J. Agric. Sci., Tokyo Univ. Agric., 53 (1), 63-68 (2008)
東京農大農学集報, 53 (1), 63-68 (2008)

Common Erythrocyte Antigens Among Cattle and their Close Relatives Revealed by Analysis of Monoclonal Antibodies

By

Koh NOMURA*, Kazuhito KUROGI**, Mitsuo MORITA**, Misao KANEMAKI**, Eriko SAITOH***, Shigehisa KAWAKAMI***, Chang-Yeon CHO****, SUTOPO*****, Md. Omar FARUQUE****** and Takashi AMANO*

(Received November 30, 2007/Accepted March 14, 2008)

Summary : To detect common erythrocyte antigens among cattle and their close relatives, extensive tests on red blood cells from more than 1,000 animals including several indigenous cattle from various Asian countries, Bali cattle, Gayal, Yaks, Water buffaloes, Bezoar and some breeds of goats and sheep were screened with thirty-two bovine red blood group monoclonal antibodies. The five species except cattle, Bezoar and Gayal shared 34.4-87.5% of erythrocyte antigens with cattle. Based on the number of common erythrocyte antigens in each species, the relationships between subgenera *Bos* and *Bibos* was closer than that between subgenera *Bos* and *Poephagus*.

The A2 antigen was distributed among all the eight species screened. The presence of the Fc antigen could be used to distinguish subfamily *Bovinae* from subfamily *Caprinae* ; that is all individuals of cattle, Bali cattle, Gayal, Yaks and Water buffaloes had the Fc antigen but not goats nor sheep.

Key words : cattle blood group antigen, common antigen, *Bovinae*, *Caprinae*

Introduction

Cattle blood type antigens are specific molecular constituents of erythrocyte membranes detected by hemolytic tests. More than ninety blood type antigens controlled by 11 independent blood group genetic systems have been recognized and used internationally as genetic markers in routine parentage and identity verification for over 60 years[1,2]. Specific antibodies to blood type antigens have been prepared by absorption from alloimmunized and heteroimmunized antiserum.

The detection of these blood typing antigens among cattle and related animals has been limited due to contamination with natural or non-specific antibodies in immunized antiserum. Therefore little evidence about common erythrocyte antigens among *Bovidae* has been known. STORMONT *et al.* reported that American bison (*Bison bison*) have homologous blood group systems with cattle and proposed that the two species might be recognized as the same genus *Bos*. They also mentioned about evidence of A antigen. The red blood cell from many of *Bovidae* and *Cervidae,* i.e. American bison, Cape buffalo, nilgai, eland, antelope, gazelle, gnu, goat, sheep, axis deer and mule deer gave rise to the production of anti-A antisera when immunized to rabbits[3].

The study on common erythrocyte antigens is especially important for investigating taxonomic relationships among species and for understanding biological significance of red blood typing antigens. Only monoclonal antibodies can detect the common antigens among heterogeneous species. Lately monoclonal anti-

* Department of Animal Science, Faculty of Agriculture, Tokyo University of Agriculture
** Maebashi Institute of Animal Science, Livestock Improvement Association of Japan, Gunma, Japan
*** Gunma Safari World Company Limited, Gunma, Japan
**** National Livestock Research Institute, Rural Development Administration, Chungnam-do, Republic of Korea
***** Faculty of Animal Science, Diponegoro University, Semaramg, Indonesia
****** Department of Animal Breeding and Genetics, Bangladesh Agricultural University, Mymensingh, Bangladesh

bodies to bovine red blood group antigens were produced and their monospecific function at routine blood typing without absorption demonstrated[4-6]. Thirty-two cattle blood typing monoclonal antibodies produced by NOMURA *et al.*[6] were used in the current study for screening erythrocytes from 8 heterogeneous species and to detect common erythrocyte antigens among *Bovidae*.

