A State Program for Prevention of Sensitization to Rh Factor

IRVING UMANSKY, M.D., DOROTHY J. WORTH, M.D., M.P.H., RACHEL BENJAMIN, M.D., MORTON A. MADOFF, M.D., GEOFFREY EDSALL, M.D., and LOUIS K. DIAMOND, M.D.

ERYTHROBLASTOSIS (Rh disease) in newborn babies has been recognized as a major threat to infant health and survival for more than a generation (*1*). Early methods of treatment reduced, but did not eliminate, the risk of this disease. In the last few years, however, it has been established that the underlying cause of the disease, sensitization of the Rh-negative woman by the blood cells of her Rh-positive fetus, can be prevented by administration of human gamma globulin containing high levels of anti-Rh antibody (*2, 3*). This treatment is an application of Theobald Smith's observation, early in the century (*4*), that excess antibody could suppress the immunizing activity of an antigen.

In view of these advances, the staff of the Massachusetts Department of Public Health in 1967 examined the department's potential role in providing this gamma globulin (Rh Immune Globulin) for the citizens of Massachusetts. Based on an estimated State population of 5 million, with approximately 90,000 births per year, it was estimated that 8,000 doses of the Rh Immune Globulin would be needed annually to protect all of the Rh-negative mothers in Massachusetts who were at risk of sensitization. This protection would have required an annual outlay of more than $500,000 for this program if the globulin were obtained by purchase. Even a projected 50 percent price decrease would have placed an intolerable burden on available resources. We therefore decided to institute a local program of preparation and distribution of Rh Immune Globulin. Initial reports of similar projects underway in other countries were encouraging.

Dr. Umansky is assistant director, and Dr. Diamond is director of the Blood Grouping Laboratory of Boston. The other authors are with the Massachusetts Department of Public Health. Dr. Worth is director of the division of family health services, Dr. Benjamin is the Rh program coordinator, Dr. Madoff, formerly director of the division of biologic laboratories, is director of the bureau of adult and maternal health services, and Dr. Edsall is superintendent of the Massachusetts State Laboratory Institute.

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Massachusetts was particularly well suited for establishment of a program of this kind. The Maternity and Infant Care Project of the State department of public health was already participating in prenatal and postnatal care throughout Metropolitan Boston and had well-established channels of communications with the major obstetrical and pediatric hospitals and clinics in this area. The Biologic Laboratories of the Massachusetts Department of Public Health, which were established in 1895, had been engaged in producing gamma globulin and other biologicals for many years. The staff of the Blood Grouping Laboratory of Boston had long been concerned with the problems of Rh sensitization and erythroblastosis fetalis (*5–8*) and had participated actively in earlier studies which had demonstrated the efficacy of Rh Immune Globulin. Both the Massachusetts Red Cross Blood Program and the Children's Hospital Medical Center had well-established facilities for plasma collection. Each of these organizations agreed to enter into a voluntary cooperative program with the utlimate aim of providing Rh Immune Globulin at no cost to every Massachusetts woman at risk of becoming sensitized to the Rh factor.

Initial assistance in funding the Rh Immune Globulin Program was obtained from the U.S. Children's Bureau. A full-time physician coordinator, deemed essential to the program, was appointed. The files of numerous private obstetricians, hospitals in the Boston metropolitan area, and cooperating organizations were made available and were screened for potential donors. Local news media publicized the program's need for donors.

Criteria for Donor Selection

Certain firm criteria were established at the outset.

1. The program would be entirely voluntary. Donors of plasma would not be paid but would be assisted, when necessary, with transportation and other incidental expenses.

2. Donors could be male or female but had to have been previously sensitized to the Rh factor either as a result of pregnancy or the transfusion of Rh-positive blood. Primary sensitization would not be used.

3. Female donors had to be beyond the childbearing age or otherwise unable to have children.

4. Donors needed to reside within reasonable proximity to the blood collection center and to have sufficient motivation to insure their regular return for plasma donations.

Initially, letters were sent to potential donors, explaining the program and offering them the opportunity to help others avert the tragedies they themselves may have suffered. Personal telephone calls were made to those who responded, and interviews were arranged during which the full extent and implications of the program were described. Written consent was obtained for taking plasma and for restimulation. Those persons with any significant doubts or reluctance were discouraged from further participation.

Each volunteer to be enrolled in the program is required to undergo a complete physical examination, usually performed by his or her private physician. Liver function tests and other necessary blood tests are done to exclude any medical contraindication to blood donations. A complete Rh blood profile, including the determination of Rh-antibody titer, is performed at the Blood Grouping Laboratory. (All titers are expressed as the reciprocal of the dilution.) The Rh antibody level is boosted, if necessary, by an initial intravenous injection of 0.5 ml. of whole blood from a carefully screened Rh-positive donor. This injection is usually followed by two further injections of 0.25 ml. of whole blood at weekly intervals. The aim is to achieve an antibody level of 512 or greater as measured by the indirect antiglobulin (Coombs) method.

