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ORIGINAL ARTICLE

Blood Group Antigens Visualisation on Leukocytes

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Abstract

Background/Aim: The leukocytes have been reported to contain blood group specific antigens, that are clinically relevant, however visualisation of A and B group antigens on leukocytes is a big issue. In cases of ABO discrepancies weak blood group antigens on nuclear cells have been demonstrated by using expensive techniques. Thus, the development of the method of the detection of weak blood group antigens on leukocytes available for any laboratory technician is hardly essential. The study aimed to reveal and analyse A and B blood group specific adsorbing antigens on leukocytes and erythrocytes and to develop a method for visualisation of weak blood group antigens on leukocytes.

Methods: Polyclonal and monoclonal anti-A and anti-B antibodies, received from international laboratories according to the program of Workshop IV, held in Paris, 2000, were used for the study. Mixed agglutination reaction was performed as the method for visualisation of weak blood group antigens on leukocytes as nuclear cells.

Results: Polyclonal sera from O blood group persons without weak blood group antigens in contrast to monoclonal antibodies demonstrated the ability to reveal weak blood group specific antigens on leukocytes by the method of mixed agglutination reaction. However, the test erythrocytes from the persons with increased levels of platelets and erythrocyte sedimentation rate did not allow to visualise weak antigen expression on the studied leukocytes in contrast to the persons with normal levels of platelets and erythrocyte sedimentation rate, that successfully formed mixed agglutinates with weak blood group antigens on leukocytes in mixed agglutination reaction. The leukocytes suspended in 0.9 % saline (as a diluent) incubated with the mixture of the serum with 0.9 % saline (1:2) led to the formation of specific agglutinates with test erythrocytes. The experiments with different temperature regimes and time of incubation demonstrated the usefulness of the studied method in specific leukocytes antigen visualisation during prolonged incubation at 4 °C. The persons with weak group A and B antigens, revealed on the leukocytes by the studied method, demonstrated decreased level of erythrocytes, platelets, titre of corresponding warm agglutinating antibodies (less than 1:8) and increased erythrocyte sedimentation rate.

Conclusion: The mixed agglutination reaction with prolonged incubation at 4 °C and the use of the selected polyclonal sera and test erythrocytes from the donors with normal values of platelets and erythrocyte sedimentation rate may be used for weak blood group antigens detection on leukocytes. The donors of the sera and test erythrocytes used in mixed agglutination reaction should be investigated on common blood analysis, agglutinating titre of corresponding warm group specific antibodies and presence of weak blood group antigens.

Key words: Discrepancy; Blood group antigens; Visualisation; Leukocytes.

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Introduction

The erythrocytes and leukocytes have demonstrated different characteristics of blood group antigens, leading to the difficulties in blood group testing depending on whether erythrocytes or leukocytes are used for the investigation.[1, 2] The expression of weak blood group antigens in leukocytes has been scarcely studied. Visualisation of leukocyte antigens by means of the coating of the cells with IgG antibodies has been described. The method of mixed agglutination reaction (MAR), invented by Coombs, has recently found application in the measurement of antibody-bound cells and was approved by the World Health Organization. Similar tests for IgA and IgM antibodies have been described.[3]

Thus, spermatozoids, as nuclear cells, taken for MAR, were added to the erythrocytes carrying specifically adsorbed IgG antibodies.[4-7]

The role of the method of visualisation of the adsorbed antibodies on weak blood group antigens is expanding.[8] The researchers revealed different localisation of blood group antigens in the glycolipid or glycoprotein form (in the secretions) on erythrocytes and nuclear cells.[9] The A, B and H blood group substances have been widely investigated on the cells by MAR.[10] The success of visualisation of the adsorbed antibodies on the cells in our previous study makes it possible to learn lessons from the method used with epithelial cells and to prove it useful for blood group testing using leukocytes.[11]

The opportunity to reveal the specifically bound antibodies with the weak cell antigens that were not detected by ordinary agglutination method, was the basis of the investigation of MAR.

The study aimed to compare the group specific adsorbing antigens of leukocytes and erythrocytes, focusing on the development of the technique for leukocytes weak blood group antigens visualisation.

Methods

The volunteers aged from 18 to 70 years, examined in the laboratory of Spine and Joint Pathology with O, A, B and AB blood groups, were chosen for the study. Participants were selected according to inclusion and exclusion criteria to ensure reliable outcomes. Inclusion criteria were: age range of 18 to 70 years, absence of HIV infection, viral hepatitis, malignancy. Exclusion/non-inclusion criteria included infectious diseases, malignancy and injuries.

