Journal of Blood Disorders

Open Access

Austin Publishing Group

Research Article

Influence of pH Environment on the Agglutination Ability of Anti-A-Monoclonal Antibodies and Their Inhibition by A-Glycoconjugates of Lipid and Protein Origin with Different Isoelectric Properties

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Received: June 07, 2023
Accepted: July 06, 2023
Published: July 13, 2023

Abstract

The effect caused by medium acidification to pH 6.5 on the agglutinating ability of seven anti-A MAbs (2-8, 2-17, 2-19, 2-22, 2-23, 2-28 from Workshop IV and BRIC-145) and their inhibition by glycoconjugates obtained from the membranes of A erythrocytes by enzymatic treatment and chloroform-methanol method, followed by ion-exchange gel chromatography, was evaluated.

Medium acidification most significantly reduced the agglutination of a erythrocytes by IgM MAbs 2-17, 2-19, 2-22 and 2-28 and had a weaker manifestation in more alkaline IgM MAbs 2-8, 2-23 and BRIC-145.

The inhibition of the lipid isotypes a lp-00, A lp-0, and Alp-3 (with the isoionic pH points of 8.1, 8.0 and 6.55) and the protein ones A pr-1 and A pr-3 (with the isoionic pH points of 7.15 and 6.45) was assessed in scores. Acidification to pH value 6.5 for MAbs 2-28 and 2-17 caused a considerable reduction in inhibition with acid Alp-3 and Apr-3 with slightly increased inhibition with alkaline A lp-00; MAbs 2-22 and 2-19 insignificantly and selectively altered the inhibitory capacity by more alkaline types of glycoconjugates. MAb 2-8 hardly changed inhibition.

All the above illustrates a significant role played by both the charge of glycotopes and antibodies and the specificity - the selective avidity of MAbs to certain isotypes of a glycotopes.

Keywords: Transferase; Erythrocyte; Agglutination; Blood; Detection.

Introduction

As shown by previous studies, acidification of the medium to pH 6.8-7.0 affects the severity and rate of agglutination of erythrocytes by polyclonal group-specific antibodies. It turned out, that agglutination was weakened and slowed down only in the absence of one of the two types of antibodies in the donor's serum (there are agglutinins a, but no anti-A antibodies or agglutinins b, but no anti-B antibodies). In turn, the absence of anti-A or anti-B antibodies was observed in donors with an atypical group characteristic of the type of the ANAP phenomenon in the ABO system - agglutination is negative, absorption with complement fixation is positive, for example Ax, (or, conditionally, O(I) Ac'+) [1].

It would seem that the phenomenon is due only to the type of reacting antibodies. However, the weakening of agglutination in a slightly acidic medium was also observed with a complete set of antibodies in the serum donor (for example, a and anti-A), but provided that the donor did not have a second, non-agglutinogenic isotype A group antigen tested by absorption of antibodies and in cell electrophoresis at complement fixation, such as AB(Ac'-Bc'+). It turned out to be difficult to explain this phenomenon in terms of the nature of the antigenic determinants glycolipid or glycoprotein. In particular, when the medium was acidified, it was possible to "remove" protein-bound antigenic determinants of ABH from the surface of erythrocytes with trypsin. However, even after such treatment, erythrocytes did not lose the ability to absorb both a and anti-A antibodies, and the decrease in titer in the agglutination test when using them was small.

Citation: Delevsky YP. Influence of Ph Environment on the Agglutination Ability of Anti-A-Monoclonal Antibodies and Their Inhibition by A-Glycoconjugates of Lipid and Protein Origin with Different Isoelectric Properties. J Blood Disord. 2023; 10(2): 1077.

