Comparison Test of Antibodies for Dog Blood Grouping

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Abstract. A comparison test was carried out with 24 kinds of type specific antibodies prepared by the authors and Bull et al. for the purpose of standardizing dog blood grouping antisera in Japan. As a result, anti-D₁ and anti-E were identical with anti DEA-3, anti-A was with anti DEA-5, and anti-180a with anti DEA-8. Anti-M and anti DEA-5 possessed a common specificity. No antisera had the same specificity as anti DEA-1•1, 2, -1•1, -4, -6 or -7. Besides, anti-D₂, -B, -C, -F, -G, -L (-H, -I, -43), -44 and -2a prepared by the authors were identical with none of anti-DEA sera.

Studies of genetic markers on the dog red cell membrane have been performed by many researchers. These have been conducted by Iseki and Terashima [7], Hosoda [5], Kita et al. [11], Yoh [21], Yamada et al. [20], Iwasaki et al. [8, 9], Ikemoto et al. [6], Yoshida et al. [22], and Ejima et al. [3] in Japan, and by Swisher and Young [15], Mears et al. [12], Hall [4], Bowdler et al. [1], Suzuki et al. [14] and Vriesendorp et al. [19] in other countries. Recently, an international workshop was held, and 8 red cell antigens, consisting of DEA 1•1, 1•2, 3, 4, 5, 6, 7 and 8, were authorized in it [19].

The blood groups of dogs were classified into various systems by using naturally occurring antibodies, iso-immune antibodies, immune heterologous antibodies [3-9, 11, 12, 14, 15, 17, 19-21], naturally occurring heterologous antibody [1] and lectins [22].

The dog blood group systems have been taken into consideration in blood transfusion, organ transplantation, and judgment of the relationship between parents and off-

spring in the field of veterinary practice and experimental medicine [10, 11, 13, 15, 16, 18]. The authors applied the D blood group system [3] to judgment of the father from the blood type of puppies which had been delivered from bitches in experimental induction of superfecundation [16]. As a result, it was indicated that the D blood group system was useful for judgment of the relationship between parents and offspring.

On the other hand, no dog red cell typing antisera produced in Japan have been approved by the International Workshop of Canine Immunogenetics [17, 19]. The purpose of this paper is to compare antisera prepared by the authors with anti-DEA sera authorized by the workshop.

Materials and Methods

Blood grouping antisera: In this study, 16 antisera consisting of anti-D₁, -D₂, -A, -B, -C, -E, -F, -G, -H, -I, -L, -M, -2a, -43, -44 and -180a prepared by the authors, and 8 antisera consisting of anti DEA-1•1, 2, -1•1, -3, -4, -5, -6, -7 and -8 kindly sup-

plied by Dr. R. W. Bull, of the Michigan State University, U.S.A., were examined.

Anti-D₁ and anti-D₂ were obtained from rabbits immunized with dog red cells [3]. Anti-A, -E, -F, -G, -L, -M and -2a were iso-immune antisera [3]. Anti-B [6], -H, -I, -44, -43 and -180a were naturally occurring antisera. Anti-C [22] was lectin extracted from the seed of *Clerodendron trichotomum*. Anti-C, -H, -44 and -43 were supplied by Ikemoto, one of the authors.

Panel red cells: Dog red cells were collected by venipuncture from 15 dogs, consisting of 11 beagles and 4 mongrel dogs. Clotted blood (without anticoagulant) and heparinized blood were prepared. The former was spun down to remove serum. In the latter, cells were washed three times with 0.85% physiological saline. Two 4% cell suspensions in fresh autologous serum and 0.85% physiological saline, respectively, were used for typing.

Red cell typing: Two drops of each antiserum were combined with two drops of 4% appropriate red cell suspension in a round-bottomed glass test tube 10×75 mm in size. When the cell suspension in fresh autologous serum and antiserum were combined, the mixture was incubated at 37°C for 15 minutes, spun at 1500 rpm for 15 seconds, and read macroscopically for hemolysis and agglutination. Similarly, when the saline cell suspension and antiserum were combined, the mixture was incubated at 4°C for 30 minutes and spun at 1500 rpm for 15 seconds. The tubes were shaken weakly and then checked macroscopically for agglutination.

