Differences in expression density and molecular weight of CR1-Like in erythrocytes of landrace swine

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Background. Porcine erythrocytes express complement receptor 1-like (CR1-like), which is involved in immune adherence.

Methods. In this study, porcine erythrocyte samples were collected from fifty-five individual Landrace swine to characterize differences in porcine CR1-like. Flow cytometry analysis was performed to examine the porcine differences in CR1-like expression density and immunoprecipitation, SDS-PAGE and Western blot were performed to detect variations in porcine CR1-like molecular weights.

Results. Different mean fluorescence intensities (MFI) of porcine erythrocytes were identified in three groups as 33.016±2.889 (40.0%), 59.974±9.299 (45.5%) and 131.241±8.375 (14.5%). Under reduced condition, three porcine CR1-like molecular weight variants were identified as 85.280±0.935 kDa (9.09%), 123.939±2.752 kDa (14.55%) and 136.696±2.028 kDa (76.36%).

Discussion. CR1-like was dispersed on the surface of porcine erythrocytes and promoted immune adherence. There have been no reports on whether differences in the expression levels and/or molecular weights of CR1-like in erythrocytes represent diversity in different individuals, and if so, whether this diversity influences the immune adherence of erythrocytes and/or whether the diversity is associated with CR1-like polymorphisms. At present, five candidate genes that are related to the differences above were found. Research examining erythrocyte immune adherence and CR1-like genes is under way. These results will provide theoretical data for future studies of the immunological mechanism of CR1-like in porcine erythrocytes.

Keywords CR1-like on porcine erythrocytes, Expression level, Molecular weight, Differences

Differences in Expression Density and Molecular Weight of CR1-Like in Erythrocytes of Landrace Swine

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ABSTRACT

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INTRODUCTION

Studies in humans revealed erythrocyte complement receptor 1 (E-CR1) density polymorphisms, which vary by ethnic group (*Panda, Ravindran & Das, 2016*). Three polymorphic phenotypes, related to the expression levels of CR1 on erythrocytes, exist in Caucasian (*Opi et al., 2016*) subjects. CR1 expression is high in HH homozygotes (approximately 1,000 molecules/cell), moderate in HL heterozygotes, and low in LL (approximately 100 molecules/cell). However, CR1 density polymorphisms on erythrocytes have not been found in African subjects (*Schmidt, Kennedy & Tham, 2015*). There are four isoforms of human E-CR1 of different molecular weights: CR1-C (160 kDa), CR1-A (190 kDa), CR1-B (220 kDa), and CR1-D (250 kDa) (*Moulds, Reveille & Arnett, 1996*). The isoforms result from single nucleotide polymorphisms in the exons of CR1 (*Hansson et al., 2013*). It has been suggested that this type of mutation eliminates CR1 activity and is associated with malaria infection and certain inflammatory diseases *(Mahmoudi et al., 2015*).

In a previous study by our group, CR1-like was found to exist on the membranes of porcine erythrocytes (*Yin et al., 2015a*). In addition, results of blocked assay suggested that E-CR1-like functions were similar to those of the C3b receptor (*Yin et al., 2015a*). Restricted by previous experimental conditions, the size of E-CR1-like was estimated to be between 135 kDa and 140 kDa (*Yin et al., 2015a*). However, to the best of our knowledge, data on differences in E-CR1-

like expression have not been reported. In the current theexpression levels and molecular weights of CR1-like on erythrocyte membranes were measured. In addition, variations in CR1-like expression was characterized using flow cytometry, immunoprecipitation, SDS-PAGE and Western blot. The data obtained from this study will be used for future in-depth analyses of the immune mechanisms mediated by CR1-like on porcine erythrocytes.

MATERIALS & METHOD

Animal ethics

All experimental procedures involving animals were approved by the Animal Science and Veterinary Medicine Ethics Committee of Shanxi Agricultural University, China and they strictly adhered to the international biomedical research guidelines (CIOMS and ICLAS, December 2012).

Experimental animals

Fifty-five Landrace swine (100 days old), which were individually marked with a number from one to fifty-five, were used. Animals were allowed free access to food and water and were reared at room temperature under normal conditions. The experiments were conducted at a pig farm in Taigu County, Jinzhong City, Shanxi Province, China.

Experimental material

The peripheral blood lymphocyte separation medium was obtained from TBD (China). The mouse anti-pig CR1-like monoclonal antibody (mAb) was prepared by our laboratory (Patent number: ZL201410308534.0) (*Yin et al., 2015b*). The other antibodies used included mouse IgG_1 isotype antibody and DyLight® 488-conjugated goat anti-mouse IgG (Abcam, UK) and horseradish peroxidase-conjugated goat anti-mouse IgG (Jackson Immuno Research, USA). NP-40 erythrocyte lysis buffer and BCA Protein Assay Kit were obtained from Solarbio (China).

Blood sample preparation

Sterile blood was drawn from the anterior vena cava (5 mL/animal). Erythrocytes were separated using peripheral blood lymphocyte separation medium. Erythrocyte suspensions (3.0×10^8 /mL)

were prepared in 0.5% IgG-free BSA in phosphate buffer (PBS) and were equally divided into two vials for subsequent use (*Yin et al., 2015b*).