To date the blood cells of only one individual, a 23-year-old male student, have been used for stimulation of the antibody levels of donors previously sensitized to the Rh factor. This student has group O blood, postive for Rh_0 (D) and negative for C, E, Kell, Duffy^a, Kidd, Kp^a, M, s, and Lutheran^a. The blood that he has donated on numerous occasions has not resulted in development of hepatitis in any recipient. The results of his liver function tests have been within the normal range, and repeated tests for Australia antigen have given negative results. Since his blood has tested negative for the factors that are most antigenic, the possibility that a woman receiving his blood might become sensitized to another blood group antigen is almost entirely eliminated.

Results of Stimulation of Antibody Levels

Response to the method of stimulation described has been almost uniformly successful (table 1). Of the 73 volunteers who received injections of Rh-positive blood before May 1, 1970, a total of 67

(92 percent) achieved Rh antibody levels of 512 or greater following the series of injections. Persons with higher initial Rh antibody levels were somewhat more likely to achieve higher levels following stimulation than those with lower initial levels. Low initial levels, however, did not preclude a satisfactory response to stimulation. Data accumulated thus far indicate that many donors achieve peak antibody levels following the second injection of whole blood. The effectiveness of a two-dose course of antibody stimulation is now under study.

The persistence of peak Rh antibody titers is highly variable and does not seem to be a function of age, sex, or the initial or maximum antibody levels. Antibody levels, tested at the time of each plasma donation, usually begin to fall within 3 to 6 months after the initial stimulation. In each of 19 instances, however, in which two additional intravenous injections (0.5 and 0.25 ml.) of Rh-positive whole blood were administered, the donor's antibody level reached essentially the same peak level as achieved on initial stimulation.

Donors have shown no abnormal effects from the injections of Rh-positive blood and no evidence of other irregular erythrocyte antibody development. However, a number of Rh-negative donors sensitized to C and D have developed significant increases in anti-C antibody levels. Since the cell donor is C negative, this increase represents the formation of anti-D + G (*9*).

Plasma Collection

Throughout the program, collection of plasma has been by the technique of double plasmapheresis. Plasmapheresis was carried out under the direction of Dr. Sherwin W. Kevy of Children's Hospital Medical Center and Dr. Allan Kliman of the Massachusetts Red Cross Blood Program. Five hundred milliliters of whole blood is withdrawn into 4 percent sodium citrate anticoagulant and centrifuged rapidly in a refrigerated centrifuge. The plasma is retained; the red blood cells are suspended in 75 ml. of sterile saline and returned to the donor through the original collection needle. The entire process is then repeated, permitting acquisition of approximately 600 ml. of plasma at each of the donor's visits. This method helps to insure collection of maximum volumes of suitable plasma. Hemoglobin and plasma protein levels are determined at each visit; to date there have been no significant deviations from initial levels.

Table 2 summarizes the results of plasma collection from the donors who were enrolled in the program before May 1, 1970. These 79 donors were selected, after studies of their blood type profiles, from among 434 prospective volunteers. More than half of the potential female donors could not be accepted because they were still of childbearing age. Each of the 11 men in the program had histories of one or more surgical procedures during which blood transfusions had been given. The obstetrical histories of the 68 female donors revealed a total of 353 pregnancies, of which 56 had terminated in miscarriages and 43 in stillbirths. Ninety-three of the pregnancies had resulted in delivery of live-born erythroblastotic infants.

Two of the 79 volunteers gave plasma only once. The remainder made repeated donations on a regular schedule, usually at 3- to 4-week intervals. Each donor receives a Medicalert bracelet, indicating his or her Rh-antibody status; in the event

Table 1. Anti-Rh_0 (D) antibody levels of previously sensitized donors before and after intravenous injection of Rh-positive red blood cells

Level before injection	Total donors	Donors not injected	Donors with post-injection levels of—									
			Less than 512	512	1,000	2,000	4,000	8,000	16,000	32,000	64,000	128,000
1	1		1									
2	9		3			1	2	1	2			
4	4			3		1						
8	6		1	2	1	1		1				
16	16		1	4	2	3	4	1		1		
32	8				1	1	3	2		1		
64	11			1	1	2	4	1		1		1
128	6	1			3	1	1					
256	11	1			1	3	2	1	1	1	1	
512	6	3					1	2				
1,024	1	1										
Total	79	6	6	10	9	13	17	9	3	4	1	1

Table 2. Plasma donations of volunteers enrolled in Massachusetts Department of Public Health Rh_0 (D) Immune Globulin Program, July 15, 1968–May 1, 1970

Sex	Number of volunteers	Mean months of participation	Total donations	Total liters of plasma donated	Per month of participation	
					Mean number of donations	Mean plasma donated (ml.)
Male	11	10.0	104	61.9	0.95	562
Female	68	8.2	512	308.6	.92	553
Total	79	8.5	616	370.5	0.92	553

an emergency arises requiring a blood transfusion, this bracelet permits immediate recognition that the person is highly sensitized to Rh_0 (D).

