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RECEPTORS FOR SECRETORY COMPONENT ON HUMAN THYMIC LYMPHOCYTES: STIMULATION OF THEIR EXPRESSION BY ADENOSINE, THEOPHYLLINE, AND THYMOCYTE SUPERNATANT

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UDC 612.112.94.014.467:612.664.018].014.46:547.857

KEY WORDS: thymus; hetero-organic antigens; secretory component; thymocyte receptors.

Hetero-organic antigens characteristic of certain highly specialized tissues of the body are known to be represented in the thymus. The myoid cells of the thymus have been shown to contain antigens common with those of mouse tissues [6], and cells of the epithelial reticulum contain antigens common with epithelial tissues of epidermal type [1, 7, 8]. Cells synthesizing lactoferrin also have been found in the thymus, and a secretory component has been discovered in the membranous structures of the gland [2, 3]. These substances are known to be components of material excreted by the epithelium of several organs of ectodermal and entodermal origin (the salivary, lacrimal, and mammary glands, epithelium of the intestine and respiratory tract, etc.). It has been shown that receptors for lactoferrin are present on lymphocytes of the thymus, and their expression is stimulated by adenosine and theophylline, which raise the intracellular cAMP concentration, and also by thymocyte supernatant. Levamisole, which facilitates intracellular accumulation of cGMP, does not affect the ability of thymocytes to express these receptors [4].

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Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 67-69, July 1987. Original article submitted January 16, 1987.

 0007-4888/87/0007-0954$12.50

TABLE 1. Effect of Theophylline, Adenosine, Levamisole, and Thymocyte Supernatant on Ability of Thymus Lymphocytes of Express Receptors for Secretory Component

Preparation	K	t°	Te	A	Le	D
	%					
Sc	1,2±0,5	1,9±0,8	3,0±0,8	4,2±0,9	2,1±0,7	3,2±0,8
sIgA	7,7±1,5	7,5±1,0	16,9±1,7	14,3±1,5	10,0±1,2	14,4±1,3

Legend. Sc) Secretory component; sIgA) secretory IgA, K) initial number of thymocytes with receptors for secretory component (T_{sc} cells), t°) number of T_{sc} cells after incubation in medium for 1 h without addition of the preparation; Te, A, Le, D) treatment of thymocytes with theophylline, adenosine, levamisole, and thymocyte supernatant respectively.

The aim of this investigation was to determine whether receptors exist on thymocytes for another hetero-organic antigen, namely secretory component, and to study the effect of the above-mentioned drugs on their expression.

EXPERIMENTAL METHOD

Secretory component was isolated from human colustrum on Sephadex G-200 (the peak II fraction) and subjected to further purification by adsorption with antisera against normal human serum proteins, against serum IgA, and against secretory IgA, treated with glutaraldehyde. Antibodies to secretory component were isolated from rabbit antiserum against secretory components by means of an immunosorbent containing glutaraldehyde-treated secretory IgA. The isolated antibodies were additionally adsorbed with human serum IgA immobilized with glutaraldehyde. The preparation of secretory component and antibodies to it were used in a concentration of 400 µg/ml. Secretory IgA, adsorbed to remove antigenic determinants common with serum IgA, by antiserum to serum IgA, treated with glutaraldehyde, was used as the source of the secretory component. Antiserum to secretory IgA exhausted with serum IgA immobilized by glutaraldehyde was used in this case as the source of the antibodies.

The method of immunofluorescence on lymphocytes from the thymus of children undergoing operations at the age of 3-14 years for the treatment of a congenital heart defect (13 cases) was adopted for the investigation. The thymocytes were washed twice in Eagle's medium containing 10% bovine serum, a suspension containing $2 \cdot 10^7$ cells in 1 ml was prepared, and it was allowed to stand overnight at 4°C in an excess of medium. Next day the cells were washed, incubated for 1 h at 37°C in 0.1 ml of a solution of secretory components or secretory IgA (400 µg/ml), washed, and treated for 1 h at 37°C with the preparation of antibodies against secretory components or antiserum against secretory IgA (dilution 1:30). After washing, the thymocytes were incubated for 45 min at 37°C with globulin fraction against rabbit IgG, labeled with fluorescein isothiocyanate (FITC). The number of cells binding secretory component or secretory IgA (T_{sc} cells) was counted among 1000 small lymphocytes revealed by simultaneous observation in blue-violet light and with the phase-contrast system on the ML-2 microscope. In self-inhibition experiments the thymocytes were incubated for 1 h at 37°C with secretory component or secretory IgA and, after washing, they were again treated with the same preparation.

