Feline Red Blood Cell Groups Detected by Naturally Occurring Isoantibody

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ABSTRACT. Naturally occurring anti-Ca and anti-Cb isoantibodies that can be used for judging red blood cell groups were detected in the plasma of cats. Anti-Ca antibodies were observed in 66.7% of the plasma of the Cb(+) type cat, and anti-Cb antibodies in 27.3% of the Ca(+) type cat plasma. The agglutinin titers of anti-Ca and anti-Cb plasma were 1:64 to :128 and 1:2 or lower, but hemolysis was not seen with anti-Cb plasma. There was no relationship between the change in these titers and the season. Anti-Ca was seen in the IgM fraction. The Ca(+) type was seen in 99.0%, the Ca(−) type in 1.0%, the Cb(+) type in 10.7%, and the Cb(−) type in 89.3% of the 299 cats examined. Combining the red blood cell types, the Ca(+)Cb(−) type was observed in 89.3%, Ca(−)Cb(+) type in 1.0%, Ca(+)Cb(+) type in 9.7% of the cats, but the Ca(−)Cb(−) type was not found. There was no relationship between sex, or the breed and the red blood cell groups. The percentage of cats with agglutinin in the plasma reacting to the Ca(+) type and red cells tended to be higher with age.—KEY WORDS: blood cell group, cat, isoantibody, isoantigen, genetics.

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Antigenic characteristics of feline erythrocytes have been reported by Ikemoto *et al.* [7], Suzuki *et al.* [8], Holmes [6], Eyquem *et al.* [5], and Auer *et al.* [1]. Blood transfusion reactions caused by the incompatibility of the red cell type have also been reported by Suzùki *et al.* [8] and Auer *et al.* [2], and the histocompatibility test has been suggested to have clinical importance.

The present study was carried out to investigate naturally occurring antibodies and red blood cell groups of cats.

MATERIALS AND METHODS

Antisera: Heparinized blood samples were collected by venipuncture. The samples were centrifuged at 3,000 rpm for 10 min and the supernatant was collected. Anti-Ca and anti-Cb wre obtained by screening cat plasma for naturally occurring antibodies. The antibodies were heated at 56°C for 30 min to inactivate the complement and then stored frozen (−20°C).

Red cells: Red cells were washed three times in physiological saline, and 4% suspensions were prepared for the blood grouping tests.

Red cell group typing: Each antiserum in 0.1 m*l* was added to 0.1 m*l* of appropriate red cell suspension in a round-bottomed glass test tube, 10×75 mm in size. The mixture was then incubated at room temperature for 15 min followed by centrifugation at 1,500 rpm for 15 sec. The tube was carefully shaken and then checked macroscopically for agglutination.

Absorption test: Equal volumes of antiserum and washed packed red cells were placed together in a test tube and incubated successively at 37°C for 1 hr, at 4°C overnight, and at room temperature for 1 hr. The tube was centrifuged at 3,000 rpm for 10 min. The resulting supernatant was titrated with an appropriate red cell suspension.

Heat treatment test: Antiserum was diluted twice with physiological saline, and

Table 1. Number of cats with anti-Ca and anti-Cb agglutinin in the plasma of Cb(+) and Ca(+) type cats, respectively

Antibody	Red blood cell type	Nos. of cats		%
		Tested	Positive Nos.	
Anti-Ca	Cb(+)	3	2	66.7
Anti-Cb	Ca(+)	267	73	27.3

heated at 70°C for 10 min. The treated sample was titrated with red cells.

2-Mercaptoethanol (ME) treatment test: Phosphate buffered saline (PBS) (0.15 M NaCl, 0.01 M phosphate buffer, pH 7.4) was used as the diluent. Antiserum in 0.5 m*l* and 2-ME solution in 0.5 m*l* (0.16 m*l* of 2-ME was dissolved in 10.0 m*l* of PBS) were mixed, shaken vigorously and incubated at 37°C for 2 hr. The mixture was dialysed with PBS overnight at 4°C. For control, antiserum was diluted with PBS and treated with the same method. The resulting mixture was examined for antibody activity by using the hemagglutination test with appropriate red cell suspensions.

Gel-filtraltion treatment: Shephadex G-200 (Farmacia, Sweden) in 25 g was suspended in 1,300 m*l* of PBS (pH 7.4). Antiserum in 1 m*l* was applied to a Shephadex G-200 colum (2.6×70 cm) and eluted at 15 m*l*/hr with PBS, pH 7.4. Protein peaks were detected by absorption at 280 nm.The first and second peak fraction were separately concentrated tenfold with collodion bags (SM13200, Sartorius) at 4°C. These fractions were titrated with appropriate red cell suspension.