Persons with antigen of group A (n = 23) and of group B (n = 23) served as donors of erythrocytes and leukocytes. Importantly, the method revealed the presence of weak A blood group antigens (n = 17) and weak B blood group antigens (n = 15). The polyclonal sera of the persons with the O blood group without weak blood group antigens and anti-A, B sera from the persons with weak blood group antigens (n = 19) were used for revealing group specific antigens on leukocytes.

Immuno-haematological investigation included blood group testing performed by the use of monoclonal (anti-A antibody 2-10, received from the International laboratory according to the program of Workshop in Paris, 2000) and polyclonal (EDTA or citrated plasma and polyclonal sera) antibodies. Serological investigation and agglutination reactions were performed according to the standard protocols using anti-A and anti-B Mabs (*Tulip diagnostics*, Goa, India) according to the AABB Technical manual.[12] The tube method was used for blood group typing with polyclonal sera for forward grouping and with group A, B and O erythrocytes for reverse testing.

The degree of agglutination was graded according to the grade from - to 4+. The data were checked for completeness, consistency and accuracy. Statistical analysis was performed by the software *Statistica* 10.0. The data were considered as significant at $p < 0.05$.

Ethylenediaminetetraacetic acid (EDTA) blood served as a source of leukocytes. The leukocytes after washing three times with 0.9 % saline, centrifugation at 1000 rpm for one minute and suspension in 0.9 % saline were used in MAR.

EDTA blood served as a source of erythrocytes. The samples of group A and B erythrocytes (n = 46) were washed three times with 0.9 % saline, mixed with group AB serum or plasma (1:100 in saline) and served as test erythrocytes.

To receive IgG antibodies, the samples of the plasma from O blood group persons have been heat-inactivated for 30 minutes at 56 °C.

MAR

The leukocytes (50 µL) and the mixture of O blood group serum or heated anti-A, B plasma (50 µL) with normal saline (50 µL) (1:2) were put into the contact for 13 hours at 4 °C.[13-15] The serum was analysed on agglutination titre before and after adsorption, whereas the deposit (the leukocytes with adsorbed antibodies) has been washed three times in 0.9 % saline and test erythrocytes were added for further one hour incubation at room temperature. After centrifugation for one minute at 1000 rpm the MAR was analysed microscopically. The agglutinating titre of antibodies was investigated at 37 °C and 4 °C. The statistical analysis was performed by the software program *Statistica* 10.0.

Results

The visualisation of blood group A antigen on leukocytes

The serum from a blood group O person after contact with group A and weak B blood group antigens (B+) on leukocytes demonstrated the presence of mixed agglutinates with group A (B+) test erythrocytes (Table 1, Figure 1).

The serum from the O blood group person after the contact with leukocytes of blood group A made mixed agglutinates with group A test cells (n = 2) (Figure 2). The heated plasma from the O blood group person after contact with leukocytes of blood group A made mixed agglutinates with group A test cells. The citrated plasma of the O blood group coated leukocytes of blood group A (B+) and formed mixed agglutinates with the test cells of group A (B+).

Table 1: The results of mixed agglutination reaction in revealing strong and weak group A and B agglutinogenic and adsorbing antigens

Parameter	Adsorption on leukocytes	Positive reaction with erythrocytes	Negative reaction with erythrocytes	p-value
Anti-A, B serum	A (B+)	A (B+)	B	P (3,4) < 0.05
Anti-A, B serum	A	A: 1+ (ESR 50 mm/h, lymph 20 %)	A: - (ESR 70 mm/h, lymph 5 %)	P (3,4) < 0.05
Anti-A, B serum	A	A: 1+ (ESR 13 mm/h)	A: - (ESR 30 mm/h)	P (3,4) < 0.05
Anti-A, B serum	A	A: 1+		
Anti-A, B serum	A	A: 1+		
Anti-A, B serum	A (B-)	B (A+): -		
Anti-A, B serum	A (B+)		A (B+)	
Anti-A, B serum	A (B+)	B: 1+		
Anti-A, B serum	A		A	
Anti-A, B serum	A	A: 2+		
		A: 2+		
Anti-A, B heated serum	B (A+)	A: 2+		
		A: 1+		
		A: 2+		
Anti-A, B heated serum	A	A: 1+		
Citrated anti-A, B plasma	A (B+)	A (B+): 2+		
Anti-A, B heated plasma	B (A+)	A:1+		
Anti-A, B heated plasma	B (A+)	A:1+		
Anti-A, B heated plasma	B	B:1+	A	P (3,4) < 0.05
Anti-A, B heated plasma	A	A:1+		
Anti-A monoclonal antibody 2-10	A	A:1+	B (A+)	P (3,4) < 0.05
Anti-A monoclonal antibody 2-10	A	A:1+		
Citrated anti-A, B plasma	A (B+)	B:1+		
EDTA anti-A, B plasma	O		B at 4 °C: - at 37 °C: -	