Absorption test: Equal volumes of antiserum or agglutinin prepared by the authors 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. The resulting supernatant was titrated with an appropriate red cell suspension. The absorption of anti-DEA sera was not carried out.

Indirect anti-globulin test: When the cell suspension in fresh autologous serum and antiserum were combined, tubes presenting no agglutination, trace or plus 1 reaction were treated with anti-dog whole serum rabbit serum obtained from rabbits immunized with dog serum. The cells in each tube were washed three times with saline. Then they were resuspended in saline. Two drops of the diluted anti-dog serum rabbit serum were added to each resulting cell suspension and mixed well. The tubes were incubated at room temperature for 5 minutes and spun at 1500 rpm for 15 seconds. They were shaken weakly and then checked macroscopically for agglutination.

Results

The reaction patterns were compared between antisera prepared by the authors and anti-DEA sera against 15 panel cells. The results obtained are shown in Tables 1A and 1B. They indicated that none of the former antisera had the same specificity as anti DEA-1·1, 2 or -1·1, because the reactivity of anti-2a against 15 panel red cells was not identical with anti DEA-1·1, 2 or -1·1.

The reaction patterns of anti-D₁ and -E were identical with that of anti DEA-3, that of anti-A was with that of anti DEA-5, and that of anti-180a with that of anti DEA-8 (Table 1B). Anti-H, -I, -L and -43 prepared by the authors had the same specificity (Table 1B).

DEA 5-positive red cells (Nos. 1, 5, 6, 12 and 14) reacted with anti-M strongly, and DEA 5-negative red cells (Nos. 7 to 11, and 13) with anti-M weakly (Table 1B). When anti-M was absorbed with DEA 5-positive red cells (Nos. 1, 5, 6, 12 and 14) of strong agglutinability against anti-M, the antibody activity disappeared against both red cells of strong and weak agglutinability. On the other hand, when the absorption was carried out on anti-M with DEA 5-negative red cells (Nos. 7 to 11, and 13) of weak agglutinability against anti-M, the antibody activity disappeared against DEA 5-negative red cells of weak agglutinability against anti-M (Nos. 7 to 11, and 13), but not reduced against DEA 5-positive red cells of strong agglutinability against anti-M (Nos. 1, 5, 6, 12 and 14). Moreover, no M(−)·DEA 5(+) type red cells were observed in this study. Namely, anti-M and anti DEA-5 had a common specificity (Table 2).

In the absorption test of various antisera with appropriate red cells, each antibody activity disappeared by the absorption of

Table 1A. Comparison of reaction pattern between anti-DEA and anti-2a for 15 types of dog panel red cells

Antiserum	Panel red cells (4% suspension in autologous serum)														
	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15
2a	0	0	2H 2+	3+	0	0	0	0	0	0	0	0	0	0	0
DEA-1·1,2	1+	0	3H 1+	3+	3+	3+	3+	0	0	0	0	0	3+	3+	3+
DEA-1·1	0	0	3+	3+	3+	3+	3+	0	0	0	0	0	3+	3+	3+

0: No reaction. +: Agglutination. H: Hemolysis.

Tubes presenting no agglutination or plus 1 reaction were treated with anti-dog whole serum rabbit serum.

Table 1B. Comparison of hemagglutination between antisera prepared by the authors and anti-DEA for 15 types of dog panel red cells

Antiserum	Panel red cells (4% suspension in saline)														
	1	2	3	2	5	6	7	8	9	10	11	12	13	14	15
D ₁	0	2+	2+	2+	0	0	2+	0	0	0	0	0	0	0	0
D ₂	0	3+	3+	0	0	3+	3+	3+	3+	3+	3+	3+	3+	3+	3+
A	2+	0	0	0	2+	2+	0	0	0	0	0	2+	0	2+	0
B	1+	0	0	0	1+	0	0	1+	1+	1+	1+	1+	0	0	0
C	0	0	0	1+	0	0	0	0	0	0	0	0	0	1+w	1+w
E	0	1+	1+	1+	0	0	1+	0	0	0	0	0	0	0	0
F	0	0	0	0	1+	1+	0	0	1+	1+	1+	1+	1+	1+	1+
G	0	1+	0	1+	0	0	1+	0	0	0	0	0	0	0	1+
L (H, I, 43)	1+	0	1+	0	1+	1+	0	0	1+	1+	1+	1+	1+	1+	0
M	3+	0	0	0	3+	3+	1+	1+	1+	1+	1+	3+	1+	3+	0
44	0	1+	0	0	1+	0	0	0	0	0	0	0	0	0	1+
180a	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1+
DEA-3	0	2+	2+	2+	0	0	2+	0	0	0	0	0	0	0	0
DEA-4	1+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
DEA-5	1+	0	0	0	1+	1+	0	0	0	0	0	1+	0	1+	0
DEA-6	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+	2+
DEA-7	2+	2+	2+	2+	2+	2+	2+	0	2+	1+w	2+	2+	2+	2+	2+
DEA-8	0	0	0	0	0	0	0	0	0	0	0	0	0	0	1+w