RESULTS

Antibodies: Anti-Ca antibody was detected in the plasma of two (66.7%) of the 3 Ca(+) type cats tested, and anti-Cb antibody in seventy-three (27.3%) of the 267 Ca(+) type cats (Table 1). The agglutinating titers of anti-Ca and anti-Cb antibodies against corresponding positive red cells were 1:64 to :128 and 1:2 or lower, respectively. Anti-Ca activity disappeared after the absorption test by Ca(+) type red cells, heat treatment at 70°C for 10 min, or the 2-ME treatment (Tables 2 and 3). By gel-filtration with Sephadex G-200, agglutinating activity was detected in the first (IgM) fraction (Table 3).

When non-inactivated anti-Ca plasma and Ca(+) type red cells were combined, strong hemolysis was observed. A cat was followed for 31 months for his anti-Ca agglutinating and hemolytic activity in the plasma. Agglutinating titers ranged from 2 to 256, while the hemolytic titers ranged from 0 to 16. It was assumed that this hemolysis might have been affected by the activity of the complement in the plasma of the cat with anti-Ca, because the hemolysis were occurred with the Ca(+) type cells suspended in fresh autologous plasma (for complement) and inactivated anti-Ca plasma. No hemolytic activity was observed with anti-Cb plasma. Titer of anti-Cb were 1:2 or lower, and the anti-Cb activity disappeared after heat treatment 2-ME treatment or the gel-filtration test. Therefore, the immuno-serological characteristics of anti-Cb activity could not be clarified.

Red blood cell groups: All 299 cats were typed for RBC groups with anti-Ca and anti-Cb plasma. Table 4 shows the frequency of the red blood cell groups of cats in Tokyo, Japan. 99.0% was the Ca(+) type,

Table 2. Absorption test of anti-Ca and anti-Cb with positive and negative red cells

Antibody	Absorbing red cell	Indicator red cell	Dilution of absorbed antiserum						
			1:2	1:4	1:8	1:16	1:32	1:64	1:128
Anti-Ca	Cb(+)	Ca(+)	+++[a)]	+++	++	+	+	+	−
		Cb(+)	−	−	−	−	−	−	−
	Ca(+)	Ca(+)	−	−	−	−	−	−	−
		Cb(+)	−	−	−	−	−	−	−
Anti-Cb	Ca(+)	Ca(+)	−	−	−	NT[b)]	NT	NT	NT
		Cb(+)	+	−	−	NT	NT	NT	NT
	Cb(+)	Ca(+)	−	−	−	NT	NT	NT	NT
		Cb(+)	−	−	−	NT	NT	NT	NT

a) Grade of agglutination
b) Not tested.

Table 3. Agglutinating activity of anti-Ca bfore and after heat, 2-ME and gel-filtration treatment against Ca(+) type red cells

Antibody	Heat treatment		2-ME treatment		Gel-filtration	
	Before	After	Before	After	IgM	IgG
Anti-Ca	+++[a)]	−	+	−	+++	−

a) Grade of agglutination.

Table 4. Red blood cell types in various breeds and sex

Breed or Sex	Nos. tested	Red cell type: Nos. observed (%)			
		Ca(+)Cb(−)	Ca(−)Cb(+)	Ca(+)Cb(+)	Ca(−)Cb(−)
Japanese	238	214(89.9)	2(0.9)	22(9.2)	0(0.0)
Siamese	14	13(92.9)	0(0.0)	1(7.1)	0(0.0)
Persian	11	8(72.7)	1(9.1)	2(18.2)	0(0.0)
Himalayan	7	5(71.4)	0(0.0)	2(28.6)	0(0.0)
Abyssinian	6	6(100.0)	0(0.0)	0(0.0)	0(0.0)
Unknown	23	21(91.3)	0(0.0)	2(8.7)	0(0.0)
Total	299	267(89.3)	3(1.0)	29(9.7)	0(0.0)
Male	122	112(91.8)	1(0.8)	9(7.4)	0(0.0)
Female	140	123(87.9)	2(1.4)	15(10.7)	0(0.0)

1.0% for the Ca(−) type, 10.7% for the Cb(+) type, and 89.3% for the Cb(−) type. Combining the red blood cell type, 89.3% was the Ca(+)Cb(−) type. 1.0% was the Ca(−)Cb(+) type cat. Twenty-nine cats (9.7%) reacted with both antisera and this type was designated as the Ca(+) Cb(+) type. The Ca(−)Cb(−) type was not observed. There were no significant differences in the Ca(+) type and Cb(+)

type in the male and female populations. No significant difference was attributable to the breed (Table 4).

