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ABO BLOOD GROUPS IN A NATURAL POPULATION OF *Chiropotes satanas*

Maria Lúcia Harada-Hamel, Tereza Cristina Oliveira Corvelo and Horacio Schneider

ABSTRACT

Blood and saliva samples were collected from 72 specimens of *Chiropotes satanas utahicki* captured on the west bank of the Tocantins river, Pará, Brazil. The saliva samples were tested for the presence of human ABH antigens by the standard method of hemagglutination inhibition. All individuals were classified as belonging to blood group A. Of 15 serum samples tested for anti-A and anti-B agglutinins, 13 showed only anti-B agglutinin and 2 showed neither antibody. Red blood cells were tested by the elution method for the presence of human A and B antigens. The results confirm the presence of the B-like antigen also detected in other New World monkeys, and suggest the occurrence of the A-like antigen.

INTRODUCTION

Several reports on ABO blood groups in New World primates have demonstrated the occurrence of ABH substances in the secretions of these animals and of agglutinins in their serum. Their red blood cells, however, have A and/or B-like agglutinogens regardless of the secretion phenotype. Lectins of known activity have been used in parallel with reagents of animal origin for the study of both salivary and red blood cell antigens.

Despite the importance of studies of this type in non-human primates, both for the understanding of the evolution of the ABO system and for the knowledge about the genetic constitution of primates utilized in biomedical research, few New World monkey species have been studied from this point of view.

Departamento de Genética, Centro de Ciências Biológicas, Universidade Federal do Pará, Campus Universitário do Guamá, 66059 Belém, Pará, Brasil.

In the present study, we report for the first time on the distribution of ABH antigens in a natural population of *Chiropotes satanas utahicki*.

MATERIAL AND METHODS

According to Hershkovitz (1985), the animals belonging to the genus *Chiropotes* found in the area between the Xingu and Tocantins river belong to a single subspecies called *C. satanas utahicki*. In the present investigation, we studied 72 animals (21 males and 51 females) of this subspecies captured on the west bank of the Tocantins river during the animal rescue program covering the area to be filled as the reservoir of the hydroelectric plant of Tucurui, Pará.

Sample collection

(i) Blood: Animals were anesthetized with an intramuscular injection of 0.05 ml of 5% ketamine hydrochloride (Parke-Davis and Co.) and blood was collected from their femoral vein using EDTA as anticoagulant. Plasma and red blood cells were separated by centrifugation, stored on ice and transported to our laboratory, where they were kept at -20ºC until the time for use;

(ii) Saliva: salivation was induced with an intramuscular injection of 0.001 ml of 15% pylocarpine (Hertape) and saliva was collected into test tubes and immediatly heated to 100ºC in a water bath for 20 minutes to destroy proteolytic enzymes. After centrifugation, the supernatant was kept on ice and carried to our laboratory, where it was stored at -20ºC.

Cell suspension

Red blood cells were washed three times with saline and a 2% suspension was prepared with the cell concentrate (1 cell volume plus 49 saline volumes).

Reagents

(i) Human: Commercially available anti-A and anti-B sera of human origin (Ortho Diagnostic) were used:

(ii) Plant extracts: Seeds of *Ulex europeus* (anti-H), *Crotalaria striata* (anti-A), *Dolichos biflorus* (anti-A_1), and *Evonymus europeus* (anti-B and H) were ground and 2.5 g of the powder was suspended in 25 ml 0.9% saline. The mixture was incubated at low temperature (4ºC) for 24 hours, with constant shaking during the last 4 hours. The suspension was then centrifuged at 900 g for 30 minutes and the intermediate phase was filtered through filter paper. The extract was divided into aliquots and frozen at -20ºC.

Hemagglutination tests

Red blood cell tests were carried out in 50 x 5 mm round-bottomed glass tubes. Aliquots (0.025 ml) of the reagents and of the cell suspension (0.025 ml) were placed in each tube. After 1 hour incubation at room temperature, the tubes were centrifuged and read with the naked eye.

Serum test

For the identification of serum agglutinins, plasma samples were absorbed exhaustively with human O red blood cells to remove nonspecific antibodies and then tested with human A1, A2, B and O red blood cells obtained from the same donors.

