УДК 611-018.51:612.118.221.2

SEROLOGICAL AND TOPOGRAPHIC DIFFERENCES IN ERYTHROCYTIC ANTIGENIC MARKERS OF ABH-GLYCOLIPIDS AND GLYCOPROTEINS

Yury P. Delevsky

Kharkov Research Institute of Orthopaedics and Traumatology named after M.I. Sitenko, Laboratory of Immunology[1)]

Differences between antigens of the ABH-glycolipid and glycoprotein nature, not only caused by peculiarities in their location on the erythrocyte membrane but found out in reactions with isolated glycoconjugates - in ELISA and hemagglutination inhibition test - have been serologically revealed. The two types of ABH antigens have two types of their corresponding isoantibodies. Monoclonal anti-A and anti-B ones react with the two types of antigens in different ways: routine ones as well as BRIC 131 (9W1), BGRL 2, BGRL 1 (044) are inhibited by glycoconjugates bound with a lipid body of the antigens; while others, e.g. BRIC 145 (9W2), BRIC 250 (2A-48) are inhibited by protein-bound glycoconjugates. In a number of cases, one can observe a discrepancy of the glycolipid and glycoprotein ABH-marking, confirmed by 0 erythrocyte-isolated carbohydrate fragment of the glycoprotein with A-serological characteristic.

INTRODUCTION

The acknowledged proposition that group-specific oligosaccharide structures are covalently bound with either a protein or a lipid [1], if it were properly substantiated, could form a basis for the statement about existence of two types of A and B group antigenic markers on the erythrocyte membrane in man. But antigenic differences between the same determinants, having the same structure of the nucleus but bound with a lipid rather than with a protein, are not known **[2].**

Meanwhile, certain serological evidence in favour of the above fact has already been published and a hypothesis about a dual character of the group antigenic marking of erythrocytes in ABO system has been proposed [3,4]. Of the two types of the group antigenic marking, as a rule, only one is well-known and tested - i.e. revealed in the reaction of direct hemagglutination. The second one, "concealed", can be revealed by absorption of group isoantibodies by erythrocytes and by a change of z-potential of erythrocytes (their electrophoretic mobility) under effect of group-specific antibodies and the complement. It is the purpose of this report to describe some serological and biochemical data, which confirm the conception about two types of location and nature of ABH antigens.

MATERIALS AND METHODS

ANTIGENS. ABO belonging was determined with use of both polyclonal human isoantibodies and standard monoclonal reagents - anti-A and anti-B ("Hematologist", Moscow). The nonagglutinogenic type of the antigenic determinants was revealed by absorption of group isoantibodies and in a cell electrophoresis test, as it was described before [5].

Release of glycolipids from erythrocyte membranes was performed by the chloroform-methanol method [6], and that of glycoprotein fragments - by treatment of erythrocytes with 1 % solution of trypsin ("Spofa", Czechoslovakia). Further separation of glycoconjugates was done on DEAE cellulose ("Reanal", Hungary), with elution in gradient of NaCI and decreasing pH. Fraction output was assessed photometrically (with 200 nm, 260 nm, 280 nm and full UV spectrum) on SP-26 and Specord spectrophotometers, serologically and in ELISA. The study covered 118 antigenic fractions of membranes. Purity of the samples was determined by the method of a highly effective gel chromatography on Woters chromatograph with filling of Silasorb-biol-400 column manufactured by "Elsico Co." (Russia). For taking IR spectra, 1.0 ml of the sample was lyophilized in vacuum and kept over a layer of phosphorus pentoxide for 3 days; then 1-2 mg of the residue received were mixed with 200 mg of well-ground potassium bromide, and a disk was pressed. IR spectra were recorded on Specord device.

[1)] 80, Pushkinskaya st., Kharkov, 310024, Ukraine; tel.: (380-572) 476-594 (off), (380-572) 724-189 (home); fax: (380-572) 431-105

ANTIBODIES for ELISA were obtained from sera with help of sorption-elution on typed erythrocyte membranes with subsequent purification on DEAE cellulose, as well as by dissociation of immune complexes on an anion exchanger with use of preselected fractions of glycoproteins or glycosphingolipids with A or B specificity.

