Jap. J. vet. Sci., **40**, 309～313 (1978)

Blood Grouping of Chimpanzees by Isoantibody

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(Received for publication December 9, 1977)

Abstract. Seventeen blood samples of chimpanzee were examined for the presence of homologous antibody. All of them contained anti-B but not anti-A antibody. They also possessed A-like antigen on the red cell membrane. After complete absorption of the antibody by human B and AB red cells, the existence of homologous antibody was demonstrated in chimpanzee serum by the bromelin method. This antibody was detected from eleven of the 17 samples tested and tentatively designated anti-Ch antibody. With this antibody chimpanzees could be divided into two types, Ch (+) and Ch (−). Of the 17 chimpanzee samples, two were of Ch (+) type and the other fifteen of Ch (−) type.

A genetic marker on the red cell membrane of chimpanzees has been demonstrated by various methods with human blood typing antisera, lectins, immune chimpanzee antibodies and immune heterologous antibodies [3, 6, 7, 9–12]. Studies on genetic polymorphism in leukocytes [2], serum protein [1] and enzyme [4] have also been reported. Landsteiner and Miller [5] found that chimpanzees had naturally occurring anti-A antibodies. There have been, however, few reports on isoantibodies in chimpanzees. The purpose of this paper is to describe serological aspects of isoantibodies observed in the serum and blood grouping of chimpanzees by using these antibodies.

Materials and Methods

Red cells: Human and chimpanzee blood samples were collected by venipuncture. Human panel red cells were obtained from the Ortho Diagnostics, Inc. They were washed three times in physiological saline, and 2% suspensions were prepared from them for the blood grouping tests.

Antisera: Chimpanzee sera were collected from blood samples by centrifugation at 3,000 rpm for 10 minutes. Human anti-A and anti-B, and anti-H from *Ulex europaeus* L. were used for ABO blood typing.

Hemagglutination: The ABO grouping and the reactions between human panel cells and chimpanzee sera were performed by the saline method. One drop of antiserum and one drop of 2% cell suspension were placed together on a hollow glass slide and incubated at room temperature for 30 minutes. Agglutination was read macroscopically. Chimpanzee blood grouping was done by the

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Table 1. Cross-hemagglutination test of 17 chimpanzee sera and red cells by bromelin method

Chimpanzee red cells	Chimpanzee sera		
	17	3 7 8 10 11 12 13 15 16 22	18 20 21 23 24 25
17	+*	+	—
18	+	+	—
3 7 8 10 11 12 13 15 16 20 21 22 23 24 25	—	—	—

Remarks.

These isoantibodies and antigens were undetectable by the saline method. For the ABO blood grouping, all the sera contained anti-B. A-like antigen was observed on red cells.

*: Spontaneous hemagglutination was observed.

bromelin method of Tokunaga [8].

Absorption tests: When the isoantibodies of chimpanzees were absorbed with chimpanzee red cells, the bromelin method [8] was used. In absorption with human red cells, this method was not used. Equal volumes of chimpanzee serum, bromelin solution, and washed packed red cells were placed together in a test tube and incubated successively at 37°C for 1 hour, at 4°C overnight, and at room temperature for 1 hour. They were centrifuged and the resulting supernatant was titrated with an appropriate red cell suspension by the bromelin method.

Table 2. Hemagglutination reaction between chimpanzee serum No. 22 and human red cells

ABO blood group system of human red cells	Chimpanzee serum No. 22
A	—
B	++
AB	+
O	+*

Remarks.

*: Antibody which agglutinated human O type red cells was observed in chimpanzee serum No. 22.

Results

Cross-hemagglutination was performed by the bromelin and saline methods. Of the 17 chimpanzee serum samples, isoantibody was detected from eleven and isoantigen from two. The 17 chimpanzee sera contained anti-B, but not anti-A, while chimpanzee red cells reacted with human anti-A but not with human anti-B or anti-H. Eleven sera, Nos. 3, 7, 8, 10 to 13, 15 to 17, and 22, contained anti-B and homologous antibody. In the case of two samples of red cells, Nos. 17 and 18, isoantigens and A-like antigen were observed on the

Table 3. Absorption test of chimpanzee serum No. 22 by human B, AB, and O, and chimpanzee Ch positive and negative type red cells

Chimpanzee serum	Type of absorbing red cells	Indicator cells					
		A	B	AB	O	Ch(posi.)	Ch(nega.)
No. 22	B	—	—	—	$+_w$	+	—
	AB	—	—	—	$+_w$	+	—
	O	—	+	+	—	+	—
	Ch(posi.)	—	+	+	$+_w$	—	—
	Ch(nega.)	—	+	+	$+_w$	+	—

Table 4. Agglutination of human panel red cells (Ortho Diagnostics) with chimpanzee serum No. 22 absorbed by human B and AB type red cells