ABH substance inhibition test

To identify the highest saliva dilution capable of inhibiting the agglutination of indicator cells by anti-A, anti-B and anti-H reagents, three sets of at least 12 tubes each (50 x 5 mm) were prepared. In each tube of one set, we placed 0.025 ml of non-diluted saliva or of saliva diluted 1:2, 1:4, ..., 1:2048 and added 0.025 ml of the appropriate antiserum. After incubation at room temperature for 20 minutes, 0.025 ml of the appropriate red cell suspension was added. The mixture was incubated at room temperature for 20 minutes and centrifuged at 900 g for 1 minute, and agglutination was examined with the naked eye. The anti-A and anti-B sera employed in this test were diluted so as to obtain a titer of 8 agglutinating units with A2 and B red cells, respectively. The anti-H reagent extracted from *Ulex europeus* seeds was adjusted for 4 agglutinating units with human O red cells. The human red cells were obtained from the same donors.

Elution test

One volume of fresh *C. s. utahicki* red cells and human A1 and B red cells was washed three times with saline and mixed with 1.5 volumes of anti-A or anti-B serum of human origin and incubated at 4ºC for 1 hour, with shaking performed at 15-minute intervals. The mixture was then washed exhaustively with cold saline until the supernatant showed no agglutinating activity. Saline was added to the agglutinated red cells at the proportion of half the red cell volume, and a 1:1 solution of chloroform and trichloroethylene was added in a volume corresponding to twice the volume of the red cell-saline mixture. The material was incubated in a water bath at 56ºC for 15 minutes with constant shaking, and centrifuged at 2000 g for 5 minutes. The upper phase was separated and tested with human and *Chiropotes* red cells. This technique is a modification of that described by Massuet and Armengol (1980).

Statistical analysis

Means, standard deviations, and the student t test statistic were calculated using the Statistical Package for Social Sciences (SPSS) program.

RESULTS AND DISCUSSION

On the basis of the saliva inhibition tests, all 72 *C. s. utahicki* specimens were assigned to the A blood group. This fact suggests that the subspecies is monomorphic for the ABO system. Substance A titers (reported as log base 2) ranged from 6 to 12, with a mean value (+ SD) of 8.75 + 1.22. Mean substance A titers did not show statistically significant differences between sexes, with 8.86 + 0.96 for males and 8.71 + 1.32 for females (t test = 0.476; P = 0.32) (Table I).

Table I - Mean substance A and H titers in bearded Saki monkeys.

A and H titers versus sex		Number of individuals tested	Mean (X)	Standard deviation (SD)
A	Males	21	8.86	0.96
	Females	51	8.71	1.32
	Total	72	8.75	1.22
H	Males	21	1.24	1.64
	Females	51	0.98	1.52
	Total	72	2.92	1.06

The mean substance H titer was 2.92 + 1.06, with only 34.7% of all individuals inhibiting the anti-H reagent (*Ulex europeus*). Since this substance may be present in very small amounts, as reported by Wiener *et al.* (1967), we retested 47 H-negative saliva samples with the highest dilution of the reagent (1 agglutinating unit) and noted that even so no sample succeeded in inhibiting the anti-H reagent, suggesting that substance H was absent or present at levels not detectable by the technique employed.

To determine whether the salivary phenotype corresponded to the serum phenotype, we tested 15 serum samples with human A1, A2 and B red blood cells. Thirteen of these reacted as expected, i.e. they only showed the anti-B antibody. However, the remaining two samples did not react with the human red cells utilized. Even though the absence of antibodies could be attributed to environmental factors or even to differences between the human cells and the antigen specificities recog-

nized by the monkey's antibodies, we cannot rule out the possibility of occurrence of antibodies in both samples, which were not detected due to the inevitable dilution of serum caused by previous absorption with human O red cells.

