MECHANISM OF SENSITIZATION OF ERYTHROCYTES BY BACTERIAL ANTIGENS.

QUANTITATIVE REGULARITIES OF Salmonella typhi BY Vi-ANTIGEN

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The indirect hemagglutination reaction has recently been widely used in microbiology and immunology. However, the procedures of sensitization of erythrocytes are often based on insufficiently defined methods. This communication deals with a study of the mechanism involved in the sensitization of erythrocytes by Vi-antigen of Salmonella typhi.

EXPERIMENTAL

Fresh and formalized [5,6] Rh negative, group O human and sheep erythrocytes were used. The sensitizing agents were supernatant fluid from suspensions of S. typhi, heated for 1 h at 100° C, and a preparation of the Vi-antigen obtained as a result of trichloroacetic acid extraction of the same culture. The sensitization was done in a medium with 0.145 M NaCl solution, having a pH of 7.2, at different temperatures for 1 h. The degree of sensitization of erythrocytes was determined by means of Vi-hemagglutination. The amount of antigen not bound by erythrocytes was determined by means of inhibition of Vi-hemagglutination [2]. The experimental results were treated statistically by means of dispersion analysis and by individual comparison of series. The results were regarded as significant if the probability of the error did not exceed 0.05 ($P \leq 0.05$).

RESULTS

The experiments have shown that with an increase of the concentration of Vi-antigen in the medium the degree of sensitization of erythrocytes increases up to a certain limit. The relationship of the degree of sensitization to the original concentration of Vi-antigen can be satisfactorily expressed by Langmuir's equation $a = -kbc/(1+bc)$. In our experiments the maximal level of Vi-sensitization of erythrocytes was obtained with the TCA extract of Vi-antigen in a concentration of 10 mg/ml. A further increase in the Vi-antigen concentration caused a lowering of the level of sensitization of erythrocytes.

The effect of the antigen concentration on the sensitization of erythrocytes was dependent on temperature (Table 1). With sufficiently high concentrations of the sensitization was more intense at a low temperature. At concentrations from 2.5-5 mg/ml the effect of temperature was not noted, while at low concentrations of antigen (0.625-1.25 μg/ml) the process of sensitization was seen to be more intense at a high temperature. Similar results were obtained with fresh sheep erythrocytes (Table 1).

When the original concentration of Vi-hemosensitin was increased to more than 10 mg/ml, the titer of hemagglutination of both formalized and fresh erythrocytes did not increase. Consequently it is advisable to increase the concentration of antigen only to 10 mg/ml in the process of Vi-hemosensitization.

The level of sensitization of erythrocytes apparently depends on the quantity of antigen bound by the cells. The total amount of antigen bound by the cells may be determined as the difference between the original and the

Table 1. Data on Sensitization of Erythrocytes at Different Temperatures and with Different Initial Concentrations of the Vi-antigen

Erythrocytes	Concentration of Vi-antigen (mg/ml)	Temperature in deg	n	$\overline{X}$	S^2	t	P	Comparison of experimental series differing in the temperature of the medium			
								F_{exp}: $F_{0,05}$	K	t (d)	P
Human (formalized)	10,0 — 20,0	4	10	1/469	$1,587 \cdot 10^{-7}$	16,9	$\ll 0,001$	< 1	18	17,2	$\ll 0,001$
		37	10	1/390	$4,713 \cdot 10^{-7}$	11,3	$\ll 0,001$				
	2,5 — 5,0	4	10	1/394	$3,820 \cdot 10^{-7}$	13,0	$\ll 0,001$	< 1	18	0	1,0
		37	10	1/394	$3,820 \cdot 10^{-7}$	13,0	$\ll 0,001$				
	0,625 — 1,25	4	10	1/21,4	$1,739 \cdot 10^{-9}$	3,55	$0,01 > P > 0,001$	> 1	9 and 9	2,28	$0,05 > P > 0,01$
		37	10	1/79,8	$5,236 \cdot 10^{-4}$	1,73	$0,2 > P > 0,01$				
Sheep (fresh)	10,0 — 20,0	4	10	1/345	$3,452 \cdot 10^{-7}$	15,6	$\ll 0,001$	< 1	18	0,763	$0,5 > P > 0,4$
		37	10	1/372	$4,283 \cdot 10^{-7}$	13,0	$\ll 0,001$				
	2,5 — 5,0	4	10	1/68,8	$2,586 \cdot 10^{-4}$	2,856	$0,02 > P > 0,01$	> 1	9 and 9	2,103	$0,1 > P > 0,05$
		37	10	1/264	$2,123 \cdot 10^{-6}$	8,221	$\ll 0,001$				
	1,25*	4	5	1/12,0	$1,389 \cdot 10^{-3}$	7,071	$0,01 > P > 0,001$	> 1	4	4,485	$0,02 > P > 0,01$
		37	5	1/143	$6,011 \cdot 10^{-5}$	28,55	$\ll 0,001$				
	0,625*	4	5	1/10,7	$2,222 \cdot 10^{-4}$	14,0	$\ll 0,001$	> 1	5	3193	$0,05 > P > 0,02$
		37	5	1/23,8	$1,570 \cdot 10^{-3}$	2,370	$0,1 > P > 0,05$				

*These groups of experiments could not be treated as one series because the results of sensitization of erythrocytes at 37° C at these concentrations were significantly different ($d = 9.663$, $k = 4$, $P < 0.001$).