Based on our experience in Massachusetts, an average volunteer donor can be expected to contribute approximately 550 ml. of plasma each month that he or she remains active in the Rh Immune Globulin Program (table 2).

Plasma Fractionation

Fractionation of the plasma has been carried out by the cold-ethanol fractionation procedure described by Oncley and associates (10), resulting in the acquisition of subfraction II–1,2, rather than the complete Cohn fraction II usually derived for use as ordinary immune serum globulin (see chart). This subfraction has unusually high levels of anti-Rh IgG antibody activity and is free of detectable IgM and saline anti-Rh antibodies. The details of the use of this method for the preparation of Rh_0 (D) Immune Globulin, originally suggested by Lewis Larsen, senior chemist at the Massachusetts State Laboratory Institute, will be published in a separate report.

Modification of cold-ethanol plasma fractionation procedure to yield fraction II–1,2 for use as Rh_0 (D) Immune Globulin

Supernatant III
(filtered)
Ethanol ---------- 17 percent (mole fraction 0.058).
Λ/2 ---------- 0.05.
pH ---------- 5.2.
temperature ------ −6° Centigrade.
protein ---------- 0.8 percent.

↓ *Supernatant II–3*
Ethanol ---------- 25 percent.
Λ/2 ---------- 0.05.
pH ---------- 7.4.
temperature ------ −5° Centigrade.
protein ---------- 0.4 percent.

↓ *Fraction II–3* γ-globulin

↓ *Supernatant II–1,2* (discarded)

↓ *Fraction II–1,2* γ-globulin

NOTE: Modification based on method of Oncley and associates, reference 10.

Initial experience indicates that an average liter of plasma collected in a program of this kind will yield approximately 51 1-ml. doses of Rh Immune Globulin, each having approximately 300 micrograms of anti-Rh antibody activity. Dr. Neville C. Hughes-Jones of St. Mary's Hospital Medical School, London, England, performed the Rh_0 (D) radioimmunoassay antibody determinations on numerous plasma and globulin samples.

Two lots of plasma have been fractionated to date, yielding 5,418 doses of Rh_0 Immune Globulin (based on initial assays of antibody activity). An additional 273 liters of plasma, which we estimate will yield 13,650 more doses of globulin, are available for fractionation. Thus, if the Massachusetts efforts to date can be continued, it should be possible to supply the entire need of this State from the output of this wholly voluntary program. We estimate that the material produced thus far has cost an average of $5.67 per 300-microgram dose.

Initial Administration of Rh Globulin

With the informed consent of the patients, Rh Immune Globulin that was prepared in our program has been administered in five university-affiliated hospitals in the Boston area in the course of a clinical trial conducted under an Investigational New Drug Application filed with the Division of Biologics Standards, National Institutes of Health. Within 72 hours of delivery of ABO-compatible Rh-positive infants, injections have been given to Rh-negative mothers who showed no evidence of sensitization to the Rh factor at the time of delivery. Blood samples at 3 or 6 months following these injections, or at both times, were obtained from 207 of the 346 women who received this preparation before May 1, 1970. There were no adverse reactions. Antibody tests using saline, albumin, indirect

Coombs, and enzyme (ficin) methods have shown no evidence of Rh_0 (D) sensitization in any recipient. Followup studies at 3 or 6 months following injection, or at both times, as well as 1 year after the injection and during subsequent pregnancies, will be continued until sufficient evidence of efficacy accumulates to permit making the Rh Immune Globulin available for general use in Massachusetts.

The goal of elimination of erythroblastosis fetalis is clearly in sight for Massachusetts, mainly because of the corps of volunteer donors whose sense of community service and human compassion have motivated them to make this program possible. Sufficient Rh Immune Globulin is available to provide for each woman at risk of sensitization. We hope that the example of this cooperative voluntary program will stimulate other States to take whatever steps are necessary to assure the full availability of this important material.

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The Massachusetts Department of Public Health and the Blood Grouping Laboratory of Boston have undertaken a program to produce Rh Immune Globulin on an entirely voluntary basis and distribute it at no charge to all unsensitized Rh-negative women in the State who deliver Rh-positive babies and are at risk of becoming sensitized to Rh.

The 79 volunteer plasma donors, all previously sensitized to Rh, were stimulated by injections, at 1-week intervals, of 0.5 ml., 0.25 ml., and 0.25 ml. of Rh-positive blood, and they then underwent plasmapheresis at frequent intervals. Pooled plasma was fractionated by the Cohn fractionation method, and the anti-Rh_0 (D)-rich gamma globulin fraction II-1, 2 was prepared in 1 ml. doses containing a minimum of 300 micrograms of anti-D per dose. Two lots of plasma have been fractionated thus far, and the yield indicates that it should be possible to supply fully the State's requirement for Rh Immune Globulin at an average cost of $5.67 per dose.

The clinical trial is now underway in five hospitals in the Boston area. Before May 1, 1970, 346 women were treated with the Rh Immune Globulin with no adverse effect. Of the 207 women who have already reached the 3-month and 6-month post partum test period, not one has shown evidence of Rh sensitization. Continued followup studies are in progress.