Flow cytometry analysis of erythrocytes

The erythrocyte suspension from each animal was divided into 3 equal parts, labeled a, b, and c. In group a, 0.5 mL of the original CR1-like McAb solution was added to 1 mL of erythrocyte suspension (3.0×10^8/mL) and the samples incubated at 37°C for 1 h. The samples were then washed three times with 0.5% IgG-free BSA/PBS, resuspended in 1 mL 0.5% IgG-free BSA/PBS and centrifuged at 1,000 rpm for 5 min. A 0.5 mL volume of DyLight® 488-conjugated goat anti-mouse IgG solution was then added to the samples and the above testing procedures were repeated. The control groups, b and c, were used simultaneously for the assay. In group c, the erythrocyte suspension was incubated with 0.5 mL of mouse IgG_1 isotype antibody solution and above testing procedures were repeated. In group b, only 0.5 mL of 0.5% IgG-free BSA/PBS was added to the erythrocyte suspension and incubated at 37°C for 1 h. Finally, samples were analyzed by flow cytometry and each sample was read three times.

Immunoprecipitation of CR1-like

Immunoprecipitation was performed as previously described (*Yin et al., 2015a*). Erythrocyte suspensions (5 mL) were prepared from blood, adjusted to a density of 3.0×10^8/mL and mixed well with NP-40 erythrocyte lysis buffer. The samples were stored at 4°C for 30 min and then centrifugated at 15,000 rpm for 30 min. Pellets were washed with PBS, dissolved in 2% SDS, and resuspended in TCA/acetone. The samples were stored at -20°C overnight, then centrifuged at 15,000 rpm for 1 h at 4°C. Supernatants were discarded and the pellets were washed with pre-cooled acetone, followed by centrifugation at 15,000 rpm for 5 min at 4°C. The final protein pellets were dissolved in 2% SDS, aliquoted, and stored at -80°C until later use. Latex beads (100 µL) were resuspended in 0.1 M MES, to which mouse anti-pig CR1-like mAb (10 µL) and 10 mg/mL EDAC (50 µL) were sequentially added. The pH of the suspension was adjusted to 6.5, and then vortexed. The suspension was stored at room temperature for 15 min, then washed with MES buffer solution and centrifuged at 10,000 rpm for 5 min. The washing step was then repeated. The latex beads were resuspended in MES buffer solution and 5% IgG-free BSA

solution was added. The suspension was again stored at room temperature for 30 min and the latex beads were then washed with MES buffer (at 10,000 rpm for 5 min) and then resuspended for later use. IgG-free BSA/PBS (0.5%) was added to the latex beads and the suspension incubated at room temperature for 30 min. The latex beads were then washed twice with MES buffer, centrifuged at 10,000 rpm for 5 min and resuspended with 500 μL MES buffer for later use. Erythrocyte membrane protein solution (1 mL) from each porcine sample was subsequently mixed with the same volume of the latex bead suspension and incubated at 4°C overnight. Samples were washed twice with MES (10,000 rpm for 5 min) for later use. The beads were subsequently resuspended in elution buffer, washed twice (10,000 rpm for 5 min) and the supernatants collected. The pellets were repeatedly washed twice with the elution buffer, and the supernatant was collected, combined, concentrated by ultrafiltration, and aliquoted for later use.

SDS-PAGE and Western blot analysis of CR1-like

A BCA Protein Assay Kit was used to determine the concentration of CR1-like; the final protein concentration of CR1-like was adjusted to 25 mg/mL. Each sample were repeated three times for each individual and were subjected to reducing SDS-PAGE. Stacking gels (5%) and resolving gels (8%) were prepared and the individual samples were mixed with the reducing sample buffer for loading onto the gels. Electrophoresis was performed at 80 V for 30 min, followed by 120 V for 2 h. Electrophoretic transfer of proteins from each gel to the membrane was conducted at 60 V for 2.5 h. The membrane was immersed in blocking solution for 2 h, then placed in a hybridization bag along with the anti-porcine CR1-like mAb for incubation overnight at 4°C. The membranes were then washed three times with TBST for 10 min and then incubated with TBST-diluted horseradish peroxidase-goat anti-mouse IgG (Jackson ImmunoResearch) in a new hybridization bag for 1 h at 37°C. The membrane was then washed 3 times and each time for 10 min with TBST. The chemiluminescence solution was evenly applied to the PVDF membranes, which were then exposed to film in a dark room. The results were scanned and saved. Alternatively, gels were stained with Coomassie Brilliant Blue for 20 min, then scanned. A Vernier callipers was used to measure the migration distance of the molecular weight markers in order to construct a molecular weight standard curve. The migration distance of each CR1-like protein band was measured and the molecular weight calculated and recorded.

156 **Statistical analysis**

157 Fluorescence values and the molecular weights for CR1-like protein were detected in 55
158 individuals. Sample normality was determined using a one-sample Kolmogorov-Smirnov test.
159 Statistical significance was determined using a one-sample *t*-test. Statistical analysis was
160 performed using SPSS Statistics V21.0 (IBM).

161

162 **RESULTS**

163 **Difference analysis of porcine CR1-like expression density**

164 Histograms representing results of flow cytometry from groups a, b and c are shown in Fig.1B, C,
165 and D. The mean fluorescence intensity (MFI) of group a was significantly higher compared
166 with groups b and c ($P<0.01$) as shown in Fig.1A and Table 1, indicating that CR1-like exists on
167 porcine erythrocyte membranes as was found in a previous study (*Yin et al., 2015a*).

168 The normal distribution for the MFI data is shown in Table 2. The K-S test indicated that
169 the MFI data did not follow a normal distribution for group a, though the MFI data did follow a
170 normal distribution for groups b and c. Therefore, the MFI of group a is represented by the
171 median value (50^{th} percentile, P_{50}).

