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The Determination of Human-ABO Blood Groups in Captive Cynomolgus Macaques (*Macaca fascicularis*)

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ABSTRACT.–In human, we can determine the ABO blood group by hemagglutination of red blood cell (RBC) with anti-A and anti-B antibodies due to the expression of antigen A/B on the surface of RBC. However, in non-human primate, except for apes, the RBC does not express the antigen A/B on the surface, but synthesize and secrete the antibody A/B into the serum. Therefore, in order to determine the human-ABO blood type in non-human primate, the agglutination of human A and/or B red blood cells with the antibody in monkey sera under the light microscope is observed.

In this study, the aim is to determine the human-ABO blood groups in cynomolgus macaques (*Macaca fascicularis*), one species among other non-human primates that do not express antigen A/B on the RBC surface. Seventy-two cynomolgus macaques (8->20 years old) reared at the Primate Research Unit, Faculty of Science, Chulalongkorn University, were used. The result showed that the ratio of O:A:B:AB of ABO blood group was 1.0:1.2:3.5:1.5 in 43 female monkeys, 1.0:1.6:6.0:1.0 in 29 male monkeys, and 1.0:1.3:4.3:1.3 in all monkeys. From this study, it can be concluded that most of cynomolgus macaques in our laboratory have type-B blood group.

KEY WORDS: ABO blood group; *Macaca fascicularis*; Primate Research Unit

INTRODUCTION

The ABO blood group was the first blood group system discovered in humans by Landsteiner in 1901. Today, it has been found in all primates that have been examined. Primates have four phenotypes of blood groups: A, B, O and AB, with two agglutinable antigens on the red cells, A and B, and two anti-A and anti-B substances present in the sera, which determine an animal's blood type (Socha and Ruffie, 1983). Wiener et al. (1972) summarized the survey result of human-type ABO blood groups of various species of primates, demonstrating that A and B antigens are not detected in the red cells but are in the saliva of macaques (*Macaca spp*). Thus, ABO blood groups of macaques are determined by the detection of both A/B antigen in the saliva and antibodies in sera.

Cynomolgus macaque (*Macaca fascicularis* (Raffles, 1821)) is an Old World monkey in Order Primates, Suborder Anthropoidea, and Infraorder Catarrhini. They are widely found in Indochina, Thailand, Myanmar, Malaya, Sumatra, Java, Borneo, Philippines, and many

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small islands (Lekagul and McNeely, 1988; Groves, 2001). In Thailand, they are widely distributed from the lower northern part (Nakorn Sawan Province) to the southernmost part (Yala Province) (Aggimarangsee, 1992; Varavudhi et al., 1989). There are published data of ABO blood group in cynomolgus macaques from Malaysia, Indonesia, Philippines, Japan and USA, but no reports of those data from Thailand (Terao et al., 1981; Socha et al., 1995). It was found that cynomolgus macaques are polymorphic in ABO blood groups, with the percentage of B > AB > A > O, respectively (Socha et al., 1995). Accordingly, it is interesting to study the ABO blood group of captive cynomolgus macaques that were originated from the various parts of Thailand and reared at the Primate Research Unit, Chulalongkorn University.

METHODS

Animals

Forty-three female and twenty-nine male cynomolgus macaques (*Macaca fascicularis*), 8- > 20 years of age and weighing 3.5-11.0 kg, were housed in individual cages at the Breeding Colony of the Primate Research Unit, Chulalongkorn University, Thailand. The temperature and humidity are under the natural condition while the photoperiod is being controlled (0600-1800 h light). The monkeys were fed daily in the morning with monkey chow (purchased from Pokphan Animal Feed Co., Ltd., Thailand) and supplemented in the afternoon with fresh fruits and vegetables, and occasionally a boiled chicken egg.

Blood collection from monkeys

Each animal was given 10 mg/kg of ketamine hydrochloride (Sankyo, Japan) intramuscularly. After an injection for 5-10 minutes, the animal was anesthetized, a 5-ml blood sample was collected by femoral venipuncture between 0800-1200 h. Immediately after the blood sample clotted, the serum was separated by centrifugation at 2,500 rpm for 20 minutes, then kept frozen at –20 °C until determination.

Preparation of 5% human red blood cell (RBC) suspension

Two-milliliter blood samples were collected from known human blood group of A and B with a heparinized syringe. The red blood cells were separated by centrifugation at 2,500 rpm for 10 minutes. One-hundred microliters of RBC was transferred into the 15 ml - test tube and resuspended with 10 ml of physiological saline. After that, the RBC suspension was once centrifuged at 2,500 rpm for 10 minutes and discarded the supernatant. Five-percent RBC suspension was performed by adding 2 ml of physiological saline.

