living and one dead may be responsible for the greater or lesser accumulation of diploforms in culture and in vivo.

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RECEPTORS FOR GROUP A STREPTOCOCCAL POLYSACCHARIDE ON HUMAN THYMUS LYMPHOCYTES. STIMULATION OF THEIR EXPRESSION BY ADENOSINE, THEOPHYLLINE, AND THYMOCYTE SUPERNATANT

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In previous investigations the writers demonstrated the presence of antigens characteristic of epithelia of epidermal type in the epithelial tissue of the thymus [2, 10], and also showed that one of the epidermal antigens represented in the thymus has a common determinant with the group A streptococcal polysaccharide [1, 15].

Besides antigens of the skin epithelium, the thymus also contains factors characteristic of cells of the secretory epithelium. For instance, it contains numerous cells producing lactoferrin [3], and the membrane system of the gland contains a secretory component [4]. Receptors for lactoferrin and the secretory component are found on thymocytes, and their expression is stimulated by adenosine, theophylline, and by thymocyte supernatant, but levamisole has no action on it [5, 6].

The aim of this investigation was to study the ability of thymocytes to bind group A streptococcal polysaccharide (A-PSC), which has a crossed determinant with a heterophil antigen of the thymus characteristic of the basal layers of skin epithelia; to study the effect of the above-mentioned preparations and of thymocyte supernatant on binding of A-PSC by thymocytes.

EXPERIMENTAL METHOD

Immunofluorescence experiments were carried out on thymus lymphocytes from children undergoing surgery at the age of 7-14 years for congenital heart defects (13 cases). The thymocytes were washed twice in Eagle's medium with the addition of 10% inactivated bovine serum, a suspension containing 10^7 cells/ml was prepared, poured into test tubes with an excess of medium, and allowed to stand overnight at 4°C. Next day the cells were washed and the percentage of viable lymphocytes was determined with the aid of trypan blue. This parameter varied in different individuals from 98 to 90%; its fluctuations did not affect the ultimate result. To detect lymphocytes binding A-PSC the cells were incubated consecutively for 1 h at 37°C in 0.1

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TABLE 1. Effect of Theophylline, Adenosine, and Thymocyte Supernatant on the Ability of Human Thymus Lymphocytes to Express Receptors for Group A Streptococcal Polysaccharide (M±m)

Nature of treatment	average number of T_{ps}-cells	Character of change in T_{ps}-cells		
		stimulation of expression of R_{ps}	inhibition of expression of R_{ps}	no effect
No treatment	5,7±1,2	—	—	—
Control	3,8±1,2	5,3±2,7 (15)	2,3±0,8 (46)	— (39)
Theophylline	8,1±1,7	12,2±2,2 (54)	1,0±0 one occurrence (8)	— (38)
Adenosine	6,0±1,6	11,05±0,8 (61)	2,5±0,5 (15)	— (24)
Levamisole	3,6±1,1	5,6±2,5 (38)	2,4±1,3 (23)	— (39)
Supernatant	8,5±2,0	8,4±2,3 (91)	—	— (9)

Legend. T_{ps}) Thymocytes with receptors for A-PSC, R_{ps}) receptors for A-PSC. In control thymocytes were incubated for 1 h in medium at 37°C without addition of preparations. Data on average number of T_{ps}-cells during stimulation, inhibition, or absence of effect are given. Number of subjects in whom number of T_{ps}-cells increased, decreased, or remained unchanged, shown in parentheses. All data are percentages.

ml of a solution of A-PSC (concentration 300 μg/ml), washed, and then incubated in 0.1 ml of rabbit antiserum against A-PSC or antibodies to that antigen (concentration 300 μg/ml), and finally, incubated in 0.1 ml of a solution of the globulin fraction against rabbit IgG, labeled with FITC. In a parallel series, before addition of A-PSC the thymocytes were incubated for 1 h at 37°C in 0.1 ml of solution of theophylline (3 mM), adenosine (0.5 mM), levamisole (24.5 g/ml), or 0.2 ml of thymocyte supernatant. In the control, the thymocytes were incubated for 1 h at 37°C in 0.1 ml of medium without addition of the preparations, and the number of thymocyte binding A-PSC (T_{ps}-cells) was determined as described above. In the self-inhibition experiments the cells were incubated for 1 h at 37°C with A-PSC, and after washing, they were again treated with A-PSC and with the other preparations in order to determine the number of T_{ps} cells. The number of these cells was counted among 1000 lymphocytes by observation in blue-violet light and with the phase-contrast system for the ML-2 microscope.

A-PSC was isolated by the formamide method [11]. Antibodies to A-PSC from the sera of rabbits immunized with group A streptococcus, type I, were isolated by affinity chromatography [7] (the antibodies were generously provided by V. Yu. Sanina, of the Imminology of Streptococcal Infections Group, N. F. Gamaleya Research Institute of Epidemiology and Microbiology). To obtain the supernatant, human thymocytes were incubated for 3 h at 37°C in the proportion of 10^7 cells to 1 ml of culture medium. The cells were removed by centrifugation and the supernatant had stimulating activity for 2 months when kept at −20°C.