1+,2+ - strength of agglutination; lymph - level of lymphocytes in common blood test; ESR: erythrocyte sedimentation rate; EDTA: Ethylenediaminetetraacetic acid;

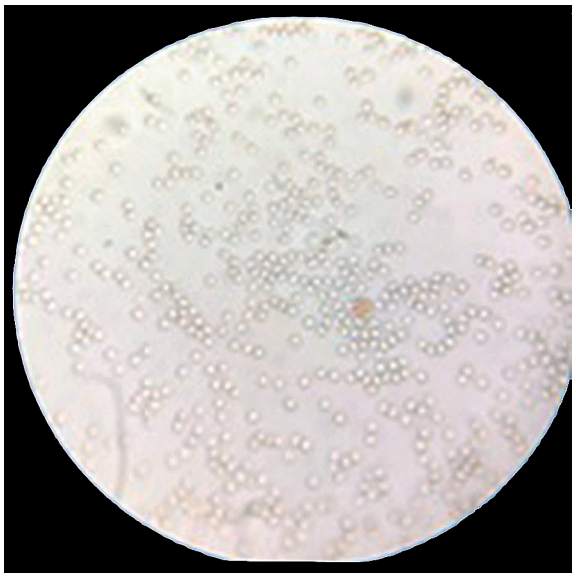

Figure 1: Group A leukocytes coated with the heated anti-A, B serum and contacted with group A erythrocytes

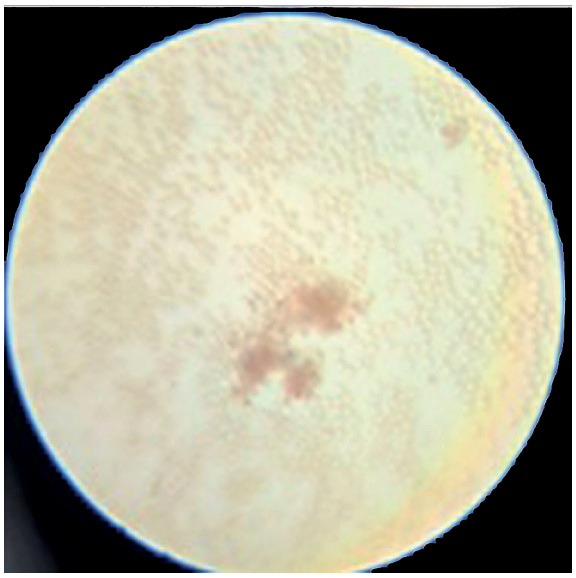

Figure 3: Group B leukocytes coated with the heated anti-A, B plasma and contacted with group B erythrocytes

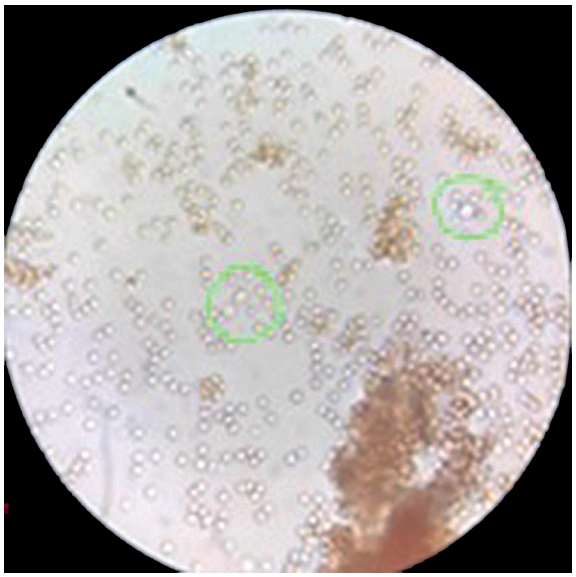

Figure 2: Group A leukocytes coated with anti-A, B serum and contacted with group A erythrocytes

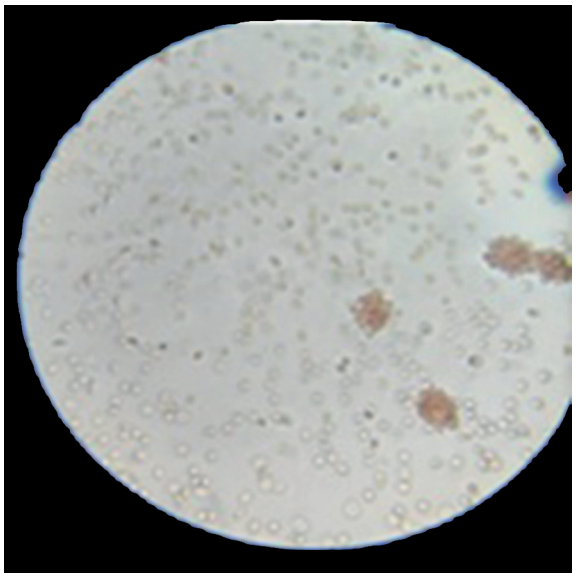

Figure 4: Group B leukocytes coated with the heated anti-A, B plasma and contacted with group B erythrocytes

The visualisation of blood group B antigen on leukocytes

The heated plasma from the O blood group was adsorbed on leukocytes of blood group B with weak A antigen (A+) and made mixed agglutinates with group B test cells (n = 2).

