SPECIFICITY OF ADHESION OF ERYTHROCYTES TO THE LYMPH CELL SURFACE IN VITRO

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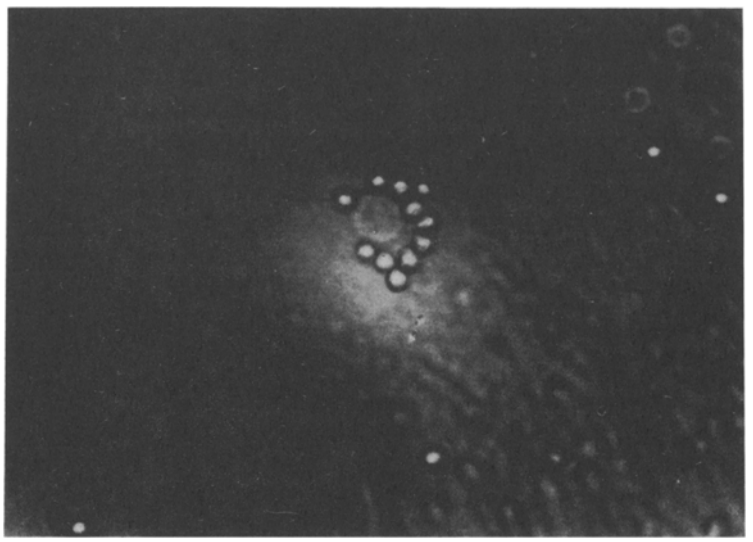
The production of antibodies by certain lymphoidal cells can be demonstrated by several methods [1-7]. One procedure used to confirm the presence of antibodies in the plasma cells is bacterial agglutination by lymphoidal cells in vitro, proposed in 1950 by Reiss et al. [8]. The authors demonstrated that bacterial contact in vitro with a suspension of lymphoidal cells of an immunized animal leads to bacterial agglutination on the surface of the plasma cells.

Because of the small size of bacteria and because of the frequently poor contrast of the preparations used in this investigation, we used erythrocytes, which have good immunogenicity and are of considerable size, as antigens.

MATERIALS AND METHODS

White rats weighing 150-180 g were used in this work. Sheep and horse erythrocytes were the antigens. Fresh blood was washed 3 times with physiological saline and different concentrations of erythrocytes in physiological solution were prepared from the sediment. The suspension was mixed with an equal volume of Freund's adjuvant,



The phenomenon of specific adhesion on the 7th day after immunization (phase contrast, objective 90 ×, ocular 7 ×).

TABLE 1. Quantitative Indicators of the Adhesion Phenomenon of Erythrocytes to Lymphoidal Cells (in vitro) from Rats Immunized with Different Concentrations of Erythrocytes

No. of days after immunization	Immunization with 0.5% erythrocyte suspension			Immunization with 5% erythrocyte suspension		
	Blood hemolysin titer	% of adhesion	adhesion index	Blood hemolysin titer	% of adhesion	adhesion index
2	2	0	0	<2	0	0
4	2	0.08	2.6	32	0.1	5.0
6	128	0.72	36.2	128	0.95	28.7
8	128	0.72	27.8	64	1.4	70.0
12	32	0.5	19.0	32	2.3	168.0
20	32	0.34	13.0	32	1.75	81.0

diluted 10-fold in normal rat serum. 0.2 ml of the mixture was introduced under the foot pads of both hind legs. The control animals received 0.2 ml of the adjuvant. At different periods after immunization the animals were exsanguinated, their popliteal lymph nodes were removed and placed in medium No. 199. The lymph node was teased with specially prepared 3-pointed needles, and the lymphoidal cells were released into the medium. The cell suspension was strained through a double layer of gauze, centrifuged for 5 min at 1000 rpm, washed once with medium No. 199 and a suspension containing 50,000 cells per ml was prepared from the sediment. To a given volume of this suspension was added an equal volume of twice washed erythrocytes, containing 100,000 cells per ml.

TABLE 2. Quantitative Indicators of the Adhesion Phenomenon (in vitro) of Erythrocytes to Lymphoidal Cells of Rats Immunized with Different Erythrocytes

Antigen used for immunization of rats	Indicator	Erythrocytes used in the experiment	
		sheep	horse
Sheep erythrocytes	Blood hemolysin titer	128	0
	% adhesion	2.0	0
	Adhesion index	173	0
Horse erythrocytes	Blood hemolysin titer	0	128
	% adhesion	0	4.1
	Adhesion index	0	270

The mixture of lymphoidal cells and erythrocytes was kept in test tubes at room temperature for 40-45 min; after careful agitation a drop of the mixture was placed on a slide and covered with a cover-slip, which was rimmed with paraffin to prevent drying. Two or three such preparations from each mixture were examined through a phase-contrast microscope. The lymphoidal cells in 20 fields were counted, and the number with adhering erythrocytes as well as the number of these erythrocytes were determined. From 3000 to 4000 lymphoid cells were counted using this procedure. Then the percent of lymphoidal cells with no fewer than 2 erythrocytes adhering and the adhesion index (the number of erythrocytes adhering to one thousand lymphoidal cells) were calculated. Control preparations, made from a mixture of erythrocytes and lymphoidal cells obtained from the popliteal lymph nodes of rats treated only with the Freund adjuvant were examined at the same time. Preparations made from lymphoidal cells of immunized rats and heterogenous erythrocytes served as second controls.