EXPERIMENTAL RESULTS

During consecutive treatment of the human thymocytes with A-PSC, antibodies or antiserum to it, and the luminescent globulin fraction against rabbit IgG, fluorescence of single point formations was observed on the surface of the cells. The number of TPS-cells in the human thymus exhibited considerable individual fluctuations, varying from 0.4 to 12%. The average number of T_{ps}-cells in the human thymus was 5.7 ± 1.2% (Table 1). In the cell inhibition experiments, during two consecutive treatments of the thymocytes with A-PSC the number of T_{ps}-

cells was reduced by half. In the control experiments, i.e., on incubation of the lymphocytes in medium without addition of the preparations, a very small increase in the number of T_{ps}-cells was observed in 15% of cases, a decrease in their number compared with the initial value was found in 46% of cases, and in 39% it remained unchanged. It will be clear from the data given in Table 1 that under the influence of theophylline an increase in the number of T_{ps}-cells was observed in 54% of cases, on average to 12.2 ± 2.2%. This value differs significantly from the average initial level ($p \leqslant 0.01$) and from the average number of T_{ps}-cells in the control ($p \leqslant 0.02$). A stimulating effect of adenosine was observed in 61% of cases. The mean number of T_{ps}-cells increased under its influence to 11.0 ± 0.8%, and differed significantly from the original value ($p \leqslant 0.01$) and from the control ($p \leqslant 0.001$). A stimulating effect of levamisole on T_{ps}-cells was observed much less often (38% of cases), an inhibitory effect rather more often (23%) than under the influence of theophylline and adenosine; however, quantitatively speaking both effects of levamisole are negligibly small (Table 1). As Table 1 shows, thymocyte supernatant had a stimulating action on the ability of the thymocytes to express receptors for A-PSC most frequently of all (91%). The average number of T_{ps}-cells under these circumstances was 8.4 ± 2.3%, which differed significantly from the control ($p \leqslant 0.05$), but not from the average initial level of T_{ps}-cells in the thymus.

The results are thus evidence that the human thymus contains a lymphocyte subpopulation binding A-PSC, evidently because of the presence of specific receptors on their surface. In favor of the specific, i.e., receptor, character of A-PSC binding is the fact that an increase in the duration of contact of the thymocytes with A-PSC during repeated treatment of the cells with this antigen did not lead to an increase in the number of T_{ps}-cells. The results of experiments with theophylline, adenosine, and thymocyte supernatant are evidence that a larger number of thymocytes has potential ability to express receptors for A-PSC, and that their expression depends on the intracellular cAMP level and also on the action of factors secreted by thymus lymphocytes. Similar patterns also were observed in a study of thymocyte receptors for lactoferrin and secretory component [5, 6]. We know that theophylline, like certain thymus hormones, stimulates expression of receptors for sheep's (human) red blood cells and mouse Thy-1-antigen on the surface of prethymocytes, a feature characteristic of more highly differentiated thymus lymphocytes and of T-cells [9, 12, 14]. It has also been shown that theophylline and thymulin stimulate proliferation of bone-marrow precursors of thymocytes, and this is accompanied by disappearance of SC-1-antigen (a marker of thymocytes) on their surface, and that during acquisition of the characteristic thymocyte phenotype, the cells lose their ability to proliferate under the influence of theophylline, but not of thymulin [9]. Thus besides receptors for sheep's red blood cells and Thy-1 (BAT)-antigen, receptors for A-PSC and also for lactoferrin and secretory component are c-AMP-dependent markers of thymocytes. The problem of whether precursors of T_{ps}^-, T_{lf}^-, and T_{sc}^--cells belong to the category of prethymocytes or whether they are more mature lymphocytes of the gland awaits further study.

The discovery of receptors for such heterologous thymus factors as lactoferrin and secretory component on thymocytes suggests that receptors for the epithelial tissue factor of the thymus, cross-reacting with A-PSC, also are present on their surface. In this case binding of A-PSC by thymocytes may take place as a result of interaction of its crossed determinant with a receptor intended for this epithelial factor of the thymus. At the same time the possibility cannot be ruled out that fixation of A-PSC is due to the presence of a lectin, with affinity for other sites on its molecule, and different from the crossed determinant, on the surface of the thymocytes. The presence of lectins binding various carbohydrates on thymocytes has been demonstrated by several investigations [13]. The study of the mechanisms of fixation of A-PSC by thymocytes is important in principle because of existing views on the important role of cross-reacting antigens of group A streptococcus in the development of the autoimmune process in rheumatic fever [8, 15]. It must also be pointed out that identification of a T-cell subpopulation with receptors for A-PSC will allow its changes in the thymus and peripheral lymphoid organs in diseases of streptococcal etiology to be studied.

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SHEDDING OF MOUSE THYMOCYTE RECEPTORS FOR AUTOLOGOUS ERYTHROCYTES UNDER THE INFLUENCE OF THYMIC OLIGOPEPTIDE FACTOR

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A small percentage of mouse thymocytes can interact with autologous and syngeneic erythrocytes to form rosettes [7, 8, 11]. Thymocytes forming rosettes with autologous or syngeneic erythrocytes (ARFC) are probably in the early stages of maturation [8, 9].

A hormone of the thymus detectable in serum (serum thymic factor), which controls T-cell differentiation, reduces the percentage of ARFC among thymocytes and their cytotoxicity against autologous erythrocytes [4]. A similar action on thymocytes has been observed after incubation for a short time with the low-molecular-weight oligopeptide factor isolated from a dialyzable thymus extract by adsorption on immobilized bovine serum euglobulins [2, 3]. The mechanism of reduction of affinity of the thymocytes for autologous erythrocytes in the presence of this factor is not clear.

The aim of this investigation was to study the properties of the supernatant obtained after incubation of thymocytes with thymic oligopeptide factor (TOF).

EXPERIMENTAL METHOD

Thymus glands from CBA mice aged 2 months were used. Thymocytes were isolated by a coarsely ground glass homogenizer in an excess of Hanks' solution. Autologous rosette formation was studied by the method in [8].

TOF was isolated from a saline extract of the thymus by chromatography on euglobulins, bound to sepharose 4B, as described previously [2, 3]. Activity of the preparation was tested by determining the decrease in the ability of mouse thymocytes (10^7 cells in 1 ml) to form

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