INDIRECT HEMADSORPTION REACTION*

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The immunological reactivity of cells of the lymph system, in particular the regularities of the synthesis of antibodies by these cells, is one of the basic problems of present-day theoretical immunology. However, the methodological possibilities of such investigations are rather limited. Fine methods which have been proposed for the direct study of the synthesis of antibodies by single competent cells are rather complex and require special equipment [1, 2]. It is possible to use a simpler method, the bacterial attachment reaction [4, 5] with sufficient reliability only when studying the synthesis of antibodies to antigens of motile bacteria.

The data in the literature convincingly attest to the high sensitivity and specificity of the indirect hemagglutination reaction. Therefore it is considered expedient to use erythrocytes sensitized by bacterial haptens as a test antigen when studying the formation of antibodies by isolated cells. By analogy with the indirect hemagglutination reaction this method is called the indirect hemadsorption reaction.

The principle of the indirect hemadsorption reaction is that erythrocytes on which the haptens are adsorbed acquire the capacity to be specifically attached to the surface of cells synthesizing antibody.

Antigens. The experimental animals were immunized with complex antigens of Salmonella cerro and Shigella flexneri and Shigella sonnei. As antigens for sensitization of the erythrocytes we used extracts of bacterial cultures. For the preparation a 24-hour-old agar culture of bacteria was washed from a plate with 5 ml of physiological salt solution and heated in a boiling water bath for $1^1/_2$ h. The bacteria were removed by centrifugation and the supernatant liquid was used for treating the erythrocytes.

Erythrocytes and their treatment. Sheep blood was collected in an equal volume of glucose-citrate preservative, stored at 4°, and used for $1\text{-}1^1/_2$ months. The erythrocytes were washed three times with physiological salt solution. To one volume of 10% suspension of erythrocytes we added ten volumes of the bacterial extract. After incubation for an hour at 37° the erythrocytes were washed twice with 20 volumes of physiological salt solution and a 5% suspension was prepared from the precipitate on a physiological solution. The suspension of sensitized erythrocytes was stored at 4° and used in the experiments for a week.

Animals. In all experiments we used white rats of both sexes weighing 200-250 g as experimental animals. The animals were immunized 1-3 times with an interval between injections of 3-4 weeks. We injected 0.1 mg of complex antigen diluted in 0.2 ml of physiological solution into the plantar surface of each hind foot.

Test technique. The animals were killed on the 4-5th day after the last injection. We extracted the popliteal lymph nodes and prepared from them a suspension of solitary cells on a balanced salt solution prepared after the manner of Kern and Eisen [3]. After filtration through Capron cloth the cells were washed once on a centrifuge (600 rpm, 5 min) and the precipitate was diluted in a ratio of 1 : 8 with the same salt solution; 0.1 ml of the cell suspension was combined with an equal volume of 5% suspension of sensitized erythrocytes and incubated 30 min at room temperature. A slide was covered with a layer of mineral oil. By means of a Pasteur pipette with a finely drawn out end

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Characteristics of the Sensitivity and Specificity of the Indirect Hemadsorption and Bacterial Attachment Reactions

Antigen for immunizing animals	Test antigen	No. of studied cells	Cell-producers found from the hemadsorption reaction		Cell-producers found from the attachment reaction	
			abs.	%	abs.	%
Complex antigen of Shigella sonnei	Erythrocytes sensitized by antigen of Shigella sonnei	3060	75	2.4	–	–
The same	Erythrocytes sensitized by antigen of Shigella flexneri	3000	0	0	–	–
The same	Unsensitized erythrocytes.	3192	0	0	–	–
"	Shigella sonnei	3030	–	–	72	2.3
"	Shigella flexneri	2000	–	–	5	0.25
Complex antigen of Shigella flexneri	Erythrocytes sensitized by antigen of Shigella flexneri	3234	45	1.5	–	–
The same	Erythrocytes sensitized by antigen of Shigella sonnei	4020	0	0	–	–
The same	Unsensitized erythrocytes.	3155	0	0	–	–
"	Shigella flexneri	2966	–	–	59	2.0
"	Shigella sonnei	2104	–	–	8	0.4
Complex antigen of Salmonella cerro	Erythrocytes sensitized by antigen of Salmonella cerro	3800	194	5.1	–	–
The same	Salmonella cerro	4250	–	–	223	5.2

a small quantity of the mixture of the cells of the erythrocytes was applied to the slide under the layer of oil and the excess was sucked off by the same pipette. The specimen was covered by the covered glass and studied under a microscope by means of immersion phase-contrast optics. In each field of the microscope there were usually 20-30 cells and 200-300 erythrocytes. The cover glass "floated" on the layer of mineral oil. As a result of this the cells and erythrocytes freely moved in the medium under the effect of Brownian movement. This permitted distinguishing the erythrocytes attached to the cells from those randomly found around the cells.

Bacterial attachment reaction. The bacterial attachment reaction was used for comparison with the hemadsorption reaction (with respect to sensitivity and specificity). To accomplish the attachment reaction 0.1 ml of a cell suspension was combined with drops of a 3-hour-old broth culture of bacteria and drops of a salt solution. The mixture of the cells and bacteria was incubated 30 min at room temperature. The method of preparing the specimen and its study was the same as that in the hemadsorption reaction.

We divided the cell suspension of each animal into two portions and simultaneously investigated them in the indirect hemadsorption reaction and the bacterial attachment reaction. In each specimen we studied from 500 to 1000 cells. In the control experiments we used cells of normal animals or of those immunized with antigens which did not have general determinants with the test antigens. In certain control experiments the cells of the immunized animals were combined with unsensitized erythrocytes.

The results of the observations are given in the table.

As we see from the table the reaction caused by hemadsorption is not inferior in sensitivity to the bacterial attachment reaction which, as follows from the literature data, is a quite sensitive immunological test. An analysis

of the results of studying the specificity of both reactions shows that in this respect the indirect hemadsorption reaction has definite advantages which are especially evidenced in experiments with antigens of immotile bacteria. Thus, Shigella sonnei in 0.4% of the cases was attached to cells of animals immunized with the antigen of Shigella flexneri, and in 0.4% to cells of normal animals. Similar results were obtained in the experiments in which we used Shigella flexneri as the test antigen. At the same time the cells of normal and immune animals did not give nonspecific adsorption with erythrocytes sensitized by extracts of the dysentery bacteria.

Thus, the new method of studying the synthesis of antibodies by isolated cells of immunized animals – the reaction of adsorption of erythrocytes sensitized by bacterial haptens – is not inferior in sensitivity to the bacterial attachment reaction and excels it in sensitivity, especially when the formation of antibodies to antigens of immotile bacteria is studied.

SUMMARY

A new method has been suggested for the study of the synthesis of antibodies by isolated cells of immunized animals – indirect hemadsorption reaction. The reaction essentially consists of the fact that erythrocytes sensitized by bacterial haptens become capable of specific attachment to the surface of cells synthesizing antibodies. For sensitivity this test is not inferior to the bacterial attachment reaction and is superior to the latter with regard to specificity, particularly when the object of study is the formation of antibodies to antigens of immotile bacteria.

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