# COMPARATIVE STUDY OF THE EFFECT OF SOME NONSPECIFIC STIMULATORS ON THE IMMUNE RESPONSE

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Much attention has been paid in recent years in the combined treatment of malignant neoplasms to the use of various agents weakening immunodepression and effectively potentiating the immune responses of the body to tumors [1, 2, 4, 12, 14]. An intensive search is also in progress for new natural and synthetic compounds with high immunostimulant activity. Accordingly, comparative studies of the action of nonspecific stimulators of varied nature on the immune status of the body and the general effect of treatment are of great importance.

In the investigation described below the action of various nonspecific stimulators on the response of immune rosette formation of lymphocytes and on the reaction of hyposensitivity of delayed type (HDT) in normal animals was studied.

## EXPERIMENTAL METHOD

Male BALB/c mice weighing 18-20 g were used. Living liquid BCG vaccine containing $3 \times 10^8$ to $9 \times 10^8$ bacterial cells/ml (from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR) freeze-dried BCG vaccine (from Stavropol' Research Institute of Vaccines and Sera), the bacterial lipopolysaccharide prodigiosan (from the Laboratory of New Antibiotics, Department of Microbiology, Central Postgraduate Medical Institute), the yeast cell membrane polysaccharide zymosan (from Tallin Pharmaceutical Chemical Factory), the polyanion dextran sulfate with a molecular weight of 500,000 (from Loba-Chemie, Austria), the imidazole derivative levamisole (from Gedeon Richter, Hungary), and the fluorene derivative tiloron (from the Physicochemical Institute, Academy of Sciences of the Ukrainian SSR, Odessa) were used as immunostimulants. All these substances were injected intraperitoneally in 0.5 ml physiological saline in the following doses: living BCG vaccine $10^7$ bacterial cells per mouse, freeze-dried BCG vaccine 0.1 mg per mouse, prodigiosan 2.5 mg/kg, levamisole 15 mg/kg, dextran sulfate and zymosan 25 mg/kg, tiloron 50 mg/kg.

To determine rosette-forming cells (RFC) mice were immunized intraperitoneally with sheep's red blood cells (SRBC) in a dose of $5 \times 10^8$ cells. The antigen was injected 2-3 h after the immunostimulant. The number of RFC was determined by the method described previously [10] on the 5th day after injection of the antigen. RFC were counted in $10^3$ lymphocytes from thymus and spleen and were differentiated with respect to functional activity: those formed by 4-6 SRBC were classed as $T_1$-RFC, by 7-10 SRBC as $T_2$-RFC, and by more than 10 SRBC as B-RFC [3, 10].

The HDT test was set up by the method described in [11]. The intensity of the reaction was expressed as the degree of swelling of the paw 24 h after injection of the reacting dose of the test antigen (SRBC). The weight of the thymus was determined and the splenic index calculated as integral indices.

## EXPERIMENTAL RESULTS

Data showing the effect of seven nonspecific stimulators on the quantitative and qualitative composition of RFC of the thymus and spleen of the immunized mice are given in Table 1. It will be clear from Table 1 that the mean number of RFC in the thymus of the control group of mice was $50 \pm 4.5/10^3$ cells; they consisted mainly of $T_1$-RFC, with weak antigen-binding power [3, 10].

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TABLE 1. Effect of Nonspecific Stimulators on Level of Immune RFC in Mouse Spleen and Thymus (M ± m)

| Preparation | Number of RFC (per $10^3$ cells) | | | | | | | |
|---|---|---|---|---|---|---|---|---|
| | in thymus | | | | in spleen | | | |
| | $T_1$ | $T_2$ | B | total | $T_1$ | $T_2$ | B | total |
| BCG (living) | 99±6,5 | 17,1±1,6 | — | 116±7,0 | 173±5,1 | 28±1,6 | 2±0,4 | 203±6,5 |
| *P* | <0,01 | <0,01 | | <0,01 | <0,01 | <0,01 | >0,05 | <0,01 |
| BCG (freeze-dried) | 65±4,2 | 3±0,1 | — | 68±4,8 | 73±4,9 | 5±1,2 | 21±2,3 | 99±7,0 |
| *P* | <0,01 | >0,05 | | <0,05 | >0,05 | >0,05 | <0,01 | >0,05 |
| Zymosan | 78±5,7 | 9±1,3 | — | 87±5,9 | 98±6,7 | 16±1,7 | 3±0,4 | 117±11,8 |
| *P* | <0,01 | <0,01 | | <0,01 | <0,01 | <0,01 | >0,05 | <0,05 |
| Prodigiosan | 101±1,8 | 3±0,7 | — | 104±1,7 | 86±2,5 | 7±0,5 | 48±2,8 | 141±5,4 |
| *P* | <0,01 | >0,05 | | <0,01 | >0,05 | >0,05 | <0,01 | <0,01 |
| Dextran sulfate | 92±6,7 | — | Single cells | 92±6,7 | 75±6,1 | 6,0±0,4 | 35±2,1 | 116±8,4 |
| *P* | <0,01 | | | <0,01 | >0,05 | >0,05 | <0,01 | <0,05 |
| Tiloron | 62±6,1 | 2±0,3 | — | 64±6,3 | 87±6,6 | 9±1,1 | 49±3,0 | 145±10,1 |
| *P* | >0,05 | >0,05 | | >0,05 | >0,05 | >0,05 | <0,01 | <0,01 |
| Levamisole | 102±5,3 | 18±1,2 | — | 120±6,6 | 136±7,5 | 27±2,0 | 6±1,7 | 169±10,5 |
| *P* | <0,01 | <0,01 | | <0,01 | <0,01 | <0,01 | >0,05 | <0,01 |
| Physiological saline (control) | 48±3,6 | 2±0,2 | — | 50±4,5 | 79±5,0 | 7±0,4 | 3±0,2 | 89±6,3 |

Legend. Value of P calculated relative to control.