Materials and Method

Red blood cell samples

A total of 1,216 blood samples from animals that belong to 8 species of *Bovidae* family were collected between 1988 and 2006. The details of samples used in this study and sampling locations are shown in Table 1.

Blood typing

The blood typing reagents are 32 cattle blood typing monoclonal antibodies (mAbs) classified by 6 genetic systems developed previously[6,7]. These antibodies are all mouse origin mAbs and have been ascertained for their specificity at biennial comparison tests of 1989/90 and 1995/96 organized by the International Society of Animal Genetics (ISAG). The specificities detected by these monoclonal antibodies are listed in Table 2.

Hemolytic tests for blood typing were performed in each country using fresh blood samples as the standard method, utilizing 20μl of culture supernatant of antibody, 10μl of 3% red blood cell suspensions and 10μl of rabbit complement[6].

Results and Discussion

Distribution of common erythrocyte antigens

Among the seven *Bovinae* species, Bali cattle (*Bos (Bibos) javanicus*) shared 87.5% of the antigens with cattle, but lacked four antigens, I1 and Ylike in the B system, and Fa and V1 in the F system. It can be estimated that Bali cattle have the closest relationships to cattle (*Bos (Bos) taurus*). Gayal (*Bos (Bibos) gaurus frontalis*) which belongs to the same subgenus with Bali cattle, that is these species, showed similar reaction pattern to Bali cattle on the A, F and Z systems, and was estimated to possess similar antigens to Bali cattle in the remaining systems. Yak (*Bos (Poephagus) grunniens grunniens*) which belongs to a separate subgenus shared only 46.7% of the antigens with cattle. From these results, it can be concluded that the relationships between subgenera *Bos* and *Bibos* is closer than that between *Bos* and *Poephagus*.

Two types of Water buffalo classified by ecotype are

Table 1 Animal samples of the family *Bovidae*

Species	Scientific name	Type	Breed or population (sampling place)	No. of sample
	Subfamily *Bovinae*			
Cattle	*Bos (Bos) taurus*	Humpless	Chinese yellow (Shantung)	38
			Korea	109
			Mongolia	43
			Nepal (mountain)	76
		Humped	China (Yunnann)	74
			Nepal (lowland)	24
			Filial ongole (Indonesia)	26
Bali cattle	*Bos (Bibos) javanicus domesticus*		(Bali, Indonesia)	156
Gayal	*Bos (Bibos) gaurus frontalis*		(Yunnann, China)	3
Yak	*Bos (Poephagus) grunniens grunniens*		(Nepal)	30
			(Mongolia)	10
Water buffalo	*Bubalus bubalis*	River buffalo	Nepal	56
			Pakistan (Vietnam)	21
			Bangladesh	20
		Swamp buffalo	China (Yunnann)	64
			Thailand	33
			Vietnam	54
			Taiwan	28
			Japan	6
			Indonesia	16
			Philippine	20
	Subfamily *Caprinae*			
Sheep	*Ovis aries*		Corridale	24
			Suffolk	7
			Northern Vietnam	34
Goat	*Capra hircus*		Japanese saanen	60
			Shiba	38
			Tokara	3
			Southern Vietnam	43
			Northern Vietnam	35
			Kambing Katjang (Indonesia)	55
Bazoar	*Capra aegagrus*		(Gunma safari world, Japan)	10

Table 2 Observed frequencies of bovine red blood cell antigens in cattle, Bali cattle, gayal and yak

