79. *Heredity of the Agglutinin C and the Anti-C Agglutinin in Human Blood.*

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From some experiments (1-9) made by means of immunization and absorption tests it had been supposed that there exists a common antigenic structure in human red cells of group A and group B, not found in group O. In 1935, HIBINO⁽¹⁰⁾ established this common antigen and designated it as agglutinin "C",* explaining that group A cells have a structure of AC, group B cells BC and group AB cells ABC. Later by KOBAYASHI's extensive work⁽¹¹⁾ it was revealed that the antigen C is widely distributed in blood and saliva of various animals and is constructed from the partial antigens C₁, C₂, and C₃ as the antigen B is from B₁, B₂, and B₃. At present, in addition to the known agglutinogens A and B, the agglutinin C can not be neglected for the study of the blood groups.

On the other hand, TERAJIMA⁽¹²⁾ observed the occurrence of the anti-C agglutinin in normal pig sera, and UYAMA⁽¹³⁾ in normal rabbit and fowl sera. Later on ISEKI and KOBAYASHI⁽¹⁴⁾ found that it appears also in some normal human sera of group O as an irregular isoagglutinin. The author has in this work confirmed their results, and furthermore investigated the hereditary relation of this agglutinin as well as the heredity of the isoagglutinogens in 100 families with 220 children.

The purpose of my own study is to show a certain evidence for the inheritance of normal antibody, and to discuss it in connection with the heredity of antigen.

Material and Methods.

The material for this study was obtained from 100 families in Tokyo, in which the relationship of parents and children was obvious. Samples were taken at random, but babies under two years old, patients suffering from any disease or persons who had received blood transfusions, the decrepits and women in pregnancy or just after delivery were all excluded from these samples, because in their sera the formation of antibodies could be insufficient or

* This letter "C" should not be confused with the one which recently Race et al. have used for the nomenclature of Rh blood types.

changed, and they are unsuitable for investigating normal antibodies. In analysing the serum properties, human and rabbit bloods for absorption and testing blood suspension for titration came from the same individuals throughout this experiment.

As to the isoagglutinogens all the bloods were examined for the properties A, B, M, N, and Q. In order to demonstrate the anti-C agglutinin in group O sera, the following procedures were carried out according to the method described by ISEKI and KOBAYASHI⁽¹⁴⁾. (Table I)

Table I.
Agglutination for Human Red Cells of Group B.

Human Sera of Group O	Serum No.	Unabsorbed	Absorbed with									
			Rabbit red cells					Rabbit cells and human A cells				
		1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32	1:2 1:4 1:8 1:16 1:32
$\alpha + \beta_2\beta_3$	3	## ++ + + +	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —	— — — — —
$\alpha + \beta_1\beta_2\beta_3$	7	## ++ ++ + +	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —
$\alpha + \beta_2\beta_3$ + anti-C	5	## ++ ++ + +	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —	++ + — — — —

In the first place, 0.2 c.c. of a 1:2 dilution of each serum is mixed with the 1/4 amount of the blood cells of rabbit (B_2B_3) and the mixture was allowed to stand for 2 hours at room temperature, after which the titer of anti-B agglutinin in the supernatant was tested by human B cells. Then in some sera the anti-B agglutinin was completely absorbed ($\beta_2\beta_3$), the others only partly absorbed. In the second place, the supernatant of the latter was separated by centrifuging, and now the 1/4 amount of human A cells were added to it, left for 2 hours at room temperature, and again the titer was tested by human B cells. If the residual anti-B agglutinin, which previously could not be absorbed by rabbit cells, still remained in the supernatant, the serum contains $\beta_1\beta_2\beta_3$, while if it was completely absorbed by human A cells, it is revealed that the serum contains the anti-C agglutinin in addition to $\beta_2\beta_3$.

According to KOBAYASHI⁽¹¹⁾, the red cells of rabbit have a structure of $B_2B_3 + C_2C_3$. Therefore, the anti-C agglutinin in human serum proved by this method is, strictly speaking, anti-C₁ agglutinin.

Experimental Results.

As to the heredity of the properties A, B, M, N, and Q in the

red cells, my results agree with the established fact that they are inherited as Mendelian dominants, and no contradiction or exception to the generally accepted theories could be found.

