

CHANGES IN THE ANTIGENIC PROPERTIES OF ERYTHROCYTES UNDER THE INFLUENCE OF CERTAIN CHEMICAL AGENTS

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There are many indications in the literature about changes in certain antigenic properties of human erythrocytes under the influence of proteolytic enzymes [3, 5-7], sodium periodate [4] and tannin.

The study of changes in the antigenic properties is of twofold interest. On the one hand, data obtained might be useful in the search for ways to decrease the heterogeneity of transplants for homoplastic transplantations. On the other hand, the effects of different factors on the antigens is an original way to study their similarities and differences.

In this paper we studied the action of different concentrations of trypsin, sodium periodate and tannin on the isoantigenic properties of rabbit erythrocytes and on various antigenic systems in human erythrocytes.

METHODS

The investigation of antigenic properties of erythrocytes was accomplished with the aid of specific sera which contain antibodies against various blood factors A, B, O, H, M, N, Rho, rh', rh'', hr', Duffy, Kell and Cellano. The antigenic properties of rabbit erythrocytes were tested with isoimmune sera obtained from animal-recipients after homoplastic transplants had been made in them [1, 2]. The erythrocytes underwent treatment after preliminary washing three times with physiological saline. Incubation with trypsin was for one hour at 37° in an isotonic NaCl medium at pH 7.6 with enzyme concentrations of one and five mg/ml.

A similar medium was used for treatment of erythrocytes with tannin. The tannin concentration for erythrocyte treatment ranged from 0.01 to 0.05 mg/ml. To prevent the spontaneous agglutination of the tanned erythrocytes in physiologic saline, the specific sera containing antibodies were diluted with single-group normal non-immune serum. In work with rabbit erythrocytes we used serum obtained from normal (nonimmunized) animals for this purpose.

For treatment of erythrocytes sodium periodate was used in concentrations from 0.035 to 0.14 mg/ml in isotonic medium at pH 5.4.

Incubation of erythrocytes with solutions of tannin and sodium periodate was made for one hour at room temperature. It is necessary to note that after such treatment the erythrocytes possessed the capacity for nonspecific agglutination by serum from that group. Therefore for testing of erythrocytes we used serum which was absorbed with rhesus-negative erythrocytes 0 (1) blood group, previously treated with sodium periodate. Such absorption removed from the serum the property of nonspecific agglutination but preserved the antigroup and other serologic specificity. In a similar manner the rabbit isoimmune serum, before investigation, was absorbed with periodate treated erythrocytes of the host organism. Testing of serologic properties was carried out by agglutination on a surface, in a tube at 37°, by the indirect Coombs method. In addition, tests of serologic properties were carried out by absorption of serum for certain antigens.

TABLE 1. Changes in Antigenic Properties of Erythrocytes as an Effect of Trypsin

Specificity of serum	Trypsin concentration (in mg/ml)	No. of experimental samples	Serum titer in relation to erythrocytes		Nature of serologic reaction
			fresh	treated	
A	1	15	64	64	Agglutination on surface
	5	10	64	512	"
B	1	15	128	128	"
	5	8	64	512	"
O	5	12	16	64	"
H	5	11	4	256	"
M	1	16	16	8	"
	5	20	16	0-2	"
N	1	16	16	8	"
	5	21	16	0-2	"
Rh ₀	1	15	64	4096	Agglutination in tube
	5	8	64	16584	"
rh'	1	5	16	128	"
rh''	1	8	16	128	Agglutination in tube
hr'	1	6	0	128-256	"
Duffy	1	5	8	0	Indirect Coomb's test.
	5	8	8	0	"
Isoimmune	5	6	4	32	"
Cellano	5	6	16	64	"
Isoimmune serum of rabbits	1	10	0-2	32-64	Agglutination on surface
	5	39	2-16	64-128	Same

RESULTS

With trypsin treatment human erythrocytes altered their agglutination properties. In the first place, they lost the capacity to be agglutinated by anti-Duffy serum. These changes occurred with use of trypsin in a concentration of one mg/ml. However, this concentration had little effect on the behavior of type and group-specific antigens (Table 1).

A concentration of trypsin of five mg/ml had a more profound effect and led to the loss of immunologic activity not only of the Duffy antigen but also the type-specific antigens M and N. In parallel with the decrease in the agglutination properties was a decrease in the adsorption activity of the treated erythrocytes. The rhesus system antigens and the isoantigens of the rabbit were not damaged by trypsin treatment. On the contrary, a considerable increase in the specific agglutinability of the human erythrocytes by anti-Rho, rh', rh'', hr' sera occurred with concentration of one mg/ml. A similar phenomenon occurred with trypsin treatment of rabbit red cells. The isoimmune serum titer rose by 3-6 dilutions. The activity of the human group antigens A, B, O, H, increased as a result of treatment of red cells only when a trypsin concentration of five mg/ml was used.

