 enough plasma had been collected, processed to gamma globulin and delivered. Even before this, with the new information accessible on the English and American trials, the desirability of having untreated controls was questioned. The studies that are here reported, therefore, differed somewhat in final plan:

1. Winnipeg, Vancouver and Calgary: Treated and control groups as originally arranged. The dose of Rh immune globulin would be 1.5 ml., assayed by Dr. Nevin Hughes-Jones as containing approximately 435 μg. of anti-D.

2. Edmonton and London: All women would be treated but they would be divided into two equal groups: one group would receive 1.5 ml. (435 μg.) and the other 0.5 ml. (145 μg.). There would be no concurrent untreated controls.

3. Saskatoon: Once the program was fully accepted by the profession, all women would be treated as in Edmonton and London. However, such women as were delivered while the study was in progress but were not treated either be-

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TABLE I.—WOMEN WHO HAD NO DEMONSTRABLE ANTI-D AT DELIVERY AND WHOSE SERUM WAS AGAIN EXAMINED SIX MONTHS LATER. ALL BABIES WERE ABO-COMPATIBLE, RH-POSITIVE AND COOMBS NEGATIVE. NONE OF THE TREATED WOMEN DEVELOPED ANTI-D BY SIX MONTHS.*

1. Studies with concurrent controls throughout

(a) *Treated*	*Primiparas*	*Multiparas*	*Total*	(b) *Controls*	*Primiparas*	*Immunized*	*Multiparas*	*Immunized*
Vancouver	88	86	174	Vancouver	53	6	99	12
Calgary	45	57	102	Calgary	37	4	66	4
Winnipeg	92	140	232	Winnipeg	103	6	123	2
				Saskatoon	10	2	9	0
Totals.....	225	283	508		203	18	297	18

2. Dosage studies. No concurrent controls except Saskatoon (see text and above).

	Dose					*Dose*			
Primiparas	*1.5 ml.*	*1.0 ml.*	*0.5 ml.*	*Total*	*Multiparas*	*1.5 ml.*	*1.0 ml.*	*0.5 ml.*	*Total*
Vancouver	19		32	51	Vancouver	51		54	105
Saskatoon	15	3	9	27	Saskatoon	13	3	18	34
Edmonton	61		52	113	Edmonton	121		126	247
London	32		33	65	London	32		34	66
Totals.....	127	3	126	256		217	3	232	452

Grand totals

	Treated				*Untreated controls*		
	Primiparas	*Multiparas*	*Total*		*Primiparas*	*Multiparas*	*Total*
With 1.5 ml.	352	500	852		203	297	500
With 1.0 ml.	3	3	6	Immunized	18	18	36
With 0.5 ml.	126	232	358		(8.9%)	(6.1%)	(7.2%)
Total.....	481	735	1216				
Immunized	0	0	0				

*The time limit of six months is used because although development of Rh antibodies may be quite slow, it never, so far as known, takes longer than six months.

cause they refused treatment or because their doctors did not want them treated were classed as "concurrent controls". The Saskatoon dosage data are included in the table with the Edmonton and London data, and the Saskatoon control data with those of the Vancouver, Calgary and Winnipeg controls.

4. In Calgary, Vancouver and Edmonton, second "in hospital" specimens were routinely taken and examined on day 4 or 5: the anti-D titre in the treated women ranged from 1:2 to 1:8 in the second specimen. In Winnipeg the second specimen was taken on day 3; for the women who were treated this was immediately before injection.

RESULTS

The results of this main trial are set out in Table I. It may be seen that none of the 1216 women who were treated became immunized, whereas of 203 untreated primiparas 18 (8.9%) and of 297 untreated multiparas 18 (6.1%) did become immunized, making a total of 36 out of 500 (7.2%) who became immunized.

FURTHER DEVELOPMENTS

1. More Rh immune globulin than was needed for the planned studies became available. As a result the Vancouver group, while continuing their central study in the Vancouver hospitals, added a dosage study of 1.5 ml. versus 0.5 ml. in Victoria, Vernon, Penticton, Trail, Prince George, Nanaimo, Kamloops and Kelowna. The Winnipeg group made Rh immune globulin available to all women in Manitoba outside metropolitan Winnipeg and to all women in northwestern Ontario. Saskatoon extended its study to other cities and towns in Northern Saskatchewan. The results of these studies will be reported later.