Blood type		Cattle							Bali	Gayal	Yak	
System	Antigen	China Shantung	Korea	Mongolia	Nepal mountain	China Yunnan	Nepal lowland	Indonesia	cattle		Nepal	Mongolia
A	A1	0.632		0.667		0.757		0.615	0.677	0.667		0.000
	A2	0.711	0.972	0.810	0.750	0.824	0.958	0.692	0.677	0.667	0.933	1.000
	A2dose	0.711		0.810	0.750	0.824	0.958	0.692	0.871	0.667	0.767	1.000
	D	0.815		0.167	0.316	0.514	0.333	0.423	0.129	0.333	0.067	0.100
	H	0.868						0.308	0.419			0.000
	Z'	0.000		0.071	0.145	0.324	0.292	0.385	0.065	0.333	0.000	0.000
B	I1	0.158		0.024				0.077	0.000			0.400
	O1	0.263			0.276		0.500	0.538	0.484		0.067	0.000
	O1like	0.368		0.381				0.577	0.548			0.000
	O4	0.658		0.667	0.842		0.917	0.885	0.903		0.633	0.800
	Ylike	0.132						0.385	0.000			
	E'2like	0.237	0.138		0.184		0.167	0.308	0.258		0.000	
	E'x	0.605	0.761	0.429	0.618		0.875	0.731	0.258		0.000	0.000
C	C2like	0.211		0.119				0.807	1.000			0.000
	X2	0.684		0.714				0.961	0.258			0.200
F	Fa	0.526	0.404	0.881	0.653	0.132	0.708	0.692	0.000	0.000	0.100	
	F1	0.895		0.881	0.868	0.851	0.833	0.807	0.839	1.000	0.167	
	Fc	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000	1.000
	Fcdose	0.895	0.862	0.881	0.855	1.000	0.833	1.000	0.839	1.000	0.167	1.000
	V1	0.289	0.266	0.190	0.092	0.041	0.042	0.000	0.000	0.000	0.000	0.000
	V2	0.526	0.440	0.405	0.342	0.716	0.458	0.462	0.484	0.667	1.000	1.000
S	S1	0.105		0.190				0.462	1.000			0.000
	S2	0.053		0.286				0.538	1.000			0.000
	H'	0.789						0.923	0.419			0.200
	H'like	0.658		0.714	0.802		0.833	0.923	0.355		0.000	
	S1U1	0.158		0.262	0.513		0.458	0.462	1.000		0.000	0.000
	U1	0.105		0.071	0.263		0.250	0.038	0.161		0.000	0.000
	U2	0.368						0.385	0.935			0.000
	S2U2	0.368						0.115	0.290			
Z	Z1	0.684			0.934	0.811	0.958	0.962	0.774	0.663	0.767	1.000
	Z1dose	0.342						0.769	0.677	0.333		
	Z2	0.526		0.357		0.757		0.923	0.226	0.667		0.000

recognized : Swamp buffalo that is used for draft and meat, and River buffalo that is used for dairy purposes. Both types of water buffaloes lacked V antigens of the F system, and all antigens of the Z system. River buffaloes lacked the S system antigens, but shared 35.5% antigens with cattle. Swamp buffalo on the other hand had S1, S2 and U2 antigens of the S system, which did not differentiate the three antigens and were inherited together as a unit. Swamp buffalo shared 50% of the other antigens with cattle. The two types of Water buffaloes have been given one scientific name *Bubalus bubalis,* but their erythrocyte antigenic components were independent from each other.

Goats shared 50% of antigens with cattle, but the compositions of the antigens differed with breeds or populations. Therefore the number of common antigens between sheep and cattle must increase more than 34.4% if sufficient number of breeds are tested. Bezoar that have been regarded as the wild ancestor of domestic goats, were also screened for common antigens. No discrepancy was observed between the results of domestic goats and those of Bezoar.

Comparisons between two linear subtype antigens revealed that subtype2 antigen had larger distribution range than subtype1 antigen (Tables 2, 3, 4 and 5). The V1 antigen in the F system was specific to cattle, while the V2 antigen occurred in cattle, Bali cattle and Yak. Similar distributions were observed between A1 and A 2 antigen in the A system, and S1 and S2 antigen in the S system (Table 5).