The heated plasma from the O blood group after adsorption on leukocytes of blood group B made mixed agglutinates with the test cells of group B (Figure 3, Figure 4).

The visualisation of weak leukocytes antigens of blood group A

Mixed agglutinates with the test cells of blood group A were revealed after the heated serum from O blood group had been adsorbed on leukocytes of blood group B with weak A antigen (A+) (previously detected by adsorption technique on erythrocytes on the contrary to the failure in detection by agglutination method) (Figure 5). The serum of O blood group person was incubated with leukocytes of blood group A and made mixed

agglutinates with the test cells of group B with weak group A antigen expression (A+) (n = 4) (Figure 6). The heated plasma from O blood group person (containing IgG antibodies) after incubation with leukocytes of group B with weak group A antigen (A+) made mixed agglutinates with group A test cells.

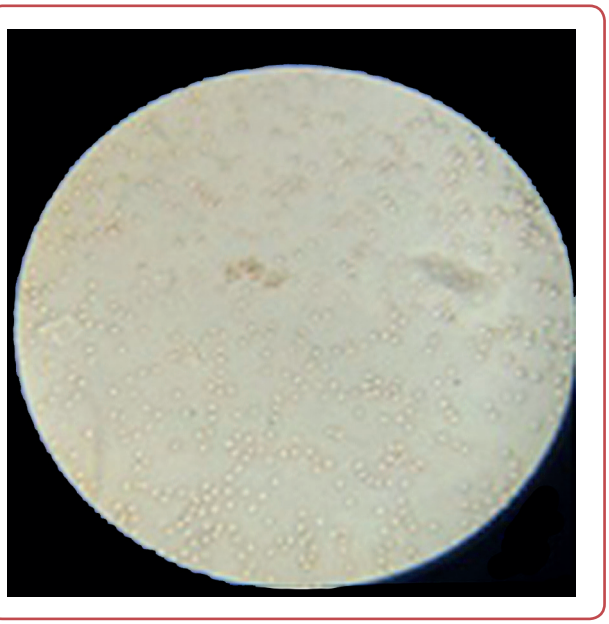

Figure 5: Group B (A+) leukocytes coated with the heated anti-A, B serum and contacted with group A erythrocytes

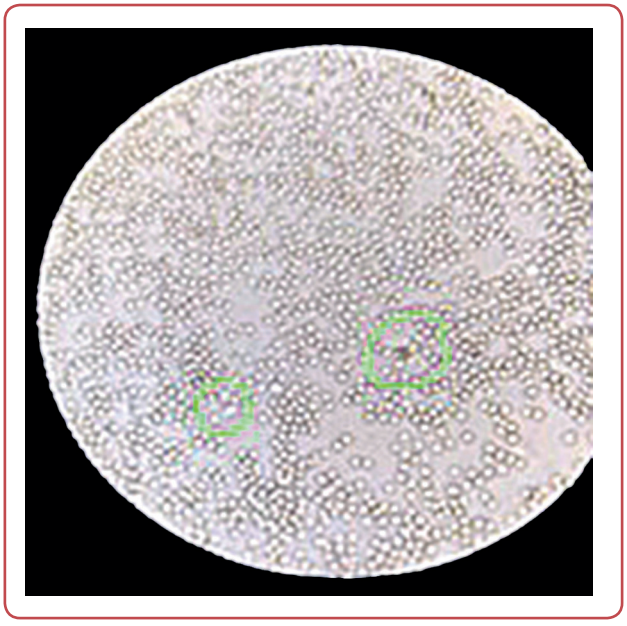

Figure 6: Group A leukocytes coated with anti-A, B serum and contacted with group B (A+) erythrocytes (detection of weak group A antigen)

The visualisation of leukocytes antigens of weak blood group B

O blood group serum coated the leukocytes of group A with weak B blood group antigen expression (B+) and made mixed agglutinates with group B test cells.