A reaction of hemagglutination inhibition with regard for reduction of the titer of hemagglutinins (in 0,9 % solution of NaCI, while for monoclonal reagents - with 1 % albumin), 60 min after a contact with antigenic substrates, was used [7]* There were sera of nonimmunized donors with the titer of 1:16 - 1:64 and monoclonal reagents with the titer of 1:32-1:1024.

The monoclonal reagents (MABs), received from IBGRL, were: A-specific - BRIC 131 (9W1) and BRIC 145 (9W2); A/Tn-specific - BRIC 66; B-specific - BRIC 250 (2A-48), BGRL1 (044), BGRL2; H 1+2-specific -BRIC 39 (9W7); H2-specific - BRIC 198, BRIC 231.

Immunoenzymatic analysis was performed by Tijssen's procedure [8] on panels with use of reagents in equalized concentration (20 mg/ml for glycolipids and at least 200 mg/ml for isoantibodies). Peroxidasemarked immunoglobulins against human IgG (H+L) (Moscow Research Institute of Epidemiology and Microbiology) were used. Readings were taken on IFKO-2 microphotometer.

RESULTS

A. Serological preconditions

It is not difficult to surmise that if there are two types of group antigenic markers with one ABH-specificity (e.g. A) and only one of them (glycolipid) manifests itself as the main agglutinogen, the human blood serum must also contain two "types" of corresponding antibodies (e.g. a agglutinin and anti-A). Hence the question how one subject can combine these two types of antigens and antibodies. For instance, when only the second one (nonagglutinogenic) is manifested, e.g. 0-cell electrophoresis reveals nonagglutinogenic A (conditionally 0, A_{pr}^{+})[1]. Obviously, in this case the serum should contain only a agglutinin, without anti-A, according to the rule (formulated by Erlich) of "horror autotoxicus".

We have made this supposition ahead of account of the facts to clarify our interpretation of the results of the experiment by the manifestation and appearance rates of agglutination under various pH values. It turned out that when the same test erythrocytes were used, agglutination was markedly weakened and delayed with pH lowering down to 6,8-7,0 only if the serum donor (in erythrocytes) lacked one of the two antigens, i.e. supposedly when not two but only one type of serum antibodies (corresponding to the missing type of antigen) was available (table 1).

Table 1 - Manifestation of hemagglutination according to pH of medium and type of group-specific antibodies.

Serum donor (antigens)	Reacting antibodies (supposedly)	Test erythrocytes	PH of medium in buffered saline solution				Time of registration of reaction
			6,8	7,0	7,2	7,4	
O, $A_{pr}^{+} B_{pr}^{-}$	α	A_1	-	-	-	+++	1'
			+M	+M	+	++++	3'
B, $A_{pr}^{+} B_{pr}^{+}$	α	A_1	-	-	+	+++	4'
			+M	+	+++	+++	8'
B, $A_{pr}^{-} B_{pr}^{+}$	α+anti-A	A_1	++	+	++	+++	1'
			+++	++	+++	++++	3'
O, $A_{pr}^{-} B_{pr}^{+}$	β	B	-	-	+M	++	2'
			hem	hem	+M	+++	10'
A_1, $A_{pr}^{+} B_{pr}^{+}$	β	B	-	+M	+	++++	2'
			-	+	+	++++	4'
O, $A_{pr}^{-} B_{pr}^{-}$	β+anti-B	B	-	+M	++	+++	20"
			+	++	+++	++++	3'

[2] Without any encroachment on the traditional symbolics of A and B ag-glutinogens, earlier we suggested to designate type II of antigenic markers as $A^{c'+}$ and $B^{c'+}$ [9]. More rightful, nevertheless, is designation of type II of antigens as A_{pr}^{+} and B_{pr}^{+} (or A_{pr}^{-} and B_{pr}^{-}); the latter designation is used in this work.

A similar weakening of agglutination in a weak-acid zone was also observed when anti-A_{hel} protectin was used, as if therewith confirming that this phenomenon was caused by absence of type II of antibodies which could have compensated for the agglutinizing ability of the serum with a decrease in pH of the medium. But the phenomenon proved to be observed with the whole set of antibodies (e.g. a and anti-A) too, if only the donor of reacting erythrocytes lacked type II of the group antigen which is tested by electrophoresis (e.g. AB, $A_{pr}^{-}B_{pr}^{+}$).