The Z' antigen which had been reported to be carried by the Zebu type (humped) cattle occurred in Asian native cattle with a frequency of 0.071 (Mongolia, humpless type) and 0.385 (Filial ongole, humped type). Chinese yellow cattle from Shantung did not have Z' antigen. Although classified as humped cattle, many local types of Chinese yellow cattle are known in China. In fact, the individuals tested in this study were seemingly humpless. Bali cattle had the Z' antigen but at low frequencies.

A2 antigen

Only the A2 antigen was distributed among all the seven species screened and is presumed to be one of the most ancient antigens that existed before speciation. All species/populations had the A2 antigen occurring with a high frequency (0.7–0.8 in cattle and Bali cattle, 0.9–1.0 in Yaks and 0.8–1.0 in goat and sheep) except for Swamp buffaloes. Swamp buffaloes from Indonesia, Philippines and Taiwan lacked the A2 antigen. However, 4–12.5% of the Swamp buffaloes from China, Thailand and Vietnam possessed the A2 antigen. In contrast, River buffaloes had the A2 antigen occurring at very high frequencies. In fact all River buffaloes in Bangladesh and Vietnam had the A2 antigen.

In cattle, the A2 antigen consists of two kinds of

Table 3 Observed frequencies of bovine red blood cell antigens in two types of water buffaloes

Blood type		River type			Swamp type						
System	Antigen	Nepal	Bangladesh	Vietnam	China	Thailand	Taiwan	Japan	Indonesia	Philippines	Vietnam
A	A1		0.750	0.762	0.065	0.061	0.0		0.0	0.0	0.0
	A2	0.982	1.0	1.0	0.125	0.061	0.0		0.0	0.0	0.037
	A2dose	0.179	0.2	0.524	0.031		0.0		0.0		0.019
	D	0.0	0.0			0.0	0.0				
	H			0.667		0.818				0.55	0.519
	Z'	0.0	0.0		0.016	0.0	0.0		0.0		
B	I1		0.0			0.0					
	O1	0.179	0.3	0.429		0.0				0.0	0.0
	O1like		0.4	0.381		0.182				0.0	0.204
	O4	0.0	0.0			0.0					
	Ylike		0.0			0.0					
	E'2like	0.0	0.0			0.0					
	E'x	0.607	0.15	0.381		0.273				0.0	0.167
C	C2like		0.0	0.095		0.0				0.0	0.0
	X2			0.0		0.061				0.0	0.0
F	Fa	0.0	0.0		0.0	0.0		0.0	0.0		
	F1	0.232		0.619	0.234			0.0		0.2	0.0
	Fc	1.0			0.938			1.0	1.0	1.0	
	Fcdose		0.65		0.938	0.575		1.0	1.0		
	V1	0.0	0.0		0.0	0.0	0.0	0.0			
	V2	0.0	0.0		0.0	0.0	0.0	0.0			
S	S1		0.0	0.0		0.061			0.625	0.0	0.13
	S1like		0.0			0.0			0.0		
	S2		0.0	0.0		0.061			0.625	0.0	0.13
	H'		0.0			0.0					
	H'like	0.0	0.0			0.0					
	S1U1	0.0	0.0	0.0		0.061			0.625	0.0	0.13
	U1	0.0	0.0	0.0					0.0		
	U2		0.0	0.0		0.061			0.625	0.0	
	S2U2								0.625	0.0	
Z	Z1	0.0	0.0		0.0		0.0				
	Z1dose		0.0		0.0		0.0				
	Z2		0.0		0.0		0.0				

Table 4 Observed frequencies of bovine red blood cell antigens in goats and sheep