To determine the number of T_α cells, i.e., cells with receptors for the Fc-fragment of serum IgA, the thymocytes were incubated for 1 h at 37°C in 0.1 ml of a solution of IgA (400 µg/ml) and, after washing, they were treated for 45 min at 37°C with FITC-labeled globulin fractions against human IgA. In the experiments with theophylline the thymocytes were incubated for 1 h at 37°C in 0.1 ml of a 0.3 mM solution of the preparation, and washed twice, after which the number of T_{sc} cells was counted. The thymocytes were similarly incubated in 0.1 ml of a 0.5 mM solution of adenosine, in 0.1 ml of a solution of levamisole (24.5 µg/ml), or in 0.2 ml of thymocyte supernatant. To obtain the supernatant the thymocytes were incubated for 3 h at 37°C at the rate of $2 \cdot 10^7$ cells to 1 ml of medium; the cells were sedimented by centrifugation and the supernatant was kept at -20°C.

EXPERIMENTAL RESULTS

On successive treatment of the thymocytes with secretory component, with rabbit antibodies to it, and with FITC-labeled globulin fraction against rabbit IgG, fluorescence of single punctate structures could be observed on the surface of the cells. The number of T_{sc} cells in the human thymus on average is 1.2 ± 0.4%. On treatment of the thymocytes with secretory IgA and antiserum to it the number of T_{sc} cells was much greater, and it averaged 7.7 ± 1.5%. Differences in the number of thymocytes binding secretory component and secretory IgA were observed also in experiments on lymphocytes isolated from a single thymus. In self-inhibition experiments, during consecutive treatment of the thymocytes twice with the secretory components, the number of cells binding it was unchanged compared with the control (a single treatment with secretory component). Similar results also were obtained in experiments with secretory IgA.

The number of T_{α} cells in the human thymus averaged 1.75 ± 0.75. It will be clear from Table 1 that preincubation of thymocytes with theophylline and adenosine was accompanied by a marked increase in the number of T_{sc} cells compared with their number in the control. Similar results were obtained in experiments with secretory IgA, the only difference being that in this case the number of T_{sc} cells in the control, as also in the experiment, i.e., after treatment of the cells with theophylline and adenosine, was greater than in the experiment with secretory component. Thymocyte supernatant also had a stimulating effect on the ability of the thymocytes to bind secretory components and secretory IgA. In the experiments with levamisole the number of T_{sc} cells was unchanged. The number of T_{α} cells likewise was unchanged under the influence of these preparations.

The results thus showed that the human thymus contains a subpopulation of lymphocytes with receptors for secretory component. Evidence in support of the suggestion that binding is specific in character is given by the self-inhibition experiments, in which an increase in the duration of contact between thymocytes and secretory component was not accompanied by any increase in the number of T_{sc} cells. The absence of a summation effect in these experiments suggests that receptors for the secretory component are present only on a certain number of thymocytes. Meanwhile experiments with theophylline, adenosine, and thymocyte supernatant showed that a much larger number of thymocytes are potentially capable of expressing receptors for secretory component, and that their expression may be influenced by factors secreted by other subpopulations of thymus lymphocytes. Similar rules were found in a study of thymocyte receptors for lactoferrin [4]; this finding evidently indicates unity of the mechanisms controlling expression of receptors for factors of the internal medium of the gland on its lymphocytes.

