108. A New Example of the Rare $D\text{--}(\overline{R}^0)$ of Rh Blood Groups in Japan

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Since the first example of the rare blood having the genotypic formula $D\text{--}/D\text{--}$ was discovered by Race and his associates^{1),2)} in 1950, many investigators have exhibited a particular interest in this subject from the standpoint of practical medicine and immunogenetics. The blood cells of a person having $D\text{--}$ are characterized by lacking any representation of the other Rh antigens such as $C(rh')$, $E(rh'')$, $c(hr')$, $e(hr'')$, and $f(hr)$. In Japan, up to date, five pedigrees having the $D\text{--}$ have been described by Yokoyama and Furuhashi (1957),³⁾ Kuniyuki and Takahara (1958),⁴⁾ Ose and Bush (1960),⁵⁾ Tsuchiya *et al.* (1962),⁶⁾ and Nakajima *et al.* (1965).⁷⁾ In the present communication the authors wish to report the sixth example of a family having the chromosome of $D\text{--}$ and to propose at this opportunity to organize the mutual supply circle of blood within the persons of $D\text{--}/D\text{--}$ living in Japan.

Rh genotypes of the propositus and her family. In Feb. 1965, the blood sample of the propositus, Mrs. M. O., aged 30, was brought to the Blood Center of Nihon University in order to examine her blood groups. She lost her second infant, three weeks before, from hemolytic disease of newborn at the 2nd day after birth. Blood grouping tests were performed with available standard antisera and gave the following results:

A_1B , $NsNs$, P_2 , q , $Le(a-b+)$, $K-k+Kp(a-b+)$, $Fy(a+)$,
 $Jk(a+)$, $Xg(a+)$ and $C-C^w-D+E-c-e-$.

As her red cells were found to lack C , C^w , E , c , e antigens, her Rh genotype was identified to be $D\text{--}/D\text{--}$.

Furthermore, for the purpose of elucidating the genetic relationship of the $D\text{--}$, we carried out the blood grouping tests of the other members of her family. In consulting with the frequencies of Rh gene-complexes in Japanese and the data of avidity tests of red cells, the Rh genotypes were established. The pedigree of the family in relation with the Rh and ABO blood groups was shown in Fig. 1. The propositus (II-8) has five brothers and one sister. One of them

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Black = homozygous $D-/-$. Half-black = heterozygous $D-/-$.
 Blank = apparently normal genotype or not tested.
 Hatched = probably heterozygous, but not tested.

Fig. 1. Pedigree of the family members and their probable Rh genotypes.

(II-4) was also homozygous $D-/-$. Three sibs (II-1, 6, 9) were heterozygous $DCE/D-$. The other two (II-2, 3) were proved to be ordinary genotypes of DCE/DCE . The first child of the propositus was normally delivered and had no clinical signs of hemolytic disease. The genotype of this child (III-3) was heterozygous $DCE/D-$. Any noticeable abnormalities of the other blood groups of the family members could not be recognized.

Serological notes. (1) The red cells: As reported by previous workers on the serological characteristics of the red cells having $D-$, some comparative tests, using three types of homozygous, heterozygous and ordinary Rh positive cells, were done and the results obtained were tabulated in Table I. Only two of 8 incomplete anti- D sera exhibited positive reactions in saline in the degree of agglutination from 2+ to 3+ with $D-$ homozygous cells (II-4, 8) and from 1+^w to 1+ with heterozygous cells (II-1, 6, 9, III-3), while they could not agglutinate the red cells of ordinary Rh positive cells. No significant differences in the titration scores within three types of red cells, showing from 20 to 40 by bromelin technic, could be confirmed. Then, the avidity of red cells to the adequately diluted anti- D serum was examined by means of serum or bromelin technics. The $D-$ homozygous cells needed 24–40 seconds until visible agglutination appeared, and heterozygous cells 30–80 seconds in both methods. On the other hand, the ordinary cells required longer time of about 90–180 seconds. The above-mentioned differences of avidity within these three types could be reversely available to presume the genotypes of tested blood. As shown in Table I, the incomplete anti- D antibody was more inten-

Table I. Serological reactivities of the $D--$ red cells to incomplete anti- D serum

	agglutination saline bromelin (titr. score)		avidity (R.T.) serum, bromelin		absorption* (titr. score)	Rh genotypes presumed
II-8	++	34	24''	35''	0	$D--/D--$
II-4	+++	40	30''	40''	0	$D--/D--$
II-1	+	32	50''	65''	2	$DCe D--$
II-6	+	34	60''	50''	2	$DCe/D--$
II-9	—	33	80''	45''		$DCe/D--$
II-3	+	34	30''	50''	1	$DcE/D--$
II-2	—	23	100''	120''	8	DCe/DCe
II-3	—	29	120''	95''	8	DCe/DCe
cont. 1	—	23	100''	105''	7	DCe/DCe
" 2	—	20	150''	180''		DCe/DCe
" 3	—		67''			DcE/DcE
" 4	—	20	100''	120''	8	DCe/DcE
" 5	—	0			32	dCe/dce

* Agglutinin in the absorbed serum was titrated with the red cells of DCe/DCe in bromelin.

sively absorbed with the $D--$ -homozygous and heterozygous cells than the ordinary cells.

