BIKEN JOURNAL Vol. 8, 171-174, 1965

SHORT COMMUNICATION

THE RELATIONSHIP BETWEEN THE ATP CONTENT OF HUMAN ERYTHROCYTES AND THEIR SUSCEPTIBILITY TO IMMUNE HEMOLYSIS

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(Received July 13, 1965)

It is now well established that sheep red cells must be aged prior to use in the immune hemolytic reaction (MAYER, 1961). Morphologically these aged sheep red cells appear to be spherical. However, it is also well known that when fresh red cells (pigeon, guinea pig, or rabbit) interact with an excess of antibody and complement they quickly change from disks to crenated disks and then to crenated spheres and to spheres which finally lyze to form ghosts (FUKUHARA, 1902). NAKAO *et al.* (1959) reported that aging of human erythrocytes in Alsever's solution caused a decrease in their adenosine triphosphate (ATP) content and at the same time they changed from a discoidal to a spherical shape.

This communication reports findings which suggest a close relationship between immune hemolysis and the ATP content of human red cells. Human blood (type 0) from healthy adults was collected with citrate, immediately washed with saline containing veronal buffer, 0.1 per cent gelatin, 0.0005 M $MgCl_2$ and 0.00015 M $CaCl_2$ (GVB^{2+}) and stored in GVB^{2+} containing 0.01 M inosine, 0.002 M adenine, 0.002 M glucose and 0.002 M K_2HPO_4 (IAG^{2+}) until use. The ATP content of these red cells did not change and they kept their discoidal form. NAKAO *et al.* (1961) reported the specific depletion of the ATP of red cells and their concomitant change to a spherical form on treatment with 0.02 M NaF. Therefore, the susceptibilities of discoidal and spherical red cells to immune hemolysis were compared.

Human red cells (E) were incubated in GVB^{2+} containing 0.02 M NaF at 37°C until they became spherical. When incubated without NaF, they remained discoidal. These two suspensions of E were thoroughly washed and used at a concentration of 4×10^8 cells per ml. They were sensitized with an equal volume of rabbit anti human 0 serum (1: 25) at 0°C for 15 min. and then at 37°C for 30 min. (EA). The EA was removed by thorough washing with GVB^{2+} and then their rates of lysis were measured in the presence of guinea pig complement (C′) at a final concentration of 1: 187.5. The lysis of NaF-treated E (NaF-E) by excess antibody and a limiting amount of C′ was found to be almost twice that of normal E under identical conditions (Fig. 1).

Since NaF-E can be rejuvenated to the discoidal form by treatment with IAG^{2+}, it was

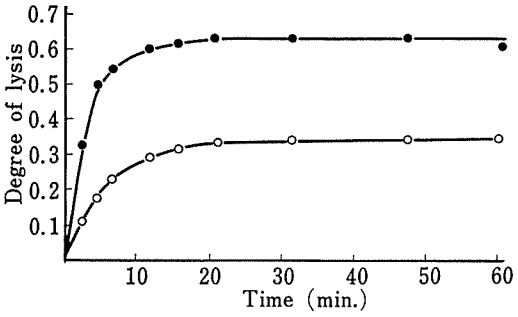

FIGURE 1 Comparison of hemolytic responses of NaF treated and normal human erythrocytes.
○———○ Control cells
●———● NaF treated cells

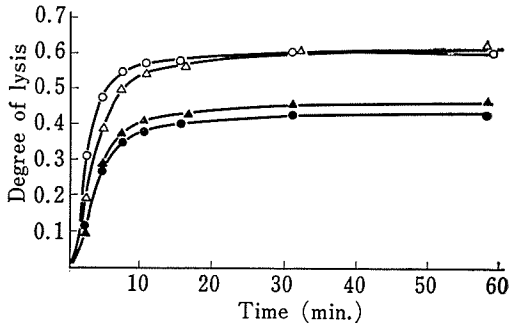

FIGURE 2 Hemolytic response of EA with spherical and discoidal forms.
○———○ NaF-EA
●———● IAG^{2+}-NaF-EA
△———△ NaF-IAG^{2+}-NaF-EA
▲———▲ IAG^{2+}-NaF-IAG^{2+}-NaF-EA