172 Next, the fluorescence intensity was analysed by *t*-test. The population mean value (PMV)
173 of E-CR1-like fluorescence intensity from the 55 individuals was 59.557±33.153. The MFI of E-
174 CR1-like was poorly represented by the PMV due to the large discrete level (*SD*=33.153). A
175 scatter diagram of the MFI was generated by SPSS Statistics V21.0 (IBM) and is shown in Fig. 2.
176 E-CR1-like fluorescence intensities of the 55 individuals were grouped into three intervals. The
177 [A–B] interval included 22 individuals, the [B–C] interval included 25 individuals, and the [C–D]
178 interval included 8 individuals (Table 3).

179 A one-sample *t*-test was performed using the population mean value (PMV) (59.557) as the
180 test value, and 95% confidence intervals (CI) were added. For interval [A–B], $PMV_{[A–B]}$
181 =33.016±2.889, $P<0.05$ (*Sig.*=.000). Therefore, the $PMV_{[A–B]}$ of the E-CR1-like MFI from 22
182 individuals should be 33.016±2.889. Similarly, the $PMV_{[C–D]}$ =131.241±8.375, $P<0.05$

(*Sig.*=.000), and the $PMV_{[C–D]}$ the eight individuals was 131.241±8.375. In contrast, $PMV_{[B–C]}$ =59.974±9.299, $P>0.05$ (*Sig.*=0.824), thus the overall PMV (59.5571) was very close to the mean value of the twenty-five individuals (Tables 3 and 4).

Taken together, the data obtained by flow cytometry demonstrate that the E-CR1-like expression levels were different among the 3 groups as follows: 33.016±2.889(40.0%), 59.974±9.299 (45.5%), 131.241±8.375 (14.5%).

Variations analysis of molecular weight of porcine CR1-like

Erythrocyte membrane protein samples were prepared from all 55 individual swine. SDS-PAGE and Western blot analysis were performed to examine diversities in molecular weight of porcine E-CR1-like under reduced conditions. It was previously suggested that the CR1-like on porcine erythrocytes contained at least one disulfide bond and three bands were observed (at 70 kDa, 50 kDa, and 15 kDa) via SDS-PAGE. Of these, two (50 kDa, 15 kDa) were further analyzed by Western blot under reduced conditions and the antigen binding sites were found to be in the 50 kDa and 15 kDa peptide fragments (*Yin et al., 2015a*). In this study, three main protein bands were detected via SDS-PAGE under reduced conditions, in 42 of the 55 samples, at approximately 70 kDa, 50 kDa and 15 kDa (Fig. 3a). Two positive bands were also observed via Western blot at approximately 50 kDa and 15 kDa (Fig. 3b). In 8 of the 55 samples, two main protein bands were detected by SDS-PAGE at approximately 70 kDa and 50 kDa (Fig. 3c) and one positive band was detected at approximately 50 kDa by Western blot (Fig. 3d). In 5 of the 55 samples, two main protein bands were detected under reduced conditions by SDS-PAGE at approximately 70 kDa and 15 kDa (Fig. 3e) and one positive band was detected at 15 kDa by Western blot (Fig. 3f).

A standard curve was generated based on the molecular weights of the marker proteins (a rrepresentative curve is shown in Fig. 4). The standard curves of the suspension array and the corresponding coefficients of determination (R^2) were good (R^2> 0.987). The molecular weights (M_rs) of porcine CR1-like from 55 individuals were calculated and subjected to normality analysis via *K-S* test, z =2.345, $P<0.05$ (*Sig.*=.000) (Table 5). The population mean value (PMV) of the porcine CR1-like M_rs was 130.166±15.181 (Table 5). A scatter diagram of the molecular weights, generated using SPSS Statistics V21.0 (IBM) to observe the distribution of CR1-like M_rs, is shown in Fig. 5. The E-

CR1-like M_rs of the 55 individuals were grouped into three intervals. The [a–b] interval included 5 individuals, the [b–c] interval included 8 individuals and the [c–d] interval included 42 individuals (Table 6).

The E-CR1-like M_rs were then analyzed using a one-sample *t*-test. The *t*-test was performed using the PMV (130.166±15.181) as the test value, and 95% CI were added. For interval [a–b], the $PMV_{[a–b]}$ =85.280±0.935 kDa, $P<0.05$ (*Sig.*=000). Similarly, for interval [b-c], the $PMV_{[b–c]}$ =123.939±2.752 kDa, $P<0.05$ (*Sig.*=000). In contrast, for interval [c-d], the $PMV_{[c–d]}$ =136.696±2.028 kDa, $P>0.05$ (*Sig.*=0.824), thus the $PMV_{[c–d]}$ and overall PMV (=130.166±15.181) were very close (Tables 6 and 7).

The data demonstrated that the molecular weights of porcine E-CR1-like were distributed as follows: 85.280±0.935 kDa (9.09%), 123.939±2.752 kDa (14.5%), 136.696±2.028 kDa (76.36%).

DISCUSSION

The erythrocyte rosette test and flow cytometry were previously used to study chicks artificially inoculated with infectious bursal disease virus, swine naturally infected with eperythrozoon, as well as mice and rats infected with adjuvant arthritis (*Li et al., 2006*; *Li, Reeve-Johnson, Wang, 2007*; *Ma et al., 2003*; *Yang et al., 2009*). It was also discovered that E-CR1-like is dispersed on the surface of porcine erythrocytes and promotes immune adherence (*Yin et al., 2015a*; *Sun et al., 2012*). After erythrocytes were opsonized by exogenous antigens and fresh serum, the plasma membrane fluidity increased and the distribution of E-CR1-like changed from dispersed to clustered, resulting in multivalent binding to C3b-IC (*Yin et al., 2015b*). However, to the best of our knowledge, there was no regarding differences in expression levels and molecular weights of CR1-like on porcine erythrocytes.