2. The investigators in Winnipeg, Vancouver and Edmonton began reporting the occurrence of weak anti-D, demonstrable by sensitive (particularly enzyme) techniques, in the blood of women at or immediately after the termination of their first pregnancy. The frequencies reported were: Vancouver, 3 out of 144, Edmonton, 10 out of 179; Winnipeg, 5 out of 210.

A comparison of these frequencies with those for the control primiparas in Table I indicates that something of the order of a quarter of the women who became immunized by a first Rh-positive, ABO-compatible pregnancy had evidence of beginning immunization at delivery. This gave rise to two questions:

1. What will happen if these women are treated at delivery?

2. Is it perhaps necessary to treat primiparas

during pregnancy as well as at delivery if one is to attain 100% protection?

The Edmonton group have reported their observations on treatment of women with weak antibodies,[1] and the Winnipeg group have submitted theirs for publication. However, the two studies are not in agreement and more information is needed.

At the April 1968 meeting of the Western Canadian Committee on Rh Prevention it was decided to compare the results of treating one group of primigravidas at 34 weeks and again at delivery, with another group treated only at delivery. The Edmonton group had already started such a program, and one is now also under way in Vancouver, in London, in Winnipeg and in Hamilton (Dr. Alvin Zipursky). It will probably be at least two more years before it will be possible to draw sound conclusions.

TABLE II.—OUTCOME OF SECOND ABO-COMPATIBLE, RH-POSITIVE PREGNANCIES OF WOMEN FIRST STUDIED AS PRIMIPARAS

	Treated after first pregnancy		*Not treated after first pregnancy*	
	Number	*Developed anti-D during second pregnancy*	*Number*	*Developed anti-D during second pregnancy*
Vancouver	4	0	3	0
Edmonton	9	1*	5	0
Winnipeg	15	0	10	0
Total	28	1	18	0

*Antibody first found in seventh month of second pregnancy.

Table II presents the results of second ABO-compatible, Rh-positive pregnancies in women first coming under study as primiparas, in whom no anti-D was identified either at delivery or six months later.

FETAL CELLS

Because of staff shortages, fetal cell counts were recorded throughout the study only in Vancouver and Winnipeg. Although the Vancouver counts are recorded as the number of fetal cells seen in 300 low-power fields and the Winnipeg figures as the number in 600 fields, the scoring distribution is curiously similar between the two (Table III).

TABLE III.—FETAL CELL COUNTS (PERCENTAGE DISTRIBUTION)

	0	*1 - 5*	*6 - 25*	*26 - 50*	*50*	*Specimens examined*
Vancouver	74.1	14.9	4.8	1.5	4.7	326
Winnipeg	74.1	17.5	5.2	0.7	2.6	454
Totals	90.3		6.1		3.65	908

The frequencies of nil and low counts, like the counts by Zipursky and Israels[2] in their earlier Winnipeg studies, are significantly lower than in the English studies.[3] There was no clear correlation between the number of fetal cells present and the frequency with which anti-D appeared within the next six months.

Shelf-life of Rh Immune Globulin (Connaught)

Lot 4 Rh Immune Globulin was put in vials by the Connaught Medical Research Laboratories in February 1967 and was used in the main study until April 1968. As indicated in Table I, no woman in that study who received Lot 4 became immunized. In the doses used it was still effective after 14 months. Further studies to determine how long Rh Immune Globulin (Connaught) retains its protective power are in progress.

GENERAL CONCLUSIONS

Rh Immune Globulin (Connaught) given in the doses used in this study to unimmunized Rh-negative women within three days of the birth of an ABO-compatible, Rh-positive baby gives practically 100% protection against Rh immunization by the pregnancy just concluded, although there is still a problem to be solved relative to primigravidas.

