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RESEARCH ARTICLE

Determination of blood group antigens using electrophoresis of erythrocytes incubated with specific antibodies

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There is a need for specific determination of blood group antigens on erythrocytes for improvement of the quality of hemotransfusion therapy. O, A, B, AB, and weak variants of A and B antigen (O washed erythrocytes with anti-A or anti-B antibody absorbing ability) were investigated for electrophoretic mobility with immunoglobulin G complement-dependent antibodies. The contact of anti-A heated serum and complement with A erythrocytes resulted in their decreased electrokinetic potential the contrary to the serum without anti-A and anti-B antibodies and increased after addition of the serum from O blood group person with anti-A and anti-B absorbing ability. Anti-B heated serum with complement decreased electrokinetic potential of B erythrocytes. Whereas both anti-A heated serum and serum from AB person with complement did not decrease the electrokinetic potential of O erythrocytes. Anti-B heated serum, in the same manner, decreased the electrokinetic potential of O blood group erythrocytes with anti-B absorbing ability. Anti-A immunoglobulin G complement-dependent antibodies decreased the electrokinetic potential of washed O erythrocytes with anti-A absorbing ability contrary to immunoglobulin M antibodies. Evaluation of the electrokinetic potential of erythrocytes with a set of immunoglobulin G complement-dependent antibodies might be considered for blood group type detection.

KEYWORDS
antibody, antigen, charge, electrophoresis, erythrocyte

1 | INTRODUCTION

Different methods of detection of blood group antigens exist in hematology [1]. Nevertheless, hemolytic reactions still occur despite the accurate performance of standard methods of blood group detection [2]. The analysis of electrophoretic mobility (EM) of red blood cells (RBCs) reflects the presence of a negative charge on erythrocyte [3, 4] and is reduced after the treatment with the antibody, as was first demonstrated by Coulter [5] and after the addition of Coomb's reagent [6]. The first antibody is unable to project

Article Related Abbreviations: EM, electrophoretic mobility; IgG, immunoglobulin G; IgM, immunoglobulin M; RBC, red blood cell.

beyond the glycocalyx of the cell and causes a significant decrease in the negative charge [7]. The addition of a second antibody was reported to allow the positive charge to extend the glycocalyx and resulted in an effective charge reduction. In the study, we aimed to investigate the possibility of the complement penetrating the membrane in a case of antigen-antibody-specific binding. CE is an excellent technique for identifying and quantifying the contents of single cells and allows researchers to study cellular function in the areas of neuroscience, oncology, enzymology, immunology, and gene expression [8, 9]. The potential across the surface of shear of the double layer is referred to as the zeta potential (ζ) and is changed once antibody molecules are attached to the cell, making it possible to detect the attachment of antibody molecules to the surface of individual RBCs by EM measurement [10].

The aim of the study was to assess the use of electrophoresis of erythrocytes reacted with immunoglobulin G (IgG) complement-dependent anti-A and anti-B antibodies for the blood group type detection.

2 | MATERIALS AND METHODS

O, A, B, AB, and weak variants of A and B antigen (O washed erythrocytes with anti-A or anti-B antibody absorbing ability) were included in the investigation. We suspended human erythrocytes in normal saline and centrifuged them at 1000 g for 10 min. To obtain IgG antibodies the serum was heated for 30 min at 60°C. IgG antibodies with a complement of guinea pig were added to the reaction for 1 h of incubation. Briefly, 50 μl of control RBCs (blood type A) were added to 100 μl of anti-A serum with complement and incubated at 20°C, as well as to 100 μl of anti-B typing serum and serum from AB blood group type (negative controls). HEPES (4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid) and sucrose buffer pH 7.0 were used for the EM measurement. EM was evaluated by MicroMed XS-3330 microscope with a digital camera Sigeta DCM -900 9.1 Mpixels, recorded on a hard disk, and the speed of each RBC was fixed in seconds. In vitro and in vivo RBCs were reported to self-associate, giving rise to RBC 'stacks' (Rouleaux formation) [11, 12]. EM of RBCs without any surface reactions was measured. EM of 20 RBCs reacted with IgG molecules and complement was assessed to receive the mean value by observing at least 10 individual cells in each direction after polarity reversal and the standard deviation was smaller than 3 %. Statistical analysis was performed using Friedman's criterion [13]. The linear velocity is calculated by the formula: $v = S/t$, where v is the linear velocity, S is the distance passed by the erythrocyte, and t is the time during which the erythrocyte passed the distance S. The calculation of the EM was performed by taking into account the voltage and the distance between the electrodes by the formula:

$$u = \frac{v \times l}{U},$$

where u is EM, $m^2/V \times s$; v is the linear velocity; l is the distance between the electrodes; and U is voltage. To determine the electrokinetic potential (ζ-potential) of cells Smoluchowski's equation was used:

$$\xi = \frac{4 \times \pi \times \eta \times u}{\varepsilon_r},$$

where ζ is electrokinetic potential, V; η is the viscosity of the solution, Pa × s; u is EM, $m^2/V \times s$; and ε_r is the permittivity of the solution. The values of η and ε for distilled water (viscosity: 1.0016 mPa × s; relative permittivity: 80.2 [14]) were used to calculate the ζ-potential.

3 | RESULTS AND DISCUSSION

The contact of anti-A heated serum and complement with A erythrocytes resulted in a significant decrease of electrokinetic potential (from -13.88 to -3.09 mV, from -14.0 to -5.30 mV, from -10.26 to -4.77 mV). Whereas the contact with the serum from the AB blood group person (without anti-A and anti-B antibodies) did not show a decrease of electrokinetic potential (from -4.5 to -5.81 mV) (Table 1).

Anti-B heated serum and complement in the same manner significantly decreased the electrokinetic potential of B erythrocytes (from -11.26 to -5.43 mV, from -13.92 to -4.31 mV). Whereas anti-A heated serum did not decrease the electrokinetic potential of O erythrocytes (from -6.84 to -6.46 mV, from -7.52 to -8.3 mV, from -8.48 to -8.48 mV), as well as the serum from AB blood group person with complement (from -6.84 to -8.55 mV). Anti-B heated serum decreased the electrokinetic potential of O blood group erythrocytes with the anti-B absorbing ability (from -8.45 to -4.31 mV) and less decrease was observed in the reaction without complement (from -8.45 to -7.64 mV).

Moreover, anti-A heated serum with complement decreased the electrokinetic potential of O erythrocytes with the anti-A absorbing ability (from -14.58 to -6.59 mV, from -7.19 to -3.88 mV, from -9.91 to -2.78 mV, from -5.17 to -3.01 mV) on the contrary to the serum from AB blood group person (from -9.91 to -7.55 mV). The electrokinetic potential of unwashed erythrocytes with anti-A absorbing ability was not decreased after the contact with anti-A heated serum and complement (from -6.77 to -6.73 mV).

The reports of the presence of inhibitory substance able to modify the electrokinetic potential of erythrocytes in the

TABLE 1 Zeta potential of erythrocytes under the influence of specific blood group antibodies (mV, M ± SD)

Erythrocytes	Intact	IgManti-A	IgManti-A+C'	Anti-A heated+C'	Anti-B heated+C'	From AB heated+C'	Anti-A heated	Anti-BIgM	From O heated + C'
A	−13.88 ± 2.87			−3.09 ± 0.4(−6.86 ± 0.5)					
A	−13.29 ± 2.84			−5.30 ± 0.4					
A	−10.76 ± 1.72			−4.77 ± 0.3					
A	−3.03 ± 1.4					−5.81 ± 0.43			
A	−9.67 ± 1.4			−5.55 ± 0.5					
A	−7.91 ± 1.5			−4.97 ± 0.32	−6.70 ± 0.65	−6.94 ± 0.70			
B	−7.32 ± 0.7				−5.38 ± 0.44				
B	−12.79 ± 2.1				−5.57 ± 0.54				
B	−11.26 ± 1.8				−5.43 ± 0.47				
B	−7.95 ± 1.2				−6.01 ± 0.4 (tree times loading: −5.08 ± 0.32)				
B	−15.05 ± 2.0				−4.31 ± 0.42				
O with anti-A, anti-B absorbing ability	−8.17 ± 1.6	−9.85 ± 0.67	−9.85 ± 0.64	−3.88 ± 0.34					
O with anti-A, anti-B absorbing ability	−5.05 ± 0.94			−4.0 ± 0.41					
O with anti-A absorbing ability	−14.58 ± 1.8			−6.59 ± 0.44					
O with anti-A absorbing ability	−7.19 ± 1.8	−9.85 ± 0.94	−9.12 ± 0.86	−3.88 ± 0.34	−11.1 ± 2.4	−10.7 ± 2.4	−5.7 ± 0.45		