Under the influence of all the stimulators except tiloron a statistically significant increase was observed in the number of T-RFC in the thymus; the greatest increase in the number of these cells was observed after injection of living BCG vaccine and levamisole. These preparations, and also zymosan, led to some increase in the absolute and relative numbers of RFC formed by the more mature $T_2$-subpopulation of lymphocytes. Under the influence of dextran sulfate no $T_2$-RFC were found, and the solitary rosettes were differentiated as B-RFC.

In mice of the control group (Table 1) the mean number of RFC in the spleen was 89 ± 6.3/$10^3$ cells. When expressed as a percentage the number of $T_1$-RFC was greater, but rosettes with a high density of antigenic receptors ($T_2$- and B-RFC) also were observed.

All the stimulators studied except freeze-dried BCG vaccine caused a statistically significant increase in the RFC level in the spleen. The highest total number of RFC was found after administration of living BCG vaccine and levamisole; more than 95% of the RFC were differentiated as $T_1$- and $T_2$-RFC. After injection of zymosan the total number of RFC in the spleen increased by a lesser degree than after injection of living BCG vaccine and levamisole, but in this case also most of the rosettes formed belonged to the $T_1$- and $T_2$-RFC categories.

By contrast, after administration of prodigiosan, tiloron, and dextran sulfate over 30% of the rosettes formed were of the B-RFC type, whereas the number of T-RFC was almost unchanged compared with the control. Freeze-dried BCG vaccine, which has a lower content of living mycobacteria than the BCG culture, led to a statistically significant increase only in the B-RFC level in the spleen.

The results are in agreement with data in the literature [8, 9, 13] showing the stimulating effect of certain bacterial polysaccharides, dextrans, and tiloron on the humoral immune response. This does not contradict the fact established by the writer previously that these same substances affect the $T_1$-RFC level. Moreover, considering the helper function of $T_1$-cells [6], it can be tentatively suggested that these stimulators have a positive action on cooperation between T and B lymphocytes, which precedes immunogenesis. At least for synthetic polyelectrolytes, such as dextran sulfate, this mechanism is now firmly established [5]. It is evidently of definite importance also in immunotherapy of tumors, for it has been shown that inhibition of the immune response to thymus-dependent antigen in mice with tumors is associated with disturbance of cooperative interaction between T and B cells [7].

As regards living BCG vaccine, levamisole, and zymosan, as the results show, these substances regularly increased the number of T-RFC, i.e., the subpopulation to which the principal effector cells of lymphocyte-mediated immunity belong [6]. In all probability, the stimulators mentioned above facilitate accumulation of a clone of sensitized lymphocytes, responsible for immune responses of cellular type.

TABLE 2. Effect of Nonspecific Stimulators on HDT Reaction in Sensitized Mice

| Preparation | Response of inflammatory swelling of paw (M ± m), mm | Intensity of HDT reaction, mm | P |
|---|---|---|---|
| BCG (living) | 0,58±0,038 | 0,24 | <0,01 |
| BCG (freeze-dried) | 0,42±0,047 | 0,08 | >0,05 |
| Zymosan | 0,57±0,045 | 0,23 | <0,01 |
| Prodigiosan | 0,38±0,025 | 0,04 | >0,05 |
| Dextran sulfate | 0,42±0,061 | 0,08 | >0,05 |
| Tiloron | 0,45±0,060 | 0,11 | >0,05 |
| Levamisole | 0,54±0,043 | 0,20 | <0,01 |
| Physiological saline (control) | 0,34±0,034 | — | — |

This hypothesis is supported by the results of an investigation of the effect of nonspecific stimulators on the HDT reaction in sensitized mice (Table 2). As Table 2 shows, living BCG vaccine, levamisole, and zymosan significantly increased the intensity of the HDT reaction, assessed by measuring the inflammatory swelling of the paw, whereas the potentiation of the response by the other preparations was not statistically significant. The results of these experiments are indirect evidence of activation of killer lymphocytes by living BCG vaccine, levamisole, and zymosan, for the effector component of all HDT reactions is linked with accumulation and enchancement of the functional activity of precisely these lymphocytes [6, 11].

An increase in the splenic index was found under the influence of all the immunostimulants, but the increase was greatest after prodigiosan, dextran sulfate, and tiloron (from 1.43 to 1.93; $P < 0.01$), i.e., those agents which affect mainly humoral immunity. Characteristically these substances did not significantly change the weight of the thymus, whereas under the influence of levamisole (45.4 ± 2.11 mg; $P < 0.01$) and living BCG vaccine (37.0 ± 1.51 mg; $P < 0.01$) it was increased significantly (from 21.6 ± 0.81 mg in the control).

The results as a whole, indicating the preferential action of living BCG vaccine, levamisole, and zymosan on T-cell immunity, and of prodigiosan, dextran sulfate, and tiloron on the humoral immune response, provide a basis for the more differential use of nonspecific stimulators during immunotherapy of tumors and of immunodeficient states of other etiology.

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