thought that these rejuvenated cells, like fresh cells, might be less sensitive to immune hemolysis than treated cells. This was found to be the case in experiments in which NaF-E was incubated with IAG^{2+} at 37°C for 2 hours and then subjected to immune hemolysis. The differential sensitivity of the two types of E to immune hemolysis might be explained by a difference in the number of hemolysin molecules combining with antigenic sites on these two types of cells. NaF-E sensitized with antiserum (NaF-EA) was treated in order with IAG^{2+}, NaF, and IAG^{2+} with thorough washing between each step, saving a sample of cell suspension at each step of the treatment. The incubation time at 37°C was 2 hours for IAG^{2+} and 4 hours for NaF treatment. The rates and extents of lysis of these cells were compared in the presence of a limiting amount of C′. It was found that the maximum lytic responses of NaF-EA and NaF-IAG^{2+}-NaF-EA (NaF-EA treated with IAG^{2+}, and then NaF) were the same, while IAG^{2+}-NaF-EA was lyzed to the same extent as IAG^{2+}-NaF-IAG^{2+}-NaF-EA (NaF-EA treated in order with IAG^{2+}, NaF, and IAG^{2+}), and that the extents of maximum lysis of both NaF-EA preparations were significantly higher than those of the IAG^{2+}-EA preparations (Fig. 2). These observations show that there is no significant difference in the number of antibody molecules on the surface of red cells regardless of their shape, and so the difference in susceptibility of the different EA preparations to immune lysis is probably due to the ATP contents of the cells and/or the amount of C′ fixed.

On the basis of the above results, attempts were made to see whether the lower susceptibility of ATP-rich cells to immune lysis is caused by the interaction of EA with C′. $EAC'_{1,4,2}$ was prepared by incubation of 2 ml of a suspension of NaF-EA (5×10^8 cells per ml) with 1 ml of whole C′ at 0°C for 15 min. The $EAC'_{1,4,2}$ was thoroughly washed and warmed at 37°C for 2 hours to destroy the C'_2. The resulting $EAC'_{1,4}$ preparation was put into 3 tubes A, B, and C. Tubes A and B were incubated with IAG^{2+} at 37°C for 2 hours, while tube C was incubated with GVB^{2+} in the same manner. Some NaF-$EAC'_{1,4}$ cells incubated in GVB^{2+} (tube C) were observed to alter in shape and become discoidal. This was probably due to the IAG^{2+}-like effects of serum components absorbed on EA. However, the transformation of $EAC'_{1,4}$ in IAG^{2+} (tubes A and B) was completed during the incubation. Rejuvenated $EAC'_{1,4}$ in tube A was treated with NaF for 4 hours at 37°C. As controls rejuvenated $EAC'_{1,4}$ in tube B and NaF-$EAC'_{1,4}$ in tube C were incubated in GVB^{2+} for