(Continues)

TABLE 1 (Continued)

Erythrocytes	Intact	IgManti-A	IgManti-A+C'	Anti-A heated+C'	Anti-B heated+C'	From AB heated+C'	Anti-A heated	Anti-BIgM	From O heated + C'
O with anti-A absorbing ability	−9.91 ± 1.1			−2.48 ± 0.23(−3.88 ± 0.31)	−4.83 ± 0.44	−7.55 ± 0.62			
O with anti-A absorbing ability	−5.17 ± 1.1			−3.0 ± 0.4					
O with anti-B absorbing ability	−8.45 ± 0.8				−4.31 ± 0.46				
O	−6.84 ± 0.94			−6.46 ± 0.56					
O	−7.52 ± 0.9			−8.3 ± 0.76					
O	−9.3 ± 1.0			−9.3 ± 0.76					
O	−6.84 ± 1.0					−8.55 ± 0.64			
Unwashed with anti-A absorbing ability	−6.77 ± 1.0	1:10: 7.55; 1:100: 8.88		−6.73 ± 0.56					
A	−12.88 ± 2.1			−9.02 ± 1.0					
A	−20.62 ± 4.1(with C':−8.19 ± 1.1)			−5.13 ± 0.4					
B with anti-A absorbing ability	−14.88 ± 1.3	1:10:−7,55 ± 1.1 1:100 −8,88 ± 1.2			−6.73 ± 0.3			1:10:−13.92 ± 1.4 1:100: −8,15 ± 1.0,	
B	−10.84 ± 1.2								−5.97 ± 1.0 after three times loading: 4.55 ± 1.1

Note: () - zeta potential after addition of serum from O blood group with anti-A, anti-B absorbing ability, C ' - complement.

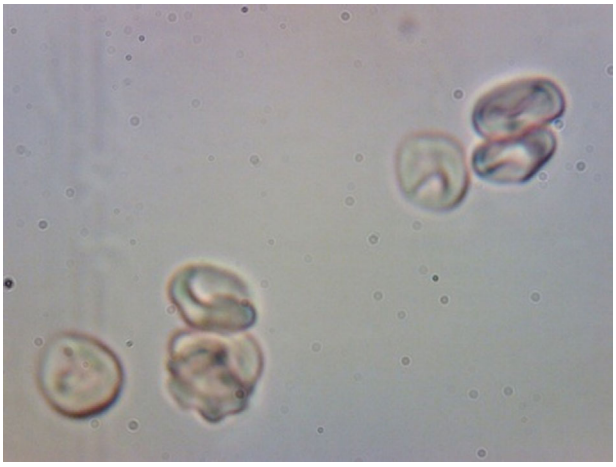

FIGURE 1 B erythrocytes contacted with anti-B immunoglobulin G (IgG) and complement

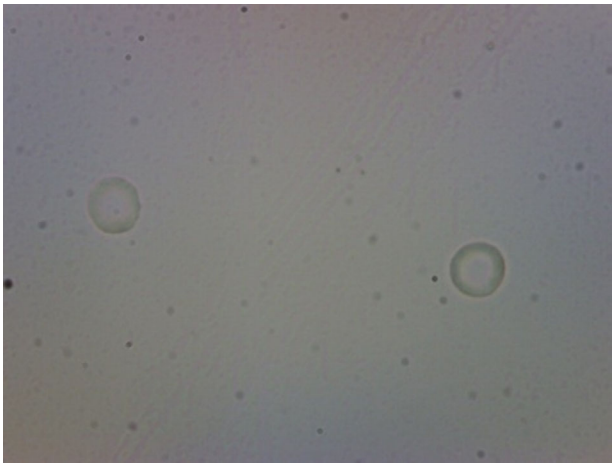

FIGURE 2 B erythrocytes with heated AB serum and complement

serum of some persons [10] encouraged investigation of the influence of the sera of the persons of O blood group with anti-A and anti-B absorbing ability on zeta potential of RBCs. Thus, the electrokinetic potential of O erythrocytes with anti-A absorbing ability decreased after the contact with anti-A heated serum and complement from -9.85 to -5.48 mV and increased after the addition of the serum from O blood group person with anti-A and anti-B absorbing ability: -8.3 mV. The electrokinetic potential of A erythrocytes decreased after the contact with anti-A heated serum with complement (from -7.19 to -3.09 mV) and increased after the addition of the serum from O blood group person with anti-A and anti-B absorbing ability (-6.71 mV).