It seems that the cause of this phenomenon is more complex and may be in a change of the topography of determinants on the surface of the erythrocyte membrane, and first of all those of type I of antigens which as if "conceal" themselves in an acid medium, while antigens of type II become uncovered. This phenomenon can be explained by the fact that group-specific glycolipids, located asymmetrically on the outer side of the membrane, change their orientation because a more acid medium (6,6-6,8) is formed outside the cell, if compared with pH of the cytoplasm of erythrocytes (7,19). But glycoproteins do not change their orientation as they are integral proteins.

B. Enzymatic treatment of erythrocyte membranes

The above supposition has helped to set conditions for treatment of erythrocytes with trypsin aimed at obtaining carbohydrate determinants of one of the group antigens - the glycoprotein one. Is it not this antigen that under blood pH manifests itself practically agglutinogen-negative, concealed and is revealed by absorption of antibodies or in cell electrophoresis?

It turned out that treatment of erythrocytes with 0.1 % trypsin according to a generally accepted technique under pH=7,2 did not change their group ABH characteristic. No profound effect was produced either by both an increased concentration of trypsin up to 1 % and pH increase up to 7,8, or by pH decrease down to 7,0.

Results of trypsin effect under pH=6,8 were quite different. Electrophoretic mobility of the enzyme-treated erythrocytes was slowed down, but after a contact with group-specific antibodies no further slowing down in the mobility was observed. But the supernatant, after trypsinization and precipitation of erythrocytes, began to manifest its serological activity which corresponded to specificity of type II of antigens (table 2). This ability to cause inhibition of hemagglutination by isoantibodies was equally characteristic for supernatants after trypsinization of both A and 0 erythrocytes, provided their electrophoresis revealed this glycoprotein type of antigenic marking (i.e. O, A_{pr}^{+} B_{pr}^{-} or A, A_{pr}^{+} B_{pr}^{-}).

At the same time, no inhibition of hemagglutination by supernatants when using standard monoclonal anti-A and anti-B reagents was observed, it principally confirming the idea about two types of groupspecific antibodies, since for a standard testing those reagents are selected which correspond in their agglutinative characteristic to type I of antibodies of human origin (IgM-a,b) and therefore react with determinants of type I (glycolipid) of antigens rather than with determinants of type II.

Interestingly, that after treatment with trypsin the erythrocytes themselves, with removed determinants of glycoprotein antigens, lessened their ability to a usual group agglutination only insignificantly (by 1 titer grade). Their ability to

Table 2 - Inhibition of hemagglutination by supernatants after trypsinization of erythrocytes

Antibodies	Supernatant of erythrocytes	Test erythrocytes (2 %)	Titer in saline solution	n
Anti-A + α isoantibodies	-	A_1, A_{pr}^{+} B_{pr}^{-}	64	12
	O, A_{pr}^{+} B_{pr}^{-}	A_1, A_{pr}^{+} B_{pr}^{-}	8	
Anti-B + β isoantibodies	-	B, A_{pr}^{-} B_{pr}^{+}	32	8
	O, A_{pr}^{+} B_{pr}^{-}	B, A_{pr}^{-} B_{pr}^{+}	32	
Monoclonal anti-A (α) (routine)	-	A_1, A_{pr}^{+} B_{pr}^{-}	256	12
	O, A_{pr}^{+} B_{pr}^{-}	A_1, A_{pr}^{+} B_{pr}^{-}	512	
Anti-A + α isoantibodies	-	A_1, A_{pr}^{+} B_{pr}^{-}	64	8
	O, A_{pr}^{+} B_{pr}^{-}	A_1, A_{pr}^{+} B_{pr}^{-}	64	
Anti-A + α isoantibodies	-	A_1, A_{pr}^{+} B_{pr}^{-}	32	10
	A_1, A_{pr}^{+} B_{pr}^{-}	A_1, A_{pr}^{+} B_{pr}^{-}	8	
Monoclonal anti-A (α) (routine)	-	B, A_{pr}^{-} B_{pr}^{+}	256	8
	A_1, A_{pr}^{+} B_{pr}^{-}	B, A_{pr}^{-} B_{pr}^{+}	256	

change agglutinin abundance in a weak acid pH of the medium went on manifesting itself, but to a less degree.

