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Human-Type Blood Group Activities on Chimpanzee Erythrocytes with Special Reference to M and N*

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Summary. Human-type blood group activities on the red blood cells (RBCs) of three chimpanzees were individually examined with commercial mouse monoclonal antibodies (anti-A, -B, -H, -M, -N, -Le^a, and -Le^b) as well as lectins (UEA-I and VGA) and conventional polyclonal antisera for the systems ABO, MN, Lewis, Rh-Hr, P, Kell, Kidd, Duffy, and Lutheran. For further analysis of the MN antigens, treatment of the RBCs with sialidase, trypsin, and chymotrypsin were employed. The activities recognized among the three chimpanzees were A, H, M, N, Le^b, c, S, k, and Jk^a. The RBCs of the three individuals possessed the A antigen which showed the same serologic activity as the human A_1. Those chimpanzee RBCs showed higher H-activity than the human A_1 RBCs. The Lewis b activity was revealed by the absorption-elution method. The RBCs of the three individuals showed a reactivity to the polyclonal anti-M reagents, which was affected by both the sialidase and trypsin treatment. The RBCs of two individuals were agglutinated with the monoclonal anti-N. The receptor was sensitive to sialidase and chymotrypsin. The RBCs of the three individuals, however, did not react with the monoclonal anti-M or with one of the polyclonal anti-N. These results indicate structural differences in the glycophorins and MN antigens between the human and chimpanzee.

Key words: Human-type blood group, activities on chimpanzee erythrocytes – Monoclonal antibody, forensic serology

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Zusammenfassung. Die Blutgruppenaktivitäten in humanen Systemen auf den Erythrozyten dreier Schimpansen wurden individuell untersucht mittels kommerziell erwerbbarer monoklonaler Antikörper (Maus IgM, Anti-A, -B, -H, -M, -N, -Le^a und -Le^b) sowie mittels Lektine (UEA-I und VGA) und polyklonaler Anti-Seren zur Typisierung der Systemen ABO, MN, Lewis, Rh-Hr, P, Kell, Kidd, Duffy und Lutheran. Die Antigene MN wurden durch Behandlung der Erythrozyten mit den Enzymen Sialidase, Trypsin und Chymotrypsin ausführlich untersucht. Die Blutgruppenaktivitäten A, H, M, N, Le^b, c, S, k und Jk^a wurden unter den drei Schimpansen nachgewiesen. Das Antigen A der Schimpansen-Zellen zeigte die gleiche serologische Aktivität wie das humane A_1. Die A-positiven Schimpansen-Zellen hatten höhere H-Aktivität als humane A_1 Zellen. Die Lewis-Aktivitäten konnten durch die Absorption-Elutions-Methode nachgewiesen werden. Die Schimpansen-Zellen zeigten positive Reaktionen mit den polyklonalen Anti-M Seren; die 'M' Aktivität wurde durch die Sialidase- bzw. Trypsin-Behandlung reduziert. Aber die Zellen konnten auf den monoklonalen Anti-M nicht reagieren. Die Erythrozyten von zwei Schimpansen wurden mit dem monoklonalen Anti-N agglutiniert; die 'N' Aktivität wurde durch die Sialidase- bzw. Chymotrypsin-Behandlung reduziert. Aus diesen Befunden ergab sich, daß die Glykophorine und MN Antigene von Menschen und Schimpansen sich strukturell unterscheiden.

Schlüsselwörter: Monoklonale Antikörper, forensische Serologie – Blutgruppen-Systeme, Aktivitäten auf Schimpansenerythrozyten

Introduction

We have investigated the human-type blood group activities (BGAs) among animals from the forensic point of view of species identification [1, 2]. A great concern in this series of investigations is the existence of BGAs very similar to those of the human on the RBCs of the chimpanzee [3–5]. The MN activities should be examined with special reference because they are carried on the glycophorins which show a great species diversity.

