EFFECT OF BACTERIAL POLYSACCHARIDE ON NUMBER OF ROSETTE-FORMING CELLS IN MICE WITH DEPRESSED IMMUNOLOGICAL REACTIVITY

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UDC 612.112.94-06:576.8.098.345.8

The effect of bacterial polysaccharide on the number of rosette-forming cells (RFC) was studied in mice with depressed immunological reactivity (irradiation with γ rays in a dose of 200 mg/kg). Bacterial polysaccharide was shown to increase the number of RFC in the intact, immunized, and irradiated animals. However, the polysaccharide had no stimulant action on RFC formation in mice treated with cyclophosphamide, confirming differences in the nature of depression of immunological reactivity after irradiation and cyclophosphamide.

KEY WORDS: rosette-forming cells; bacterial polysaccharide; irradiation; cyclophosphamide.

Lymphocytes are known to form "rosettes" because of the receptors located on their outer membrane [3, 1]. Rosette-forming cells (RFC) can be macrophages, plasma cells, lymphocytes, etc. [11, 13]. Since cells which form rosettes are responsible for immunological reactions, it is possible to use the rosette formation method to judge the immunological reactivity of the animal at the cell level. Bacterial polysaccharides (PS) are also known to stimulate natural and artificial immunity by increasing nonspecific resistance to infection, the number of antibody-forming cells, and the antibody titer [1, 6, 7, 10]. Rosette formation can be used as an objective test for the study of immunological reactivity of the organism and its changes under the influence of PS.

The object of this investigation was to study the effect of PS on the number of RFC in animals with depressed immunological reactivity.

EXPERIMENTAL METHOD

Experiments were carried out on 280 CBA mice weighing 18-20 g. To depress the immunological reactivity of the animals they were irradiated or treated with cyclophosphamide (CP). The mice were given a single session of whole-body irradiation in a dose of 400 rad on the EKU-50 γ-ray source with a dose rate of 69 rad/min. This dose did not cause death of the animals in these experiments. Cyclophosphamide was injected intraperitoneally in a dose of 200 mg/kg.

The PS used for stimulation were obtained from somatic O antigen of typhoid bacilli [2]. PS was injected intraperitoneally in a dose of 50 μg per mouse 24 h before irradiation and injection of CP. Some animals were immunized with sheep's red cells (RBC), $5 \cdot 10^8$ of which were injected intraperitoneally 3 days after irradiation. Animals receiving CP were immunized 3 h and 3 days after the injection of CP.

The animals were divided into 10 groups: 1) intact; 2) animals receiving PS; 3) immunized with RBC; 4) receiving PS and immunized with RBC 24 h after injection of PS; 5) irradiated and immunized with RBC on the third day after irradiation; 6) treated with PS 24 h before irradiation and immunized with RBC 3 days after irradiation; 7) immunized with RBC 3 h after injection of CP; 8) treated with PS 24 h before injection of CP

Laboratory of Natural Immunity, N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 58-59, January, 1977. Original article submitted February 19, 1976.

TABLE 1. Number of RFC in Spleens of Mice Receiving Various Treatments (arithmetic mean values and confidence limits, $P < 0.05$)

Index	Group of animals									
	1-	2-	3-	4-	5-	6-	7-	8-	9-	10-
Number of RFC per 1000 nucleated cells	0,6	0,9	20,2	23,6	0,8	1,2	0,8	0,8	4,5	4,6
Confidence limits	(0,5—0,7)	(0,7—1,1)	(18,6—21,8)	(22,4—24,8)	(0,6—0,9)	(1,0—1,4)	(0,6—0,9)	(0,6—0,9)	(2,7—6,3)	(3,7—5,5)
Number of animals	30	25	30	30	30	30	30	25	15	20

and immunized with RBC 3 h later; 9) receiving CP and immunized with RBC 3 days later; 10) receiving PS 24 h before treatment with CP and immunized with RBC 3 days after injection of CP.

The number of RFC was determined by Zaalberg's method [14]. The number of RFC was counted in 1000 nucleated cells in a "squashed drop" preparation under the phase-contrast microscope. Nucleated cells with five or more adherent red cells on their surface were regarded as rosette-forming cells.