In this study, the expression levels of E-CR1-like in Landrace swine E-CR1-like were varied. Among the 55 individuals tested, 22 fluorescence intensity detection values were significantly lower (33.016±2.889) and 8 fluorescence intensity values were significantly higher (131.241±8.375) than the population mean value. Accordingly, the expression of CR1-like on the porcine erythrocytes was different under natural conditions. In addition, E-CR1-like molecular weights were variable among the 55 individuals as analyzed by SDS-PAGE under reduced

conditions. A total of 42 individuals were A type (approximately 70 kDa, 50 kDa, 15 kDa), whereas 8 individuals were missing the 15 kDa active band, and 5 individuals were missing the 50 kDa active band. Consequently, as the calculated results show, the M_rs of E-CR1-like were 85.280±0.935 kDa (9.09%), 123.939±2.752 kDa (14.55%), and 136.696±2.028 kDa (76.36%) among the 3 groups. Therefore, the difference in CR1-like expression levels and molecular weights might affect the immune adherence functions of porcine erythrocytes. Furthermore, the diversity in CR-1-like expression and molecular weight may be related to the CR1-like polymorphism in erythrocytes. Recently, our research has focused on the CR1-like gene and 5 candidate genes associated with the above diversity have been found. Research on immune adherence function, the effects of CR1-like genes on erythrocytes, and the effects of the absence of the CR1-like active fragments on adhesion activity is ongoing.

CONCLUSIONS

Porcine erythrocyte CR-1-like expression and molecular weight is different in different individuals. The data obtained in this study provide a basis for investigating the molecular mechanisms governing the immune adherence function of porcine E-CR1-like.

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ADDITIONAL INFORMATION AND DECLARATIONS

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Competing Interests

The authors declare no competing financial interests.

Author Contributions

● Hongquan Li designed all the experiments, contributed reagents/materials/analysis tools, performed and reviewed the manuscript.

● Jingjing Zhang and Wei Yin performed the experiments, wrote the main manuscript text, prepared figures and/or tables and reviewed the manuscript.

● Chun Wang, Ruipu Jia, Kuohai Fan, Na Sun and Yaogui Sun performed the experiments and reviewed the manuscript.

Data Availability

The following information was supplied regarding data availability.

The research in this article did not use, analyze, or collect raw data.

REFERENCES

Hansson, HH, Kurtzhals JA, Goka BQ, Rodriques OP, Nkrumah FN, Theander TG,Bygbjerg IC, Alifrangis M. 2013. Human genetic polymorphisms in the Knops blood group are not associated with a protective advantage against Plasmodium falciparum malaria in Southern Ghana. *Malaria Journal* **12:** 400–408.

Li HQ, Wu CH, Gao H, Song JD. 2006. Effect of astragalus polysaccharides on immune function of erythrocytes of chickens infected with IBDV. *Veterinary Science in China* **36:**

74–79.

Li HQ, Reeve-Johnson L, Wang JD. 2007. Effect of Astragalus polysaccharides on Erythrocyte Immune Adherence of Chickens Inoculated with Infectious Bursal Disease Virus. *Journal of Integrative Agriculture in China* **6:** 1402–1408.

Ma HL, Han HY, Wang S, Zheng MX, Li HQ, Ning GB. 2003. Effect on erythrocyte of swine with natural infection of Eperythrozoon suis. *Chinese Journal of Preventive Veterinary Medicine* **25:** 44–49.

Mahmoudi R, Kisserli A, Novella JL, Donvito B, Dramé M, Réveil B, Duret V, Jolly D, Pham BN, Cohen JH. 2015. Alzheimer's disease is associated with low density of the long CR1 isoform. *Neurobiology of Aging* **36:** 1766.e5–1766.e12. DOI 10.1016/j.neurobiolaging.2015.01.006.

Moulds JM, Reveille JD, Arnett FC. 1996. Structural polymorphisms of complement receptor 1 (CR1) in systemic lupus erythematosus (SLE) patients and normal controls of three ethnic groups. *Clinical & Experimental Immunology* **105:** 302–305. DOI 10.1046/j.1365-2249.1996.d01-748.x.

Opi DH, Uyoga S, Orori EN, Williams TN, Rowe JA. 2016. Red blood cell complement receptor one level varies with Knops blood group, α(+) thalassaemia and age among Kenyan children. *Genes & Immunity* **17:** 171–178. DOI 10.1038/gene.2016.2.

Panda AK, Ravindran B, Das BK. 2016. CR1 exon variants are associated with lowered CR1 expression and increased susceptibility to SLE in a Plasmodium falciparum endemic population. *Lupus Science & Medicine* **3:** e000145. DOI 10.1136/lupus-2016-000145.

Schmidt CQ, Kennedy AT, Tham WH. 2015. More than just immune evasion: Hijacking complement by Plasmodium falciparum. *Molecular Immunology* **67:** 71–84. DOI 10.1016/j.molimm.

Sun YG, Yin W, Fan XF, Fan KH, Jiang JB, Li HQ. 2012. The Cytological Observation of Immune Adherence of Porcine Erythrocyte. *Cell Communication & Adhesion* **19:** 79–84. DOI 10.3109/15419061.

316 **Yang LH, Qiu JD, Li HQ. 2009.** Effects of Astragalus heteropolysaccharides on
317 erythrocyte immune adherence function of mice with adjuvant-induced arthritis. *Acta*
318 *Pharmaceutica Sinica* **44:** 1364–1370.

319 **Yin W, Cui JY, Jiang JB, Zhao JX, Fan KH, Sun N, Wang ZR, Sun YG, Ma HL, Li**
320 **HQ, 2015a.** The immune adherence receptor CR1-like existed on porcine erythrocytes
321 membrane. *Scientific Reports* **5:** 13290–13296. DOI 10.1038/srep13290.