Blood type		Goat							Sheep		
System	Antigen	Saanen	Shiba	Tokara	Vietnam		Indonesia	Bezoar	Corriedale	Suffolk	Vietnam
					Nothern	Southern					Northern
A	A1	0.0	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0
	A2	1.0	1.0	1.0	1.0	1.0		0.1	0.792	0.857	0.882
	A2dose	0.2	0.579	0.0	0.2	0.047		0.0	0.0	0.0	0.0
	D	0.017	0.079	0.667	0.429	0.023		0.0	0.083	0.143	0.0
	H	1.0	1.0	0.667		0.977			1.0	1.0	1.0
	Z'	0.717	1.0	0.667	0.457	0.581		0.0	0.0	0.0	0.0
B	I1	0.183	0.026	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.294
	O1	0.0	0.0	0.333	0.143	0.0	0.291	0.0	0.0	0.0	0.0
	O1like	0.083	0.026	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	O4	0.2	0.0	0.0	0.029	0.0	0.0	0.0	0.917	1.0	0.735
	Ylike	0.117	0.0	0.0	0.0	0.0	0.273	0.0	0.0	0.0	0.0
	E'2like	0.0	0.026	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	E'x	0.85	0.026	0.0	0.0	0.721	0.655	0.4	0.083	0.0	0.0
C	C2like	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.25	0.286	
	X2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
F	Fa	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	F1	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	Fc	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	Fcdose	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	V1	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
	V2	0.0	0.0	0.0	0.0		0.0	0.0	0.0	0.0	
S	S1	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0	0.0
	S2	0.517	0.132	0.0	0.886	0.395	0.455	0.5	1.0	1.0	0.971
	H'	0.0	0.0	0.0	0.543	0.0	0.0	0.0	0.0	0.0	0.0
	H'like				0.0		0.0	0.0			
	S1U1	0.0	0.079	0.0	0.0	0.14	0.236	0.2	0.0	0.0	0.0
	U1	0.0	0.0	0.0	0.171	0.0	0.0	0.0	0.0	0.0	0.0
	U2	0.0	0.105	0.0		0.721			0.0	0.0	0.118
	S2U2	0.0	0.0			0.721			0.0	0.0	0.059
Z	Z1	0.0	0.053	0.0	0.0		0.0	0.0	0.0	0.0	
	Z1dose			0.0	0.0		0.0	0.0			
	Z2	0.0	0.079	0.0	0.0		0.036	0.0	0.065	0.0	

antigenic molecule. One is a qualitative antigen that is recognized by anti-A2 antibody, while the other is a quantitative antigen that is recognized by anti-A2 dose antibody[6]. These two antigens occurred in parallel in cattle, but in Bali cattle and Yaks, 10 to 20% of A2 antigen positive animals lacked the quantitative A2 ; similar to the relationships of subtypes. Of the two types of Water buffaloes, almost all individuals of River buffalo had qualitative A2 antigen, while 50-80% of these animals lacked quantitative A2.

Table 5 Interspecies distribution of two linear subtype antigens and Fc antigen

Scientific name								
Subfamily		*Bovinae*					*Caprinae*	
Genus		*Bos*			*Bubalus*		*Capra*	*Ovis*
(Sub genus)		*(Bos)*	*(Bibos)*	*(Poephagus)*				
Species		*taurus*	*javanicus*	*grunniens*	*bubalis*		*hircus*	*alies*
Common name		Cattl	Bali cattle	Yak	River buffalo	Swamp buffalo	Goat	Sheep
Blood system	Antigen							
F	V1	+	-	-	-	-	-	-
	V2	+	+	+	-	-	-	-
	Fc	+	+	+	+	+	-	-
A	A1	+	+	-	+	+	-	-
	A2	+	+	+	+	+	+	+
S	S1	+	+	-	-	+	-	-
	S2	+	+	-	-	+	+	+

F system

As shown in Table 5, Fc antigens were distributed in cattle, Bali cattle, Yaks and Water buffaloes. Anti-Fc reacted parallel to anti-F1, but hemolysed all cells at high concentration. Using anti-Fc at high concentration, we confirmed positive reactions with all individuals of cattle, Bali cattle, Yaks, Swamp and River buffaloes. Therefore the presence of the Fc antigen could be used to distinguish animals in the subfamily *Bovinae* from those of *Caprinae*. That is, all *Bovinae* individuals possessed the Fc antigen that lacked in *Caprinae*. The Fc antigen was detected in a molecule of 250 kDa, sialic acid related bovine specific glycophorin[8]. This molecule is supposed to be one of the basically common component of *Bovinae* species specific molecule.

