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RHESUS-LIKE ANTIGENIC ACTIVITY MANIFESTED IN RED BLOOD CELLS OF RHESUS-NEGATIVE BLOOD DONORS AND INCREASED EXPRESSION OF ABO ANTIGENS AFTER UV-IRRADIATION OF BLOOD

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The writers showed previously that UV irradiation causes partial destruction of the outer juxtamembranous layer in autonomous mammalian tissue cells [2, 3, 6, 7]. In lymphocytes this is accompanied by changes in expression of membrane receptors [4, 5].

The aim of the present investigation was to discover whether expression of membrane antigens of the ABO and rhesus systems in human red blood cells (RBCs) is modified by UV irradiation.

EXPERIMENTAL METHOD

RBCs were obtained from freshly prepared packed red cells from blood obtained from 23 donors of different groups, stabilized with "Glyugitsir" solution. There were three series of experiments: I) on isolated RBCs washed three times with buffered NaCl solution (0.9%) and resuspended in it in a concentration of 5×10^7 cells/ml; II) on RBCs from packed cells diluted to a concentration of 5×10^7 cells/ml with solution TsOLIPK No. 8b; III) on RBCs from undiluted (intact) packed red cells. UV irradiation (254 nm) was carried out by DB-30 tubes in doses which increased hemolysis of RBCs by 5-32% after 2-3 h. Agglutinating activity of antigens of the ABO and rhesus systems was investigated in accordance with current instructions, using standard isohemagglutinating sera. The degree of agglutination of RBCs was estimated by microscopy on a 4-point system (4 points denotes the maximal reaction).

EXPERIMENTAL RESULTS

Agglutinating activity of antigens of the ABO system of unirradiated isolated RBCs from different donors varied: The minimal concentration of antibodies in which RBCs began to agglutinate was 1:64 for some blood samples and 1:8 for others. Antigens of the ABO system 2-3 h after irradiation began to be detected in the presence of a lower concentration of antibodies in the serum: antigens A and B in a titer of 1:128, H antigen in a titer of 1:32 (Table 1); this indicated an increase in agglutinating activity of the antigens by 2-4 times. The stimulating action of irradiation was exhibited in all blood samples tested, but was strongest in samples with the lowest initial antigenic activity (Table 1); in these cases their expression was increased by 8-16 times. The effect decreased appreciably after 24 h. Similar results were obtained when RBCs from diluted and intact packed cells were subjected to UV irradiation, but the effect of irradiation in this case was still present 24 h after exposure. The explanation for this could be that irradiated RBCs survive better in the form of packed cells than when diluted in physiological saline.

An increase in agglutinating activity of the $Rh_0(D)$ antigen of the rhesus system was found in RBCs of only one of six samples of RH^+ blood tested (Table 1) which was distinguished by the low initial activity of this antigen (1:32) compared with the rest (1:512). A stimulating action of UV irradiation was recorded in this case in isolated RBCs and in RBCs of in-

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TABLE 1. Changes in Agglutinating Activity of A, B, H, and $Rh_0(D)$ Antigens of Isolated RBCs 2-3 h after UV Irradiation

Antigen studied	No. of blood samples and batch no.	Dose of UV irradiation, J/m²	Degree of agglutination of RBCs with different dilutions of anti-A, anti-B, anti-H, and anti-Rh sera (mean data)						
			1:2	1:4	1:8	1:16	1:32	1:64	1:128
A	10	0 (control)	4.0	4.0	3.2	2.6	1.4	0.1	0
		248	4.0	4.0	3.8	3.4	2.4	1.3	0.2
		620	4.0	4.0	3.9	3.8	2.3	1.0	0.1
		1240	4.0	4.0	4.0	3.7	2.2	1.1	0.1
		2480	4.0	4.0	4.0	3.3	2.2	1.2	0.2
	1	0 (control)	4.0	3.0	1.0	0	0	0	0
	27 450	620	4.0	4.0	4.0	4.0	2.0	1.0	1.0
		1240	4.0	4.0	4.0	4.0	2.0	1.0	1.0
B	6	0 (control)	4.0	4.0	3.9	2.9	1.9	0.6	0
		248	4.0	4.0	4.0	3.9	3.1	1.8	0.5
		620	4.0	4.0	4.0	4.0	3.4	2.1	0.3
		1240	4.0	4.0	4.0	4.0	2.8	1.8	0.2
		2480	4.0	4.0	3.8	3.6	2.9	1.8	0.2
	1	0 (control)	4.0	4.0	4.0	2.0	0	0	0
	34 186	620	4.0	4.0	4.0	4.0	4.0	3.0	1.0
		1240	4.0	4.0	4.0	4.0	2.0	2.0	1.0
H	2	0 (control)	2.5	2.0	1.3	0	0		
		248	3.5	3.5	2.3	0.5	0.5		
		620	3.5	3.5	2.5	08.	0.5		
		1240	3.5	3.5	3.0	1.0	1.0		
		2480	3.5	3.5	3.0	1.3	1.0		
$Rh_0(D)$	1	0 (control)	2.0	2.0	2.0	1.0	1.0	0	
	27 784	248	3.0	3.0	2.0	1.0	1.0	0	
		620	3.0	3.0	3.0	2.0	1.0	1.0	
		1240	3.0	3.0	2.0	2.0	2.0	2.0	
		2480	3.0	3.0	3.0	2.0	1.0	0	