322 **Yin W, Xue YP, Jiang JB, Fan KH, Sun N, Sun YG, Ma HL, Li BG, Wang ZW, Li HQ,**
323 **2015b.** Initial study of CR1-like expressed on porcine erythrocytes surface. *Veterinary*
324 *Science in China* 9: 985–990. DOI 10.16656/j.issn.

325

Figure 1

The fluorescence intensity of CR1-like contrast between groups (a, b and c) erythrocytes.

(A) The population mean value of CR1-like from group a compare with group b and c. (B) The flow cytometry histogram of group a ; (C) The flow cytometry histogram of group b; (D) The flow cytometry histogram of group c (The flow cytometry histograms of Fig. 1B, 1C and 1D are one group of all histograms).

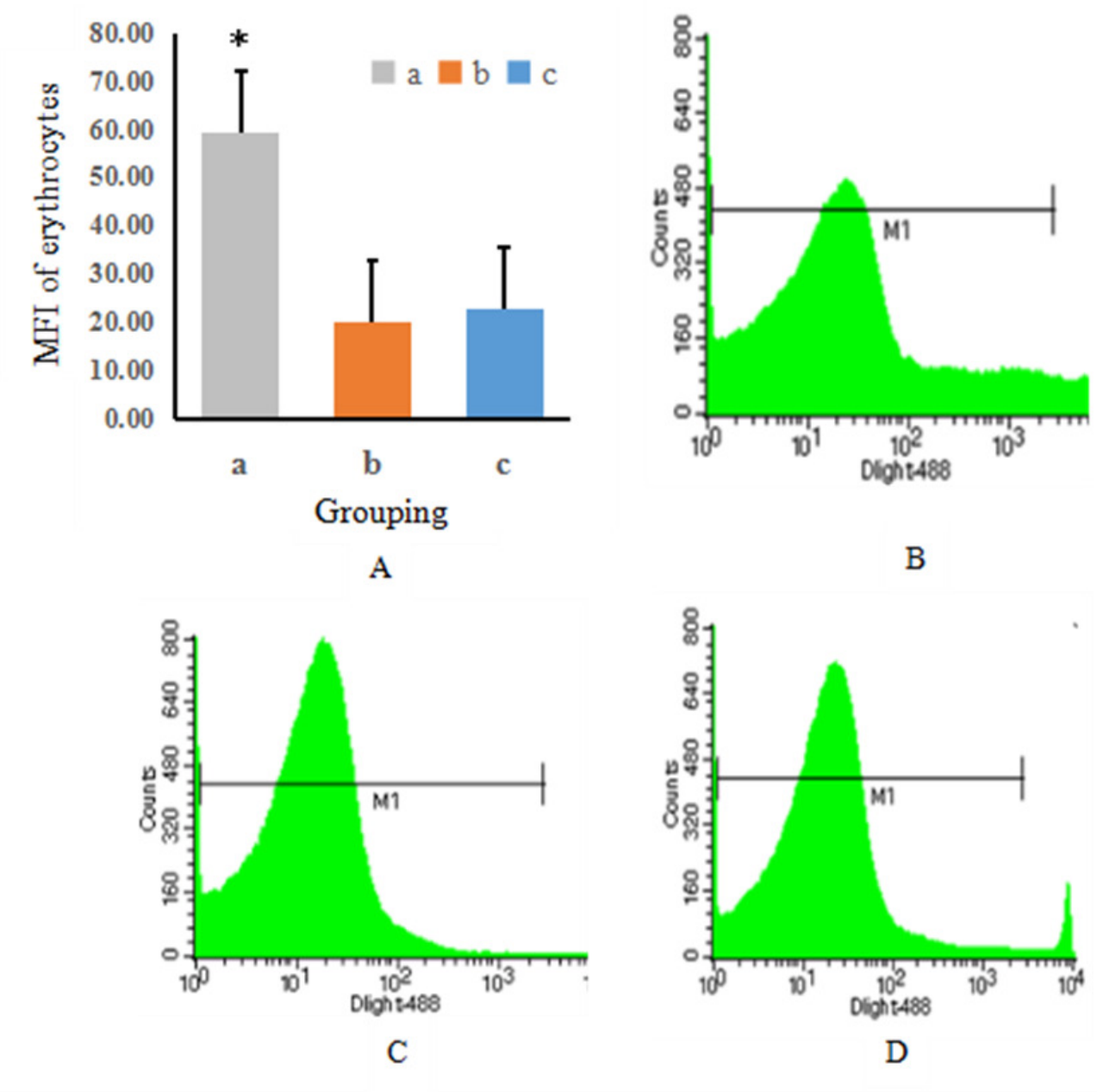

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Figure 2

The fluorescence intensity of CR1-like on porcine erythrocytes grouped broadly into 3 intervals([A-B], [B-C], [C-D]).

Each circle represented one individual, plotted by MFI on the horizontal axis and individual number on the vertical; A, B, C and D represented interval codes.