Summary In a study of the effectiveness of Rh Immune Globulin (Connaught) in the prevention of primary Rh immunization of childbearing women, 1216 were treated by one injection by the third day after delivery and 500 were not treated. Of the treated, 852 received 1.5 ml. (approximately 435 μg. of anti-D immune globulin), six received 1.0 ml. (290 μg.) and 358, 0.5 ml. (145 μg.). All were retested six months later. None of the treated but 36 (7.2%) of the untreated had become immunized, with the development of anti-D. A higher percentage of primiparas became immunized (8.9%) than of multiparas (6.1%).

Twenty-eight women who, as primiparas, had been treated and 18 who had not, went through a second ABO-compatible, Rh-positive pregnancy. One of the 28 who had been treated, but none of the 18 who had not, developed anti-D during the course of that second pregnancy.

The anti-D (Rh_0) Immune Globulin (Connaught) was 14 months old at the conclusion of the study and was apparently still effective as a prophylactic agent.

Résumé Dans l'étude destinée à évaluer l'efficacité de l'immunoglobuline Rh (Connaught) pour prévenir l'immunisation Rh primaire, on a traité 1216 femmes par une injection donnée le troisième jour après l'accouchement, et 500 n'ont pas été traitées. Parmi les 1216 femmes traitées, 852 ont reçu une injection de 1.5 ml (environ 435 mcg d'immunoglobuline anti-D), six ont reçu 1.0 ml (290 mcg) et 385 ont reçu 0.5 ml (145 mcg). Toutes ces

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femmes ont été réexaminées six mois plus tard. Aucune des femmes traitées, mais 36 des femmes non traitées (soit 7.2%) sont devenues immunisées avec le développement de l'anti-D. Un pourcentage plus élevé de primipares sont devenues immunisées (8.9%) que de multipares (6.1%).

Vingt-huit femmes qui, comme primipares, avaient été traitées et 18 qui ne l'avaient pas été, eurent une deuxième grossesse à Rh positif, ABO compatible. Une des 28 femmes traitées, mais aucune des 18 femmes non traitées, ont développé l'anti-D durant le cours de cette deuxième grossesse.

Signalons que l'immunoglobuline (Connaught) anti-D (Rho) était vielle de 14 mois à la fin de notre étude et semblait encore efficace comme agent prophylactique.

Dr. Alvin Zipursky does not appear among the authors of this report, for, although he had played a key role in the original Winnipeg studies[2] and an active part in the organization of the present one, he had been translated to the Professorship of Pediatrics of McMaster University some months before the clinical study got under way. We are happy to have had him continue as a member of the Western Canadian Committee for the Study of Rh Immunization and its Prevention.

Dr. Jean Webb, at that time the Director of the Division of Maternal and Child Health, Department of National Health and Welfare, was of great help in the organization of the study. It was through the active interest of Dr. Albert Fisher of the Connaught Medical Research Laboratories that the anti-D (Rh_o) immune globulin was prepared, both for the earlier Winnipeg studies and for this one. The Canadian Red Cross Society generously allowed the plasmapheresis of donors to be carried out, where desired, in the regional depots of its Blood Transfusion Service, donating the use of space and equipment and the time of personnel.

To the many women, here nameless, who were the donors of the plasma from which the anti-D immune globulin was extracted, we are in permanent debt.

APPENDIX

1. A baby is said to be ABO-compatible with its mother when their ABO groups are such that the mother's serum does not contain an antibody (anti-A or anti-B) that will react with the baby's red cells.

Compatible		*Incompatible*	
Mother	*Baby*	*Mother*	*Baby*
O	O	O	A, B
A	O, A	A	B, AB
B	O, B	B	A, AB
AB	A, B, AB	AB	None

2. In the study, only those women whose babies were ABO-compatible were treated because Rh immunization is more common in this situation than when the baby is ABO-incompatible with its mother. With a more than adequate supply of Rh Immune Globulin (Connaught) now available, the ABO status of mother and fetus needs no longer be considered in deciding on treatment.

2. In the study a "cross-match" between the Rh Immune Globulin and the red cells of the woman to be treated was not done. There were no reactions save occasionally a slight local soreness at the site of injection.

4. In the study no special precautions were taken to make certain that the woman was not a D^u (weak Rh-positive) rather than a true Rh-negative.

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