The experiments that demonstrated the absence of mixed agglutinates of the leukocytes with the test cells in MAR

The EDTA plasma from O blood group incubated with leukocytes of group O did not show the presence of agglutinates of the test cells of blood group B (both at 37 °C and 4 °C). The plasma from O blood group person contacted with leukocytes of group B did not show the presence of mixed agglutinates with the test cells of group A. The heated serum from O blood group person contacted with the leukocytes of blood group A did not show the presence of mixed agglutinates with the test cells of group B. Thus, the studied leukocytes of group O did not make mixed agglutinates after adsorption with anti-A, B serum and further contact with test erythrocytes if group A and B.

However, some sera failed to detect blood group antigens on leukocytes. Thus, the sample of serum from O blood group person contacted with leukocytes of group A with weak B blood group antigen expression (B+) did not show mixed agglutinates with the test cells of group A (B+). A sample of O blood group serum being adsorbed on leukocytes of group A did not agglutinate group A test cells. O blood group heated serum contacted with leukocytes of group A did not show mixed agglutinates with the test cells of group A. Meanwhile, the nonheated serum of the person without weak blood group antigen expression after contact with leukocytes of group A made mixed agglutinates with group A test cells.

The extended study revealed, sera, that failed to reveal blood group antigens expression on leukocytes belonged to the persons with the presence of weak blood group antigens. Moreover, these sera demonstrated the presence of warm antibodies with low agglutinating titre (less than 1:8).

The visualisation of blood group antigens using monoclonal antibodies in MAR

Monoclonal antibody (Mab) 2-10 contacted with leukocytes of blood group A showed the presence

of mixed agglutinates with the test cells of group A. However, Mab 2-10 failed to detect weak leukocyte blood group antigen A.

The O blood group persons (according to the method of agglutination, while the adsorption technique demonstrated the presence of weak blood group antigen) showed weak leukocyte antigen of group A by visualisation of the adsorbed antibodies from polyclonal serum in MAR. Thus, MAR helped to detect the presence of weak blood group A antigen with the use of polyclonal sera from the selected donor, that was not revealed by ordinary agglutination.

In blood group B persons according to the agglutination method, the leukocyte antigen of group B was confirmed by MAR. The O blood group type in persons according to the agglutination method was confirmed by MAR technique.

Blood group antigens are widely distributed in body fluids and tissues. Both strong (agglutinogenic) and weak (adsorbing) blood group substances have been revealed by the method of MAR. The method may be used in the case of difficulties in blood group testing in pre-transplantation investigation.

Different temperature regimes have been investigated in MAR. The study revealed the necessity to perform successful weak blood group antigens detection on leukocytes at low temperature: the leukocytes and sera incubated for 13 hours at 4 °C, and the binding of test cells (erythrocytes) with the leukocytes coated by antibodies performed at 23 °C resulted in successful formation of specific mixed agglutinates on the contrary to the regimes of shorter incubation at warm temperature. Moreover, the incubation of the heated serum of O blood group with leukocytes suspended in group AB serum as a diluent led to the formation of weaker mixed agglutinates as compared to the incubation with leukocytes suspended in 0.9 % saline.

The incubation of the mixture of 0.9 % saline and anti-A, B heated serum (1:1) with leukocytes resulted in the appearance of specific mixed agglutinates as compared to the use of the serum without adding normal saline. Group A leukocytes incubated with the heated O blood group serum demonstrated the appearance of mixed agglutinates with group A cells in contrast to the O blood group serum without heat inactivation. Negative mixed agglutination reaction was revealed while the use of test erythrocytes from the donors with higher levels of platelets (408.25 ± 27.40 × 10^9/L) ($p < 0.05$), leukocytes (9.92 ± 2.40 × 10^9/L) ($p > 0.05$) and neutrophils (65.0 ± 7.5 %) ($p > 0.05$) as compared to the cases with positive mixed agglutination reaction (the level of platelets from the donors of test erythrocytes: 240.6 ± 12.5 × 10^9/L, leukocytes: 7.33 ± 0.82 × 10^9/L, neutrophils: 57.6 ± 6.2 %).

To evaluate if any polyclonal serum may be taken for MAR, the donors of the sera were analysed on the presence of weak blood group antigens (anti-A and anti-B adsorbing abilities of the donor erythrocytes). Thus, O blood group serum from the persons with the absence of weak erythrocyte antigen of group A incubated with leukocytes of group A made mixed agglutinates with cells of blood group A and B (A+) (with weak group A antigen) ($n = 2$). In turn, O blood group serum taken from the person with weak erythrocyte antigen of group A not always successfully detected group A antigen: after incubation with leukocytes of group A the serum showed the presence of mixed agglutinates with cells of one sample of group A on the contrary to the other sample of the cells of group A and B (A+). The O blood group serum with anti-A antibodies from the person without weak A antigen was strongly adsorbed on leukocytes of blood group A. Meanwhile, the antibodies from O blood group person with weak antigen of group A demonstrated better adsorbing ability on the red blood cells of group A. Thus, these sera failed to detect leukocytes blood group antigens in MAR.