New opportunities in the study of the phenomenon opened up with the use of a wide range of monoclonal antibodies presented at Workshop IV, and especially in connection with the discovery of isoelectric differences in A, B and H glycotopes (glycoconjugates) associated with both lipids and erythrocyte membrane protein [2]. Significant differences in the isoionic point of glycoconjugates (from pH 8.1 to 6.5) indicated differences in the charge of glycotopes acquired at blood pH [3,8]. There are grounds to suggest that the change in the charge of glycotopes upon acidification of the medium (contrary to the previously expressed opinion about the absence of charged groups in antigens representing group-specific substances [4]) is one of the significant factors in changing the agglutinability of erythrocytes. The foregoing determined the task of this study - to evaluate the effect of medium pH on the agglutinating properties of various anti-A MAbs and their inhibition by A glycoconjugates of various nature and with different isoelectric properties.

Materials and Methods

Monoclonal Antibodies (MAbs) were obtained for testing under the program of the 2nd section of the IV International Workshop on monoclonal antibodies to red blood cell antigens (Paris, 2001). Among the used MAbs anti-A 2-17, anti-A 2-19, anti-A 2-22, anti-A 2-23, anti-A 2-8, anti-A 2-28, anti-A BRIC - 145.

Antigens: Isolation of glycosphingolipids in the composition of polar lipids from erythrocyte membranes was carried out by the chloroform-methanol method according to J. Folch et al. (1957) in the modification of M. Brockhaus [5], and glycoprotein fragments, using the treatment of erythrocytes with 1% trypsin solution (Spofa, Slovakia) under the previously described conditions at pH 6.6 [7]. Further isolation and purification of glycoconjugates was carried out on DEAE-cellulose (Reanal, Hungary) with elution in a NaCl gradient and decreasing pH and on DEAE-sephadex A-25 (Farmacia, Fine Chemicals, Sweden). Chromatography of glycolipid antigens on DEAE cellulose was performed using 33% ethanol solutions.

The yield of fractions was evaluated photometrically (at 205, 280 nm and full UV spectrum) on an SF-26 spectrophotometer, Specord and serologically.

Serological studies: The hemagglutination reaction was performed on 96-well panels according to the standard method using O, A, and A2 erythrocytes [6] and taking into account visually (+) and under a microscope (+m) both the titer and the severity of the reaction and dilutions (Score). A2-erythrocytes were tested by the nature of the reaction with polyclonal antibodies, with MAB anti-H and the absence of agglutination (visually) with MAB anti-A 2-24.

Score was calculated according to W. Marsh [9] with an indication of the degree of agglutination at dilutions (points - pt), using the scale: and a 60 minute exposure with a suspension of test erythrocytes.

4+	12 pt	1+	5 pt
3+	10 pt	w+	3 pt
2+	8 pt	w+	1 pt
		-	0 pt

Results and Discussion

Analyzing the change in the agglutinating ability of seven MAbs anti-A with a change in pH from 7.4 to 6.5, one has to state significant differences in the nature of the effect of pH on Mabs. Nevertheless, in all cases, there was a decrease in titer and especially Score in the agglutination test. The only difference, although significant, was the degree of this decrease. By arranging Mabs according to the degree of decrease (Table 1), we were able to distinguish 2 groups of Mabs - with a significant weakening of the ability to agglutinate (inhibition index > 5 — **MAbs 2 – –28, 2 – –22,2–19, and 2 – –17) and with less sig**nificant weakening (inhibition index < 5 - MABs 2-8, BRIC-145).

Despite the fact that the groups were formed randomly only according to the degree of decrease in agglutination under the influence of a drop in the pH of the medium, it turned out that the first group with a strong decrease in agglutination included antibodies exclusively of the IgM class, and the 2nd group with a weaker decrease - IgG class (MAb 2-8: IgG 3; Mab 2-23: IgG 3, Mab BRIC-145: IgG 1).

