AN ANTIBODY WHICH SUBDIVIDES THE HUMAN MN BLOOD GROUPS

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Received 8.viii.47

AN antibody has been found in a sample of human serum which subdivides the MN blood groups, as we have already shown (1947).

The donor of the serum containing this agglutinin was a woman whose fifth pregnancy had just terminated in the delivery of a still-born macerated fœtus. She had never been transfused, but had had one normal child followed by a second which died, aged one day, of erythroblastosis fœtalis ; the third and fourth pregnancies had ended in early miscarriages. The blood groups of the family are as follows :—

Mother :	A cde/cde	MN
Father :	A CDe/CDe	M
Child :	A CDe/cde	

The serum contains anti-D of the "incomplete" type, which undoubtedly was responsible for the disease of the two infants, but it also contains the agglutinin whose reactions are the subject of this paper.

The following table shows the results of agglutination tests on blood from 190 unselected English people, the tests were carried out in saline and at room temperature. (The agglutinin is also active at 37° C.)

M		MN		N		Total
+	−	+	−	+	−	...
36	14	57	38	15	30	190
18·95%	7·37%	30·00%	20·00%	7·89%	15·79%	100·00%

* Supported by the Australian Red Cross.

The association of the " new " antibody with the MN system is clear from the following 2 × 2 table :—

	New antibody +	New antibody −
M+MN	93	52
N	15	30

for which the χ^2 equals 13 for 1 d.f., corresponding to a probability of less than 0·001. No significant association with the A_1A_2BO groups, nor the P groups, nor any of the Rh antigens can be found. The antibody does not give the reactions of the rare agglutinins " anti-Lutheran," " anti-Kell " nor " anti-Lewis."

A reasonable genetical interpretation is that there are four allelomorphs at the locus responsible for these groups M, MS, N and NS, the mutation S being a change which can happen both to M and to N genes, and which makes the resulting red cells agglutinable by the new antibody.

On the other hand, it is possible that S is a separate linked gene, presumably having an allelomorph *not S* (or s). If an antibody corresponding to *not S* were found, this interpretation of a separate locus would seem the more probable. Anti- *not S* would agglutinate about 88 per cent. of English bloods. The situation would then be very similar to that of the C, D and E antigens of the Rh system. If the interpretation of linked genes is correct, the linkage must be very close, otherwise crossing over would presumably have resulted in an equilibrium in which the ratio of MS to M would equal that of NS to N.

The calculations given below lend strong support to one or other of these genetical interpretations of the observed reactions.

The simple postulation of a third gene allelomorphic to M and N, say L, will not meet the facts, for MN blood is frequently agglutinated by the new antibody, and the presence of three allelomorphs in one person would have to be invoked.

GENE FREQUENCIES

In the calculations shown below both the symbols and the figures will fit the idea of 4 allelomorphs or of separate but closely linked genes.

M		MN		N		Total
+	−	+	−	+	−	
MS MS MS M	MM	MS N MS NS M NS	MN	NS N NS NS	NN	
36 0·1895	14 0·0737	57 0·3000	38 0·2000	15 0·0789	30 0·1579	190 1·0000

We are greatly indebted to Professor Fisher who has estimated the gene frequencies by his method of maximum likelihood (1946), with the following results as percentages :—

MS . . .	25·0487
M . . .	26·2671
NS . . .	9·2121
N	39·4721
	100·0000

These gene frequencies can be used to calculate the expected frequencies of bloods in the various phenotype groups.

	Expected	Observed	χ^2
MS MS MS M	36·9236	*36*	0·02310
MM	13·1093	*14*	0·06052
MS N MS NS M NS	55·5351	*57*	0·03864
MN	39·3991	*38*	0·04970
NS N NS NS	15·4300	*15*	0·01198
NN	29·6029	*30*	0·00533
	190·0000	*190*	0·18927 d.f. 2 $p = 0·9$

The agreement between expected and observed frequencies is seen to be very close.

FAMILY INVESTIGATIONS

The subdivisions of the MN groups defined by this serum greatly increase the number of distinguishable matings. There are only 6 phenotypically and genotypically distinct types of classical MN matings, but with the help of the new antibody there are theoretically 21 phenotypically and 55 genotypically different matings. A list of these matings is given in table 1, with the approximate frequencies of their occurrence.

The results of the family investigations are given in table 2. In the second column of this table the classification of the type of mating refers to the catalogue of matings given in table I. Sometimes the exact type of mating is known as in families 3, 4, 6, 9, 13, 14, 20, 22, 23, 24, 25, 26, 27, 29 and 30. Sometimes only the main group to which the mating belongs can be recognised. Sometimes the main group is known but one of the constituent matings can be excluded ; for example, family 15 belongs to type 13, but cannot be 13*a* for the latter mating does not produce MN children.