Convincing data were obtained in our study of supernatants after trypsinization of B, A_{pr}^{+} B_{pr}^{+} erythrocyte sample. The fact is that if pH = 6,8, it is possible, as a rule, "to remove" determinants mostly with A specificity. Only in case of trypsinization with pH=6,6 the supernatant (after precipitation of erythrocytes) became more active in inhibiting hemagglutination by anti-B isoantibodies (by 4 grades). It should be noted that it is a common rule for a glycoprotein with B_{pr} activity whose protein structures seem to be located on the erythrocyte membrane deeper than A_{pr} and more covered with glycolipids, since their uncovering and contact with trypsin require a more acid medium.

Selectivity of the reaction of inhibition was also manifested when we used sera with an incomplete set of isoantibodies - when they interacted with trypsinized erythrocytes, and with the supernatant received during this trypsinization. The sera having the both types of antibodies do react, by liter decrease, with the both types of antigenic reagents, unlike a scrum having antibodies only of type I (e.g. a from B, $A_{pr}^{+}B_{pr}^{+}$ donor); the latter scrum does not decrease the titer when contacting with the supernatant (which contains a glycoconjugate with A-specificity from glycoprotein), but is absorbed by trypsinized erythrocytes.

C. Analysis of purified antigenic fractions

Serological differences after chromatographic purification of glycoconjugates, bound with glycolipids and glycoproteins, were not smoothed away but, on the contrary, increased in proportion to a higher serological activity of the preparations on the stages of purification. For instance, final preparations of the 2nd stage of purification (A_{pr}31 and A_{lp}21) revealed a more definite selectivity in reactions with the standard monoclonal reagent of anti-A and isoantibodies. Thus, A_{pr}31 glycoprotein preparation decreased the titer of isoantibodies from 1:64 to 1:8 (and caused the phenomenon of prozone) but did not change the titer of monoclonal antibodies, while A_{lp}21 glycolipid preparation, on the contrary, almost did not change the titer of isoantibodies of the same serum but decreased the titer of monoclonal antibodies from 1:1024 to 1:32. It should be noted that A_{lp}21 was got from erythrocyte membranes of A_1, A_{pr}^{+} B_{pr}^{-} donor, while A_{pr}31- from 0, A_{pr}^{+} B_{pr}^{-} donor. Fig. 1 shows IR spectra of absorption of these preparations, typical in a way. Without going into their detailed analysis, we would like to note their rather high purity (over 90 % by data of a highly effective liquid chromatography) and a close similarity in the IR spectrum associated with carbohydrate structures.

But often the purity of antigenic substrates for such a sensitive test as ELISA proved to be insufficient. The problem became even more complica-

