IMMUNOBIOLOGICAL PROPERTIES OF STAPHYLOCOCCAL ENTEROTOXINS OF TYPES A, B, C, D, AND E

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Staphylococcal enterotoxins (SE) are polyfunctional in nature: they have a marked enteropathogenic action and, in sublethal doses, they actively influence various immunologic processes in the host organism. The method whereby staphylococcal enterotoxins enter the animal or human body is an important factor for manifestation of their activity. The speed with which the toxic protein finds the corresponding target cells, an ultimately the end result of their interaction, will evidently depend on this factor.

Staphylococcal enterotoxins of types A and B in vitro stimulate mitoses in a thymus-dependent lymphocyte population. Besides their ability to induce blast transformation of lymphocytes, enterotoxins of these types can also induce immune interferon synthesis [6, 7, 8]. Other serologic types of SE have virtually not been studied from the standpoint of their immunobiological properties.

The aim of this investigation was to compare the mitogenic properties of five serologic types of SE, and also their ability to induce immune interferon production in a culture of healthy human peripheral blood lymphocytes.

EXPERIMENTAL METHOD

Preparations of SE of types A (SEA), B (SEB), C (SEC), D (SED), and E (SEE) were obtained by methods described previously [1, 2, 5]. Mitogenic activity of the SE was studied in the short-term cultures of healthy human peripheral blood lymphocytes, isolated by centrifugation in a Ficoll–Verografin gradient. The cells were cultured for 72 h in complete medium RPMI-1640. Proliferation was counted on the basis of ^{3}H-thymidine incorporation. The index of stimulation (IS) was calculated relative to spontaneous proliferation. The experiments were carried out in three repetitions. To study the interferon-inducing activity of SE, a lymphocyte culture was prepared by the method in [3]. To stimulate the lymphocytes, control inducers were used: con A (Serva, West Germany) and PHA (Gibco, USA), together with SE of types A, B, C, D, and E. Activity of induced interferons and their antigenic properties were determined by methods described previously [3, 4]. To determine the antigenic properties of interferons induced by SE, human α- and β-interferons and sera against them, obtained from the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, were used as the control. Antiserum to human γ-interferon was obtained by immunizing rabbits with a concentrated interferon preparation.

EXPERIMENTAL RESULTS

The mitogenic activity of the SE preparations was studied on the basis of their ability to stimulate the proliferative response of healthy human peripheral blood lymphocytes, and comparing this with their response to PHA and con A. DNA synthesis was monitored as ^{3}H-thymidine incorporation. The cells were treated with PHA and con A in concentrations of 5 and 10 μg/ml, in which maximal stimulation of proliferation was observed. The SE preparations were used in concentrations of 1, 2, 2.5, 5, 10, 15, and 20 μg/ml. The results showed

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TABLE 1. Stimulation of Proliferative Activity of Healthy Human Peripheral Blood Lymphocytes by Staphylococcal Enterotoxins of Different Types

Mitogens	Experiment (cpm/0.5 × 10^6 cells/ml)	Index of stimulation (IS)
PHA	763±190	10,7±2,5
con A	979±245	13,7±3,7
SEA	782±195	10,9±2,2
SEB	753±185	10,6±3,07
SEC	825±206	11,6±2,9
SED	1143±286	16,1±4,02
SEE	722±180	10,2±2,9

Control: spontaneous incorporation of ^{3}[H-thymidine was 71 ± 20.6.

TABLE 2. Interferon Production by Human Lymphocytes Stimulated by PHA, con A, SEA, SEB, SEC, and SEE

Concentration of inducers (μg/ml)	Titer of induced interferons					
	PHA	con A	SEA	SEB	SEC	SED
0,01	—	—	320	20	20	80
0,1	—	—	640	80	80	160
1,0	80	40	1280	160	160	160
10,0	160—320	160	320	320	160—320	320
100,0	160—320	160	320	320	160	320

that the SE preparations in a dose of 5 μg/ml caused maximal stimulation of proliferative activity of the lymphocytes, and that an increase in concentrations of the preparations did not lead to any increase in IS. When the cells were treated with the toxic proteins in concentrations of 10, 15, and 20 μg/ml, in some experiments the ^{3}H-thymidine incorporation was below the control level. The results are given in Table 1.

