THE BINDING OF AUTOLOGOUS γ-GLOBULIN WITH ISOHEMAGGLUTININ ACTIVITY TO HUMAN RED BLOOD CELLS *

By

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γ-globulin forms a substantial fraction of the serum proteins. It accounts for most if not all of the naturally occurring antibodies and those produced by infection or generated experimentally. A significant portion of γ-globulin may well have biological properties other than antibody activity and additional roles have been proposed (Boyd, 1956). In this connection, a recent theory of antibody induction has been advanced (Najjar, 1963; Harshman and Najjar, 1963) which leads to the view that a portion of the γ-globulin is induced by cell membrane determinants and patterned to associate with them, forming a protein coat of possible functional significance. According to this theory, the naturally occurring isohemagglutinins fall into this category and associate with the autologous blood group determinants without agglutinating the cells. In contrast, a current assumption is that the isohemagglutinins arise from natural immunization (Springer et al, 1959.). The purpose of this communication is to show that, of the serum proteins, only γ-globulin is bound to autologous red cells. The bound γ-globulin can be readily eluted and shows isohemagglutinin activity.

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Human blood groups A, B and O from citrated blood, were used for these studies. All manipulations were carried out at 4°C and cells were separated by centrifugation for 10 minutes at 1,000 x g. In all cases, cells were washed three times with 2.5-5 volumes of a sucrose solution composed of sucrose 0.27 M, $CaCl_2$ 1×10^{-4}M, NaH_2PO_4 2×10^{-3}M, and Na_2HPO_4 3×10^{-3}M, pH 7.0. The sucrose washings showed successive diminution of plasma proteins such that in the third washing albumin was no longer detectable in the supernatant. The bound γ-globulin was then eluted with 0.15 M NaCl (saline). The three successive sucrose washings and the salt eluates were then subjected to protein analysis, (Lowry, 1951), electrophoresis, (Block, 1958), isohemagglutinin (Boyd, 1956) and anti-influenza PR8 and Lee titration (Jensen, 1956).

Figure I shows a typical electrophoretic pattern of the original plasma, the first sucrose washing and the salt eluate. The relative concentrations of the various components, estimated from the electrophoretic pattern, in the first sucrose washing were identical with those of the plasma, while the sodium chloride eluate contained only γ-globulin. (see Table I) The total protein concentration and relative content of γ-globulin, in each fraction, along with the various antibody titers (1/dilution) are shown in Table I. It is apparent that the sucrose washings showed progressive diminution of isohemagglutinins which were absent or present in trace amounts in the third washing. The sodium chloride eluate however, showed substantial isohemagglutinin titers in all instances studied. By contrast, the anti-viral antibodies in this fraction were either completely absent or negligible.

Figure I →

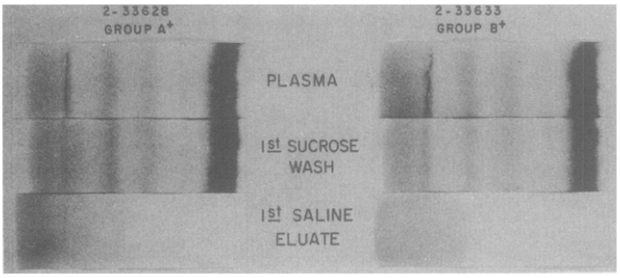

TABLE I

The presence of adsorbed γ-globulin with isohemagglutinin activity on sucrose washed autologous red blood cells and its elution with NaCl 0.15 M.

		Isohemagglutinin titer		Anti-influenza virus titer		Total protein	γ-globulin
Blood group	sample	anti-A	anti-B	anti-PR8	anti-Lee	mg/ml	%
30354 A^+	plasma	0	2048	256	512	64.00	18.8
	sucrose wash no.*						
	1		320	80	80	9.90	17.4
	2		10			0.32	
	3		0			0.13	0.
	saline eluate *		160	0	0	0.27	100.
28722 B^+	plasma	1024	0	64	64	68.00	18.6
	sucrose wash no.*						
	1	320		20	20	10.95	18.7
	2	5				0.65	
	3	0				0.12	0.
	saline eluate *	80		0	0	0.27	100.
67672 O^+	plasma	512	512	64	256	55.00	11.6
	sucrose wash no.*						
	1	160	80	20	80	7.70	10.7
	2	0	0			0.47	
	3	0	0			0.09	0.
	saline eluate *	40	40	0	0	0.21	100.

* Titers corrected to one volume per volume of cells (observed titer x 2.5). Protein values not corrected.

The results indicate that γ-globulin with isohemagglutinin activity to the reciprocal red blood cell type, is adsorbed and can be eluted from autologous red blood cells in contrast to anti-influenza virus antibodies. Cells stripped of γ-globulin by prior washing with 0.15 M NaCl are capable of readsorbing about the same amount of γ-globulin from their own sucrose diluted plasma and effectively reducing the isohemagglutinin titer. The adsorbed hemagglutinins also are retained after sucrose washings and quantitatively eluted with sodium chloride. Thus the property of red cells to associate with autologous γ-globulin, which appears to be ionic in character, remains quantitatively and qualitatively unaltered.

In the sucrose solution used, the equilibrium is greatly in favor of adsorption. The separated cells were washed with sucrose and eluted with 2.5 volumes of saline as above. The saline eluate was then dialyzed against several changes of sucrose solution for 18-20 hrs. at 4°C. This γ-globulin sucrose solution, freed of NaCl, was brought up to its original volume with sucrose solution and mixed at 0°C with 0.4 volume of red blood cells. These cells were previously stripped of γ-globulin by three washings with saline and the salt concentration reduced by one further washing with sucrose. Aliquots of the supernatant were then obtained at 1, 10, 30 and 60 minutes after mixing. Table II shows that under these conditions practically all the isohemagglutinin activity was adsorbed by the first minute leaving little or not activity in the supernatant. This adsorbed γ-globulin which remained bound throughout the 60 minute test period, was recovered in substantial quantity from these cells by a subsequent elution with 0.15 M NaCl.

TABLE II

The extent of readsorption of isohemagglutinins to autologous red blood cells (RBC). γ-globulin solutions were obtained from saline eluates of sucrose washed RBC followed by dialysis in sucrose solution.

Blood no. and group	Type	Isohemagglutinin titer: Plasma	Sample before adsorption	Supernatant 1 min. after adsorption	Saline eluates of RBC used for adsorption
12153 A^+	anti-B	256	80	0	40
31993 A^+	anti-B	512	80	0	40
33376 A^+	anti-B	512	80	0	40
31900 O^+	anti-A	1024	80	0	40
31900 O^+	anti-B	2048	320	0	80

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