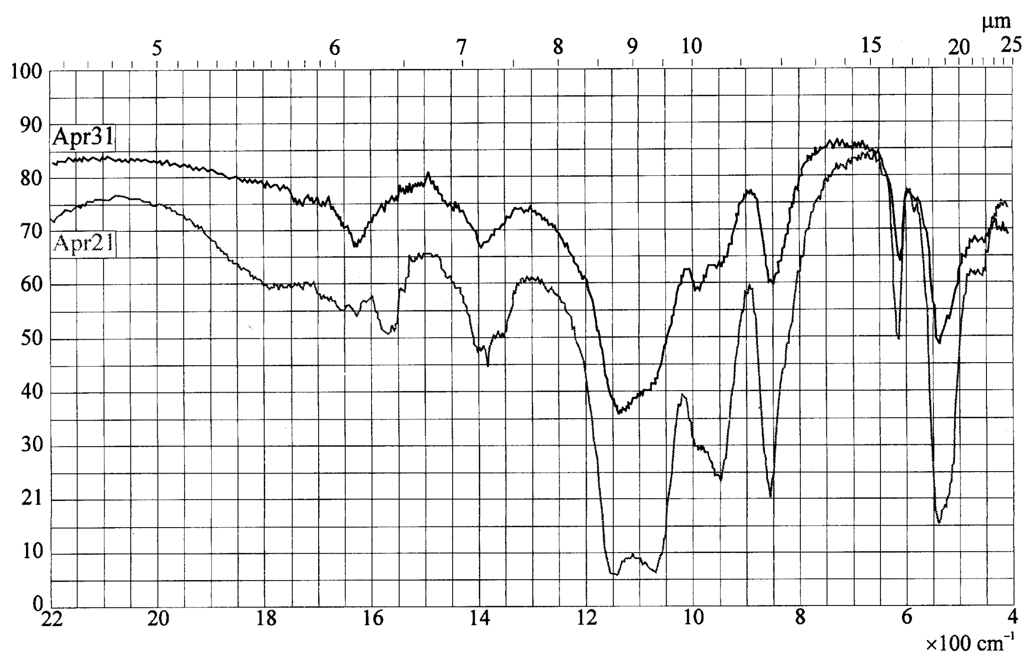

Figure 1 - IR spectra of absorption by glycolipid glycoconjugate A_{lp}21 (from erythrocyte membranes of A_1,$A_{pr}^{+}B_{pr}^{-}$ donor) and by glycoprotein preparation A_{pr} 31 (isolated after enzymatic treatment of erythrocytes of O,$A_{pr}^{+}B_{pr}^{-}$)

Table 3 - Differences in ELISA of glycolipid and glycoprotein A and B antigenic fractions

Antibodies		Fractions of glycolipid antigens			Fractions of glycoprotein antigens (pr)			Control of antigen
		A_{i-5}	A_5	B_{18}	A_8	A_{11-02}	B_{20}	
Anti-A_{pr}	№ 134	0,70±0,08	0,39±0,08	0,35±0,04	-	0,87±0,10	0,43±0,05	0,33±0,03
	№ 121	0,75±0,09	0,74±0,16	0,33±0,05	1,03±0,07	-	-	0,26±0,03
Anti-A_{lp}	№ 131	0,91±0,09	0,36±0,07	0,39±0,03	-	0,49±0,07	0,36±0,03	0,37±0,08
	№ 133	0,97±0,13	0,83±0,13	-	0,35±0,08	0,44±0,05	-	0,38±0,06
	№ 136	1,12±0,18	0,92±0,16	0,44±0,05	-	0,42±0,06	0,43±0,02	0,42±0,07
Anti-B_{pr}	№ 35	-	0,48±0,07	1,07±0,14	0,37±0,04	-	1,13±0,15	0,39±0,08
	№ 115	0,35±0,05	-	0,96±0,13	-	-	2,13±0,29	0,35±0,07
Control of antibodies		0,32±0,04	0,28±0,03	0,35±0,05	0,38±0,08	0,42±0,02	0,40±0,03	Background 0.02

ted due to heterogeneity of isoantibodies. The results were better when we used products of immune complexes after their separation and purification, rather than those of the primary purification.

Table 3 represents preliminary summary results of measurements when, in particular, some fractures of antibodies (Nos 134 & 136) and antigens (A_{11-02}), isolated from immune complexes, were used.

Differences between the two types of antibodies can be seen. Therewith antibodies of type I - to glycolipid antigens - manifest a higher selectivity and react with carbohydrate-containing glycoprotein fractions significantly weaker. It cannot be said about antibodies of type II - both anti-A and anti-B specific - which under conditions of the reaction on the panel are able to strongly react with determinants of both the glycoprotein antigens and glycolipid ones.

D. Inhibition of MABs by glycoconjugates of two types of ABH markers

Thanks to the kindness and creative participation of Prof. D.J. Anstee and Dr. Ph.A. Judson in selection of the monoclonal reagents, we had an opportunity to study and compare specificity of the glycoconjugates isolated from glycoproteins and glycolipids. Of course, the analysis of results stated below must be considered as preliminary, as a detailed study of specificity of MABs is the prerogative of international Workshops. But these results are very interesting from the aspect of this work and trustworthy, because in the standard technique [7] we used antibodies from IBGRL with the best characteristics, including those ones which were approved at the 1st Workshop (Paris, 1987 - 9W1, 9W2, 9W7), the 2nd Workshop (Lund, 1990 - 044), and the 3rd Workshop (Wantes, 1996 - 2A-48).

Table 4 - Inhibition of agglutination activity of MABs by glycoconjugates isolated from glycolipids (Ip) or glycoproteins (pr) of erythrocyte membranes.