TABLE 1

The possible MNS matings

	Phenotypically distinguishable	Genotypically different	Expected frequency per cent.	Children
M × M	1. MM × MM	(*a*) MM × MM	0·48	All MM
	2. MM × (MM)S	(*a*) MM × MS MS	0·87	All MS M
		(*b*) MM × MS M	1·82	½ MS M ½ MM
	3. (MM)S × (MM)S	(*a*) MS MS × MS MS	0·39	All MS MS
		(*b*) MS MS × MS M	1·65	½ MS MS ½ MS M
		(*c*) MS M × MS M	1·73	¼ MS MS ½ MS M ¼ MM
M × MN	4. MM × MN	(*a*) MM × MN	2·86	½ MM ½ MN
	5. (MM)S × MN	(*a*) MS MS × MN	2·60	½ MS M ½ MS N
		(*b*) MS M × MN	5·46	¼ MS M ¼ MS N ¼ MM ¼ MN
	6. MM × (MN)S	(*a*) MM × MS NS	0·64	½ MS M ½ M NS
		(*b*) MM × MS N	2·73	½ MS M ½ MN
		(*c*) MM × M NS	0·67	½ MM ½ M NS
	7. (MM)S × (MN)S	(*a*) MS MS × MS NS	0·58	½ MS MS ½ MS NS
		(*b*) MS MS × MS N	2·48	½ MS MS ½ MS N
		(*c*) MS MS × M NS	0·61	½ MS M ½ MS NS
		(*d*) MS M × MS NS	1·22	¼ MS MS ¼ MS NS ¼ M MS ¼ M NS
		(*e*) MS M × MS N	5·20	¼ MS MS ¼ MS N ¼ M MS ¼ MN
		(*f*) MS M × M NS	1·27	¼ MS M ¼ MS NS ¼ MM ¼ M NS
M × N	8. MM × NN	(*a*) MM × NN	2·15	All MN
	9. (MM)S × NN	(*a*) MS MS × NN	1·95	All MS N
		(*b*) MS M × NN	4·10	½ MS N ½ MN
	10. MM × (NN)S	(*a*) MM × NS NS	0·12	All M NS
		(*b*) MM × NS N	1·00	½ M NS ½ MN

	11. (MM)S × (NN)S	(*a*) MS MS × NS NS	0·11	All MS NS
		(*b*) MS MS × NS N	0·91	½ MS NS ½ MS N
		(*c*) MS M × NS NS	0·22	½ MS NS ½ M NS
		(*d*) MS M × NS N	1·91	¼ MS NS ½ MS N ¼ M NS ¼ MN
MN × MN	12. MN × MN	(*a*) MN × MN	4·30	¼ MM ½ MN ¼ NN
	13. (MN)S × MN	(*a*) MS NS × MN	1·92	¼ MS M ¼ MS N ¼ M NS ¼ NS N
		(*b*) MS N × MN	8·20	¼ MS M ¼ MS N ¼ MN ¼ NN
		(*c*) M NS × MN	2·01	¼ MM ¼ MN ¼ M NS ¼ NS N
	14. (MN)S × (MN)S	(*a*) MS NS × MS NS	0·21	¼ MS MS ¼ MS NS ¼ NS NS
		(*b*) MS NS × MS N	1·83	¼ MS MS ¼ MS N ¼ MS NS ¼ NS N
		(*c*) MS NS × M NS	0·45	¼ MS M ¼ MS NS ¼ M NS ¼ NS NS
		(*d*) MS N × MS N	3·91	¼ MS MS ½ MS N ¼ NN
		(*e*) MS N × M NS	1·91	¼ MS M ¼ MS NS ¼ MN ¼ NS N
		(*f*) M NS × M NS	0·23	¼ MM ½ M NS ¼ NS NS
MN × N	15. MN × NN	(*a*) MN × NN	6·46	½ MN ½ NN
	16. (MN)S × NN	(*a*) MS NS × NN	1·44	½ MS N ½ NS N
		(*b*) MS N × NN	6·16	½ MS N ½ NN
		(*c*) M NS × NN	1·51	½ MN ½ NS N
	17. (MN)S × (NN)S	(*a*) MS NS × NS NS	0·08	½ MS NS ½ NS NS
		(*b*) MS NS × NS N	0·67	¼ MS NS ¼ MS N ¼ NS NS ¼ NS N
		(*c*) MS N × NS NS	0·34	½ MS NS ½ NS N
		(*d*) MS N × NS N	2·87	¼ MS NS ¼ MS N ¼ NS N ¼ NN
		(*e*) M NS × NS NS	0·08	½ M NS ½ NS NS
		(*f*) M NS × NS N	0·70	¼ M NS ¼ MN ¼ NS NS ¼ NS N
	18. MN × (NN)S	(*a*) MN × NS NS	0·35	½ M NS ½ NS N
		(*b*) MN × NS N	3·02	¼ M NS ¼ MN ¼ NS N ¼ NN
N × N	19. NN × NN	(*a*) NN × NN	2·43	All NN
	20. (NN)S × (NN)S	(*a*) NS NS × NS NS	0·01	All NS NS
		(*b*) NS NS × NS N	0·12	½ NS NS ½ NS N
		(*c*) NS N × NS N	0·53	¼ NS NS ½ NS N ¼ NN
	21. (NN)S × NN	(*a*) NS NS × NN	0·26	All NS N
		(*b*) NS N × NN	2·27	½ NS N ½ NN

