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Antigenic Properties of Human and Animal Bloodstains Studied by Enzyme-Linked Immunosorbent Assay (ELISA) Using Various Antisera Against Specific Plasma Proteins*

H. Tsutsumi[1], H. H. Htay[2], K. Sato[2], and Y. Katsumata[2]

[1] Criminal Science Laboratory, Aichi Prefectural Police Headquarters, 2–1–1 Sannomaru, Nagoya 460, Japan
[2] Department of Legal Medicine, Nagoya University School of Medicine, Nagoya 466, Japan

Summary. Antigenic properties of bloodstains of human and non-human primates as well as other animal bloodstains were investigated by the inhibition ELISA using commercially available anti-human albumin (Alb), α_2-macroglobulin (α_2-M), fibrinogen, transferrin, and immunoglobulin G. In general, chimpanzee bloodstains showed strong cross-reactions with these antisera, and the extent of the cross-reactions of other animal bloodstains decreased largely with the phylogenic order, i.e., agile gibbon (ape), Old World monkeys (Japanese monkey and hamadryas baboon), New World monkeys (night monkey and tufted capuchin monkey), prosimians (grand galago and ring-tailed lemur) and other animals (rat, cattle, swine, goat, dog, cat, and chicken). Among these antisera, anti-human α_2-M showed the weakest cross-reaction with chimpanzee bloodstains, and anti-human Alb showed next.

Key words: Bloodstains, species identification – Species identification, immunoassay (ELISA)

Zusammenfassung. Die Antigeneigenschaften von Blutspuren menschlicher und nichtmenschlicher Primaten und anderer Tiere wurden mit Hilfe des Inhibitions-ELISA unter Benutzung käuflich erhältlicher Anti-Human-Albumin- und Anti-Alpha$_2$-makroglobulin-, Fibrinogen-, Transferrin- und IGG-Seren untersucht. Allgemein kann festgestellt werden, daß Blutspuren von Schimpansen die stärksten Kreuzreaktionen mit diesen Antiseren aufwiesen und daß das Ausmaß der Kreuzreaktionen von Blutspuren anderer Tiere deutlich in Verbindung mit dem phylogenetischen Rang, wie folgt, ab-

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Offprint requests to: H. Tsutsumi (address see above)

nahm: agiler Gibbon, Hundsaffen (Japanischer Affe, Mantelpavian), Breitnasenaffen (Nachtaffen und Kapuziner), Halbaffen (Groß-Galago und Katta) und andere Tiere (Ratte, Rind, Schwein, Ziege, Hund, Katze und Huhn). Unter diesen Antiseren zeigten Anti-Human-Alpha$_2$-Makroglobulin-Seren die schwächsten Kreuzreaktionen mit Blutspuren von Schimpansen; Anti-HumanAlbumin folgte hiernach.

Schlüsselwörter: Blutspuren, Antigeneigenschaften – Speziesidentifikation, Immunoassay (ELISA)

Introduction

Enzyme-linked immunosorbent assay (ELISA) has been employed for medicolegal examinations of bloodstains [1–4] and body fluid stains [5–7]. We demonstrated previously that human bloodstains could be differentiated from chimpanzee by the inhibition ELISA using commercially available raw anti-human serum [8,9]. In the present study, human and animal bloodstains were analyzed by the inhibition ELISA using five kinds of commercially available antisera against specific human plasma proteins to investigate which protein is mainly responsible for the immunologic difference between human and animal bloodstains.

Materials and methods

Antisera

Rabbit anti-human albumin (Alb), α_2-macroglobulin (α_2-M) and transferrin (Tr) were purchased from Behring Institute (Marburg, FRG). Rabbit anti-human fibrinogen (Fb) was purchased from DAKOPATTS (Copenhagen, Denmark) and rabbit anti-human immunoglobulin G (IgG) was purchased from MBL Laboratory (Nagoya, Japan). Alkaline phosphatase labeled goat anti-rabbit IgG was purchased from Kirkegaard and Perry Laboratory (Gaithersberg, MD, USA).

Preparation of Coating Antigens

From pooled human plasma, crude Alb was prepared by the method of Peters [10], and crude α_2-M by the method of Harpel [11]. Crude human IgG and crude human Tr were also prepared from pooled human plasma by a standard ammonium sulfate precipitation method; 33% saturation fraction for IgG [12] and 40–60% saturation fraction for Tr [13]. Human lyophilized Fb was purchased from Kabi Diagnostica (Stockholm, Sweden).