4 hours at 37°C. The final suspensions of $EAC'_{1,4}$ in tubes A, B, and C will be denoted as NaF-IAG^{2+}-NaF-$EAC'_{1,4}$, IAG^{2+}-NaF-$EAC'_{1,4}$, and NaF-$EAC'_{1,4}$, respectively. Assuming that Mayer's " one hit theory " for the immune hemolysis of sheep red cells also holds for the kinetics of the human E system, it is possible to compare the number of $SAC'_{1,4}$ sites reacting with C'_2 on the two types of cells by measuring the peak times (Tmax) of their $SAC'_{1,4,2}$ generation (BORSOS, *et al.*, 1961). A suspension of $EAC'_{1,4}$ (6×10^7 cells per ml) at 30°C was mixed with an equal volume of C'_2 at 30°C. At intervals 1 ml samples of reaction mixture were mixed with 1.5 ml of cold C'-EDTA (1: 20) and the mixture were incubated at 37°C for 90 min. to allow lysis of $EAC'_{1,4,2}$. The results demonstrated that the peak times of the 3 types of $EAC'_{1,4}$ at a concentration of 3×10^7 cells per ml were the same regardless of their ATP contents. However, NaF-IAG^{2+}-NaF-$EAC'_{1,4}$ was lyzed much more than IAG^{2+}-NaF-$EAC'_{1,4}$ (Fig. 3). From the established theory on the $SAC'_{1,4,2}$ generation curve (BORSOS, *et al.* 1961), preparations with the same Tmax have the same number of $SAC'_{1,4}$ sites per cell. Therefore, a possible explanation for these results would be that a) $SAC'_{1,4}$ sites remained reactive regardless of whether the cells were spherical or discoidal and b) as the amounts of C'_2 or C'-EDTA used in this experiment were the same with all the types of cells employed, the difference in the extents of lysis of IAG^{2+}-NaF-$EAC'_{1,4}$ and NaF-IAG^{2+}-NaF-$EAC'_{1,4}$ might be caused by differences in the reactivities of these cells with C'-EDTA.

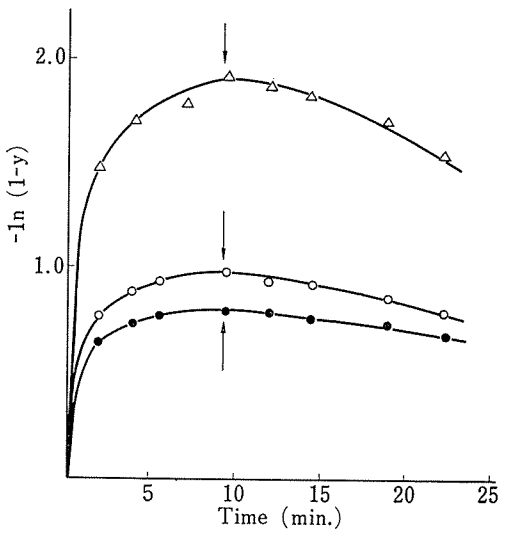

FIGURE 3 Tmax of 3 types of $EAC'_{1,4}$
○——○ NaF-$EAC'_{1,4}$
●——● IAG^{2+}-NaF-$EAC'_{1,4}$
△——△ NaF-IAG^{2+}-NaF-$EAC'_{1,4}$

Preliminary experiments showed that the E* produced from NaF-EA or NaF-$EAC'_{1,4,2}$ in the presence of 4 mM Dextran 40 (molecular weight 40,000, Pharmacia Uppsala, Sweden) (SEARS, *et al.*, 1964) can be lysed in GVB^{2+} to the same extent as the E* rejuvenated with IAG^{2+} in Dextran protected medium (Table 1).

From the above results, it can be stated that the difference in susceptibility to immune hemolysis depends upon the ATP content of either an intermediate cell which has reacted with certain component(s) of the C'_3 group, or E* precursor reported by FRANK, *et al.* (1965) which has reacted with all the C' components.

TABLE 1 *The lysis of E* with discoidal or spherical forms*

		Extent of lysis			
		IAG^{2+}—NaF—E*		NaF—E*	
		OD at 541 mμ	y	OD at 541 mμ	y
E*1	Lysis in GVB^{2+}	.143	1.00	.184	0.990
	Lysis in water	.143		.186	
E*2	Lysis in GVB^{2+}	.112	.548	.100	.547
	Lysis in water	.204		.183	

E*1 was prepared from NaF—EA with excess C' and 4 mM Dextran 40.
E*2 was prepared from NaF—$EAC'_{1,4,2}$ with C'EDTA (1 : 20) and 4 mM Dextran 40.
Incubations for lysis of E* were carried out for 90 min. at 30°C.

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