When the position of S is not stipulated, it is written, for example, thus (MM)S ; when the position is known—as in the theoretical genotypes—it is written MS M or MS MS.

TABLE 2

The results of family investigations

No.	Type of Mating	Parents		Children						
		Father	Mother	1	2	3	4	5	6	7
1*	2	MM B R_2R_2	(MM)S A_1 R_1R_2	MS M O R_2R_2	MS M O R_2R_2	MS M A_1B R_1R_2				
2	3	(MM)S A R_1R_0	(MM)S A rr	(MM)S A R_0r	(MM)S A R_0r					
3	6*b*	MS N A R_1R_1	MM O $R''r$	MS M O R_1R''	MN O R_1R''	MN A_1 R_1r	MN A R_1r	MS M O R_1R''	MN O R_1R''	MS M O R_1R''
4†	6*b*	MM A_2 R_1R_1	MS N O R_1r	(MM)S A_1 R_1r	MN O R_1R_1	MN O R_1r	MN A_2 R_1R_1			
5	5	(MM)S O $R_1{}^wR'$ Lewis− Kell+ P	MN A_1 R_1r Lewis+ Kell− P	MS N O $R'r$ Lewis+ Kell− P	MS M A_1 $R'r$ Lewis+ Kell+ P	MS N A_1 R_1R' Lewis+ Kell+ P				
6	7*e*	MS M O R_1R_1	MS N A R_1R_1	(MM)S O R_1R_1	MN A R_1R_1	(MM)S O R_1R_1				
7	7	(MN)S A_1B R_1R_1	(MM)S O R_1R_2	(MM)S B R_1R_1						
8	7	(MN)S A R_1R_1	(MM)S A R_2r	(MM)S O R_1R_2	(MM)S A R_1R_2					
9	9*b*	MS M O R_2R_2	NN O R_2r	MS N O R_2R_2	MN O R_2r	MN O R_2r				
10	9	NN O R_1r	(MM)S O rr	MS N O rr						
11	11	(NN)S O R_1r	(MM)S A R_1R_2	(MN)S A R_2r	(MN)S A R_1r	(MN)S A R_1R_2				
12	11	(NN)S O R_1r	(MM)S B R_1r	(MN)S O R_1r	(MN)S B R_1r	(MN)S O R_1r				
13	12*a*	MN O R_1r	MN O rr	MN O rr	MN O rr	MN O rr				
14	12*a*	MN A_1 R_1r	MN A_1 R_1r	MN A_1 R_1R_1						
15	13 not *a*	(MN)S O R_1R_1	MN O R_2R_2	MN O R_1R_2	(MN)S O R_1R_2	(MN)S O R_1R_2				
16	14 not *d*	(MN)S A_1B R_1r	(MN)S O rr	(MN)S B R_1r	(NN)S B rr					

* 1st child is father in family 9. 2nd child is mother in family 21.

† 1st child by previous husband.

