Immunologic Tolerance in Rats to Type Specific Antigens of Human Erythrocytes.*† (24433)

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Reduced ability of an animal, exposed early in life to a specific foreign antigen, to react immunologically to the same antigen in later life is known as "actively acquired tolerance"(1). Most studies using erythrocytes as antigen have involved identical or closely related species(2,3). The possibility of producing tolerance to antigens of erythrocytes of distant species has been investigated by injecting red cells from rabbits into chicks(4), sheep into rats(5) and humans into birds(6), all with negative results. However, other investigators have reported depression or delay of antibody formation by injecting human erythrocytes into rats, rabbits and chicks(7, 8). The feasibility of inducing tolerance to human blood antigens is relevant to bone marrow transplantation studies and conditions involving blood group incompatibility. It was the purpose of this investigation to determine whether human erythrocytes could confer type specific immunologic tolerance when injected into prenatal or neonatal rats.

Material and methods. Twenty litters of rats of the Sprague-Dawley strain were used. Seven of 8 litters injected pre-natally at laparotomy were lost at birth and one additional litter injected at birth died in immediate postnatal period. Preliminary studies on erythrocytes from 50 adult rats of this strain revealed, in all instances, strong agglutination of these cells by commercial human anti-B serum and no agglutination with Anti-A_1 or anti-D (Rh_o) serum. Human erythrocytes of types A, Rh-pos. (A+), A, Rh-neg. (A–) or O, Rh-neg. (O–), stored in Alsever's solution, were washed in saline and used as antigen. To induce tolerance, one intraperitoneal injection of 0.2-0.4 cc of a 50% erythrocyte suspension was given during the peri-natal period. Later, immunization injections consisting of 0.5 cc of a 50% A+ erythrocyte suspension were administered subcutaneously. Five litters totaling 38 rats (Table II) received a single immunizing injection, another 5 litters totaling 44 rats (Table I) received 7 injections on alternate days over a period of 2 weeks. Two-fold dilutions of inactivated serum (56°C for 30 minutes) were prepared in 0.1 ml amounts. To this an equal amount of a 2% suspension of washed A+ erythrocytes in .15M saline was added. The tubes remained at room temperature (24 ± 4° C) for 20 minutes and centrifuged at 200 G for one minute. A reading of 1+ agglutination was considered the end point. Agglutinin titers of rat sera with human A+ cells are expressed as $\log_2$ of the reciprocal of the final dilutions. A difference of 2 or more titer units (equivalent to a four-fold dilution) was considered meaningful. Wherever possible erythrocytes from the same individual were used for injections and titrations. Tests for "incomplete antibodies" were carried out in the usual manner employing both 22% bovine albumin as protein diluent and chicken anti-rat globulin serum. Absorption experiments were performed at room temperature (24 ± 4°C) by adding 2 successive volumes of 0.2 ml of packed O– human erythrocytes, washed in .15 M saline, to 0.4 ml of test serum. All the rats were weighed at weekly intervals. No difference was observed between those receiving and those not receiving the "tolerance" injection.

Results. Table I and Fig. 1 show the findings for the group of animals receiving multiple immunization injections over a 2-week period. Animals which had received a "tolerance" injection of A+ erythrocytes during the peri-natal period and challenged as late as 83 days after birth showed appreciably lower titers than the animals which had not received a "tolerance" injection. Injection at birth of O– erythrocytes (litter T-6) appar-

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TABLE I. Animals Receiving Immunizing Injections on Alternate Days over a Period of 2 Weeks.

Litter No.	No. of animals	Age at tolerance inj.	RBC's inj. at tolerance inj.	Age at immunization with A+ RBC's (days)	Avg unabsorbed titers against A+ erythrocytes (reciprocal of dilutions expressed as $\log_2$)*						
					Immed. before immunization	Wk after final immunizing inj.					
						0	2	3	4	5	8	10
T-7	4	3 days	A+	40	.8	6.5				8.8	7.5	7.3
	2		None	40	1.5	12.0				12.0	12.0	13.5
	2	3 days	A+	100	3.0	9.5		9.0				
	2		None	100	2.5	11.5		12.5				
4	7	3 days before birth	A+	83	1.0	7.3			9.4			8.6
6	7	At birth	O−	68	.6	10.7		11.0			10.3	
8	8	4 days	A+	65	1.5	7.1	9.3					9.1
	3		None	65	.0	10.3	11.7					11.0
12	5	At birth	A+	30	.8	7.2	9.6				9.6	
	4		None	30	.5	10.5	11.3				11.5	

* The difference of 2 or more titer units (a 4-fold dilution) was considered meaningful.

ently had little or no effect on later immunization with A+ cells. An apparent loss of tolerance with time is illustrated by comparing the response of the animals immunized at 40 days with their litter-mates immunized at 100 days (litter T-7).

Table II and Fig. 2 demonstrate a similar inhibition of agglutinin response for the tolerant animals which received a single immunizing injection. Again injection of O− cells at birth conferred no immediate protection to later immunization but the response obtained was less sustained than in the controls. The results observed in litters T-14 and T-17 (Table II) seem to indicate that absence of the D (Rh_0) antigen in the initial injection reduced degree of tolerance obtained.