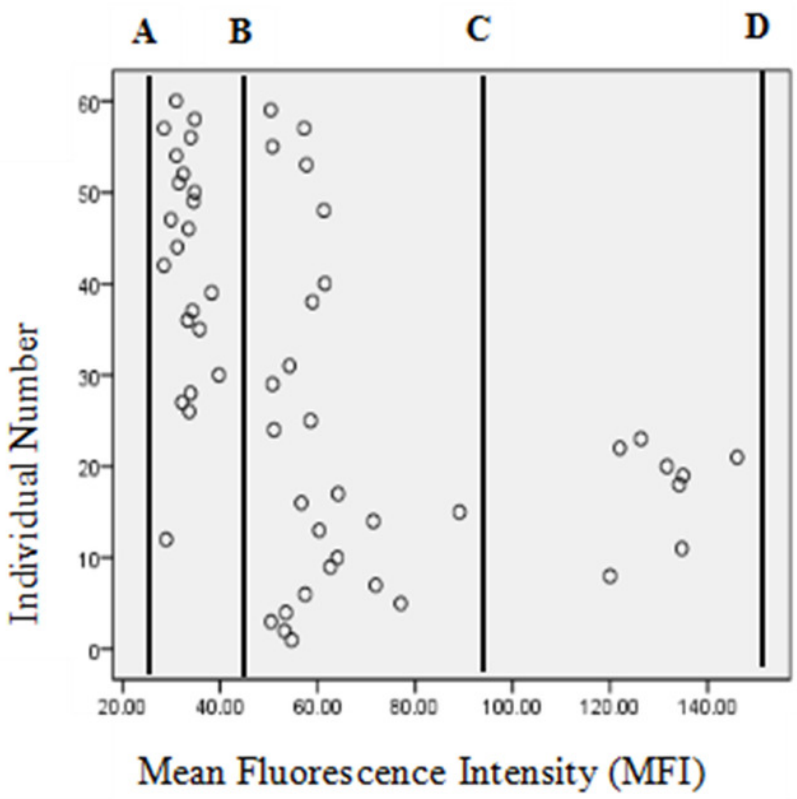

Fig. 2 The fluorescence intensity of CR1-like on porcine erythrocytes grouped broadly into 3 intervals([A-B], [B-C], [C-D]). Each circle represented one individual, plotted by MFI on the horizontal axis and individual number on the vertical; A, B, C and D represented interval codes.

Figure 3

The representative samples' bands of CR1-like on erythrocytes by SDS-PAGE(a, c and e) and western blot(b, d and f).

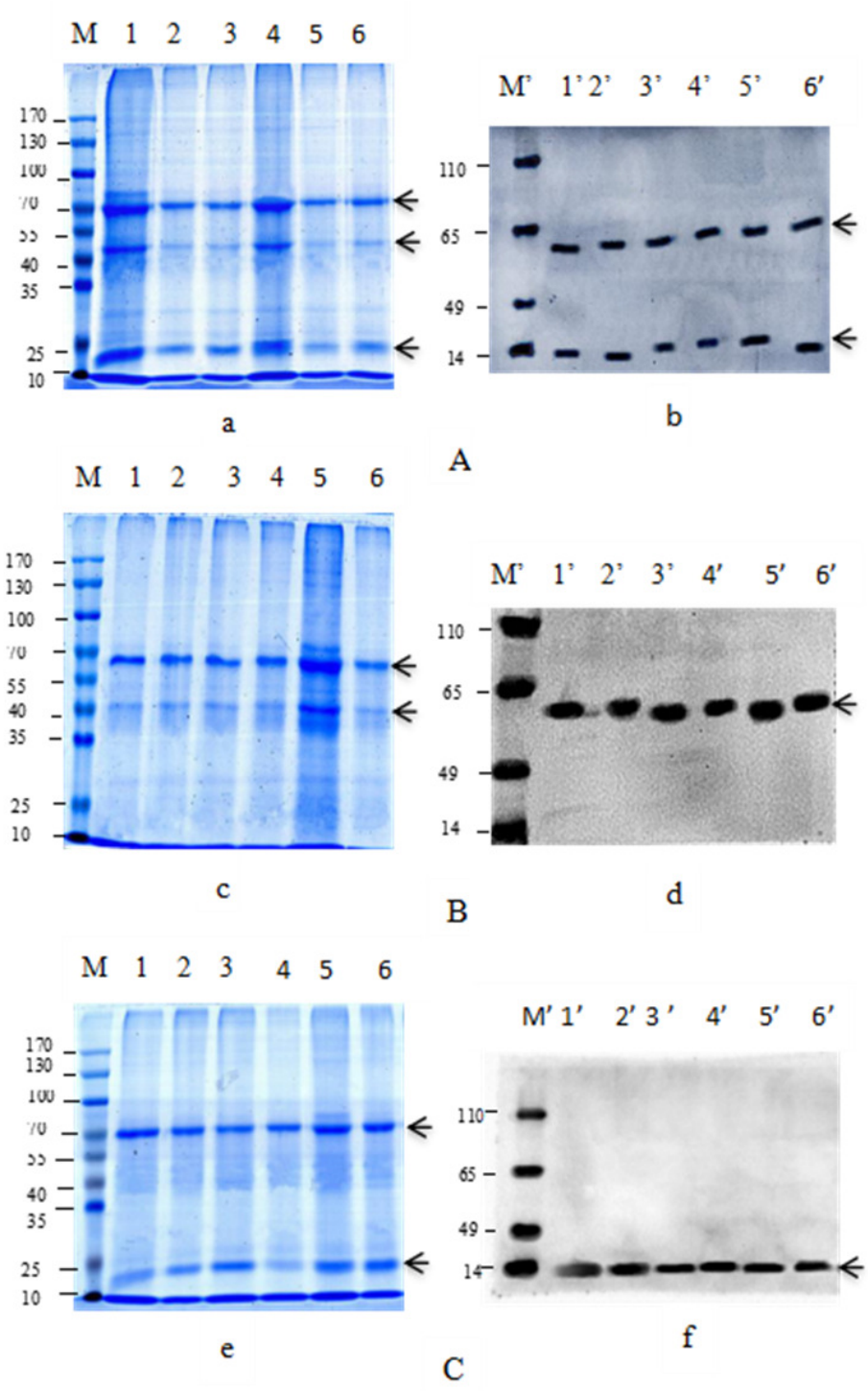

Fig. 3 The representative samples' bands of CR1-like on erythrocytes by SDS-PAGE(a, c and e) and western blot(b, d and f). M, SDS–PAGE protein markers. Themolecular weights of markers (kDa) were 10, 25, 35, 40, 55, 70, 100, 130, and 170 kDa from bottom to top. M', protein markers shown on the film after exposure. The molecular weights of markers were 14, 20, 35, 49, 60, 65, and 110 kDa from bottom to top, and only the bands about 14, 49, 65, and 110 kDa appeared in the exposure blots, others couldn't appear in the blots. Line 1-6 showed the porcine ECR1-like bands were picked randomly from all bands by SDS-PAGE, and line 1'- 6' showed the corresponding CR1-like bands by western blot.