The common blood tests of the persons served as serum donors with the presence of weak blood group antigens were analysed. Thus, persons with weak B blood group antigen demonstrated decreased levels of erythrocytes, platelets and increased level of erythrocyte sedimentation rate in the common blood test ($p < 0.05$) (Table 2).

The study of the samples of plasma on agglutinating activity at 37 °C and at 4 °C according to the adsorbing abilities of erythrocytes.

The donors' anti-B plasma without weak blood group antigen (additional adsorbing ability of erythrocytes) at 4 °C demonstrated strong agglutinating activity of anti-B antibodies: 2.85 ± 0.30+ in 1:4 titre as compared to the plasma of the persons with weak blood group B antigen: 2.01 ± 0.24+ ($p < 0.05$).

Table 2: Common blood analyses of the persons with weak blood group antigens and positive mixed agglutination reactions (MAR)

Parameter	Persons with group A and weak B blood group antigen	Persons with group A	Test erythrocytes, that gave negative MAR	Test erythrocytes, that gave positive MAR	p-value
Erythrocytes, × 10^{12}/L	4.0 ± 0.3	4.9 ± 0.3	4.2 ± 0.3	4.5 ± 0.3	P (1,2) < 0.05
Haemoglobin, g/L	125.0 ± 10.5	142.4 ± 15.6	135.5 ± 14.1	133.5 ± 12.4	
Haematocrit (%)	36.7 ± 5.1	42.0 ± 6.3	37.5 ± 5.4	36.5 ± 5.0	
Platelets, × 10^{9}/L	254.0 ± 13.1	343.2 ± 17.1	408.3 ± 27.4	240.6 ± 12.5	P (1,2) < 0.05 P (3,4) < 0.05
Leukocytes, × 10^{9}/L	7.1 ± 1.2	9.5 ± 2.6	9.9 ± 2.6	7.3 ± 0.8	
ESR, mm/h	32.4 ± 4.5	12.2 ± 1.7	30.5 ± 4.2	20.5 ± 4.0	P (1,2) < 0.05 P (3,4) < 0.05
Eosinophils, %	4.2 ± 0.4	5.3 ± 0.5	4.2 ± 0.4	5.4 ± 0.5	
Young neutrophils, %	2.8 ± 0.5	1.7 ± 0.3	2.2 ± 0.4	3.0 ± 0.7	
Segmented neutrophils, %	63.6 ± 8.5	60.0 ± 7.0	65.0 ± 9.3	57.2 ± 6.2	
Lymphocytes, %	26.0 ± 5.0	27.6 ± 6.1	26.5 ± 5.3	30.3 ± 7.4	
Monocytes, %	3.8 ± 0.3	6.8 ± 0.6	4.5 ± 0.3	4.5 ± 0.4	

ESR: erythrocyte sedimentation rate;

Table 3: The decreased agglutinating activity of plasma of the persons with additional adsorbing ability of erythrocytes (presence of weak blood group antigen)

Parameter	Strength of agglutination				p-value
	Plasma of A blood group (n = 18)	Plasma of A blood group with anti-B antibody adsorbing ability (n = 15)	Plasma of B blood group (n = 16)	Plasma of B blood group with anti-A antibody adsorbing ability (n = 17)	
With group B erythrocytes at 4°C	2.85 ± 0.30 +	2.01 ± 0.24 +			P (1,2) < 0.05
With group B erythrocytes at 37°C	3.07 ± 0.40 +	1.60 ± 0.20 +			P (1,2) < 0.05
With group A erythrocytes at 4°C				3.60 ± 0.20 +	P (3,4) < 0.05
With group A erythrocytes at 37°C				3.70 ± 0.32 +	P (3,4) < 0.05

Table 4: Hemagglutination inhibiting activity of plasma with weak A blood group antigen

Reagents	Strength
Anti-B plasma from donor with weak A group antigen + anti-B usual plasma + B er.	2+
Anti-B plasma 1:2 from donor with weak A group antigen + anti-B usual plasma + B er.	4+
Anti-B plasma 1:4 from donor with weak A group antigen + anti-B usual plasma + B er.	4+
Anti-B plasma from donor without weak A group antigen + anti-B usual plasma + B er.	4+
Anti-B plasma 1:2 from donor without weak A group antigen + anti-B usual plasma + B er.	4+
Anti-B plasma 1:4 from donor without weak A group antigen + anti-B usual plasma + B er.	4+

er. – erythrocytes; Strength: strength of agglutination;

At 37 °C the donors of anti-B plasma in 1:4 titre demonstrated stronger activity of anti-B antibodies: 3.07 ± 0.4+ as compared to the persons with the presence of weak blood group B antigen: 1.6 ± 0.2+ ($p < 0.05$). Similarly, anti-A plasma demonstrated stronger agglutinating properties at 4 °C in 1:4 titre: 3.6 ± 0.2+ as compared to the persons with the presence of weak blood group antigen of group A: 3.00 ± 0.23+ ($p < 0.05$).