Thus, the decrease in agglutinating ability under the influence of medium acidification was most pronounced for more acidic IgM antibodies, which can be explained by a weakening of their charge (somewhere at the pH 7.2 —7.1 boundary, judging by the change in the avidity of IgMa antibodies [7] up to a change in the sign of their charge from negative (at blood pH) to positive at pH 6.5. In more alkaline antibodies of the IgG class, the positive charge did not change, but even increased, which, apparently, affected a small decrease in their agglutinating properties. Incidentally, Dausset [6] drew attention to the beneficial effect of acidification of the medium during fixation of incomplete agglutinins.

Especially since the isolated A glycoconjugates, as already mentioned, differ significantly in isoelectric properties, which can significantly affect the final result when the pH of the medium changes.

In order to inhibit MABs, we used lipid glycoconjugates of conventionally designated isotypes A lp-00, A lp-0 and A lp-3) (with isoionic pH 8.1, 8.0, and 6.55, respectively) and protein A pr-1and A pr-3 (with isoionic pH 7.15 and 6.45, respectively) [8]. Thus, the range of A glycoconjugates used in this study, unfortunately, did not include alkaline (A pr-00 and A pr-0) glycoprotein fractions. All five Mabs used were previously analyzed for changes in agglutination properties without inhibition (Table 1). The results of this study are presented in Table 2.

Having arranged MAbs in the sequence corresponding to the degree of decrease in their ability to be inhibited by acidic protein glycoconjugates, we obtained an order that is partly similar to the data in Table 1 - with the maximum decrease in the inhibition index in an acidic medium for MAb 2-28 and the minimum index for MAb 2-8.

Table 1: Influence of Ph medium on agglutinating ability of Mabs. A1 test erythrocytes, Score.

pH medium	Anti-A IgM Mabs			
	2-28	**2-22**	**2-19**	**2-17**
7.4	x1=47.5±4.9	x1=56.3±3.8	x1=63±4.5	x1=52.8±4.5
6.5	x2=34.7±1.1	x2=50±6.1	x2=57±2.6	x2=47±3.8
	x1-x2=12.8	x1-x2=6.3	x1-x2=6	x1-x2=5.7
pH medium	Anti-A IgG Mabs			
	2-23	BRIC-145	2-8	
7.4	x1=26±4.7	x1=35.7±4.8	x1=45.8±3.8	
6.5	x2=21.3±4.71	x2=32.3±4.0	x2=43±6.4	
	x1-x2=4.7	x1-x2=3.2	x1-x2=2.8	

Table 2: Inhibition ofanti-A Mabs by glycoconjugates of lipid (Alp) and protein (Apr) origin from erythrocyte membranes (Score, A1 test erythrocytes).