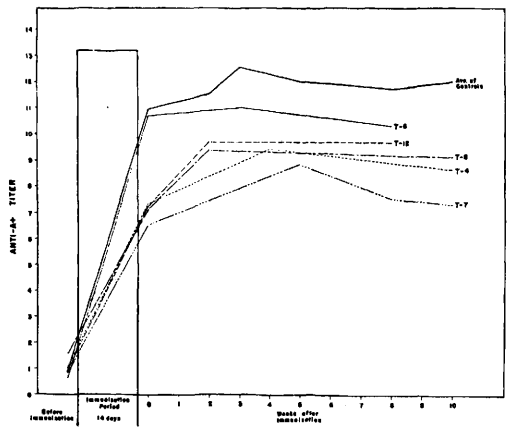

FIG. 1. Avg unabsorbed titers (reciprocal of dilution expressed as $\log_2$) against A+ erythrocytes for rats receiving immunizing inj. on alternate days over a period of 2 wk. Interrupted lines represent values for rats which received "tolerance" inj. of A+ cells in early life. Rats in T-6 received O− erythrocytes at birth. Controls were given no inj. in perinatal period. (See Table I for details.)

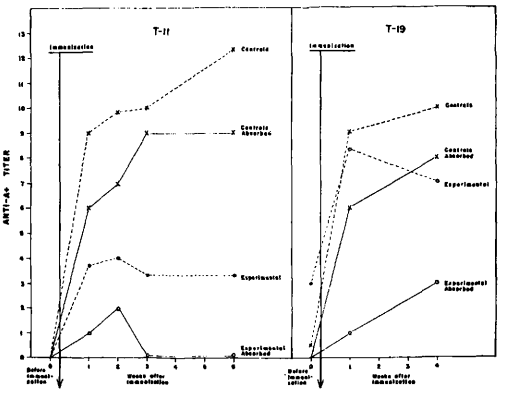

FIG. 2. Titers expressed as in Fig. 1. All rats received one immunization inj. at arrow. Experimental animals were inj. in neonatal period with A+ erythrocytes; controls did not receive this inj. Interrupted lines signify avg unabsorbed titers; solid lines represent titers obtained with same sera after absorption with O− human erythrocytes.

It should be emphasized that ability to induce tolerance in rats to human erythrocytes is by no means a uniform phenomenon. Marked differences were observed among individual litter-mates and between litters. This has been reported in a similar system(7) and was observed also for mouse skin homografts (1).

In 2 other litters (T-10 and T-20) the initial "tolerance" injection of A+ erythrocytes was delayed until 2 weeks after birth. All 10

TABLE II. Animals Receiving Single Immunizing Injection.

Litter No.	No. of animals	Age at tolerance inj.	RBC's inj. at tolerance inj.	Age at immunization with A+ RBC's (days)	Avg unabsorbed titers against A+ erythrocytes (reciprocal of dilutions expressed as $\log_2$)* Immed. before immunization	Wk after immunizing inj. 1	2	3	4	6
T-11	3	At birth	A+	30	0	3.7	4.0	3.3		3.3
	4		None	30	0	9.0	9.8	10.0		12.3
14	4	1 day	A+	80	0	4.3		8.0		
	3	"	A−	80	0	7.0		8.2		
17	6	At birth	A+	73		6.2		5.8		
	6	"	A−	73		7.2		7.7		
16	7	"	O−	67	.4	10.0			8.4	
19	3	1 day	A+	56	3.0	8.3			7.0	
	2		None	56	.5	9.0			10.0	

* The difference of 2 or more titer units (a 4-fold dilution) was considered meaningful.

injected animals showed an immune response.

To investigate the possibility that the apparent depression of immune response may have been due to formation of antibodies other than saline agglutinins(7), tests for "incomplete antibody" were performed. No evidence for the presence of "incomplete antibodies" was obtained.

Absorption with fresh human O− erythrocytes reduced the antibody titer against A+ cells in the non-tolerant animals. In similarly absorbed sera from tolerant rats, agglutinins against A+ cells were either markedly reduced or not demonstrable (Fig. 2). Thus. it appears that another antigen or group of antigens probably related to species differences, was responsible for most of the antibody observed in the tolerant animals. Very early embryonic injections may be required to produce complete tolerance to these species-specific factors. It is not unlikely that some of the inconsistencies reported in experiments dealing with wide species differences may be explained on this basis, although other variables such as dose or route of injection, age of animals at tolerance or immunization injections, and at time of bleeding for serum, undoubtedly play an important role.

Summary and conclusions. Intraperitoneal injection of human erythrocytes in pre-natal or neo-natal rats reduces antibody response to subsequent immunizations. No ill-effects were observed in any of the experimental animals surviving the initial injection and there was no difference in growth rate from the uninjected controls. These studies suggest that it is possible to confer type specific tolerance to subsequent immunization of A and D (Rh_0) antigens of human erythrocytes.

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