Acknowledgement

This research was supported by a Grant-in-Aid for Scientific Research, no. 1456023 (2002–2005) from the Japan Society for the Promotion of Science and The Advanced Research Project Type A, no. 02 (2006, 2007) from Tokyo University of Agriculture.

References

1) Ferguson, L.C. 1941, Heritable antigens in the erythrocytes of cattle. *The Journal of Immunology,* **40** : 213–242.
2) Ferguson, L.C., C. Stormont and M.R. Irwin, 1942, On additional antigens in the erythrocytes of cattle. *The Journal of Immunology,* **41** : 147–164.
3) Stormont, C. 1962, Current status of blood groups in cattle. *Annals New York Academy of Science,* **97** : 251–268.
4) Tucker, E.M., L. Metenier, J. Grosclaude, S.W. Clarkeand and L. Kilgour, 1986, Monoclonal antibodied to bovine blood group antigens. *Animal Genetics* **17** : 3–13.
5) Tucker, E.M., S.W. Clarke and L. Metenier, 1987, Murine/bovine hybridomas producing monoclonal antibodies to bovine red cell antigens. *Animal Genetics* **18** : 29–39.
6) Nomura, K., T. Amano, K. Tanaka, M. Kanemaki and T. Hohdatsu, 1991, The production and characterization of monoclonal antibodies to bovine blood group antigens. *Animal Science and Technology,* **62** : 336–342.
7) Kurogi, K.T. Akimoto, K. Nomura and T. Amano, 1997, Analyses on goat and sheep red cell antigens by 32 kinds of monoclonal antibodies. *The Journal of Animal Genetics,* **25** : 87–93.
8) Nomura, K., Mori, E., Amano, T. and Mori, T., 1995. Immunological analysis of bovine red blood cell F1 antigen. *The Journal of Animal Genetics,* **23** : 13–17.

ウシとその近縁種の共通赤血球抗原

野村こう*・黒木一仁**・森田光夫**・印牧美佐生**・斉藤恵理子***・
川上茂久***・趙　昌衍****・ストポ*****・
モハメド オマー ファルーク******・天野　卓*

（平成19年11月30日受付/平成20年3月14日受理）

要約：ウシとその近縁家畜に共通する赤血球抗原を特定するために，アジア在来牛，バリウシ，ガヤ－ル，ヤク，スイギュウ，ベゾア－，ヤギおよびヒツジの計1,000頭以上について，32種類のウシ血液型判定用モノクローナル抗体を用いた試験を行なった。これらの家畜はウシと34.4～87.5％の赤血球抗原を共有していた。共通抗原の数から推定すると，ウシ亜属（*Bos*）とガウア亜属（*Bibos*）間は，ウシ亜属（*Bos*）とヤク亜属（*Poehagus*）間より近縁であった。

A2抗原は調べた8種全てに分布する抗原だった。Fc抗原の存在はウシ亜科とヤギ亜科の動物を区別した。すなわちウシ，バリウシ，ガヤ－ル，ヤク，スイギュウのすべての個体がFc抗原を持つのに対し，ヤギやヒツジでFcをもつ個体は認められなかった。

キーワード：ウシ血液型抗原，共通性抗原，ウシ亜科，ヤギ亜科

* 東京農業大学農学部畜産学科
** 社団法人家畜改良事業団家畜改良技術研究所
*** 群馬サファリパーク動物研究所
**** 韓国農村振興局畜産技術研究所
***** ディポネゴロ大学
****** バングラデシュ農業大学