TABLE 2. Exhibition of Agglutinating Activity of $Rh_0(D)$ Antigens in Isolated RBCs and RBCs from Diluted Packed Cells from 10 Samples of Rhesus-Negative Blood at Various Times after UV Irradiation

Sample tested	Dose of UV irradiation,	Conglutination reaction with different dilutions of anti-Rh serum						Indirect Coombs' test
		1:2	1:4	1:8	1:16	1:32	1:64	
Isolated RBCs	0 (control)	0	0	0	0	0	0	0
	248	0.2 (0)	0.3 (0)	0.7 (0)	0.6 (0)	0.4 (0)	0.1 (0)	0.2
	620	0.5 (1.0)	0.7 (1.0)	1.2 (1.0)	0.9 (1.3)	1.0 (1.0)	0.3 (1.0)	0.5
	1240	1.0 (2.0)	1.3 (2.0)	1.4 (1.5)	1.4 (1.5)	1.2 (1.8)	0.9 (1.7)	0.5
	2480	1.1 (1.5)	1.2 (1.5)	1.4 (1.5)	0.9 (1.0)	1.0 (0)	0.6 (0)	0.4
Diluted packed red cells	0 (control)	0	0	0	0	0	0	0 (0)
	248	0.9	0.8	1.1	1.4	1.4	—	1.8 (1.0)
	620	0.8	1.2	1.5	1.5	1.6	—	1.0 (2.5)
	1240	1.6	2.2	2.6	2.6	2.8	—	2.4 (4.0)
	2480	2.6	2.9	3.0	2.8	2.9	—	2.6 (3.5)
	3720	2.6	3.4	3.6	3.6	3.3	—	3.1 (4.0)

Legend. Mean data shown. Degree of agglutination of RBCs 2-3 h and 24 h after irradiation given in parentheses.

tact packed cells, both immediately and 24 h after irradiation.

RBCs from Rh^- blood also were irradiated. Unirradiated RBCs of all 10 samples studied in the experiments of series I and II were not agglutinated by anti-D sera, either in the test with gelatin or in the indirect Coombs' test (Table 2). Maximal manifestation of the agglutination reaction of RBCs of Rh^- blood with anti-$Rh_0(D)$ serum was found after irradiation of diluted packed cells: All 10 samples gave a positive response in the test with gelatin and in the indirect Coombs' test; sometimes the degree of agglutination reached 4 points. Similar data were obtained in this series of experiments 24 h after irradiation also, and the degree of agglutination of the irradiated RBCs actually increased somewhat (Table 2). This manifestation of the ability of RBCs of Rh^- blood to give a positive agglutination test with anti-$Rh_0(D)$ serum was specific in character. This is confirmed by the results of experiments on the same samples of RBCs with standard group AB(IV) serum not containing anti-$Rh_0(D)$ antibodies or in gelatin solution: No agglutination of the RBCs was found. Furthermore, in a parallel investigation the writers found that irradiation does not change the electrophoretic mobility of the RBCs, and it is therefore unlikely that it could effect their electrostatic interaction, which is an important factor in agglutination.

The increased expression of antigens of the ABO and rhesus systems in Rh^+ RBCs induced by UV irradiation and the manifestation of specific activity of the $Rh_0(D)$ antigen in Rh^- RBCs took place against the background of partial destruction of the outer juxtamembranous layer and escape of 10-15% of its components (evidently glycoproteins) into the medium surrounding the cell [2]. Since glycoproteins of the cell surface may mask certain antigens and agglutination sites [1], we postulate that their removal from the surface of UV-irradiated RBCs contributes to the demasking of antigenic determinants, including the determinant of the $Rh_0(D)$ antigen in Rh^- RBCs. This hypothesis correlates closely with results obtained on isolated membranes of human RBCs, according to which the content of $Rh_0(D)$ antigen in membranes of Rh^- RBCs is the same as in membranes of Rh^+ RBCs; however, because they are dis-

tributed in deeper layers of the cell surface than in Rh^+ RBCs they cannot exhibit their activity in the presence of specific antibodies [9, 10].

The results of this investigation provide a new argument in support of the writers' hypothesis regarding the modulating influence of UV irradiation on the immune properties of blood cells [8] and the role of this phenomenon in the formation of the immune response of the organism as a whole when exposed to the action of UV irradiation and solar radiation [5]. The possibility that activity of the $Rh_0(D)$ antigen may be manifested in persons with Rh^- blood must be taken into account in clinical practice during the treatment of various pathological states by autotransfusion of UV-irradiated blood.

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