Figure 4

The representative samples' molecular weight standard curve based on the marker proteins.

Distence (cm), the migration distance of the protein marker's molecular weight. Marker (kDa) showed the molecular weights of the protein marker.

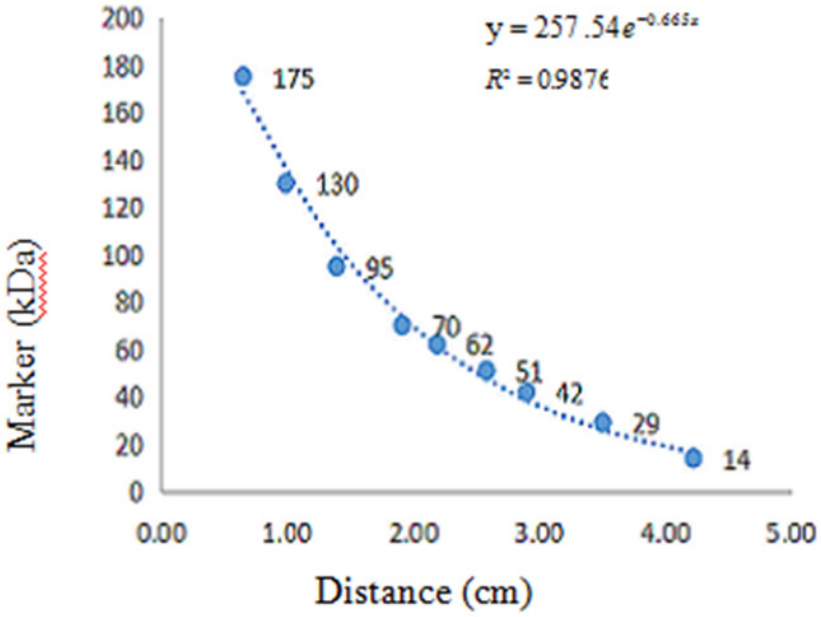

Fig. 4 The representative samples' molecular weight standard curve based on the marker proteins. Distence (cm), the migration distance of the protein marker's molecular weight. Marker (kDa) showed the molecular weights of the protein marker.

Figure 5

The molecular weight of CR1-like on porcine erythrocytes grouped broadly into 3 intervals([a-b], [b-c], [c-d]).

Each circle represented one individual, plotted by molecular weight (kDa) on the horizontal axis and individual number on the vertical; a, b, c and d represented interval codes.

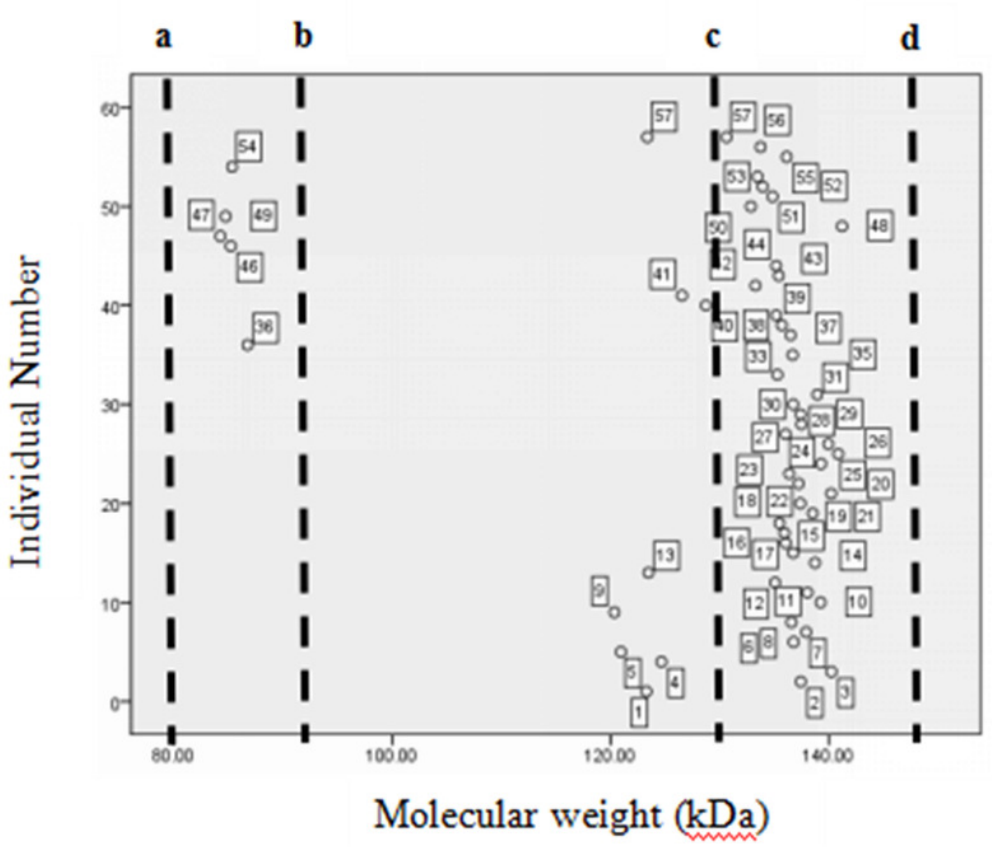

Fig. 5 The molecular weight of CR1-like on porcine erythrocytes grouped broadly into 3 intervals([a-b], [b-c], [c-d]). Each circle represented one individual, plotted by molecular weight (kDa) on the horizontal axis and individual number on the vertical; a, b, c and d represented interval codes.