(2) Antibodies in the serum of *propositus*: It was demonstrated in cross-matching test that her serum agglutinated all 45 samples containing 3 samples of Rh negative blood. Therefore it seemed that multiple antibodies for the lacking antigens but D of the known Rh antigens were contained in her serum. Then, the analysis of antibodies was done by the absorption and elution technics applying Weiner's method,⁽¹¹⁾ using the panel cells of DCe/DCe , DcE/DcE , dce/dce etc. From the results shown in Table II, it was apparent that her serum possessed the separable anti- E and anti- c , and besides the cross reactive antibodies of anti- Cc and anti- Ee to react respectively with red cells having both of antigens C , c and E , e . The agglutinin titres for DcE/DcE cells were 1:1024 in bromelin and 1:512 in saline; for DCe/DCe cells 1:64, 1:32; for DCe/DcE 1:2048, 1:1024 respectively. It was noteworthy that such differences in the agglutinin titers were found in accordance with the antigen structures corresponding to anti- c , anti- Cc , anti- E and anti- Ee .

Discussion. In the explaining the question--how to generate the $D--$, Race *et al.*^(1),2) postulated a short deletion of chromosome in their first report. Recently, Yokoyama *et al.*⁽⁸⁾ interpreted the $D--$ -complex to have arisen by mutation of several sub-elements (mutons)

Table II. Analysis of antibodies in the serum of propositus by absorption and elution technics

Fractions	Tested in	Reaction of the fraction with the red cells of							
		R_2R_2	R_1R_1	R_1R_2	$R_1^wR_1$	$R'r$	$R''r$	rr	
1) Unabsorbed serum	sal.	++++	++++	++++	++++	++++	++++	++++	
2) Sup. of (1) abs. with R_1R_1 cells	sal. brom.	++++	- -	++++	- -	+ +++	++++	++++	
3) Eluate from (2)	brom.	++++	++++	++++	+++	++	++	++++	
4) Sup. of (2) abs. with rr cells	sal. brom.	++++	- -	++++	- -	- -	++	- -	
5) Eluate from (4)	sal. brom.	++ +++	- -	- ++	- -	- +	- +	+ +++	
6) Sup. of (3) abs. with rr cells	brom.	-	-	-	-	-	-	-	
7) Sup. of (3) abs. with R_2R_2 cells	brom.	-	-	-	-	-	-	-	
8) Sup. of (1) abs. with R_2R_2 cells	brom.	-	-	-	-	-	-	-	

according to the cistron theory. The precise genetical background of generating the $D--$, however, is still unknown. Of special interest was the fact that the consanguinity rate in the parents of propositi, as described by Race and Sanger,^{9),10)} was very high and probably over 80 per cent. The parents of the propositus in the present case were also the first cousins. Four of six $D--/D--$ propositi discovered in Japan was revealed to be the product of consanguineous marriages between cousins. Since the parents of the remaining two came from the same village, consanguinity could not be excluded. Most of parents of propositi, as mentioned in the previous reports, were confirmed to be heterozygotes having the $D--$ chromosome. Accordingly, one quarter of sibs might be genetically expected to be $D--/D--$. Contrary to such an expectation, it was clarified, however, that 15 out of 31 sibs in the six examples were homozygous $D--/D--$. The high rate of homozygous persons is considered by Race and Sanger¹⁰⁾ to be the consequence of a trans effect suppressing the production of the other antigens by normal partner chromosome.

Serological characteristics of the red cells having $D--$, established in the previous investigations,¹⁻⁸⁾ are as follows: 1) the red cells can be agglutinated in saline suspension by incomplete anti- D serum, 2)

higher reactivity than ordinary Rh positive cells, 3) increased capacity to absorb anti- D than the ordinary cells. The above-mentioned features might be attributed to have more D antigens than have the cells of ordinary genotypes, and to the absence of allosteric inhibition due to the adjacent antigens such as C , c , E , e . However, in the present study some different behaviors of red cells having D^{--} were observed. The D^{--} cells have been agglutinated in saline by only two of 8 incomplete anti- D sera used. No significant differences in the titration scores between the D^{--} red cells and ordinary cells could be demonstrated. On the other hand, the avidity of red cells, expressing as time needed until the appearance of agglutination became stronger in order of D^{--}/D^{--} , D^{--}/D^{--} and ordinary genotypes. Thereupon, this avidity test could be available to recognize heterozygote within a family having D^{--} .

It is natural that D^{--}/D^{--} persons have more opportunity to be immunized than have persons of ordinary genotypes. The antibodies contained in the serum of the propositus were identified to be anti- c , anti- E , anti- Ce , and anti- Ee . These multiple antibodies are considered to be produced in response to the antigens originated from her two children at the period of incompatible pregnancies, because she had no history of blood transfusion.

The propositus Mrs. M.O. is now pregnant and desires eagerly to rescue newborn from erythroblastosis which may happen at high probability. So we are considering to prepare about 1,000 ml. of compatible blood having D^{--}/D^{--} for exchange transfusion and general transfusion to deal with unexpected accident in delivery. The best hope is to take blood from her brother ($AB, D^{--}/D^{--}$). As the blood taken from a person, however, is limited in volume, we have encountered with some difficulties to obtain the blood of D^{--}/D^{--} . Whoever has the genotypes D^{--}/D^{--} may be faced with opportunity requiring blood transfusion either in pregnancy or disease. Summarizing the data reported up to date, there are about ten persons having the genotypes D^{--}/D^{--} in Japan. Under the circumstances, we are in hope of the mutual supply circle of blood among them to be organized.

Summary. A new example of the family having the rare $D^{--}(\bar{R}^0)$ has been presented as the sixth case discovered in Japan. The propositus, Mrs. M.O., aged 30, had the genotype D^{--}/D^{--} . One of her six sibs was also homozygous D^{--}/D^{--} . The other three sibs were heterozygous D^{--}/D^{--} and the remaining two had the ordinary genotypes D^{--}/D^{--} . Their deceased parents were first cousins. The anti- c , anti- E , anti- Ce , and anti- Ee could be detected in the serum of

the propositus. As she had received no blood transfusion, these multiple antibodies were probably produced due to the incompatible pregnancies of her two children.

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