According to the results, the number of T_{α} cells was unchanged under the influence of theophylline, adenosine, and thymocyte supernatant, unlike the number of T_{sc} cells. This rules out the possibility that, when secretory IgA is used, the higher percentage of labeled cells was due to the simultaneous detection of T_{α} and T_{sc} cells under these conditions. Differences in the number of T_{sc} cells may perhaps be due to the fact that the thymocyte receptor has greater affinity, not for free secretory component, but for the bound form, i.e., in the composition of the secretory IgA molecule. In this connection there are some interesting data in the literature [5] to show that free secretory component differs significantly from bound in its antigenic properties.

The writers' previous investigations showed that lactoferrin affects differentiation of precursors of T_{μ} and T_{γ} cells of the thymus, inducing receptors on their surface for the Fc-fragment of IgM and IgG. Another possibility is that the secretory component present in the thymus is also a factor for the differentiation of various thymocyte subpopulations, including T_{α} and T_{sc} cells, which repopulate in the organs of local immunity to regulate immune responses in them. The data showing that theophylline, adenosine, and thymocyte supernatant can enrich the T_{sc}-cell subpopulation provide a basis for a technique to isolate these cells, study their function, and elucidate the role of secretory component in their differentiation. It must also be pointed out that evaluation of the state of the T_{sc} cells in the thymus and peripheral lymphoid organs in diseases affecting the epithelium of the gastrointestinal and respiratory tracts may provide an additional diagnostic criterion and may be used as an indicator of the effectiveness of their treatment.

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EFFECT OF CHOLINERGIC STIMULATION ON SPONTANEOUS ADHESION OF LYMPHOCYTES IN VITRO

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UDC 612.112.94.014.46:615.217.32

KEY WORDS: spontaneous adhesion of lymphocytes; muscarinic and nicotinic acetylcholine receptors.

Potentiation of some immunologic reactions in the presence of cholinergic agonists [9] provided a basis for the hypothesis that acetylcholine receptors are present on the surface of lymphocytes. In most cases investigated the response to cholinergic drugs was depressed or completely abolished by atropine, a specific blocker of muscarinic acetylcholine (ACh) receptors. Direct investigations of binding of ^{3}H-quinuclidinyl benzylate, a specific ligand of muscarinic ACh receptors, showed that the reaction of lymphocytes to ACh is determined by the presence of muscarinic ACh receptors [5]. Choline-dependent potentiation of the cytotoxic activity of killer T cells, increased production of macrophage migration inhibition factor (MIF), and stimulation of proliferation of T lymphocytes induced by the graft versus host reaction, have been described [6, 7]. However, the effect of cholinergic stimulation on spontaneous lymphocyte adhesion has not previously been studied.

The aim of this investigation was to study the effect of the neurotransmitter ACh and its synthetic analog carbachol (CCh) on spontaneous adhesion of human lymphocytes in vitro, using blockers of muscarinic (atropine) and nicotinic (hexamethonium) ACh receptors.

EXPERIMENTAL METHOD

Peripheral blood lymphocytes were isolated from healthy donors and their spontaneous adhesion studied by the method developed by the writer previously to study inhibition of lymphocyte adhesion without the addition of specific antigens [2, 3].

The wells of 96-well plastic plates (No. 3040, Falcon, USA) contained 0.1 ml of healthy human lymphocyte suspension ($2 \cdot 10^6$ cells/ml), 0.05 ml of medium 199 with 20% embryonic calf serum (ECS, from Gibco, England), inactivated by heating to 56°C for 30 min, 0.05 ml of a solution of the preparation in the concentration to be tested, and 0.05 ml of a solution of the corresponding antagonist (medium 199 in the control). The plates were incubated (37°C, 5% CO_2) in a humid chamber for 1.5 h. The plates were then accurately turned over and incubated in the horizontal inverted position for another 30 min [1]. When the plates were

Laboratory of Clinical Radioimmunology, All-Union Oncologic Scientific Center, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR N. N. Trapeznikov.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 104, No. 7, pp. 69-71, July, 1987. Original article submitted June 12, 1986.