This paper describes an investigation of the human-type BGAs on the chimpanzee RBCs with commercially available monoclonal antibodies (mAbs) for the ABO, MN, and Lewis typing as well as conventional polyclonal reagents and lectins.

Materials and Methods

Blood Specimens. Whole blood was freshly collected from three individuals of the chimpanzees *(Pan troglodytes)* named "Gon", "Puchi", and "Reiko" at the Primate Research Institute of Kyoto University (Japan). The blood relationship among the three chimpanzees is unknown. The chimpanzee and control human RBCs were suspended at a concentration of 2% for the hemagglutination tests. The blood was stained on cotton cloths for the absorption-elution (A-E) tests.

Table 1. Human-type blood group activities on chimpanzee erythrocytes by hemagglutination test (1)

Antisera		lot. (origin)	Human positive control		Chimpanzee cells		
					"Gon"	"Puchi"	"Reiko"
A	Ortho	SA931C-2(H)		2^8	2^8	2^8	2^8
	TSS	60-1(R)		2^8	2^8	2^8	2^8
	Biotest	111084(M)*		2^9	2^9	2^9	2^9
B	Ortho	SB420C-2(H)		2^8	2^1	2^1	2^1
	TSS	60-1(G)		2^8	−	−	−
	Biotest	112084(M)*		2^8	−	−	−
			(0 cells)	(A_1 cells)			
H	UEA-I EY	011711	2^{10}	2^0	2^8	2^6	2^4
	UEA-I Vector	30413	2^{10}	2^5	2^7	2^6	2^3
	Chembiomed	4235(M)*	2^9	2^1	2^3	2^1	2^1
M	Merz&Dade	300, 10(H)		2^4	2^4	2^3	2^3
	Ortho	MM503C(R)		2^4	2^3	2^3	2^4
	Ortho	MM501A(R)		2^3	2^3	2^3	2^3
	Gifu	2-14p(R)**		2^4	2^4	2^4	2^4
	Biotest	118084(M)*		2^8	−	−	−
N	Merz&Dade	302, 12(H)		2^3	2^1	2^0	2^1
	Ortho	NN607A(R)		2^4	2^3	2^1	2^1
	Ortho	NN602A(R)		2^5	2^1	−	−
	Gifu	2-14p(R)**		2^4	−	−	−
	Biotest	119084(M)*		2^6	2^6	−	2^6
	VGA EY	0430D		2^2	2^1	−	2^1
Le^a	Ortho	LA339F(G)		++	−	−	−
	Biotest	113084(M)*		+++	−	−	−
Le^b	Ortho	LB535A(G)		+	+	+	+
	Biotest	124084(M)*		++	−	−	−

2^n, agglutination titer ($1:2^n$); + ~ +++, positive reaction in test tube method; −, negative reaction; *, monoclonal antibody; **, purified by absorption-elution technique. Origins of the reagents are of human (H), rabbit (R), goat (G), and mouse (M). See text for the results in the absorption-elution tests

Typing Reagents. Mouse mAbs, polyclonal antisera, and lectins, (cited in Tables 1 and 2) were obtained from Ortho Diagnostics (USA), Biotest (West Germany), Tokyo Standard Serum (TSS, Japan), Merz & Dade (Switzerland), Chembiomed (Canada), EY Laboratories (USA), and Vector (USA). Rabbit polyclonal anti-M and -N antibodies purified by the A-E technique [6], designated as Gifu 2-14p, were kindly provided by Prof. Dr. K. Sagisaka (Tohoku University School of Medicine).

Enzymes. L-1-tosylamide-2-phenylethylchloromethyl ketone(TPCK)-treated trypsin from bovine pancreas (Sigma, Type XIII), N-α-P-tosyl-L-lysine-chloromethyl ketone(TLCK)-treated chymotrypsin (Sigma, type VII), and sialidase from *Clostridium perfringens* (Sigma, type V) were employed.