pH medium	Isotypes of A glycoconjugates					Mab
	Alp-00	Alp-0	Alp-3	Apr-1	Apr-3	
7.4	x1=24.2±4.5 x2=23.2±3.6 -1	x1=26±2.7 x2=25±2.9 -1	x1=26.4±2.5 x2=18.2±3.7 -8.2	x1=26±2.7 x2=24.2±3.8 -1.8	x1=26.4±2.5 x2=15.4±3.5 -11	2-28
6.5	x3=20.2±4.1 x4=17.5±3.1 -2.7	x3=23.6±3.7 x4=22.6±2.8 -1	x3=23.6±4.3 x4=17.6±3.4 -6	x3=23.4±3.6 x4=21.4±3.3 -2	x3=23.6±3.7 x2=16±3.1 -7.6	
7.4	x1=59.8±1.3 x2=58.2±1.8 -1.6	x1=58±1.8 x2=58±1.8 -1	x1=58.6±1.7 x2=47.7±5.3 -10.9	x1=58±1.8 x2=57±1.9 -0.6	x1=58.8±1.9 x2=50.7±5.7 -8.1	2-17
6.5	x3=52.2±1.2 x4=49.2±1.2 -3	x3=53.8±5.1 x4=53.2±3.8 -0.6	x3=52.7±4.5 x4=44.1±5.3 -8.6	x3=53.8±3.4 x4=53.8±1.7 0	x3=53.5±4.4 x4=47.2±5 -6.3	
7.4	x1=46.2±1.1 x2=45.8±1.6 -0.4	x1=52±6 x2=50.8±6.6 -1.2	x1=48.4±1.9 x2=35.2±5.9 -13.2	x1=49.4±1.8 x2=48.6±1.5 -0.8	x1=48.4±1.9 x2=35.8±5.7 -12.6	2-22
6.5	x3=45±2.8 x4=42.2±4.8 -2.8	x3=48.6±4.8 x4=47.2±4.3 -1.4	x3=47.3±4.9 x4=34.7±6.1 -12.6	x3=48.6±4.8 x4=44.2±4.4 -4.4	x3=47.3±5.4 x4=33.3±1.3 -14	
7.4	x1=55.5±5 x2=55.8±5.8 -2.7	x1=51.8±1.6 x2=48.8±1.6 -3	x1=56.8±5.2 x2=43.5±6.2 -13.3	x1=51.8±1.6 x2=50±0.7 -1.8	x1=51.2±1.9 x2=39.2±2.7 -12	2-19
6.5	x3=57±3.8 x4=54.6±4.6 -2.4	x3=50.4±4 x4=49.82±3.9 -0.6	x3=54.2±5 x4=38.9±4.5 -15.3	x3=49.8±3.5 x4=47.6±4.5 -2.2	x3=50.3±3.4 x4=37.6±5.2 -12.7	
7.4		x1=53±3.1 x2=51±2.3 -2	x1=53±3.1 x2=43.2±2.9 -9.8	x1=53±3.1 x2=50.4±5 -2.6	x1=53±3.1 x2=43.4±2.1 -9.6	2-8
6.5		x3=52.7±2.2 x4=51±3.3 -1.7	x3=53.8±3 x4=42.4±2.8 -11.4	x3=52.7±2.2 x4=51.7±3.4 -1	x3=52.7±2.2 x4=43±2.2 -9.7	

Note:
x1 - initial activity of Mab at pH 7.4
x2- activity after inhibition at pH 7.4
x3 - activity of Mab at 6.5
x4- activity after inhibition at pH 6.5

Table 2 represents the data on the inhibition of MAb by glycoconjugates both at pH 7.4 and upon acidification to pH 6.5. It is the difference in the degree of inhibition that, in our opinion, is the most significant in assessing the nature of the effect of an acidic environment. If we take into account that acidic protein glycotopes are most responsible for the A-agglutinogenicity of erythrocytes (comparison of A1, A2, and Ax, [3]), then this is primarily the difference between inhibition by acid Apr-3 glycoconjugates at pH blood and in an acidic environment.

It turned out the of at acidic fractions of glycoconjugates of lipid and especially protein origin actually give the greatest decrease in the inhibition index, with the maximum decrease being shown by MAb 2–28, which was the leader in this regard and in the test of the direct effect of pH on agglutinating ability. Interestingly, IgM MAbs 2-28 and 2-17 showed similar and mostly typical for isoelectric relationships changes in activity under the influence of a drop in pH - the ability to be inhibited by acid glycoconjugates of both protein and lipid nature decreased. Inhibition by the alkaline type of glycoconjugates A lp-00 even increased. It should be noted, that both of these MAbs were initially characterized by a low level of inhibition by alkaline glycoconjugates, and some, although not significant, increase in their reaction with lipid glycoconjugates of this type deserves attention.

However, the individual specificity of the Mabs used, their ability to selectively react with certain types of glycotopes, apparently also affected the results. Little has changed, for example, in an acid medium, the high inhibitory ability of acidic fractions of MAb 2-22 and 2-19.

In general, MAbs 2-22 and 2-19 also showed their inherent characteristic properties under these conditions - preferential inhibition by glycoconjugates of protein origin for MAb 2-22 and lipid for MAb 2-19 [2]. This preference manifested itself upon acidification of the medium by an increase in inhibition by acidic conjugates: in MAb 2-22 of protein origin, for MAb 2-19 of lipid origin. A slight increase in inhibition by alkaline glycoconjugates was also observed in these MAbs, especially in MAb 2-22.