Specificity and name of glycoconjugates	Donor of erythrocyte membranes	Specificity and name of monoclonal antibodies, their titer								
		A/Tn BRIC 66	A BRIC 131 (9W1)	A BRIC 145 (9W2)	B BRIC 250 (2A-48)	B BGRL 1 (044)	B BGRL 2	H 1+2 BRIC 39 (9W7)	H2 BRIC 198	H2 BRIC 231
----	----	1024	256	512	256	128	256	512	32	32
$A_{1p}21$	A_1, A_{pr}^+	1024	64	512	256	128	256	512	32	32
$A_{1p}37$	A_1, A_{pr}^+	1024	64	512	256	128	256	512	32	32
$A_{pr}38$	A_1, A_{pr}^+	1024	256	128	256	128	256	512	32	32
$A_{pr}33$	A_2H,$A_{pr}^+H_{pr}^+$	1024	256	256	256	128	256	512	32	32
$A_{pr}30$	O,$A_{pr}^+H_{pr}^+$	1024	256	128	256	128	256	512	32	32
$B_{1p}17$	B, B_{pr}^+	1024	256	512	256	64	64	512	32	32
$B_{1p}28$	B, B_{pr}^+	1024	256	512	256	64	64	512	32	32
$B_{pr}25$	B, B_{pr}^+	1024	256	512	128	128	256	512	32	32
$B_{pr}27$	O, $B_{pr}^+H_{pr}^+$	1024	256	512	64	256	256	512	32	32

As it is shown on the data produced (table 4), MABs which are inhibited by determinants of the glycolipid origin (BRIC 131, BGRL1, BGRL2) do not react with glycoconjugates isolated from glycoproteins. And, on the contrary, MABs which react with glycoprotein determinants (BRIC 145, BRIC 250) are not inhibited by glycoconjugates isolated from glycolipids. In our opinion, this circumstance unidirectionally confirms existence of pronounced serological differences between the two types of ABH markers.

Another fact deserves attention, that MABs which react with the glycoprotein type of a marker do it also in the case when it did not manifest itself as agglutinogen but was revealed in erythrocytes of a donor by absorption of antibodies and in cell electrophoresis (A_{pr}30, B_{pr}27). It is not a mere chance that routine MABs for determination of the group belonging turned out to be those ones that react with a glycolipid marker, rather than MABs reacting with a glycoprotein marker.

It is known that anti-A MAB 9W2, according to the data produced by G. Hartman and M.L. Beck [10], reacts with selected B cells of donors from B group. At the same time, this reagent showed some extent of activity with A_x cells, it enabling conclusion that there is manifestation of anti-A activity with A receptors which are present on cells of some donors with B group (so-called B/A/ reaction). But such "an exaggerated sensitivity" leading to a clinically unacceptable determination of blood groups resulted in discard of this MAB from those ones which were recommended for routine use.

In our opinion, ability of MAB 9W2 to react with certain samples of B erythrocytes is due to presence of A_{pr} receptors on the above B erythrocyte samples, just as in our study this reagent was inhibited by glycoconjugates isolated by trypsinization from membranes of the donor with O, A_{pr}^{+} group (A_{pr}30, table 4), rather than A_1 and A_2 donors only.

DISCUSSION

The hypothesis about the agglutinogenic and nonagglutinogenic types of ABH marking was initially grounded only by the ability of epidermis, in certain persons, to absorb group-specific agglutinins not in accordance with their blood group, it enabling a more exact selection for skin allopathy [11]. This phenomenon proved to be universal for all tissues and organs of the subject given.

But it was difficult to combine all these facts together until erythrocytes of the same person also revealed the ability to bind anti-A or anti-B agglutinins and not to be agglutinized therein, i.e. until ANAP phenomenon, known for HLA system, was discovered; this phenomenon is clearly tested in ABO system by absorption of antibodies and in cell electrophoresis [4,5]. The notion about two types of antigenic marking has begun to form, where ANAP agglutination-negative phenomenon is a particular case of a general regularity and only reflects a variant of absence of manifestation for one of them. But the fact that it is a regularity also resulted from the genealogical analysis, including inheritance of the agglutinational and sorptional ABO characteristic in monozygotic and dizygotic twins [3,4].