No.	Type of Mating	Parents		Children						
		Father	Mother	1	2	3	4	5	6	7
17	14 not *d*	(MN)S A_1B R_2r	(MN)S O *rr*	(MM)S B R_2r	(NN)S A_1 *rr*					
18‡	14	(MN)S A_1 R_1r	(MN)S B R_1R_2	(MN)S A_1B R_1R_1	(MN)S A_1B R_1r	(MN)S A_1B R_2r				
19	14 *not f*	(MN)S A_1 R_2r	(MN)S A R_1r	(MN)S A_1 R_2r	(MM)S A R_1r					
20	12*a*	MN O R_1R_1	MN A_1 R_1^wr	MN O R_1r	MN O $R_1^wR_1$	MN O $R_1^wR_1$	MM A_1 R_1r			
21§	7*d* *e*, or *f*	(MN)S A_2 R_2r	MS M O R_2R_2	(MM)S O R_2r	(MM)S A_2 R_2R_2					
22	4*a*	MN A_2 R_1r	MM A_2 R_1r	MN A_2 *rr*	MM O *rr*					
23	16*c*	NN O R_1R_1	M NS O R_2r	MN O R_1r	NS N O R_1R_2	MN O R_1r				
24	8*a*	NN A_1 R_1R_1	MM O *rr*	MN O R_1r						
25	18*b*	NS N O R_1r	MN A_1 R_1r	MN O R_1r						
26	11*d*	MS M O *rr* *pp*	NS N A_1 R_2r P*p*	(MN)S A_1 R_2r *pp*	MN O R_2r P*p*	(MN)S A_1 R_2r P*p*	(MN)S A_1 *rr* P*p*			
27	14*e*	(MN)S A_1 R_2r	(MN)S O R_2r	MN A_1 *rr*	(MN)S O R_2R_2	MS M O R_2R_2	(MN)S O R_2R_2			
28	3	(MM)S O R_1R_2 P	(MM)S O R_1r P	(MM)S O R_1R_2 P	(MM)S O R_1R_2 P	(MM)S O R_1r P	(MM)S O R_1r P	(MM)S O R_1R_2 P		
29	4*a*	MM O *rr* *pp*	MN O R_0r *pp*	MM O R_0r *pp*	MN O *rr* *pp*	MN O *rr* *pp*	MM O R_0r *pp*	MN O *rr* *pp*		
30	15*a*	MN A_1 *rr* P*p*	NN B R_2r *pp*	NN A_1 R_2r P*p*	MN A_1 *rr* P*p*	NN A_2 R_2r P*p*	MN A_1 R_2r *pp*			

(MN)S written thus indicates that the S may be located on either or both the chromosomes. When family grouping makes clear the position of the S, it is written thus, MS N or M NS or MS NS.

The short Rh symbols used in this table have the following significance :—R_1 = CD*e*, R_2 = *c*DE, *r* = *cde*, R_1^w = C^wDe, R′ = C*de*, R″ = *cd*E and R_0 = *c*D*e*.

In the determination of the Rh genotypes the following antisera were used :—anti-C-C^w-*c*-D-E-*e*.

‡ 1st child is father of family 7. 2nd child is father of family 16. 3rd child is father of family 17.

§ Mother is 2nd child in family 1.

Families 3, 4 and 23 are of particular significance in the support of the association of S with the MN blood groups.

In family 3, the father is MN and possesses S (that is to say, his blood is agglutinated by the new antibody), the mother is MM and does not possess S. It will be seen that S is segregating with the father's M ; for the 3 children to whom he gave his M all have S, whereas the 4 to whom he gave his N have not. The probability of this segregation being due to chance is 1/35, using Fisher's exact method for 2×2 tables ; or 1 in 64, postulating, as in this case we may, the theoretical 1 : 1 ratio.

Family 4 is of the same type of mating, excluding the first child (the issue of a previous marriage of the mother) ; there are 3 children who have received their mother's N, but not her S. If these three children are added to those of family 3, the probability of the segregation being due to chance is reduced to 1 in 120. Although the group of the father of the first child in family 4 is not known, this child has received M from his mother and has S. If it is allowed that this S has come from the mother, then the probability is further reduced to 1 in 330.

In family 23 we have been fortunate in finding an example of the converse type of mating, that is to say, M NS$\times$NN. Here we see dependent segregation of N and S, for the two children who have received their mother's M have not S, and the one child who has received her N has S.

Through the finding reported in this paper a glimpse is obtained of the complexity of the MN groups. Since their discovery by Landsteiner and Levine in 1927, the MN groups remained, it seemed, singularly uncomplicated in comparison with the ABO and Rh groups. Whether this insight will have other than theoretical interest depends upon the success of attempts to reproduce the antibody at will in human beings or rabbits.

SUMMARY

The reactions of an antibody which subdivides the MN blood groups are described.

This antibody agglutinates 72 per cent. of M, 60 per cent. of MN and 33 per cent. of N bloods ; such bloods are designated by the addition of the symbol S.

Statistical analysis and family groupings are consistent with the hypothesis that either there are 4 allelomorphs MS, M, NS and N, or that S is a gene closely linked to the MN locus. The gene frequencies calculated are MS 25·0 per cent., M 26·3 per cent., NS 9·2 per cent., and N 39·5 per cent.

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