It will be clear from Table 1 that the highest value of IS was observed after treatment of cells with SED, indicating that this type of enterotoxin has higher mitogenic activity.

Thus, the SE preparations are strong polyclonal mitogens against human peripheral blood lymphocytes.

To determine the interferon-inducing activity of SE, lymphocyte suspensions were treated with the following concentrations of these proteins: 0.01, 0.1, 1.0, 10.0, and 100.0 μg/ml. The cells were cultured on a rotary shaker at a speed of 98 rpm for 72 h, after which the interferon activity in the incubation medium of the cultures was determined. The results of these investigations are given in Table 2. It will be clear from Table 2 that the most marked interferon production was observed in response to stimulation of the cells by SEA in a concentration of 1.0 μg/ml. In the same concentration of SE of types B, C, and E the interferon titers were lower and approximated to values of activity of interferons induced by PHA and con A in concentrations of 10 μg/ml. The highest titer of SEA-induced interferon was 640-1280. The fact must be noted that with an increase in SE concentration the interferon titer did not increase.

Thus, irrespective of the serologic type, SE possessed considerable interferon-inducing activity in healthy human peripheral blood lymphocyte cultures. Treatment of lymphocytes with SEA induced a higher level of interferon production than treatment with SEB, SEC, and SEE, and also such inducers as PHA and con A.

To determine the class of SE-induced interferons their physicochemical and antigenic properties were studied. Samples of SE-induced interferons, and also human α- and β-interferons were treated with 0.1 N HCl and pH of the medium was adjusted to 2.0. The samples were kept for 24 h and then their pH restored to 7.0 by means of 0.1 N NaOH. Other samples were heated to 56°C for 30 min. The results of these tests showed that treatment with acid and heat completely inactivated the antiviral action of interferons induced by PHA, con A,

and SE but did not affect activity of α- and β-interferons. Antisera to human α- and β-interferons neutralized the antiviral action only of standard homologous interferons, and did not affect activity of interferons induced by SE, PHA, and con A. The antiviral properties of the latter were neutralized on treatment with antiserum to human γ-interferon which, in turn, was inactive against standard α- and β-interferons. The results indicate that the SE preparations induce the production of an interferon, by human peripheral blood lymphocytes, whose physicochemical and antigenic properties correspond to those of human γ-interferon.

It can thus be concluded from these investigations that SE possess marked mitogenic and interferon-inducing properties.

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MONOCLONAL ENZYME IMMUNOASSAY TEST SYSTEM FOR TOTAL IMMUNOGLOBULIN E DETERMINATION IN CHILDREN AND ADOLESCENTS

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Determination of the total immunoglobulin E (IgE) concentration in the blood serum or plasma of children and adolescents can be informative for the discovery of persons predisposed to allergic diseases [1]. The serum IgE level in children is known to be lower than in adults, and in the normal neonate it is 1-2 IU/ml [5]. With age the IgE concentration rises, to reach the adult level at ~15 years of age [6]. To detect such insignificant concentrations of IgE, a reliable and highly sensitive method of determination of the IgE level is needed. The use of monoclonal antibodies (McAb) with high binding constants, specific for particular epitopes of antigen molecule (in this case the IgE molecule) enables such a method to be developed.

The aim of this investigation was to develop a test system capable of the sufficiently rapid, easy, and reliable measurement of IgE concentrations of between 1 and 100 IU/ml in undiluted blood serum, with a minimal volume of the serum sample and with one-stage conduct of the immunochemical reaction.

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