In a series of 420 specimens, a total number of the bloods of parents and children examined, 125 were group O. Among these, 28 were found to contain the anti-C agglutinin in the serum, that is, 22.4 per cent, agreeing well with 21.4 per cent obtained by KOBAYASHI⁽¹¹⁾. Therefore, its distribution in the Japanese population is 6.7 ± 0.8 per cent. The agglutinin titers were all low: 2—8.

For the convenience of explanation, the letter γ stands for persons with anti-C agglutinin, and o (zero) for those without anti-C agglutinin. Because anti-C agglutinin does not occur *a priori* in the sera of groups A, B, and AB, the letter o includes the persons of these groups besides of group O without anti-C agglutinin. The results of the heredity of the anti-C agglutinin are summarized in Table II and are arranged in three classes corresponding to the three types of unions. 17 individual families are shown as pedigrees, in the member of which the appearance of the anti-C agglutinin was observed.

Table II.

Summary of Family Material.

Mating		Number of Families	Number and Frequency of Children with Theoretical Values					
			0		γ		χ^2	
1	0×0	90	186	95.4% (95.6%)	9	4.6% (4.4%)	195	0.019
2	$0 \times \gamma$	9	17	77.3% (79.1%)	5	22.7% (20.9%)	22	0.043
3	$\gamma \times \gamma$	1			3	100% (100%)	3	0
Totals		100	203		17		220	0.062 $n=2, \text{Pr} > 0.95$

As is apparent from the result with the family in which both parents are γ , evidently the anti-C agglutinin is inherited as a recessive. In one such mating encountered in my series, all three children proved to be γ .

In analogy to the anti-Q agglutinin whose heredity has been investigated, one may presume that the anti-C agglutinin is transmitted by means of a pair of genes, R and r, where the dominant gene R determines its absence. Hence, three genotypes would exist, RR, Rr, and rr, the first two corresponding to the phenotype o, the last to γ . Assuming that the distribution of γ in

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the population is 7 per cent, namely the frequency of gene *r* is 26.4 per cent and the frequency of gene *R* 73.6 per cent, one can calculate the theoretical frequencies in the offspring of various matings as shown in round brackets in Table II. A value of χ^2 equal to approximately 0.062 is obtained, *n* being 2, and the probability larger than 95 per cent. Consequently, it can be concluded that the anti-C agglutinin is inherited as a simple Mendelian recessive.

Discussion.

From the nature mentioned in the introduction, that the antigen C is common between properties A and B which are inherited as Mendelian dominants, it is obvious that the antigen C is also dominantly inherited. Presuming that it is transmitted by means of a pair of genes, C and *c*, where the dominant gene C determines its presence, it follows from the triple allele theory that the three completely linked pairs, (AC), (BC), and (Oc) are supposed instead of the genes A, B, and O. The genotypes corresponding to each of the four blood groups are shown in Table III.

Table III.

Heredity of the Properties A, B, and C.

Phenotype	Genotype	
	Homozygous	Heterozygous
O = Oc	(Oc) (Oc)	
A = AC	(AC) (AC)	(AC) (Oc)
B = BC	(BC) (BC)	(BC) (Oc)
AB = ABC		(AC) (BC)

On the other hand, the investigation of families leads one to conclude that the anti-C agglutinin is inherited as a simple Mendelian recessive. Then, the following question might arise: in what genetical relations are the antigen and the antibody? As a matter of serological fact, the appearance of antibody in serum depends on the absence of antigen in red cells. If the genes for the antibody are not completely linked with the genes for the antigen, crossing over would occur in family materials. In the above illustrated pedigrees, not a single case of crossing over was found. However, as regards this genetical point, conclusive information cannot be obtained from my scanty data.

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Summary.

The anti-C agglutinin in human normal serum of group o appears at a rate of 6.7 ± 0.8 per cent in the population. Examination of families showed that this irregular agglutinin is inherited as a simple Mendelian recessive. From the nature that the antigen C is common between agglutinogens A and B, the agglutinin C is obviously inherited as a simple Mendelian dominant, the gene for which being completely linked with the genes for A and B. In order to ascertain the genetical relations between the formation of antigen and that of antibody, further investigation is necessary.

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