Testing of the monkey sera for isoaggutinins anti-A and anti-B

The anti-A and anti-B antibodies in the sera of cynomolgus macaques were determined by

TABLE 1. The blood grouping and agglutination of cynomolgus macaque sera against the human type-A or type-B red blood cells suspension (- and + indicate non-agglutination and agglutination, respectively).

Human type-A RBC	Human type-B RBC	Judgement
+	-	Type-B
-	+	Type-A
-	-	Type-AB
+	+	Type-O

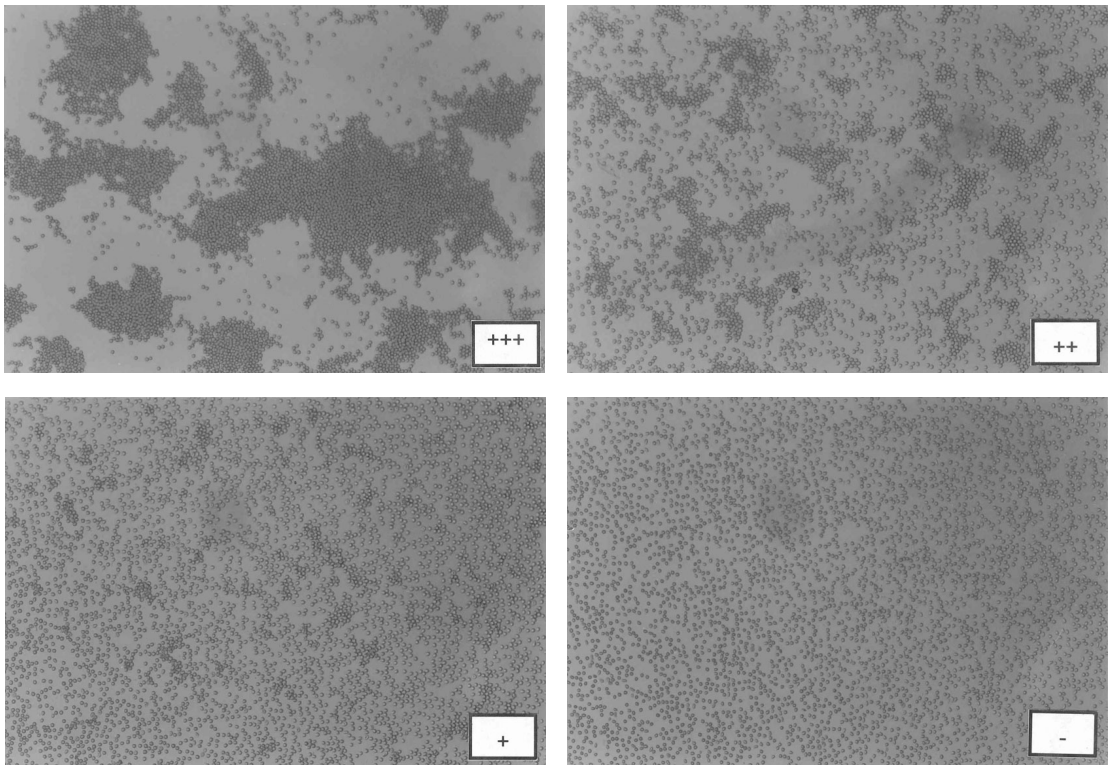

FIGURE 1. The agglutination of anti-A and anti-B agglutinins of cynomolgus macaque serum against known human red cells of group A and B (+++, ++, + and – indicate large, medium, low and none agglutination, respectively).

the saline agglutination method following Terao et al. (1979). In brief, the monkey's serum was diluted to the concentration of 1:10 with physiological saline. One-hundred microliters of diluted serum was mixed with a fifty microliters of human type-A or type-B RBC suspension, respectively, in the microfuge tube, and incubated at the room temperature for 30 minutes. The mixture was centrifuged at 1,000 rpm for 10 seconds. The agglutination was observed under the light microscope. The blood grouping was judged as in Table 1.

Statistical analysis

The Pearson correlation coefficient between anti-A and anti-B blood group antibody levels (large, medium and low agglutination) and age was determined using SPSS Program version 10.0. Significance was set at $p < 0.05$.

RESULTS

The agglutination of anti-A and anti-B agglutinins of cynomolgus macaque serum against known human red cells of groups A and B, observed under the light microscope, was divided into 4 classes: large (+++), medium (++), low (+) and none (-) (Fig. 1). There were no correlation between anti-A and anti-B blood group levels with age ($r = -0.102$ ($p = 0.453$) and 0.332 ($p = 0.165$), respectively). The ratio of O:A:B:AB of ABO blood group was 1.0:1.2:3.5:1.5 in 43 female monkeys, 1.0:1.6:6.0:1.0 in 29 male monkeys, and 1.0:1.3:4.3:1.3 in all monkeys (Table 2). No difference in the ratio of ABO blood groups was observed between both sexes. When the blood group was considered according to the originated location, it was found that most monkeys from Samut Songkhram have type-B

blood group. Coincidently, most of laboratory-bred monkeys at the Primate Research Unit, of which their parents originated from Nakhon Si Thammarat, Tak and Samut Songkhram, were also of type-B blood group (Table 3).