Table 1(on next page)

The comparison for the expression of CR1-like on porcine erythrocytes.

Table 1: The comparison for the expression of CR1-like on porcine erythrocytes

	(I) Grouping	(J) Grouping	Differences of mean value (I-J)	Standard error	*Sig.*	95% confidence intervals	
						Floor limit	Upper limit
LSD	a	b	39.420*	3.660	.000	32.192	46.648
		c	36.672*	3.660	.000	29.445	43.900
	b	a	-39.420*	3.660	.000	-46.648	-32.193
		c	-2.748	3.660	.454	-9.975	4.480
	c	a	-36.672*	3.660	.000	-43.900	-29.445
		b	2.748	3.660	.454	-4.480	9.975

The dependent variable: fluorescence intensity.

* The significance level of the mean difference is 0.05.

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Table 2(on next page)

One-sample Kolmogorov–Smirnov test for the expression level of CR1-like on porcine erythrocytes

Table 2: One-sample Kolmogorov–Smirnov test for the expression level of CR1-like on porcine erythrocytes

		Fluorescence intensity		
	N	55	55	55
Normality parameters[a,,b]	Mean value	59.557	20.137	22.885
	Standard deviation	33.153	2.148	1.201
Extreme differences	Absolute value	0.226	0.134	0.125
	Positive	0.226	0.134	0.125
	Negative	−0.174	−0.063	−0.077
Kolmogorov–Smirnov Z		1.676	0.993	0.930
Sig.		0.007	0.277	0.353

[a] A normal distribution was suggested by the test.

[b] Results were calculated based on these data.

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Table 3(on next page)

Samples distribution for the expression of CR1-like on erythrocytes in each interval (n = 55)

Table 3: Samples distribution for the expression of CR1-like on erythrocytes in each interval (n = 55)

	N	Individual number	Mean	Standard deviation	Standard error
Fluorescence intensity	22	12, 26, 27, 28, 30, 32, 33, 34, 36, 38, 42, 44, 46, 47, 49, 50, 51, 52, 54, 56, 57, 58	33.016	2.889	0.616
	25	1, 2, 3, 4, 5, 6, 7, 9, 10, 13, 14, 15, 16, 17, 24, 25, 29, 31, 35, 37, 41,48, 53, 55, 59	59.974	9.299	1.860
	8	8, 11, 18, 19, 20, 21, 22, 23	131.241	8.375	2.961

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Table 4(on next page)

The sample *t* test for the expression level of CR1-like on erythrocytes

Table 4: The sample *t* test for the expression level of CR1-like on erythrocytes

	t	df	*Sig.*	Differences of mean value	95% confidence intervals	
					Floor limit	Upper limit
Fluorescence intensity	−43.086	21	.000	−26.541	−27.822	−25.260
	.224	24	.824	0.417	−3.421	4.256
	44.325	7	.000	131.241	124.240	138.243

Test value = 59.557;

df showed the degree of freedom about n-1;

Sig. showed the *P*-value.

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Table 5(on next page)

One-sample Kolmogorov–Smirnov test for the molecular weight of CR1-like on porcine erythrocytes

Table 5: One-sample Kolmogorov–Smirnov test for the molecular weight of CR1-like on porcine erythrocytes

		Molecular weight
	N	55
Normality parameter[a,,b]	Mean	130.166
	Standard deviation	15.181
Extreme differences	Absolute value	0.316
	Positive	0.233
	Negative	−0.316
Kolmogorov–Smirnov Z		2.345
Sig.		.000

[a] A normal distribution was suggested by the test.

[b] Results were calculated based on these data.

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Table 6(on next page)

Sample distribution for the molecular weight of CR1-like on erythrocytes in each interval (n = 55)

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Table 6: Sample distribution for the molecular weight of CR1-like on erythrocytes in each interval (n = 55)

	N	Individual number	Mean (kDa)	Standard deviation	Standard error
Molecular weight (kDa)	42	2, 3, 6, 7, 8, 10, 11, 12, 14, 15, 16, 17, 18, 19, 20, 21, 22, 23, 24, 25, 26, 27, 28, 29, 30, 31, 33, 35, 37, 38, 39, 42, 43, 44, 48, 50, 51, 52, 53, 55, 56, 58	136.696	2.281	0.352
	8	1, 4, 5, 9, 13, 40, 41, 57	123.939	2.752	0.973
	5	36, 46, 47, 49, 54	85.280	0.935	0.418

Table 7 (on next page)

The sample *t* test for the molecular weight of CR1-like on erythrocytes

Table 7: The sample *t* test for the molecular weight of CR1-like on erythrocytes

	t	df	*Sig.*	Differences of mean value	95% confidence interval	
					Floor limit	Upper limit
Molecular weight (kDa)	1.976	41	0.055	0.695	-0.015	1.406
	−12.395	7	.000	−12.061	−14.362	−9.760
	−106.947	4	.000	−44.720	−45.881	−43.559

Test value = 130.166;

df showed the degree of freedom about n-1;

Sig. showed the *P*-value.

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