Genetic studies were performed in 15 incomplete families. We were unable to determine the mechanism of inheritance of the feline Ca and Cb types because of the difficulty in obtaining complete families and Ca(+) type individuals. As shown in Table 5, only a limited number of offspring [4] from known dams and sires were typed, making interpretation of the genetic pattern difficult. In the Ca(+)×Ca(+) mating, Ca(−) type offspring were not seen. In other cases, sires were either unknown of unavailable.

Agglutinin in plasma and age: Agglutinin in the plasma was examined with Ca(+) type and Ca(+) type red cells. Agglutinin reacting with the Ca(+) type RBC was detected in 67 (28.9%) of the 232 cats tested, and with the Cb(+) type RBC in 12 (5.1%) of the 235 cats. The specificities of these agglutinins were not examined due to the fact that these hemagglutination reactions were very weak. The percentage of cats possessing agglutinin reacting with Ca(+) type red cells tended to increase with age (Table 6). Studies were also done to see whether there was any relationship between age and agglutinin against the Cb(+) type red cells, and between sex and agglutinins reacting with both red cells, but no such relationship was observed.

Table 5. Distribution of the Ca(+) type in cats and their offsprings

Parents	Nos. of samples	Offspring	
		Ca(+)	Ca(−)
Ca(+)×Ca(+)	4	4	0
Ca(+)×?	11	41	0

DISCUSSION

Two (66.7%) of the 3 Cb(+) type cats possessed anti-Ca antibodies and 27.3% of the Ca(+) type cats possessed anti-Cb antibodies. It is uncertain whether the Ca(+) and Cb(+) types in this study correspond to those designated by Auer *et al.* [1] as we have not been able to carry out comparison tests. However, the red blood cell groups seem to the similar based on frequency of each red blood cell group in these two studies. Accordingly, the specificity of anti-Ca and anti-Cb antibodies is assumed to be similar to that of anti-A and anti-B, respectively. The incidence of natu-

Table 6. The relation between age and agglutinin in plasma of 232 cats detected by the Ca(+) type red cells

	Age								Total
	Foetus	0~6 m[a)]	~1y[b)]	~2y	~3y	~4y	~5y	5y~	
Nos. tested	8	45	56	48	30	15	8	22	232
Nos. observed	0	4	13	14	10	7	5	14	67
%	0.0	8.9	23.2	29.2	33.3	46.7	62.5	63.6	28.9[c)]

a) Month.
b) Year.
c) The specificity of agglutinins in Ca(+) type cat plasma was not examined and unknown, because the agglutinin activity was very weak, and the reappearance was scarce.

rally occurring antibodies was investigated in cats in Australia, with 95% of the blood type B having anti-A and 35% of the type A having anti-B. Naturally occurring antibodies were found more frequently in the plasma of cats reared in Australia than in those reared in Japan [1].

Anti-Ca activity was detected in the IgM globulin fraction, suggesting that the antibody may be produced without immunization. The titers of anti-Cb antibodies were low, and disappeared after various treatments, and hence the immunological characteristics of it could not be clarified. It may be that anti-Cb plasma also have antibody activity in the IgM globulin fraction, as reported by Ikemoto *et al.* [6].

When fresh anti-Ca plasma and red cell suspension of the Ca(+) type were combined at room temperature, strong hemolysis with or without hemagglutination was observed. The authors have been studying the seasonal levels in titers of anti-Ca and -Cb antibodies for 31 months. It is very interesting that hemolytic activity of anti-Ca plasma disappeared and reappeared repeatedly, and there was no relationship between season and titers of agglutinin or hemolysin of anti-Ca and -Cb.

Red blood cell groups were determined in 4 breeds; the Japanese, Siamese, Persian, and Abyssinian. It is still too early to conclude that particular red blood cell types are connected with certain breeds as the numbers of cats examined were small. Frequencies of feline A and B red cell types have been reported as follows; in a survey of Australian cats, 73.3% were type A, 26.3% type B, and 0.4% type AB; in England cats, 97% were type A and 3% type B; and in French cats, 85% were type A and 15% type B [3]. Our study shows that the Ca-positive type is the most frequently occurring antigen, being present in approximately 99% of the total cat population. From the results mantioned above, it can be assumed that the Ca and Cb red blood cell groups correspond to the A and B types, respectively.