Ordinary anti-A plasma donors (without weak blood group antigens) showed higher agglutinat-

ing activity of anti-A antibodies at 37 °C as well: 3.7 ± 0.32+ as compared to the persons with anti-A antibody adsorbing ability of erythrocytes: 1.8 ± 0.2+ ($p < 0.05$) (Table 3).

The plasma of the donors with weak blood group antigens exhibited agglutination inhibiting properties. Thus, anti-B plasma of the donor with weak antigen of group A inhibited agglutination of erythrocytes of blood group B by usual antibodies on the contrary to the serum of the donor without weak group antigen. This agglutination inhibiting properties of dome sera have been observed in previous studies (Table 4).[16]

Discussion

The presence of weak blood group antigens has been referred as type II discrepancy and enhancing the cell concentration or incubation at 4 °C has been suggested for the improvement of weak blood group antigens detection. The polyclonal sera in contrast to the monoclonal antibodies, have been reported to react with weak blood group antigens. The previous works demonstrated stronger anti-A or anti-B antibodies adsorbing ability of leukocytes as compared to the erythrocytes.[17] Thus, in the present study the leukocytes have been used for antibody adsorption with extended incubation. The specific binding resulted in detection of weak blood group antigens by formation of mixed agglutinates of the test cells.

However, not all polyclonal sera demonstrate the ability to form mixed agglutinates and to detect weak adsorbing blood group antigens despite the presence of agglutinating activity. Therefore, the used sera were analysed on agglutinating titre at 37 °C and at 4 °C to estimate their application in mixed agglutination reaction.

The cell localisation of the antigen of group A was investigated by immunostaining methods, showing that the antigens of group B and H of stomach and intestine may disappear in epithelial malignancies.[18] Therefore, the clinicians pointed to the necessity of weak blood group antigens visualisation on the cells for reliable blood group testing. Various techniques of weak blood group antigens detection have been invented and investigated in our previous studies.[19] MAR with immune anti-A antibodies has been proposed to visualise the antigens of group A1, A2, A, B and H. The positive or negative MAR was not associated with the quantity of saliva antigen of group A.

Blood group detection using automated methods was considered as reliable.[20, 21] However, in some cases unreliable results have been reported and manual and genetic methods have become popular for blood type testing.[22] Researchers' previous studies outlined the usefulness of manual methods in successful blood group testing in cases of discrepancies.[23] The antiglobulin-based methods also reveal the coated antibodies on the cells. The detection of the quantity of the percentage of the coated with antibody cells has been advised by World Health Organization for couple-infertility cases with 50 % of antibody-coated cells considered as significant.

The aim of the study was to visualise antibody adsorption on leukocyte weak blood group antigens by MAR. The antigens of group A, B and H usually initiate IgM mediated immunity with activity at 4 °C and group specific antibodies found in serum are predominantly of IgM type with strong agglutinating properties.

However, the studied sera that showed negative mixed agglutination reaction, demonstrated low agglutination titre of group specific antibodies at 37 °C, suggesting that antibodies responsible for the binding with weak leukocytes antigens were immune and of IgG type, that agrees with the data of previous works. Thus, Coombs with researchers demonstrated the advantage of the heated serum use for visualisation of the adsorbed antibodies.[24] The heat-inactivated serum with IgG antibodies adsorbed on leukocytes testified to the strong avidity of these antibodies for the leukocytes as compared to IgM antibodies. The researchers revealed, usual anti-A sera with strong agglutinating properties may demonstrate negative MAR. The property of inducing positive MAR was associated with haemolysins and two fractions of antibodies: 19S fractions (with strong agglutinating activity) and 7S fractions (able to sensitise the cells).

Pearlman reported 19S and 7S fractions of antibodies are able to modify antibody production and immune response after transfusion in small quantities. Thus, IgM and IgG antibodies, passively administered, demonstrated a depressive effect on immune response by binding with antigens.[25-29]

Group A antigen on the cells and erythrocytes was considered not to be identical, suggesting different reactivity of some anti-A sera. However, in most studies the differences in MAR with anti-A sera from different persons have been associated with 7S or 19S antibody fractions. It is not surprising, that the contact with 2-mercaptoetanol led to the absence of mixed agglutinating activity in some sera due to the decreased titre of incomplete antibodies.