The experimental results were subjected to statistical analysis with determination of the arithmetic mean values and confidence limits by the use of Strelkov's tables [5].

EXPERIMENTAL RESULTS

Immunization of the animals with RBC caused a marked increase in the number of RFC compared with their number in the unimmunized animals (groups 1 and 3). In groups 3-10 all the mice were therefore immunized with RBC, irrespective of their other treatment (Table 1).

Irradiation of the animals in a dose of 400 rad led to a sharp decrease in the number of RFC per 1000 nucleated cells compared with the control (groups 3 and 5). A decrease in the number of RFC also was observed after injection of CP (groups 7 and 9). Under the influence of PS the number of RFC was increased both in the unimmunized mice (group 2) and in the mice immunized (group 4) with RBC. PS had the same sort of stimulant action on the irradiated animals also (group 6). Different results were obtained on injecting PS into mice treated with CP. In that case injection of PS did not increase the number of RFC (groups 7-10).

The results indicated that PS has a stimulating effect when immunological reactivity is depressed by irradiation but no such effect after the action of CP. This is evidence that the mechanisms of depression of immunological reactivity after CP and irradiation are different. The results also show that the stimulating action of PS is manifested in the presence of cells of a certain type. Cells of lymphoid tissue belong to this type. Lymphoid tissue is the least radioresistant [4, 8], and irradiation even in doses not causing death of the animal leads to a marked decrease in the number of lymphocytes, which explains the decrease in the number of RFC in the irradiated animals. When PS was injected before irradiation, it exhibited its radioprotective action, as a result of which more cells of the lymphoid tissue remained viable than in the animals which did not receive PS. By stimulating proliferative processes in the lymphoid tissue remaining after irradiation, PS led to an increase in RFC. Considering the known fact that CP acts selectively on cells of the proliferative pool, unlike other alkylating agents or irradiation [12], it will be clear why PS does not increase the number of RFC in animals injected with CP.

Yumasheva and co-workers [9] showed that an antigenic stimulus induces proliferation of immunocompetent cells and they die selectively as a result of the action of CP, whereas other lymphoid cells, which do not react to this antigen and do not proliferate, can compensate for the damage caused by CP. Since PS was injected before treatment of the animals with CP, proliferative processes in the lymphoid tissue were intensified [9], whereas under the influence of CP all the proliferating cells died, so that the stimulating action of PS was completely abolished and the number of RFC was reduced to their level in the irradiated animals.

Whether the stimulating action of PS is manifested or not in an animal with depressed immunological reactivity thus depends on the mechanisms of its depression.

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ANTIBODIES AGAINST COMMON ANTIGEN OF EPITHELIAL TISSUE OF HUMAN THYMUS AND SKIN EPIDERMIS IN MYASTHENIA GRAVIS

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UDC 616.74-009.17-07:616.438-008.9-097.5-078.73

Experiments by the indirect immunofluorescence method showed that the sera of patients with myasthenia gravis in a high percentage of cases react with cells of epithelial type of the human thymus. By absorption of the sera with suspensions of epidermal cells and tissue homogenates of several human organs it was shown that the antigen of the epithelial cells with which the sera of patients with myasthenia react belongs to the epidermal heteroorganic antigens of the thymus, i.e., it is common to the epithelium of the thymus and the epidermis of the human skin. The presence of antibodies against epithelial tissue cells of the thymus in the blood serum of patients with myasthenia gravis suggests that in this disease an immunopathological process takes place, aimed against thymus tissue antigens, including against the heteroorganic structures of its epithelium.

KEY WORDS: myasthenia gravis; epithelium of the thymus; epidermis of the skin; cross-reacting antigens and antibodies.

The serum of patients with myasthenia gravis is known to contain antibodies against antigens of the myoid cells of the thymus common with antigens of skeletal muscle and myocardium [1, 2, 6]. In this disease, as in other autoimmune processes (rheumatic fever, ulcerative colitis, multiple sclerosis), the serum reacts in a

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(Presented by Academician of the Academy of Medical Sciences of the USSR O. V. Baroyan.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 83, No. 1, pp. 60-62, January, 1977. Original article submitted May 24, 1976.