The inheritance mode of feline red blood cell groups is not clearly understood. Family data in the present study was inadequate to be able to determine the mechanisms of inheritance of Ca and Cb red blood cell types. There are two possible explanations for the occurrence of the phenotypes, Ca(+), Cb(+) and Ca(+)Cb(+). The first hypothesis is similar to the inheritance of the D system in dogs with the D^1 and D^2 genes being allelic and codominant [4]. The second hypothesis involves a gene at another locus, and cosistent with a simple pattern of inheritance with a dominant allele at an autosomal locus. Holmes [6] has reported that the inheritance pattern of the cat red blood cell groups are similar to those of the A_1A_2O and Rh systems in man. The similarity with the ABO system and the dominant suppressor gene in man was discussed by Auer *et al.* [1]. In this study, no individual of the Ca(−)Cb(−) type was observed, and it was assumed that there was a high possibility that the genes of C^a and C^b would be a allelic and codominant at an autosomal locus.

It has been shown that the percentage of cats with agglutinins reacting with the Ca(+) type RBC tended to increase with age. Agglutinin production in the plasma may be related to acquired immunity. It has been concluded that these agglutinins are not suitable as red blood cell typing antiserum because these activities are very weak, and because the reappearance of hemagglutination reactions were scarce.

REFERENCES

1. Auer, L., and Bell, K. 1981. The AB blood group system of cats. *Anim. Blood Grps. Biochem. Genet.* 12: 287–297.
2. Auer, L., Bell, K., and Coates, S. 1982. Blood transfusion reactions in the cat. *J. Am. Vet. Med. Assoc.* 180: 729–730.

3. Cain, G. R., and Suzuki, Y. 1985. Presumptive neonatal isoerythrolysis in cats. *J. Am. Vet. Med. Assoc.* 187: 46–48.
4. Ejima, H., Kurokawa, K.,and Ikemoto, S. 1980. Comparison test of antibodies for dog blood grouping. *Jpn. J. Vet. Sci.* 42: 435–441.
5. Eyquem, A., Podliachouk, L.,and Millot, P. 1962. Blood groups in chimpanzees, horses, pigs, and other mammals. *Ann. NY. Acad. Sci.* 97: 320–328.
6. Holmes, R. 1953. The occurrence of blood groups in cats. *J. Exp. Biol.* 30: 350–357.
7. Ikemoto, S., Sakurai, Y., and Fukui,M. 1981. Individual difference within the cat blood group detected by isohemagglutinin. *Jpn. J. Vet. Sci.* 43: 433–435.
8. Suzuki, T. 1966. Experimental studies on the blood transfusion of the cat. *Bull. Azabu Vet. Coll.* 15: 131–145.

要　　約

同種自然抗体の検出によるネコ赤血球型の分類について：江島博康・黒川和雄・池本卯典[1]（日本獣医畜産大学第一外科学教室，[1]自治医科大学人間生物学研究室）——ネコ血漿中に抗-Ca および抗-Cb の2種類の自然抗体が認められた。抗体の出現頻度は，抗-Ca は Cb(+)型ネコの66.7%に，抗-Cb は Ca(+)型の27.3%に認められた。抗体の疑集素価は，抗-Ca は 1：64～1：128 倍，抗-Cb は 1：2 以下で，溶血素価は抗-Ca は 1：16 以下であり，抗-Cb には溶血素活性はみられず，力価の変動は季節とは一致しなかった。抗-Ca 活性は IgM グロブリン分画にあった。赤血球型の出現頻度は299例中，Ca(+)型：99.0%，Ca(−)型：1.0%，Cb(+)型：10.7%，Cb(−)型：89.3%であり，赤血球型の組合せにおいては，Ca(+)Cb(−)型：89.3%，Ca(−)Cb(+)型：1.0%，Ca(+)cb(+)型：9.7%であり，両赤血球型を欠損する個体はみられなかった。各赤血球型出現頻度に性差や品種差はなく，また，Ca(+)型，Cb(+)型の遺伝様式は確定できなかった。特定の Ca(+)型赤血球を用いて検索したネコ血漿中の凝集素出現頻度は加齢に伴って高くなった。