The titre of 7S antibody (IgG) after treatment with 2-mercaptoethanol was not decreased in mixed agglutination and agglutination reactions, on the contrary to the titre of 19S antibody (IgM). The failure of all anti-A sera to produce mixed agglutination reaction was associated with their properties and not with their molecular size. Some sera were reported to be able to demonstrate MAR in non-secretors. The titre was reported to be higher in secretor cells as compared to non-secretor cells.

The antigenic differences of the soluble or cellular antigens of secretor and non-secretor cells as well as different fractions of anti-A antibodies might explain the weak and strong reactions of MAR with secretor and non-secretor cells. The property of making mixed agglutinates was explored after the heating the serum. Some sera demonstrated no agglutination of erythrocytes and mixed agglutination (suggesting the presence of 19S, IgM antibodies). The other sera showed anti-A agglutinating activity and the ability to produce mixed agglutination (due to 7S globulin antibodies).

In the present study MAR revealed the presence of leukocyte antigen of group A (n = 23) and of group B (n = 23). Importantly, the method revealed the presence of weak A blood group antigens (n = 17) and weak B blood group antigens (n = 15). The polyclonal sera of the persons with O blood group without weak blood group antigens revealed group specific antigens on leukocytes as compared to anti-A, B sera from the persons with weak blood group antigens.

The study demonstrated the formation of mixed agglutinates as the point of specific interactions between adsorbed on leukocytes antibodies and bound test erythrocytes. Thus, MAR may be used in weak leukocytes blood group antigens visualisation at 4 °C by the use of antibodies from polyclonal serum of the selected donor in contrast to the use of monoclonal antibodies (possibly due to the absence of 7S globulins, IgG antibodies).

The erythrocytes of the donor of the serum for MAR need to be tested for the presence of weak group antigens. The absence of weak A and B antigens of erythrocytes of the donors makes these sera suitable for MAR.

The study revealed, that the use of MAR for reliable weak blood group antigens detection on leukocytes should be performed with the following conditions: 1. The leukocytes should be suspended in 0.9 % saline as a diluent and not in the serum of AB group person. 2. The incubation of the mixture of the heated specific antiserum with 0.9 % saline (1:2) leads to the formation of specific agglutinates as compared to serum without dilution. 3. The adsorption of antibodies should be performed at 4 °C for 13 h. 4. The serum and not citrated and EDTA plasma should be chosen. 5. The donor of polyclonal antibodies with blood group O should be investigated on the presence of weak blood group antigens, IgM or IgG class of antibodies and optimal temperature for antibody activity (the agglutination titre more than 1:8, the strength of agglutination more than 2+ in 1:4 titre). The donor of the test erythrocytes should have normal values of erythrocyte sedimentation rate and platelets. 6. The incubation of the mixture of the heated specific antiserum with 0.9 % saline (1:2) leads to the formation of specific agglutinates as compared to serum without dilution.

The study revealed different antibody adsorbing abilities of leukocytes and erythrocytes. In addition to the differences in common blood analysis the serum donors with weak variants of group A and B antigen demonstrated low titre of agglutinating antibodies with significant decrease of their activity at 37 °C. Thus, the sera of the donors of group A and weak antigen of group B (n = 15) demonstrated the decrease of anti-B agglutinating activity at 37 °C as compared to 4 °C. Similarly, persons with B blood group and weak antigen of group A demonstrated decrease of anti-A agglutinating activity at 37 °C on the contrary to the sera of the donors without weak blood group antigens, that preserved their agglutinating activity at 37 °C.

Conclusion

Mixed agglutination reaction visualised strong (agglutinogenic) and weak (adsorbing) group specific leukocyte antigens, while prolonged contact of leukocytes and sera at 4 °C and may be used for blood group testing in pre-transplantation investigations. The leukocytes suspended in 0.9 % saline as a diluent and a mixture of the serum with 0.9 % saline (1:2) should be used for the formation of specific mixed agglutinates with test erythrocytes. The polyclonal sera of the persons with O blood group on the contrary to the monoclonal antibodies visualised group specific antigens on leukocytes. Only anti-A, B sera from persons without weak antigens demonstrated the ability to reveal weak leukocytes antigens due to the stability of agglutinating properties at different temperature regimes.

The plasma from donors with weak blood group antigens did not show reliable results in MAR due to the low agglutinating activity of group specific antibodies, especially at 37 °C. The study also demonstrated, the donor of test erythrocytes for mixed agglutination reaction should have normal levels of platelets and erythrocyte sedimentation rate.

Ethics

All persons gave their informed consent for inclusion in the study. The study was approved by the Ethical Committee of the Kharkiv National Medical University, protocol No 2, dated 17 October 2023.

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Conflicts of interest

The authors declare that there is no conflict of interest.

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Data access

The data that support the findings of this study are available from the corresponding author upon reasonable individual request.

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