Table 2. Human-blood group activities on chimpanzee erythrocytes by hemagglutination test (2)

Antisera		lot. (origin)	Human positive control	Chimpanzee cells		
				"Gon"	"Puchi"	"Reiko"
P_1	Ortho	PG016B(G)	+++	−	−	−
C	Ortho	CS148A1(H)	+++	−	−	−
c		SC810A(H)	+++	+++	++	++
D		DS634B(H)	+++	++	++	++
E		ES109C(H)	+++	−	−	−
e		SE109A(H)	+++	−	−	−
S	Ortho	SS045D(H)	++(−)	++(−)	++(−)	+(−)
s		LS041A1(H)	+(−)	−(−)	−(−)	−(−)
K	Ortho	KC029A(H)	/	−(−)	−(−)	−(−)
k		LK031A(H)	++(−)	+(−)	+(−)	+(−)
Jk^a	Biotest	112083(H)	++(−)	++(−)	++(−)	++(−)
Jk^b		111083(H)	+(−)	−(+)	+(+)	−(+)
Fy^a	Ortho	FA607A(H)	++(−)	−(−)	−(−)	−(−)
Fy^b	Biotest	111073(H)	++(−)	++(+)	++(+)	++(+)
Lu^a	Biotest	113073(H)	/	−(+)	++(+)	−(+)
Lu^b		111013(H)	++(+)	++(+)	++(+)	++(+)

+ ~ +++, positive reactions; −, negative reaction; /: not tested; (−) or (+), readings before Coombs' test. Origins of the reagents are of goat (G) and human (H)

Hemagglutination Test. The slide glass method was used for the ABO and MN systems, and the test tube method for the other systems.

Absorption of Anti-M and -N Reagents with RBCs. Of each reagent 120 µl was mixed with 40 µl packed RBCs. The mixture was incubated at room temperature for 2 h and centrifuged at 2,800 rpm for 10 min. The supernatant was again absorbed under the same conditions. After recentrifugation, the supernatant was tested by the hemagglutination method.

Absorption-Elution Test. A piece of thread (ca. 5 mm in length) from the blood-stained cloth was examined for the A, B, H, and M antigens by the A-E method [7, 8]. A piece of cloth (ca. 4 mm^2) was used for detecting the Le^a, Le^b, C, c, D, E and e antigens, and half this for the N antigen.

Enzyme Treatment. Enzyme solutions were prepared as follows; sialidase (100 U/ml) in 0.05 *M* acetate − 0.9% NaCl − 0.1% $CaCl_2$ buffer (pH 5.5); trypsin (1 mg/ml), and chymotrypsin (2 mg/ml) in 0.01 *M* phosphate-buffered saline (pH 7.6) [9–11]. Of each solution 60 µl was added to an equal volume of the packed cells rinsed with the same buffer, and the mixtures were incubated at 37°C with occasional shaking. The incubation time was 30 min for sialidase, 1 h for trypsin, and 2 h for chymotrypsin. The enzyme-treated RBCs were washed 3 times with saline and suspended at a concentration of 2% for the hemagglutination tests.

Results

Human-Type Blood Group Activities on Chimpanzee Erythrocytes

ABO Group (see Table 1)

With all the anti-A reagents used, the RBCs of the three individuals of chimpanzee showed the same agglutinability as the human A_1 RBCs. The human origin anti-B weakly agglutinated the chimpanzee RBCs. Those RBCs possessed an agglutinability with the anti-H, which was higher than that of the human A_1 RBCs, although the RBCs of each individual showed a reactivity different from each other.

In the A-E tests, the blood stains of the three chimpanzees gave positive reactions with every kind of the anti-A used. The B and H activities were not detected so long as the indicator cells without papinization were used.

The chimpanzees had the anti-B agglutinin in the sera.

MN Group

The RBCs of the three were agglutinated with the human and rabbit polyclonal anti-M, but not with the monoclonal anti-M. The human anti-N as well as one of the rabbit antisera weakly agglutinated the RBCs of all the three. The monoclonal anti-N and VGA agglutinated the RBCs of two ("Gon" and "Reiko") of them. One of the anti-N (Gifu 2-14p) did not agglutinate the RBCs of any of them.