RESULTS

At first it was necessary to establish the optimal conditions for demonstrating adhesion of erythrocytes to the cells. Therefore, the initial experiments were carried out with only one antigen—sheep erythrocytes. First, it was established that the optimal time for contact of erythrocytes with the lymphoidal elements is 40 min; the adhesion phenomenon was less pronounced when shorter time intervals were used; increasing the time of contact led to agglutination of erythrocytes not amenable to quantitative estimation. In addition, it was easier to count the elements formed in the preparations which contained 50,000 cells/ml lymphoidal cells mixed with an erythrocyte suspension containing 100,000 cells/ml. In this case, from 75 to 150 lymphoidal cells were seen in each field (7 × ocular, with 1.5 × increase due to binocular microscopy; objective 40 ×). It has been also established that after a single foot pad immunization of rats with a mixture of 0.2 ml of 5% suspension of sheep erythrocytes mixed with an adjuvant, the adhesion phenomenon with lymphoidal cells from regional lymph node was demonstrated more clearly 6-12 days after immunization. Finally adhesion phenomenon was not demonstrated in either the control preparations

TABLE 3. Comparative Quantitative Indicators of the Adhesion Phenomenon of Erythrocytes to the Lymphoidal Cells, Obtained from Once-Immunized and Revaccinated Animals

Days after immunization	Lymphocytes of once-immunized rats		Days after revaccination	Lymphocytes of revaccinated rats	
	% adhesion	adhesion index		% adhesion	adhesion index
1	0	0	1	0.25	8.75
2	0	0	3	1.1	49.5
4	0.1	5.0	4	2.4	134.4
6	0.95	28.7	6	2.1	90.5
8	1.4	70.0	8	0.3	10.5
12	2.3	168.0	12	0.1	3.5
20	1.75	81.0			

obtained from lymphoidal cells of control rats and sheep erythrocytes, nor in a mixture of immune lymphoidal cells with heterogenous erythrocytes; individual leucocytes with 1-2 adhering erythrocytes were found occasionally in the field of vision. Figure 1 shows the phenomenon of adhesion of sheep erythrocytes to a lymphoidal cell from the popliteal lymph node of a rat on the 7th day after a single foot pad injection with sheep erythrocytes.

It was noted that the antibody producing cells, to which the erythrocytes adhered, were large and had a reasonably wide ring of protoplasm around the nucleus. A more detailed cytological examination of this observation was not carried out. After establishing the conditions for obtaining a clearly demonstrable adhesion phenomenon, 3 series of experiments were carried out, using differently immunized rats.

1. The relation of the adhesion phenomenon to the concentration of erythrocytes used for immunization. From the data in Table 1 it can be seen that the percent of lymphoidal cells with attached erythrocytes and the adhesion index were higher when erythrocytes were in contact with lymphoidal elements obtained from rats immunized with 5% concentration of sheep erythrocytes.

Lower indicators of the adhesion phenomenon of immune lymphocytes in contact with erythrocytes in vitro were obtained upon decreasing the immunizing dose 10-fold; using the lower immunizing dose, the adhesion index reached a peak 4-6 days earlier than when the dose of antigen was increased 10-fold. At the same time this index was significantly lower than that observed with the larger immunizing dose (36.2 compared to 168.0). In the latter case, the high adhesion phenomenon indexes were present for a longer period of time, although the blood hemolysin titers were very similar with both immunizing doses. There was no direct parallel between the expression of the adhesion phenomenon and the blood hemolysin titers; particularly with the greater immunizing dose, when during clearly evident decrease in the blood hemolysin titer the adhesion index continued to increase. Apparently, the phenomenon of specific adhesion of erythrocytes to the immune lymphoidal cells is a better indicator of the degree of immunity than the blood hemolysin titer.

2. Specificity of the adhesion phenomenon of erythrocytes to lymphoidal cells. As can be seen from Table 2, only the sheep erythrocytes adhered to the lymphoidal cells obtained from the regional lymph nodes of rats immunized with sheep erythrocytes; the lymphoidal elements of rats immunized with horse erythrocytes reacted in vitro only with horse erythrocytes. It was also noted that along with the adhering erythrocytes in the preparations made from a mixture of lymphoidal cells of rats immunized with horse erythrocytes, 40-45 min contact with the horse erythrocytes led to a significant number of agglutinated erythrocytes between lymphoidal cells. This was seldom observed after 45 min contact of the immune cells with sheep erythrocytes.

3. Demonstration of adhesion phenomenon of erythrocytes to lymphoidal cells in revaccination. In the experiments shown in Table 3, 5% sheep erythrocytes, without adjuvants, were used 2 weeks after a single injection of 5% sheep erythrocytes with Freund's adjuvants.

As was to be expected, the phenomenon of erythrocyte adhesion to the lymphoidal cells of revaccinated animals appeared and decreased earlier than adhesion to lymphoidal cells of rats immunized only once with the same antigen.

SUMMARY

The authors developed a procedure enabling the induction of a phenomenon in which erythrocytes adhere to the lymph cells obtained from regional nodes of immunized rats. Specificity in respect to erythrocytes used for immunization was demonstrated. Quantitative determination in the phenomenon under examination involving the establishment of the percentage of cells—antibody producers—(adhesion percentage) and the number of adherent erythrocytes per 1,000 lymphoid cells (adhesion index) demonstrated that the magnitude of the phenomenon in question depends on the amount of the antigen taken for immunization and the degree of the immunity perseverance.

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