0: No reaction. +: Agglutination. +_w: Weak agglutination.**Table 2. Absorption test of anti-M with M(+)-DEA 5(+) type, M(+)-DEA 5(-) type, and M(-)-DEA 5(-) type red cells**

Antiserum	Type of absorbing cells	Indicator cells		
		M(+)-DEA 5(+)*	M(+)-DEA 5(-)**	M(-)-DEA 5(-)
Anti-M	M(+)-DEA 5(+)*	—	—	—
	M(+)-DEA 5(-)**	+++	—	—
	M(-)-DEA 5(-)	+++	+	—

* M(+)-DEA 5(+) type red cells show strong agglutinability with anti-M.

** M(+)-DEA 5(-) type red cells show weak agglutinability with anti-M.

Table 3. List of red cell antisera

Antiserum prepared by the authors	Specificity
D ₁ , E	DEA 3
A	DEA 5
M	DEA 5+extra
180a	DEA 8
D ₂	
B	
C	
F	
G	
L (H, I, 43)	
44	
2a	

positive red cells, but not at all by negative red cells.

In this study, there were no antisera with the same specificity as anti DEA-1·1, 2, -1·1, -4, -6 or -7. Besides, none of anti-D₂, -B, -C, -F, -G, -H, -I, -L, -2a, -43 and -44 prepared by the authors were identical with any of anti-DEA sera (Table 3).

Discussion

The realities of investigation on dog blood groups have developed into a request for arrangement of blood typing antisera.

Comparison tests of antisera and antigens for dog blood grouping have been advanced in the International Workshops [17, 19]. Table 4 shows dog red cell antigen systems arranged by interchanges of organization by Vriesendorp et al. [17, 19] and Bull et al. [2] as leaders.

It has not been determined, however, whether the blood grouping antisera produced in Japan correspond to the antisera approved by the International Workshop or not. The authors could obtain 8 antisera which had been supplied by Dr. R. W. Bull and compared 16 antisera prepared by the authors with the anti-DEA sera for the purpose of standardizing dog blood grouping antisera.

Table 4. Nomenclature of dog red cell typing antisera authorized by International Workshops

Old	New
Anti-A	Anti DEA-1·1, 2
-A1	-1·1
-B	-3
-C	-4
-D	-5
-F	-6
-Tr	-7
-He	-8

Table 5. Phenotype and genotype of the D system

Phenotype	Genotype	Anti-D ₁ *	Anti-D ₂ *
D ₁	D ¹ /D ¹	+	—
D ₂	D ² /D ²	—	+
D ₁ D ₂	D ¹ /D ²	+	+

* Anti-D₁ and -D₂ are immune antibodies obtained from rabbits following immunization with dog red cells.

The reaction patterns were compared among 24 dog blood grouping antisera and 15 samples of dog panel red cells. None of the antisera prepared by the authors had the same specificity as anti DEA-1·1, 2 or 1·1. Anti-D₁ and -E were identical with anti DEA-3, anti-A was with anti DEA-5, and anti-180a with anti DEA-8. Anti-M and anti DEA-5 contained a common specificity.

In this comparison test, it was very interesting to note that the specificity of anti-D₁ was identical with that of anti DEA-3. Furthermore, there was no anti-DEA that had the same specificity as anti-D₂ prepared by the authors. Anti-D₁ and -D₂ were immune antibodies obtained from rabbits following immunization with dog red cells. Genetically, it was confirmed that D₁ and D₂ antigens in the D system were co-dominant factors (Table 5) [3, 7]. This D system is a historic system in Japan.