DISCUSSION

In anthropoid apes; human, chimpanzee, gorilla, orangutan and gibbon, the ABO blood group can determine by hemagglutination of RBC with anti-A and anti-B antibodies, called hemagglutination method, due to the expression of antigen A/B (ABO substances) on the surface of RBC. Their ABO substances are also found in the secretion as well. Thus, all anthropoid apes possess A-B-H factors both in their saliva and on their RBC (Socha and Ruffie, 1983). However, in Old and New World monkeys as well as prosimians, the ABO substances are found only in secretions but never on the RBC. Thus, the ordinary methods used to determine the human ABO blood groups in those nonhuman primates, including cynomolgus macaques, are the mixed cell agglutination reaction (MCAR) and the saline agglutination method (Socha et al., 1972; Terao et al., 1979). MCAR is the detection of ABO substances in the macaque saliva. This method has an advantage in that it uses only a small amount of saliva and easy to apply (Terao et al., 1979). However, this method is inconvenient to perform in the field, because it needs to determine the ABO substances in saliva immediately after the collection, with some disadvantage of running steps (rinsing the slide in the running water for 5 minutes). The saline agglutination method, the detecting of anti-A and anti-B antibodies in sera of cynomolgus macaques, has more convenient in this point of view. The blood sera could be kept frozen and determined for ABO blood groups afterward,

TABLE 2. The ABO blood groups of the cynomolgus macaques reared at the Primate Research Unit, Chulalongkorn University, were classified according to sexes.

Sex	Blood group (number)				Total number tested	Ratio (O:A:B:AB)
	O	A	B	AB		
Female	6	7	21	9	43	1.0:1.2:3.5:1.5
Male	3	5	18	3	29	1.0:1.6:6.0:1.0
Total	9	12	39	12	72	1.0:1.3:4.3:1.3

TABLE 3. The ABO blood groups of the cynomolgus macaques reared at the Primate Research Unit, Chulalongkorn University, were classified according to the originated locations.

Originated from	Blood group (number)				Total number tested
	O	A	B	AB	
Nakhon Si Thammarat (South)	-	3	2	1	6
Tak (North)	-	1	-	1	2
Samut Songkhram (Central)	7	1	17	5	30
Ratchaburi (West)	-	1	1	1	3
Prachin Buri (East)	-	-	1	1	2
Maha Sarakham (Northeastern)	-	1	-	1	2
Laboratory-bred (Primate Research Unit)	2	5	18	2	27
Total	9	12	39	12	72

with the other concomitant analysis, e.g. hormonal and lipid levels. Then, the saline agglutination method was applicable to detect ABO blood group in this study. Unfortunately, this method is possible for the detection of ABO blood groups in the older monkeys only, because the anti-B antibody was detected for the first time in the monkeys at 3 to 4 months of age (the weaning period of monkey). In cynomolgus macaques, both anti-A and anti-B antibodies levels gradually rose with the increase of age and reached a maximum at the age of 4 to 5 years (Terao et al., 1983). Seemingly, this method did not cause any problem in this study in which the monkeys are 8 to more than 20 years old. Terao et al. (1983) reported that the antibody level gradually fell with aging, however, no correlation between anti-A and anti-B levels with age was observed in our cynomolgus macaques. One possible reason is that most of monkeys tested in this study are older than 20 years old (51.38%), whereas the oldest monkeys in their study are 15 years old.

In agreement with the previous reports (Terao et al., 1981; Socha et al., 1995), the ABO system in cynomolgus macaques in Thailand are polymorphic (many forms), possessing all of the four phenotypes. It is a monomorphic in some primate species. For example, the two species of gorilla (*Gorilla gorilla* and *Gorilla beringei*) and the siamang (*Symphalangus spp*.) have only B, while the pygmy chimpanzee (*Pan paniscus*) has only A group (Socha et al., 1995). Terao et al. (1981) have reported that the distribution of ABO blood groups among cynomolgus macaques from different countries of origin showed a significant difference. The result in our monkeys is similar to those of cynomolgus macaques from Philippines, the percentage of B > AB > A > O. The distribution of ABO blood groups among our cynomolgus macaques originated from the different locations throughout Thailand also showed a significant difference. Nevertheless, some of these results must be considered provisional because of the small numbers of animals tested. The ABO blood groups in our laboratory-bred cynomolgus macaques also showed the phenotypic inheritance as in the report of Terao et al. (1981). Most of the laboratory-bred macaques were the B-type blood groups as their parents were.

In conclusion, the cynomolgus macaques in our laboratory have four blood groups and most of them are the type-B blood group.

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