In the A-E tests, the blood stains of the three to the polyclonal anti-M (rabbit MM501A and Gifu 2-14p) and those of two ("Gon" and "Reiko") to the monoclonal anti-N were positive. None of the stains reacted with the monoclonal anti-M or the purified rabbit anti-N (Gifu 2-14p).

Lewis Group

The chimpanzee RBCs were not agglutinated with the anti-Le^a goat serum or mAb. They were agglutinated with the goat anti-Le^b, but not with monoclonal anti-Le^b. Examined by the A-E tests, the blood stains of the three were revealed positive to the goat and monoclonal anti-Le^b and negative to both of the anti-Le^a.

Other Blood Groups

Table 2 shows the results in the routine hemagglutination tests with the commercial antisera for the human blood typing. The RBCs of the three individuals showed the same reactivity: They were agglutinated with the anti-c, -D, -S, -k, and -Jk^a after the Coombs' test and with the anti-Jk^b, -Fy^b, -Lu^a, and -Lu^b before the Coombs' test. They were negative with the anti-P_1, -C, -E, -e, -s, -K, and -Fy^a.

By the A-E tests, the c activity was detected in the stains of the three individuals.

Table 3. Changes of anti-M and -N reagents in the reactivity with human and chimpanzee erythrocytes after absorption

Antisera		lot. (origin)	Original titer	Absorbed with cells of	Tested with cells of			
					human positive	chimpanzee		
						"Gon"	"Puchi"	"Reiko"
M	Ortho	MM503C(R)	2^4	chimp. (P)	2^1	–	–	–
				human O-M	–	–	–	–
	Gifu	2-14p(R)**	2^4	chimp. (P)	–	–	–	–
				human O-M	–	–	–	–
N	Ortho	NN607A(R)	2^4	chimp. (G)	2^4	–	–	–
				human O-N	2^0	–	–	–
	Biotest	119084(M)*	2^6	chimp. (G)	2^1	2^1		2^0
				human O-N	–	–		–
	VGAEY	0430D	2^2	chimp. (G)	–	–		–
				human O-N	–	–		–

2^n, agglutination titer ($1:2^n$); –, negative reaction; chimp. (P) and chimp (G), chimpanzee Puchi and Gon, respectively; *, monoclonal antibody, **, purified by absorption-elution technique. Origins of the reagents are of rabbit (R) and mouse (M)

Further Investigation on the MN Antigens of Chimpanzee Erythrocytes

Absorption of Anti-M and -N with Erythrocytes (see Table 3)

The rabbit anti-M serum (MM503C) and the purified anti-M (Gifu 2-14p) lost the capacity to agglutinate the chimpanzee RBCs after absorption with human O-M RBCs. After absorption with the chimpanzee cells, the purified anti-M (Gifu 2-14p) did not agglutinate the human M RBCs, but the rabbit antiserum (MM503C) remained weakly reactive to those.

The mouse monoclonal anti-N and VGA completely or almost lost the reactivity to both the human and chimpanzee N RBCs simultaneously by the absorption with human or chimpanzee N RBCs. The rabbit anti-N serum (NN607A) did not react with the chimpanzee N RBCs after absorption with the human O-N RBCs but retained its original reactivity to the human N RBCs after absorption with the chimpanzee N RBCs.

Effects of Enzyme Treatments on M and N Activities: Sialidase (see Tables 1, 4a)

M Activity. The agglutinability of the human O-M and the chimpanzee RBCs with one of the rabbit anti-M (Gifu 2-14p) completely disappeared by the sialidase treatment, and their agglutinability with another anti-M (MM503C) showed a tendency to be somewhat reduced. The human O-M RBCs completely lost the reactivity to the monoclonal anti-M as well. On the other hand, the human O-N RBCs reacted with the rabbit anti-M (MM503C) to the same degree as the O-M RBCs.