The effect of acidic pH on the degree of MAb 2-8 inhibition was least pronounced. Here we can, perhaps, speak only of a trend of changes, however, opposite to those observed in MAb 2-28 - the reaction with acid glycoconjugates did not decrease, but even increased somewhat, with alkaline ones, on the contrary, it slightly weakened, which is quite consistent with the very modest effect of pH on the overall agglutinating ability of this anti-A IgG Mab.

Does it make sense to study in artificially created conditions that do not exist in nature? After all, blood pH is reliably kept by many buffer systems. We considered the expediency of using such a significant change in the pH of the medium in the possibility of detecting differences in A antigenic markers, and, therefore, as another confirmation of important isotopic differences in the ABO system, that has not been practically taken into account so far and remain insufficiently studied. This study

seemed all the more interesting when taking into account the previously identified iso electric differences between group-specific glycotopes of the erythrocyte membrane - glycoconjugates of both lipid and protein origin.

Indeed, it would seem that with a decrease of the pH of the medium, the negative charge inherent in red blood cells will decrease and, accordingly, their mutual repulsion will decrease, the possibility of aggregation will increase, and the severity of agglutination will increase. However, we obtained completely different results due to the use of both antibodies with different specificities and different isoelectric properties, and the two main isotypes of antigenic glycoconjugates - acidic and alkaline, that also differ in nature - glycolipid or glycoprotein.

Acidification of the medium most significantly reduced the erythrocyte agglutinating capacity of IgM MAbs and had a weaker effect on that of IgG MAbs. If we take into account the tropism of anti-A IgG MAbs to the acidic type of glycolipid determinants that do not exhibit agglutinogenic properties [3], then the weak effect of acidification on the effect of agglutination by these IgG MAbs is quite understandable. At the same time, acidic glycotopes of type A lp-3 in a slightly acidic medium lost little in the negative charge, but antibodies of the IgG type acquired an additional positive charge, which was reflected by a slight increase in the inhibition index in MAbs 2–19 and 2–8. The inhibition of MAbs are natural due to their high specificity and not only do not contradict the stated provisions, but reinforce them with a significantly pronounced selectivity in the reaction of each MAb with certain, especially acidic types of glycotopes.

In general, the difference in the nature of the influence of the taken external factor - acidification of the reactive medium to pH 6.5 - showed the ratios of antibodies and glycotopes with the opposite charge, that naturally exist at pH human blood, providing a certain selectivity of the interaction of alkaline glycotopes - and more acidic IgM agglutinins. A, acidic glycotopes and more alkaline anti-A IgG antibodies. That is why they do not coexist in one individual and in individuals with a low severity of acid complement-fixing glycolipid A determinants (conditionally Ac'-), for example, in AB (Acc'- Bc'+) anti-A antibodies are naturally present, but there are no α antibodies [1], and in persons of type Ax, [conditionally 0 (Ac'+Bc'+)], there are no anti-A antibodies, but α-antibodies are reveaed [3]. The foregoing determines the clinical significance of taking these features into account in the ABO system due to possible transfusion hemolytic complications, the relative rarity of which is due, in our opinion, not to the low frequency of these oppositions, but to the presence in individuals with the ANAP phenomenon of a natural serum immunosuppressive factor a, - globulin, which blocks both the inductive and effect or phases immune response and, most importantly, complement activation [3]. The data obtained are also methodologically interesting, taking into account, in particular, a possible change in the agglutinating properties of polyclonal group-specific antibodies upon acidification, for example, with boric acid, which was previously used to stabilize them.

Finally, it is also important that the observed effect illustrates the significant role of both the charge of glycoconjugates and antibodies and their specificity, which is selective for A glycotope isotypes.

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