We have managed to reveal some clinically significant regularities too. For instance, persons with ANAP phenomenon have proved to develop bone tumours, including sarcomas, more frequently and at a younger age [12]. Carriers of the "concealed" group have a complex of immunosuppressive factors in their blood serum, it determining a better acceptability of skin allografts[13].

The data represented in this report enable a more grounded isolation of two types of ABH marking which differ by the topography on the cell membrane and the way of their isoserological revealing, and correspond to certain biochemical structures. The phenomenon of the "concealed" group was not only explained as a specific absorption of antibodies by an antigen of the glycoprotein nature, but, so to say, materialized - A-serologically active carbohydrate fragment was isolated from membranes of 0 erythrocytes and its IR spectrum was similar to the one isolated in a donor of A_1 group.

We have also managed to make sure of two typea of group-specific antibodies, and in one of them — **a** and **b** IgM agglutinins - we revealed a more pronounced selectivity in the ability to react with one of the two types of ABH antigens (with the glycolipid one). We have determined a reverse dependence between the type of a group antigen revealed on the erythrocyte and presence of the corresponding type of antibodies in the serum.

Results of the investigation with use of the most well-known and studied MABs were another convincing argument in favour of existence of two types of ABH markers; most of these MABs reacted selectively -either with glycoconjugates bound with lipids (like routine monoclonal reagents) or with glycoconjugates bound with a protein.

ACKNOWLEDGEMENTS

We would like to express our profound gratitude to Prof. D.J. Anstee and Dr. P.A. Judson for an opportunity, so kindly afforded by them, to use typing monoclonal reagents of IBGRL in our work.

REFERENCES

1. Anstee D.J. Blood Group-Active Surface Molecules of the Human Red Blood Cell. Vox Sang., 1990; 58: 1-20.

2. Clausen H., Hakomori S. ABH and Related Histo-Blood Group Antigens; Immunochemical Differences in Carrier Isotypes and Their Distribution. Vox Sang., 1989; 56: 1-20.

3. Delewski J. Dualitat der Antigenmerkmale der Erythrozyten und des Knochegewebes // Wissenschaftliche Zeitschrift der Humboldt-Universitat zu Berlin. - 1974; R. XXIII: 219-223.

4. Delevsky Yu.P. On dual character of group antigenic cell marking of human blood and tissues // Ann Acad. Med. Sci. USSR, 1975; 5: 79-87.

5. Delevsky Yu.P. Donor selection with regard to genetically determined differences in ABO differentiation of tissues. Orthoped. Traumatol., 1971; 9: 56-63.

6. Higgins J.A. Isolation and analysis of lipid components of membranes; in Findlay JBC, Evans WH (ed.): Biological Membranes // A Practical Approach. Oxford, Washington DC, JRL Press, 1987. - P. 150-195.

7. Oriol R., Samuelsson B.E., Messeter L. ABO Antibodies - Serological Behaviour and Immunochemical Characterization //J. Immunogen. - 1990; 17: 279-299.

8. Tijssen P. Practice and Theory of Enzyme Immunoassay // New York, Elsevier Sci Publishing Co, 1985.

9. Delevsky Yu.P., Gavrilina L.V., Ishkhanova M.A., Khavkina L.V. Immuno-suppreasive factor of human blood serum associated with ABO system // Immunology and Allergy. - Kiev, 1979. - pp. 49-54.

10. Beck M.L. Summary compilation of reports from participating laboratories. // Revue Francaise de Transfusionet Immunohematologie. -1987. -T.XXX - N 5, p.489-499.

11. Delevsky Yu.P. Divergencies between Group Belonging and Isoantigenic Characteristics of Human Skin // Orthoped. Traumatol. - I966; 9: I7-2 p.

12. Delevsky Yu.P., Pachyuk A.Yu., Kulish N.I., Kononenko L.S. Effect of levamisole on some indices of immunoreactivity in patients with bone tumors // Advances in Comparative Leukemia Research (Satellite Symposium). - USSR Acad. Med. Sci. Moscow, 1980. — P. 3-7.

13. Delevsky Yu. P. Skin allografting with respect of HLA-A,B,C antigens, ANAP phenomenon in the ABO system and natural immunosuppressive factor, connected with this system //VII International Workshop on Tissue Typing. - Leningrad, 1981.- P.95-96.