Table 4. Effect of enzyme treatments on M and N activities

Antisera		lot. (origin)	Control human cells		Enzyme treated				
					human		chimpanzee cells		
			O-M	O-N	O-M	O-N	"Gon"	"Puchi"	"Reiko"
(a) Sialidase									
M	Ortho	MM503C(R)	2^4	–	2^2	2^2	2^1	2^2	2^1
	Gifu	2-14p(R)**	2^3	–	–	–	–	–	–
	Biotest	118084(M)*	2^8	–	–	–	–	–	–
N	Ortho	NN607A(R)	–	2^3	2^3	2^3	2^2	2^3	2^3
	Gifu	2-14p(R)**	–	2^4	–	–	–	–	–
	Biotest	119084(M)*	–	2^7	–	–	–	–	–
	VGA EY	0430D	–	2^0	2^0	2^2	2^1	2^1	2^2
(b) Trypsin									
M	Ortho	MM503C(R)	2^4	–	2^2	2^2	2^2	2^3	2^2
	Gifu	2-14p(R)**	2^3	–	2^1	2^1	2^1	2^1	2^1
	Biotest	118084(M)*	2^8	–	–	–	–	–	–
N	Ortho	NN607A(R)	–	2^4	2^3	2^3	2^1	2^1	2^1
	Gifu	2-14p(R)**	–	2^4	2^3	2^3	–	–	–
	Biotest	119084(M)*	–	2^8	2^4	2^5	2^7	–	2^7
	VGA EY	0430D	–	2^2	2^3	2^3	2^3	–	2^1
(c) Chymotrypsin									
M	Ortho	MM503C(R)	2^4	–	2^4	2^0	2^2	2^1	2^2
	Gifu	2-14p(R)**	2^3	–	2^3	–	2^1	2^3	2^3
	Biotest	118084(M)*	2^8	–	2^8	–	–	–	–
N	Ortho	NN607A(R)	–	2^4	2^0	2^3	2^2	2^1	2^1
	Gifu	2-14p(R)**	–	2^4	–	2^4	–	–	–
	Biotest	119084(M)*	–	2^7	–	2^8	–	–	–
	VGA EY	0430D	–	2^1	–	2^3	–	2^0	2^0

Abbreviations: refer to Table 1

N Activity. The sialidase-treated RBCs of two individuals ("Gon" and "Reiko") and the treated human O-N RBCs showed no agglutination with the monoclonal anti-N. The human O-N RBCs lost the reactivity to the purified rabbit anti-N (Gifu 2-14p), additionally. The RBCs of one chimpanzee ("Puchi") and the human O-M RBCs acquired the agglutinability with the rabbit anti-N serum (NN607A) and VGA.

Trypsin (see Tables 1, 4b)

M Activity. The reactivity of the human O-M and the chimpanzee RBCs to the purified rabbit anti-M (Gifu 2-14p) was distinctly reduced by the trypsin treat-

ment, and that of the human O-M RBCs to the monoclonal anti-M completely disappeared. The RBCs of one ("Puchi") and the human O-N RBCs were agglutinated with the rabbit anti-M (MM503C and Gifu 2-14p) after the trypsin treatment, but they remained negative in the reaction with the monoclonal anti-M.

N Activity. The human O-M RBCs acquired the agglutinability with all the anti-N used, but the RBCs of a chimpanzee ("Puchi") remained negative to the monoclonal anti-N and VGA.

Chymotrypsin (see Tables 1, 4c)

M Activity. The reactivity of the human O-M RBCs to the anti-M reagents was not affected by the chymotrypsin treatment. The chimpanzee RBCs showed a tendency to be slightly reduced in the agglutinability with the rabbit anti-M (MM503C and Gifu 2-14p).

N Activity. In contrast with the human O-N RBCs, the RBCs of two chimpanzee individuals ("Gon" and "Reiko") completely lost the reactivity to the monoclonal anti-N after treatment.

Discussion

A problem in the examination of the BGAs on animal erythrocytes by hemagglutination is the possible existence of heteroagglutinins in the typing reagents, especially in human sera. Positive reactions in the A-E tests are considered to be more reliable, since the human RBCs are used as the indicator.