Specimens

Heparinized blood samples were obtained from humans, apes (chimpanzee and agile gibbon), Old World monkeys (Japanese monkey and hamadryas baboon), New World monkeys (night monkey and tufted capuchin monkey), prosimians (grand galago and ring-tailed lemur), and other animals (rat, cattle, swine, goat, dog, cat, and chicken). Pieces of filter paper (Toyoroshi, No. 2) were stained with these blood samples, dried at room temperature, and subjected to analysis. A piece of the stains, 5×5 mm in area, was used. Plasma samples were obtained from humans and chimpanzees, and subjected to the immunodiffusion test.

Inhibition ELISA

Wells of a polystyrene microtiter plate (129 B, Dynatech Ltd., Sussex, England) were coated with 100 µl of a 100-fold diluted antigen in 50 m*M* sodium carbonate buffer (pH 9.6) for 30 min at room temperature. Other steps were performed according to the method previously reported [8, 9].

Immunodiffusion test

This was performed according to the method of Yakulis and Heller [14].

Results

Bloodstains of human, chimpanzee and other animals were analyzed with the present inhibition ELISA using antisera against Alb, α_2-M, Fb, Tr, and IgG of human origin. Tables 1–5 summarizes the results obtained. In each test, human

Table 1. Inhibition of the ELISA reactions by human and animal bloodstains using anti-human albumin serum

Species	*n*	Inhibition (%). Dilution of antiserum		
		1:100	1:500	1:1000
Human	4	88.8 ± 0.2[a]	93.0 ± 0.3	94.5 ± 0.4
Apes Chimpanzee	4	55.9 ± 2.9	72.6 ± 3.2	84.4 ± 0.7
Agile gibbon	1	28.9	45.8	62.9
Old World monkeys	2	18.1	20.5	29.6
New World monkeys	2	8.1	10.3	14.5
Prosimians	2	7.0	8.9	13.2
Other animals	7	2.9 ± 1.9	5.5 ± 2.5	10.0 ± 2.6

[a] Mean ± SD

Table 2. Inhibition of the ELISA reactions by human and animal bloodstains using anti-human α_2-macroglobulin serum

Species	*n*	Inhibition (%). Dilution of antiserum		
		1:100	1:500	1:1000
Human	4	77.1 ± 3.6[a]	93.0 ± 1.6	95.3 ± 1.2
Apes Chimpanzee	4	23.3 ± 3.1	48.5 ± 1.2	52.2 ± 2.0
Agile gibbon	1	15.3	41.7	49.7
Old World monkeys	2	15.3	34.1	40.6
New World monkeys	2	0	14.3	25.5
Prosimians	2	0	8.6	14.5
Other animals	7	0	0	2.3 ± 2.1

[a] Mean ± SD

Table 3. Inhibition of the ELISA reactions by human and animal bloodstains using anti-human fibrinogen serum

Species	*n*	Inhibition (%). Dilution of antiserum		
		1:100	1:500	1:1000
Human	4	39.5 ± 1.9[a]	76.4 ± 3.3	86.2 ± 3.0
Apes Chimpanzee	4	20.8 ± 1.7	46.6 ± 1.0	62.0 ± 1.6
Agile gibbon	1	11.9	30.7	47.8
Old World monkeys	2	9.0	18.8	30.7
New World monkeys	2	5.3	12.1	17.5
Prosimians	2	5.2	11.7	12.3
Other animals	7	3.5 ± 2.1	6.3 ± 1.9	7.0 ± 2.1

[a] Mean ± SD

Table 4. Inhibition of the ELISA reactions by human and animal bloodstains using anti-human transferrin serum

Species	*n*	Inhibition (%). Dilution of antiserum		
		1:100	1:500	1:1000
Human	4	94.5 ± 1.7[a]	95.6 ± 1.4	98.0 ± 0.6
Apes Chimpanzee	4	84.9 ± 1.4	92.0 ± 1.3	94.9 ± 0.6
Agile gibbon	1	43.5	61.7	76.7
Old World monkeys	2	31.6	48.5	62.5
New World monkeys	2	14.0	36.1	52.9
Prosimians	2	11.8	14.4	21.7
Other animals	7	5.0 ± 2.4	7.3 ± 2.6	11.5 ± 2.1

