

免疫学的定量法に用いられる緬羊赤血球の個体間差異

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BRIEF NOTE

**Individual Differences of Sheep Red Blood Cells
Used in Various Immunological Assays**

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Sheep red blood cells (SRBC) have been used most commonly as an indicator antigen or immunogen in immunological tests. It has been known that the results obtained in the tests are often influenced by the donor of SRBC. Up to the present time, differing susceptibility of SRBC to immune hemolysis [1] and two types of SRBC as regards to the responsiveness of mouse [5] have been reported.

The present experiments were undertaken to find out the correlation between individual differences of SRBC in various immunological tests and the known genetical markers of SRBC.

Adult female sheep of four breeds reared in Saitama Livestock Farming Experimental Station were used. They were fed ordinary diet. Blood samples from 26 Corriedale, 27 Suffolk, 9 Romney Marsh and 9 Border Leicester sheep were kept at 5°C in sterile Alsever's solution and used within 3 weeks after bleeding. Blood types, hemoglobin types and potassium types of all SRBC were determined by the methods of Drury and Tucker [4, 7].

1. Response of mice to SRBC

Eight C57BL/6J male mice were injected intraperitoneally with 1×10^7 SRBC

in 0.2 ml of saline at day 0, then 4 of them were killed 5 days later and their spleen were assayed for anti-SRBC plaque forming cells (PFC) by the method of Cunningham and Szenberg [3]. The remaining 4 mice received the second injection with the same dose of SRBC at day 20 and the same assay for spleen PFC was done 5 days later. The results are shown in Table 1. In the primary response, all Romney Marsh SRBC gave significantly lower response than those of the other breeds. The secondary response to each source of SRBC was approximately parallel to its primary response except the responses to Romney Marsh 1 and 3 SRBC. It was also noticed that all of Romney Marsh SRBC which gave low response in this experiment were i type at R-O blood group system, as shown in Table 2. Further experiments will be needed to determine whether the low response is due to the lack of R or O antigen, or other factors of SRBC.

2. Agglutinability of SRBC to immune serum

Individual variations of agglutinability against anti-SRBC rabbit immune serum were observed in both agglutination and Coomb's test as described by Spooner [2, 6].

Table 1. Response of mouse spleen cells to different breeds of sheep red blood cells

SRBC		Primary response		Secondary response	
Breed	No.	Direct	Direct	Indirect	
Suffolk	1	361 ¹⁾ a	586 ²⁾ a	1910 a ³⁾	
	2	141 a	283 ab	755 ab	
	3	180 a	333 ab	557 ab	
Corriedale	1	327 a	307 ab	1337 a	
	2	427 a	984 a	1107 a	
	3	216 a	52 bc	262 bc	
Romny Marsh	1	80 b	15 c	97 c	
	2	72 b	62 bc	333 ab	
	3	7 c	520 a	826 a	
Border Leicester	1	499 a	213 ab	408 ab	
	2	228 a	166 bc	51 c	
	3	337 a	186 ab	1132 a	

Remarks.

- 1): Mean PFC/10⁷ cells at 5 days after a single injection of SRBC.
- 2): Mean PFC/10⁷ cells at 5 days after the second injection of SRBC.
- 3): Means in the same column with different letters differ significantly (p < 0.05).

Table 2. Blood groups and some immunological characters of SRBC

SRBC No.	Blood group systems						Aggluti- nation	Coomb's test	50% hemolysis	
	R-O	A	C	M	Hb	[Ke ⁺]				
S.	1	O	—	—	Mb/Mb	AB	LK	5*	5*	6.4**
	2	O	—	—	Ma/Ma	BB	HK	5	4	5.8
	3	O	—	—	Ma/Ma	BB	HK	4	5	5.9
C.	1	R	—	—	Ma/Ma	AB	HK	6	5	5.9
	2	R	Aa	—	Ma/Mb	BB	LK	5	5	6.7
	3	R	—	—	Ma/Mb	AB	LK	4	5	6.5
RM.	1	i	Aa	Ca	Mb/Mb	AB	LK	4	4	6.4
	2	i	—	—	Mb/Mb	BB	LK	5	4	6.7
	3	i	—	—	Mb/Mb	BB	LK	5	5	7.4
BL.	1	R	—	—	Ma/Mb	AB	LK	5	5	6.1
	2	R	—	—	Ma/Mb	AB	LK	6	5	6.2
	3	R	—	—	Mb/Mb	BB	LK	4	5	6.2

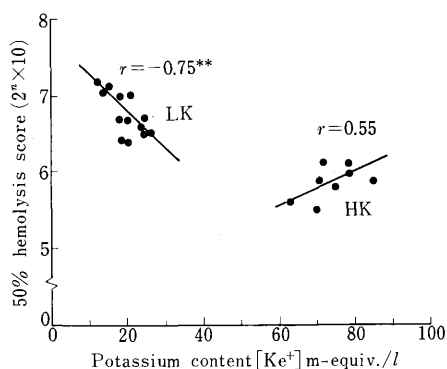
Remarks.

* : The scores were expressed by $\log_2 \frac{\text{titer}}{10}$.** : The scores were expressed by $\log_2 \frac{\text{titer}}{100}$.Hb : Hemoglobin type. [Ke⁺] : Potassium type.

S. : Suffolk. C. : Corriedale. RM. : Romny Marsh.

BL : Border Leicester.

Fig. 1. Relationship between 50% hemolysis score and potassium content of erythrocytes in Suffolk breeds



Remarks.

- LK=low potassium type.
- HK=high potassium type.
- ** : $p < 0.01$.

but no correlation between blood groups and the agglutinability of SRBC was found. SRBC could not be grouped into two categories of high and low agglutinability as found in bovine erythrocytes [2, 6].

3. Susceptibility of SRBC to immune hemolysis

Fifty percent hemolytic score of SRBC to anti-SRBC were determined by the method described elsewhere. As shown in Fig. 1, the scores of high potassium (HK) type SRBC were significantly lower than those of low potassium (LK) type SRBC. Within the LK type of SRBC, a significant negative correlation between 50% hemolysis score and potassium content of red cells was also observed. These observations agree with the results reported Bell et al. [1]. From the result, it is recommended to use the same potassium type of SRBC through in an experiment.

4. Rate of SRBC-rosette formation to lymphocytes

SRBC-rosette formation by human lymphocytes were examined by the method of Yada and Tachibana [8]. As shown in Table 3, Suffolk SRBC gave a significantly

Table 3. SRBC-rosette formation rate by a fixed human lymphocyte preparation

Breed	Number of animals	SRBC-rosette formation	
		Untreated	Neuraminidase-treated ¹⁾
Corriedale	7	36.6 ± 10.4 ²⁾	55.9 ± 2.9
Suffolk	19	48.6 ± 8.7 ^{**}	54.7 ± 7.1

Remarks.

- 1) : 1 ml of 1% SRBC was incubated with 10 units of neuraminidase (Behringwerke) at 37°C for 1 hr, then the cells were washed 3 times with saline.
- 2) : Mean % of rosette-forming cells ± SD.
- ** : $p < 0.01$.

higher rate of rosette formation than Corriedale SRBC when the same human lymphocytes preparation was used. However, neuraminidase treatment of SRBC increased the rate of rosette formation and the treated SRBC became less variable in reactivity. This result suggested that some antigenic properties of SRBC may depend on the sialic acid contents of the cells. No correlation was found between the rosette formation rate and blood group antigens of SRBC.

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