Importantly, immunoglobulin M (IgM) anti-A antibodies did not decrease the electrokinetic potential of O erythrocytes with the anti-A absorbing ability (from -7.19 to -9.85 mV). IgM anti-A blood group-specific antibodies did not decrease the electrokinetic potential of O erythrocytes with anti-A, anti-B antibody absorbing ability (from -8.17 to -9.85 mV), the addition of the complement did not decrease the electrokinetic potential (from -8.17 to - 9.85 mV), however, IgG complement-dependent antibodies significantly decreased the electrokinetic potential of RBCs (from -8.17 to -3.88 mV).

Thus, the potential of B nonheparinized erythrocytes was not decreased after the contact with specific anti-B heated serum with complement (from -7.15 ± 1.5 mV to -7.24 ± 1.6 mV). The morphology of B erythrocytes with anti-A absorbing ability was rather different after the contact with anti-A and anti-B IgG antibodies, however, was not modified after the contact with serum from the AB blood group (Figures 1–3).

The diameter of A erythrocytes was 5.32 ± 0.52 μm, after the contact with anti-A IgG and complement: 4.4 ± 0.4 μm, after the contact with anti-B IgG and complement: 5.33 ± 1.30 μm, after the contact with anti-A IgG, complement and serum from O blood group with anti-A, anti-B absorbing ability: 4.53 ± 0.40 μm (Figure S1–4).

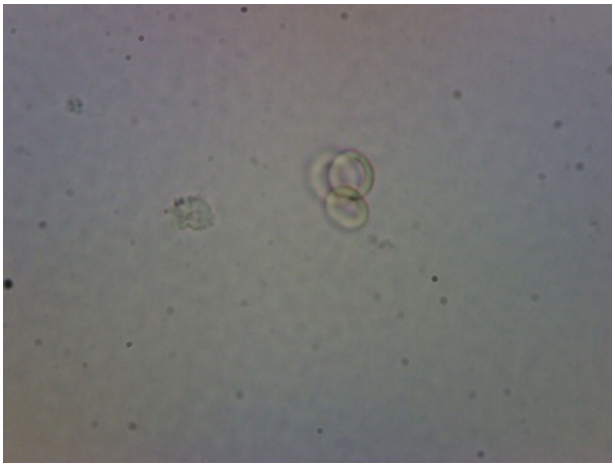

FIGURE 3 B erythrocytes with anti-A absorbance with anti-A immunoglobulin G (IgG) and complement

To reveal IgG antibody influence on the morphology of RBCs various dilutions of antibodies were used for the contact with erythrocytes. Thus, the diameter of RBCs increased after the contact with specific IgG antibodies and gradually decreased with the increase of the antibody dilution (Table 2).

The electrokinetic potential of RBCs was significantly decreased (37.2%) in the reactions of A erythrocytes with IgG complement-dependent anti-A antibodies (Figure S5), however, there was no expressed reduction with IgG anti-B antibodies (on 15.3%) or with serum without anti-A and anti-B antibodies (on 12.3 %). The electrokinetic potential was reduced in the reaction of B erythrocytes with complement-dependent IgG anti-B antibodies, as well as of

TABLE 2 Diameter of erythrocytes under the influence of immunoglobulin G (IgG) blood specific antibodies with various concentrations (μm)