Cell No.	Rh-hr								Kell		Duffy		Kidd		X Linked	Lewis		MNSs				P	Lutheran		*
	D	C	E	c	e	f	C^w	V	K	k	Fy^a	Fy^b	Jk^a	Jk^b	Xg^a	Le^a	Le^b	S	s	M	N	P_1	Lu^a	Lu^b	
1	+	+	0	0	+	0	+	0	0	+	+	+	0	+	+	0	+	+	+	+	0	+	0	+	+
2	+	+	0	0	+	0	0	0	+	+	0	+	0	+	+	0	+	+	+	+	+	+	0	+	+
3	+	0	+	+	0	0	0	0	0	+	0	+	+	+	+	0	+	0	+	0	+	+	0	+	+
4	0	+	0	+	+	+	0	0	0	+	+	+	+	0	+	0	0	0	+	+	+	0	0	+	+
5	0	0	+	+	+	+	0	0	0	+	+	+	0	+	+	0	0	0	+	0	+	0	0	+	+
6	0	0	0	+	+	+	0	0	+	+	0	+	+	0	0	0	+	+	0	+	+	+	0	+	+
7	0	0	0	+	+	+	0	0	0	+	+	0	+	+	0	0	+	0	+	+	0	+	0	+	+
8	0	0	0	+	+	+	0	0	0	+	0	+	0	+	+	+	0	0	+	+	+	+	+	+	+
9	0	0	0	+	+	+	0	0	0	+	0	+	+	0	+	0	+	0	+	+	+	0	+	+	+
10	+	+	0	0	+	0	0	0	0	+	+	+	+	0	+	+	0	+	0	+	0	+	0	+	+

Remarks.

*: The reaction pattern between human panel cells and chimpanzee serum No. 22 absorbed by human B and AB red cells was identical with those of anti-k and anti-Lu^b.

red cell membrane. The titer of isoantibody against isoantigen was 1:256–1:512 (Table 1).

In the hemagglutination reaction between chimpanzee serum No. 22 and human red cells, anti-B and antibody which agglutinated O type red cells were found in that serum (Table 2). The absorption test was carried out on that serum, using human B, AB and O type red cells. When the serum was absorbed by human B and AB red cells, the antibody activity was reduced against B and AB but not against O. Similarly, when the serum was absorbed by O type red cells, the activity was reduced only against O (Table 3). Namely, chimpanzee serum No. 22 absorbed by human B and AB red cells contained antibody which weakly agglutinated human O red cells. The hemagglutination test with human panel red cells revealed that all the panel cells reacted with serum No. 22 which had already been absorbed by human B and AB red cells, and that the reaction of this serum was identical with those of anti-k and anti-Lu^b (Table 4). It was assumed, however, that the properties of isoantibody in serum No. 22 absorbed by human B and AB red cells might be different from those of anti-k and anti-Lu^b antibody, because both k and Lu^b antigens were observed on the surface of Japanese red cells in the absorption test. These results suggest that isoantibody detected from serum No. 22 may be characteristic of chimpanzees. This isoantibody was tentatively designated anti-Ch. It could be detected only by the bromelin method.

In the absorption test of anti-Ch antibody with chimpanzee red cells, anti-Ch activity was reduced by positive red cells, Nos. 17 and 18, but not at all by negative chimpanzee or human B red cells. The titer of anti-Ch antibody against chimpanzee positive red cells was 1:64–1:128.

Table 5. Frequencies of anti-Ch and Ch antigen in 17 chimpanzee samples

	No. of tested	No. of positive	%
Anti-Ch	17	11	64.7
Ch (+)	17	2	11.8
Ch (−)	17	15	88.2

The red cells agglutinated by anti-Ch serum were tentatively designated the CH (+) type, and those not agglutinated the Ch (−) type. Anti-Ch antibody was observed in eleven (64.7%) of the 17 chimpanzee sera. The Ch (+) type was found in two, or 11.8%, and the Ch (−) type in 88.2% of the 17 samples of red cells (Table 5).

Discussion

Most of the chimpanzee blood factors have been demonstrated with human blood tying antisera. ABO, MN, Lewis, Rh, Ii and P systems in the chimpanzee blood have already been reported [7]. Among the simian blood group systems, V-A-B and C-E-F systems [9, 11] have also been recognized. The property and frequency of appearance of antigens of these systems were studied [7]. Landsteiner and Miller [5] described that naturally occurring anti-A antibody was present in chimpanzees and had properties identical with those of human anti-A antibody. There are, however, few reports on isoantibodies and antigens in chimpanzees.

In the present studies, 17 chimpanzee blood samples were used for the detection of isoantibody with the results mentioned above. Of the ABO system in chimpanzees there are several reports with different results [7]. In this investigation all the chimpanzee sera contained anti-B but not anti-A

antibody. They also possessed A-like antigen on the red cell membrane.

In the cross-hemagglutination, isoantibodies were detected from chimpanzee sera. After both anti-B antibody and isoantibody contained in these sera were absorbed by human B and AB red cells, the isoantibody was detected by the bromelin method. The isoantibody against chimpanzee positive red cells was 1:64–1:128 in titer.

This antibody was tentatively designated anti-Ch. The red cells agglutinated by it were tentatively designated the Ch (+) type, and those not agglutinated the Ch (−) type. Of the 17 chimpanzee blood samples, eleven contained anti-Ch antibody and two the Ch (+) type.

With anti-Ch antibody A type chimpanzee red cells were classified into two types, Ch (+) and Ch (−). Anti-Ch antibody activity was absorbed by chimpanzee A red cells of Ch (+) type, but not by those of Ch (−) type. From these results, it is concluded that no Ch factor is related to A-like antigen, and that anti-Ch antibody may be chimpanzee-specific isoantibody.

It has not been determined whether Ch factor is related to the blood group factors described by Moor-Jankowski and Wiener [7] or not. Further investigation may be necessary to establish a test for compatibility of the Ch system with the other systems in order to evaluate blood transfusion or transplantation in experimental medicine by using chimpanzees.

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