TABLE 2. Relative Amounts of Vi-sensitin Firmly Bound by Erythrocytes

Temperature	Antigen concentration (mg/ml)	
	1	20
4°	20	320
	20	240
	0	240
	20	80
	20	80
37°	60	480
	20	480
	60	320
	60	480
	60	480

TABLE 3. Level of Sensitization of Erythrocytes Treated with Vi-antigen

Temperature	Antigen concentration (mg/ml)	
	1	20
4°	1 280	2 560
	1 280	1 920
	1 920	1 920
	1 920	2 560
	1 920	2 560
37°	2 560	1 280
	2 560	1 920
	2 560	1 920
	1 920	1 920
	2 560	1 280

TABLE 4. Amount of Vi-antigen Firmly Bound by Erythrocytes Before and After Ether Extraction

Expt. No.	Before extraction	After extraction
1	480	400
2	480	240
3	320	160
4	480	160
5	480	240
6	480	160
7	480	240
8	480	160
9	320	160
10	480	240

resultant concentration of the Vi-hemosensitin. With a decrease of the resultant concentration the amount of bound antigen became less. However, it was not possible to determine the precise effect of temperature on the total amount of bound antigen. Consequently, there is no basis at this time for explaining the different levels of sensitization of erythrocytes by Vi-antigen at different temperatures by differences in the total amounts of sensitin bound by erythrocytes.

It may be assumed that Vi-antigen-erythrocytes bond has unequal degrees of stability. In this case it would be expected that the level of Vi-hemosensitization is determined only by the portion of firmly bound antigen. This portion of the Vi-antigen was determined by the difference in hemagglutinating activity of the Vi-serum, depleted by nonsensitized erythrocytes (in the control) and by erythrocytes sensitized at different temperatures and thoroughly washed (in the experimental). Formalized erythrocytes were treated with Vi-antigen (1 and 20 μg/ml) at 4 and 37° C.

It was found that the concentration of the antigen and the temperature of the medium had a significant effect on the amount of firmly bound antigen and that with increase of these perameters the firm binding of the antigen increased (Table 2).

Table 3 shows the levels of Vi-hemosensitization in the same experiments. The analysis of these results has shown that at a relatively low temperature of the medium the level of sensitization increased ($F_{exp} < F_{0.05}$ K = 8 t = 3,796 $0.01 > P > 0.001$) and at a relatively high temperature it decreased ($F_{exp} < F_{0.05}$ K = 8 t = 2,887 $0.05 > P > 0.02$) with increase in the concentration of the antigen.

These results show that an increase in the amount of Vi-antigen firmly bound by erythrocytes led to an increase of the level of sensitization only to a certain limit, after which a further increase in the amount of antigen could be accompanied even by a lowering in the level of Vi-sensitization of erythrocytes to the agglutinating effect of the Vi-serum. In this connection it may be noted that the Vi-antigen sharply increases the nonspecific stability of erythrocyte suspensions [4 (1963)]. Apparently when antigen becomes firmly bound by erythrocytes and reacts with them, two simultaneous processes take place (a specific sensitization of erythrocytes and a nonspecific stabilization of the cell suspension) which exert opposing effects on the level of the Vi-agglutinability of erythrocytes. This level increases with increase in the amount of firmly bound antigen only until the time that the process of specific sensitization prevails over the process of stabilization of the erythrocyte suspension by Vi-antigen.

The increase in amount of firmly bound antigen with increase of temperature indicates a predominantly chemical mechanism of interaction of the erythrocyte surface with the Vi-antigen. This also is comfirmed by the existence of a sufficiently firm erythrocyte-antigen bond. The antigen cannot be completely removed even after repeated washings of the erythrocytes. However, it may also be assumed that at initial stages of sensitization the process of physical adsorption is also effective, as a portion of the Vi-antigen is only weakly bound and may be removed by washing.

The problem of the nature of erythrocyte receptors x which are "responsible" for the binding of Vi-antigen is of interest. It is known that Vi-sensitization sharply decreases the group agglutinability of erythrocytes, the agglutinability by influenza viruses, and the agglutinability by antisera to antigens used in the preliminary sensitization [2-4, 8, 10]. We have recently shown that Vi-sensitin inhibits the agglutination of goose erythrocytes by the hemagglutinin of the tick-borne encephalitis virus. The results obtained by us, however, could be due to an increase in the physicochemical stability of the suspension of erythrocytes which have been treated with the Vi-sensitin. These expiments have helped to elucidate tha nature of receptors; formalized erythrocytes, following treatment with diethyl ether which removed a part of the lipoids from their surface, were able to bind less Vi-antigen than untreated erythrocytes ($P < 0.001$) (Table 4). Consequently, it must be assumed that in erythrocytes the receptors of Vi-antigen are lipoids, as is also known in the case of the hemagglutinin of tick-borne encephalitis virus [11] and of several other viruses [1, 7, 9].

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