On the RBCs of the three chimpanzee individuals examined, the activities A, Le^b, M, N, and c were detected by the A-E tests. The results on the MN activities depended on the reagents used, as discussed below. The positive reactions in the hemagglutination tests for the activities S, k, and Jk^a seemed to be specific. The results on the other activities, where the agglutination was seen even before the Coombs' test (Table 2), are not reliable; the existence of heteroagglutinins should be considered. It is also the case with the human anti-B.

The chimpanzee RBCs have been reported to possess the A and M antigens [4, 5] as well as the receptor to VGA [12] and to be agglutinated with the anti-D, -c, $-Fy^b$, $-Le^b$, and -k [13]. In our investigation, the possible existence of the antigens S and Jk^a is shown [5]. Among the recognized activities, only the N showed a distinct individual variation.

The serologic differences in the MN activities, especially in the N between the human and chimpanzee, have been already well known [5]. Two of the three chimpanzee individuals examined in this study were typed as MN and the other was typed as M (Table 1). The RBCs of the three, however, did not react with the mouse monoclonal anti-M or with one of the rabbit polyclonal anti-N. Each of those reagents is considered to recognize M or N determinants which are "human-specific".

The absorption of the anti-M and anti-N reagents with the RBCs (Table 3) showed that one of the rabbit anti-M (Gifu 2-14p), the monoclonal anti-N and

VGA recognized determinants which were common to the human and chimpanzee RBCs. On the other hand, the results with another rabbit anti-M (MM503C) and the rabbit anti-N (NN607A) indicated partial difference in the M and N antigens between them [14, 15].

In the analysis of the MN antigens by enzyme treatment, it was difficult to obtain clear results with the commercial polyclonal reagents. Some kinds of contaminating antibodies may have reacted with the enzyme-treated RBCs. The purified rabbit anti-M (Gifu 2-14p) and the mouse monoclonal anti-N distinctly showed the effects of the enzymes. Mainly from the results with those reagents, the changes of the MN activities by enzyme treatments are discussed below: The complete disappearance of the reactivity to the monoclonal and purified anti-M and -N by sialidase (Table 4a) indicates that sialic acid is essential for the M and N determinants of the chimpanzee RBCs as well as of the human. The remaining reactivity of the human and chimpanzee N RBCs to VGA and the rabbit anti-N (NN607A), however, shows the possible existence of sialic acid-independent N determinants [16]. The M activity of the human and chimpanzee RBCs is sensitive to trypsin and insensitive to chymotrypsin (Table 4b, c). This shows a structural similarity of the M substance between them [17]. However, the effect of chymotrypsin on the N activity was quite different between the human and chimpanzee RBCs [18]: The chimpanzee N activity (the reactivity to the monoclonal anti-N), which was insensitive to trypsin, was completely inactivated by the chymotrypsin treatment, while the human N (the reactivity to the monoclonal and the purified rabbit anti-N) was not affected. The M and N activity of the chimpanzee, respectively, are sensitive to trypsin and chymotrypsin. A reasonable speculation for this observation is that the M and N specificities are carried separately on at least two different kinds of glycophorins on the chimpanzee RBCs [19, 20].

As described above, the chimpanzee RBCs possessed the human-type BGAs A, H, Le^b, M, N, and c and possibly S, k, and Jk^a. The MN antigens of the chimpanzee are partially different from those of human in their serologic specificity. Some of anti-M and -N reagents are capable of differentiating the MN antigens of the human from those of the chimpanzee. The monoclonal anti-M (Seraclone, lot no. 118084, Biotest) is considered to be specific for the human M antigen, showing no reaction with the blood specimens of 20 species of non-human primates or other vertebrates examined (details not shown here). Additionally, the chimpanzee N activity is sensitive to chymotrypsin in contrast to the human N. These results show a possibility for the identifcation of the blood as human origin through the MN grouping.

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