	1:2	1:4	1:8	1:16	1:32	1:64
Anti-B heated and B RBCs (8.44 ± 1.0, 8-9)	13.8 ± 1.37 + 12–15	13.0 ± 1.3 +12–14	12.41 ± 1.2 −11–15	11.5 ± 1.27 −10–13	9.2 ± 1.1 −8–13	–
Anti-A heated and A RBCs (8.58 ± 1.0, 8-10)	13.1 ± 1.23 +11–14	11.6 ± 1.0 +9–14	11.8 ± 1.0 +10–14	10.12 ± 1.2 −10–13	8.4 ± 1.0 8–10	8.6 ± 1.0 8–10
Anti-B heated after absorbtion by B RBCs and B RBCs (8.44 ± 1.0, 8-9)	13.42 ± 1.0 −11–15	13.2 ± 1.3 −12–14	12.6 ± 1.3 −12–15	12.15 ± 1.3 −11–15	8.7 ± 1.0 −7–11	7.7 ± 1.0 −7-8
IgM Anti-B and B RBCs (6.33 ± 1.0, 6-7)	9.5. ± 1.1 8–10	9.3 ± 1.1 7–10	8.33 ± 1.1 8–8.5	6.8 ± 1.0 5–9	6.27 ± 1.0 6–9	
IgM Anti-A and B RBCs (6.33 ± 1.0, 6–7)	7.47 ± 1.0 6–9	6.81. ± 1.0 5–10	7.73 ± 1.0 5–10	6.5 ± 1.0 5–8	8.25 ± 1.1 6–10	
anti-A heated after absorbtion by B RBCs with anti-A absorbing ability and A RBCs (8.58 ± 1.0, 8–10)	11.77 ± 1.0 +11–13	10.44 ± 1.1 +8–12	8.0 ± 1.0 −7–9	–	8.66 ± 1.0 −8–11	8.41 ± 1.0 −8–9
anti-A heated with A RBCs 5 min.	8.85 ± 1.0 +8–12	9.71 ± 1.1 +8–15	8.14 ± 1.0 +8–9	8.55 +7–11	8.75 ± 1.0 +7–12	8.2 ± 1.0 +7–9
anti-A heated after A RBCs with A RBCs 5 min.	8.58 ± 1.0 +7–11	9.33 ± 1.0 +8–11	9.58 ± 1.0 +7–14	7.9 ± 1.0 +6–12	9.3 ± 1.0 +7–13	8.18 ± 1.0 +7–11
IgM anti-A with A RBCs 5 min.	7.25 ± 1.0 +6–9	7.57 ± 1.0 +5–9	8.57 ± 1.0 +7–10	7.66 ± 1.0 +7–8	8.11 ± 1.0 +7–9	7.66 ± 1.0 +7–8
IgM anti-A after A RBCs with A RBCs 5 min.	7.66 ± 1.0 +7–8	7.2 ± 1.0 +6–8	7.8 ± 1.0 +7–9	7.87 ± 1.0 +7–9	7.19 ± 1.0 +7–8	8.0 ± 1.0 +7–9

Note: RBCs – red blood cells.

O erythrocytes with anti-A and anti-B antibody absorbing ability after the contact with IgG antibody complement-dependent anti-B antibodies.

Thus, the decrease of the electrokinetic potential of RBCs by more than 20% after the contact with IgG blood group-specific antibodies might testify to the presence of specific blood group antigens on the erythrocyte membrane. Only washed heparinized erythrocytes should be used, as well as polyclonal heated serum (for the destruction of IgM antibodies) and complement. Interestingly, the serum of the persons with O blood group with anti-A or anti-B absorbing ability showed the presence of the inhibitory substance, able to abolish the decrease of the electrokinetic potential of erythrocytes under the influence of complement-dependent IgG anti-A and anti-B blood group antibodies.

Importantly, the zeta potential of unwashed and coagulated erythrocytes with additional anti-A and anti-B absorbing abilities was not decreased under the influence of IgG complement-dependent anti-A and anti-B antibodies, possibly due to the presence of the reported inhibitory substance in plasma. Both the erythrocytes in saline and contacted with serum without anti-A and anti-B antibodies have been found to be morphologically and functionally normal.

To illustrate the induced antibodies decrease in the electrokinetic potential of RBCs, we performed experiments with the unheated serum and serum without complement. The data are consistent with a study of the electrokinetic potential of erythrocytes [10], which shows that a single antibody contact is not enough for the modification of the erythrocyte charge. In Europe and Japan, an increasing number of clinical laboratories are using cell electrophoresis [15], therefore electrophoresis of erythrocytes might be used for blood group detection.

4 | CONCLUDING REMARKS

The decrease of the electrokinetic potential of RBCs by more than 20% after the contact with IgG blood group-specific antibodies might testify to the presence of specific blood group antigens on the erythrocyte membrane. IgG complement-dependent anti-A and anti-B antibodies binding with A and B antigens decrease the electrokinetic potential of washed erythrocytes on the contrary to IgM antibodies. The method of electrophoresis of erythrocytes may be used not only in the field of cell biology but also in the medical and pharmaceutical industries.

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CONFLICT OF INTEREST

The authors declared that they have no conflict of interest.

DATA AVAILABILITY STATEMENT

The data that support the findings of this study are available on request from the corresponding author. The data are not publicly available due to privacy or ethical restrictions.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.

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