The lectins studied, with the exception of *Evonymus europeus*, showed no activity against *C. s. utahicki* red cells, suggesting the absence of types A1 and A2 and H specificity in their blood cells, as reported in previous studies (Wiener *et al.*, 1942, 1967; Socha and Ruffié, 1983; Schneider *et al.*, 1985). The ability of *Evonymus europeus* to recognize specificities in the red blood cells of New World monkeys had been previously described by Froehlich *et al.* (1977) for *Alouatta palliata* and by Schneider *et al.* (1987) for *Saguinus midas*.

In view of the results obtained with the lectins, absorption and elution tests were carried out using anti-A and anti-B reagents of human origin to determine whether they might be fractionated and whether the agglutinin fraction thus obtained might distinguish between human and monkey agglutinogens. As shown in Table II, anti-A and anti-B reagents of human origin agglutinated both human and monkey red blood cells. However, absorption of these reagents with human red blood cells did not eliminate the reactivity against *Chiropotes* red blood cells and absorption with *Chiropotes* cells did not eliminate the reaction with human A or B red cells.

Table II - Reaction of anti-A and anti-B reagents with bearded Saki red blood cells before and after specific absorption.

Anti-A and anti-B reagents	Human cells				
	O	A1	A2	B	Cs
Commercial anti-A reagent					
nonabsorbed	-	$+^{s}$	$+^{s}$	-	$+^{s}$
absorbed with human A1 cells	-	-	-	-	$+^{s}$
absorbed with Cs cells	-	$+^{s}$	$+^{s}$	-	-
eluted from human A1 cells	-	$+^{s}$	$+^{s}$	-	-
eluted from Cs cells	-	+	+	-	$+^{w}$
Commercial anti-B reagent					
nonabsorbed	-	-	-	$+^{s}$	$+^{s}$
absorbed with human B cells	-	-	-	-	$+^{s}$
absorbed with Cs cells	-	-	-	$+^{s}$	-
eluted from human B cells					
nonabsorbed	-	-	-	$+^{s}$	+
absorbed with Cs cells	-	-	-	$+^{s}$	-
eluted from Cs cells	-	-	-	$+^{s}$	+

Cs = *Chiropotes satanas utahicki*; $+^{s}$ = complete agglutination; + = one or two large agglutinates; $+^{w}$ = a number of large agglutinates; - = negative reaction.

When tested against human and monkey red blood cells, anti-B eluates obtained from human B cells agglutinated both, suggesting that human B cells absorb antibodies consisting of two subpopulations: one recognizing only the human B antigen, and the other recognizing an antigen present both in man and in *C. s. utahicki* (B-like). Absorption tests with the human anti-B eluate confirmed this suggestion (see Table II).

When tested against human and monkey red blood cells, the human anti-A eluate obtained from human A red cells reacted only with human erythrocytes. In contrast, the anti-A eluate from *C. s. utahicki* reacted with both red cell types, suggesting that monkey red blood cells absorb an agglutinin that reacts with both species. These results are similar to those obtained with anti-B reagents, except for the anti-A eluate obtained from human erythrocytes, which unexpectedly did not react with *C. s. utahicki* red blood cells. The existence of two agglutinin populations seems to be an undoubtful fact. The anti-A-like agglutinin was not detected probably because of competition with human anti-A agglutinin or because of differences in avidity.

The occurrence of the B-like antigen in *Chiropotes satanas* agrees with observations in other New World primates, but the presence of the A-like antigen had been previously detected only in *Saguinus nigricollis* (Gengozian, 1964).

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RESUMO

Foram coletadas amostras de sangue e saliva de 72 animais da espécie *Chiropotes satanas utahicki*, capturados na margem esquerda do rio Tocantins, Pará (Brasil). As amostras de saliva foram testadas para a presença dos antígenos ABH humanos, através do método convencional de inibição da hemaglutinação. Todos os indivíduos foram classificados como pertencentes ao grupo sanguíneo A. Aglutininas anti-A e anti-B foram pesquisadas em 15 amostras de soro, dos quais 13 apresentaram apenas a aglutinina anti-B e duas não apresentaram nenhum dos anticorpos. Amostras de hemácias foram testadas pelo método de eluição, para verificar a presença de antígenos A e B humanos. Os resultados confirmam a presença de B-like, detectado em outros macacos do Novo Mundo e sugere a ocorrência de A-like.

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