Tannin has a different effect on red cell antigens of man and the rabbit (Table 2). Even in concentrations of

TABLE 2. Change in Antigenic Properties of Erythrocytes as an Effect of Tannin.

Serum specificity	Tannin concentration (in mg/ml)	No. of samples	Serum titer in relation to erythrocytes		Type of serologic reaction
			fresh	treated	
A	0.01	5	64	64	Agglutination surface
	0.025	10	64	4-8	"
B	0.01	5	128	128	"
	0.025	5	128	8	"
O	0.01	5	32	32	"
	0.025	10	32	0-2	"
H	0.025	5	8	0-2	"
M	0.01	5	16	16	"
	0.025	17	16	0-2	"
N	0.01	4	16	16	"
	0.025	12	16	0-2	"
Rh ₀	0.01	11	128	0	Agglutination in tube
rh'	0.01	5	64	0	"
rh"	0.01	5	16	0	"
Isoimmune rabbit serum	0.01	6	16	0-2	Agglutination in tube
	0.025	10	16	0-1	"

0.005-0.01 mg/ml it depressed the serologic activity of the rhesus system antigens (Rh₀, rh^I, rh^{II}). However this concentration did not reflect on the behavior of the group (A, B, O) and type-specific antigens M and N. Incubation of erythrocytes with tannin solution in higher concentrations led to a decrease in the agglutination properties of group and type antigens. The optimal tannin concentration which had the greatest activity in relation to the group and type antigens, was 0.025 mg/ml. Rabbit red cells, as the studies have shown, are characterized by a high sensitivity to the action of tannin. Rabbit antigens lose their serologic activity during treatment with tannin solutions in a concentration of 0.015 mg/ml, i.e. at such tannin concentration which inactivates the rhesus system antigens.

The effect of sodium periodate on the antigenic properties of the red cells of humans and rabbits is similar to the tannin effect (Table 3). Sodium periodate, like tannin, possesses the capacity to alter certain antigens in small concentrations. Incubations of erythrocytes with a sodium periodate solution of 0.035 mg/ml leads to the depression of serologic activity of the rhesus system and of the rabbit isoantigens. However, this concentration still does not affect the agglutinating properties of group and type-specific antigens. Treatment of red cells with much higher concentrations of periodate (0.14 mg/ml) does depress in significant degree the agglutinability of both group and type-specific antigens. It must be noted, that the depression of the serological activity of type antigens by sodium periodate occurs unequally. The N antigen is somewhat more resistant to sodium periodate than is the M antigen.

In summary, it may be said that trypsin acts selectively on different systems. It disrupts the Duffy antigen first and then the type antigens M and N. Antigens of the rhesus system, Kell-Cellano and isoantigens of the rabbit do not fall into the sphere of the trypsin proteolytic activity. The agglutination properties of these antigens increase after red cells are treated with trypsin, evidently, at the expense of the surface antigens (the Duffy and typespecific antigens) there are freed new antigens of the rhesus Kell-Cellano and other groups.

TABLE 3. Change in Antigenic Properties of Erythrocytes as an Effect of Sodium Periodate

Serum specificity	Periodate concentration (in mg./ml)	No. of samples	Serum titer in relation to erythrocytes		Type of serologic reaction
			fresh	treated	
A	0.035	4	16	16	Agglutination on a surface
	0.14	8	16	2-4	"
B	0.035	4	32	32	"
	0.14	4	32	4-8	"
O	0.035	4	32	32	"
	0.14	4	32	4-8	"
M	0.035	5	16	16	"
	0.14	6	16	0	"
N	0.035	5	16	16	"
	0.14	6	16	4	"
Rh ₀	0.035	10	32	0	Agglutination in tube
Isoimmune rabbit serum	0.035	14	8-16	0-2	Agglutination on a surface

Tannin and sodium periodate also have unequal actions on the different antigen systems. In contrast to trypsin, which acts mainly on the Duffy and type antigens, they first affect the serologic activity of the rhesus antigen system and the antigens which determine the isoserologic differences in the rabbit. Only the use of higher concentrations affects the behavior of group and type antigens.

Comparing the behavior of red cells treated with trypsin, tannin and sodium periodate in man and in the rabbit, it is not difficult to note a general resemblance between antigens of the rhesus system and rabbit isoantigens. This similarity extends to the marked rise in agglutinability of rabbit erythrocytes and human rhesus negative erythrocytes under the influence of small concentrations of trypsin and, on the contrary, the fall in serologic activity of rhesus system and rabbit isoantigen antigens as the effect of small concentrations of tannin and sodium periodate. It may be suggested that the similarity in the behavior of human and rabbit red cells occurs as a consequence of certain similarities in the rhesus system antigens and the antigen which determine isoserologic differences in the rabbit.

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