[a] Mean ± SD

Table 5. Inhibition of the ELISA reactions by human and animal bloodstains using anti-human immunoglobulin G serum

Species	*n*	Inhibition (%). Dilution of antiserum		
		1:100	1:500	1:1000
Human	4	53.7 ± 3.9[a]	75.8 ± 2.2	83.5 ± 3.1
Apes Chimpanzee	4	44.3 ± 4.5	65.7 ± 2.2	76.9 ± 2.3
Agile gibbon	1	15.1	16.0	22.1
Old World monkeys	2	15.1	15.2	20.2
New World monkeys	2	5.6	5.8	8.5
Prosimians	2	4.3	4.3	5.8
Other animals	7	2.5 ± 0.6	3.1 ± 2.4	5.6 ± 3.5

[a] Mean ± SD

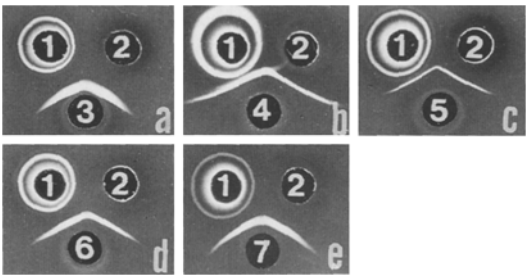

Fig. 1. Immunodiffusion patterns of human and chimpanzee plasma samples developed with various antisera against specific plasma proteins. *1* human plasma, *2* chimpanzee plasma, *3* anti-human Alb(a), *4* anti-human α_2-M(b), *5* anti-human Fb(c), *6* anti-human Tr(d), *7* anti-human IgG(e)

bloodstains showed the strongest inhibition of the ELISA reactions and second was chimpanzee. The inhibition rates of the bloodstains of the other sepcies were smaller than that of chimpanzee ones, and there appeared to be some relationship between the inhibition rate and the phylogenic order: the inhibition rates generally decreased in the following order: agile gibbon (ape), Old World monkeys, New World monkeys, prosimians, and other animals. The difference between inhibition rates of human and chimpanzee samples was greatest when anti-human α_2-M and anti-human Alb were used. Furthermore, the difference tended to increase when dilution rate of the antisera was decreased.

Human and chimpanzee plasmas were investigated by the immunodiffusion test using anti-human Alb, α_2-M, Fb, Tr, and IgG sera. With most of these sera, plasmas of the two species developed complete fusion of the precipitin lines (Fig. 1a, c, d, and e),but with only anti-human α_2-M serum, human plasma formed a spur against chimpanzee plasma (Fig. 1b).

Discussion

Recently, various ELISA systems using antisera against specific human protein were applied to species identification of human blood and its stains. Tamaki and Kishida [1] reported that, with their ELISA system, human serum and bloodstains could be differentiated from orangutan (ape) and Old World monkey but not chimpanzee (ape) using anti-human IgG (500-fold dilution) and Alb (500-fold dilution). Fletcher et al. [2] reported that human bloodstains could not be differentiated from chimpanzee and other monkey (Old World monkey) using anti-human lambda chains (500-fold dilution). Oshima and Hara [15, 16] reported that human serum samples could be differentiated from New World monkey using anti-human Alb (32000-fold dilution), IgG (16000-fold dilution), β_1-lipoprotein (2000- to 16000-fold dilution), and α_2-M (2000- to 4000-fold dilution), from Old World monkey using anti-human α_1-anti-trypsin (2000- to 6000-fold dilution) and from gibbon (ape) using anti-human Tr (16000-fold dilution). In the present ELISA, human bloodstains could be differentiated from chimpanzee using anti-human α_2-M and anti-human Alb. Using a less sensitive immunologic method, the immunodiffusion test, chimpanzee plasma and human plasma showed a spur formation only with anti-human α_2-M. From these results we consider that α_2-M is the most specific protein for human, and Alb is

the next one. Therefore, the present ELISA system using a low dilution of antisera against α_2-M or Alb can be suitable for analysis of the antigenic properties of closely related species.

The present results suggest that the human specificity of anti-human serum used in the ELISA for differentiation of human bloodstains from chimpanzee's [8, 9] is mainly ascribed to the specificity of α_2-M and Alb.

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