DEA 3 antigen was recognized by the International Workshop. It is presumed that

Table 6. Comparison of phenotype frequencies of D₁ and DEA 3 antigen on dogs reared in Japan and U.S.A.

Red cell antigen	Present authors			Swisher & Young*		
	No. of dogs tested	No. of positive dogs	%	No. of dogs tested	No. of positive dogs	%
D ₁	543	39	7.2			
DEA 3(B)				867	48	5.5

* Cited from *Physiol. Rev.* **41**, 495-520 (1961).

Table 7. Frequencies of D antigens in dogs of various breeds reared in Japan

Phenotype	% Positive dogs (No. of dogs tested)					
	Beagle	Shepherd	Mongrel	Shiba	Akita	Terrier
D ₁	0.0 (346)	0.0 (40)	7.2 (543)	7.7 (13)	57 (7)	14 (7)
D ₂	99.4 (346)	95.0 (40)	63.9 (543)	15.4 (13)	14 (7)	86 (7)
D ₁ D ₂	0.6 (346)	5.0 (40)	28.9 (543)	76.9 (13)	29 (7)	0 (7)

DEA 3 may have the same antigenic membrane structure as D₁ determinants. D₁-positive mongrel dogs bred in Japan exhibited almost the same frequency of appearance as DEA 3-positive dogs bred in the U.S.A. (Table 6). In the future, if anti-D₂ serum is presented to the workshop, the allelic relationship between D₁ and D₂ antigen will be authorized.

On the dog blood groups detected by immune rabbit sera several investigators performed studies with different results. Namely, Suzuki et al. [14] described that immune antibodies obtained from rabbits immunized with dog red cells were not so sufficiently available for use in the preparation of dog blood grouping antiserum. In Japan, Iseki and Terashima [7] and Yoh [21] divided dog blood groups into 3 types, D₁, D₂ and D₁D₂, Yamada et al. [20] into 4 types, D₁, D₂, D₁D₂ and X by using anti-D₁ and -D₂, and Iwasaki et al [8, 9] into 10 types from the combinations of antigens by using anti-D₁ to anti-D₇. From the results mentioned above, it is assumed that the International Workshop may have possessed no sufficient number of valuable materials.

The frequency of appearance of D antigen in dogs bred in Japan is shown in Table 7. That of D₂ antigen is higher in beagles, shepherds, and terriers than in mongrels, shiba and akita. That of D₁ antigen is higher in mongrels, shiba, akita and terriers than in beagles and shepherds. On the other hand, Vriesendorp et al. [19] observed no difference in that of DEA 3 antigen between beagles and mongrels.

From the results mentioned above, anti-D₂, -B, -C, -F, -G, -L (-H, -I, -43), -44 and -2a seem to be new antibodies at present, because none of them have the same specificity as any of anti-DEA sera, also each antibody activity was absorbed by appropriate positive red cells, but not by negative red cells. Anti-C was lectin extracted from the seed of *Clerodendron trichotomum*. It may be new distinguishable agglutinin against dog red cell antigens.

Vriesendorp et al. [19] found anti-N1 to -N8 sera to be new antisera for dog blood grouping. Further investigation may be necessary for the preparation of dog blood grouping antisera.

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要 約

イヌ血液型判定用抗体の比較同定試験：江島博康・黒川和雄（日本獣医畜産大学第1外科学教室），池本卯典（自治医科大学法医学教室）——日本におけるイヌ血液型判定用抗体の標準化を目的として，Bull らより入手した抗 DEA 抗体 8 種ならびに著者らにより作製分類された抗体 16 種，計 24 種類について比較同定試験を行った．その結果，抗 D₁ および抗 E は抗 DEA-3 と同一型特異性を有し，抗 A は抗 DEA-5 と，抗 180a は抗 DEA-8 と同一型特異性を示した．また，抗 M は抗 DEA-5 と共通な抗体を含有していた．著者らの作製分類した抗体の中で抗 DEA-1・1, 2, -1・1, -4, -6, -7 と同一型特異性を示す抗体は認められなかった．また，抗 D₂, B, C, F, G, L (H, I, 43), 44 および 2a はいずれの抗 DEA 抗体とも符合しなかった．