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MICROBIOLOGY AND IMMUNOLOGY

Lymphocytes with Receptors of Group A Streptoccal Polysaccharide in the Thymus of Rheumatic Patients: Altered Reaction to Adenosine, Theophylline, and Normal Thymocyte Culture-Conditioned Medium

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One of the characteristic features of the rheumatic process is the presence in patients' sera of high levels of antibodies to the polysaccharide of group A streptococci (A-PS) [2,10,11]. An important step in the understanding of the pathogenetic role of A-PS antibodies was the discovery of the determinants shared by A-PS and one of the epidermal antigens of the thymus epithelial cells. It was suggested that antibodies to the A-PS cross-reactive determinant cause damage to the thymus epithelium, thereby leading to disturbed thymocyte differentiation and to the development of the autoimmune process in rheumatism [7-9]. The involvement of the thymus in the rheumatic pathological process is evidenced by: a) focal destruction of the epithelial cells containing epidermal antigens, including determinants shared by A-PS; b) presence of bound immunoglobulins and complement in the cytoplasm of these cells [4,13]; c) an increase in both the number and functional

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activity of myoid cells (containing the muscle antigens) [5] and the presence of immunoglobulins and complement in their cytoplasm; and d) immune complex deposition in the blood vessel wall and at the margins of poorly differentiated lymphocytes of the thymus cortex [4,13]. In this connection it is interesting that the thymus contains a subpopulation of lymphocytes bearing receptors of A-PS (R-PS). Under normal conditions the percent of R-PS-bearing thymocytes increases in response to theophylline, adenosine, and a 3-hour normal thymocyte culture-conditioned medium (TCM) [6].

The aim of this investigation was to study the reaction of R-PS-bearing thymus lymphocytes from rheumatic patients to these immunomodulators.

MATERIALS AND METHODS

Immunofluorescent study was carried out on thymocytes of rheumatic patients who underwent commissurotomy at the age of 7-15 years (12 cases). As a control, thymus lymphocytes of children (13 cases) who underwent surgery for congenital heart defect were studied (they are designated in the text as normal). The lymphocytes, freed of the stroma, were washed in Eagle's medium supplemented with 10% bovine serum and stored for 18 hours at 4°C. The next day the cells were washed and consecutively incubated for 1 hour at 37°C in 0.1 ml A-PS solution (300 μg/ml), in 0.1 ml rabbit anti-A-PS serum (diluted 1:20), and in fluorescein-labeled anti-rabbit IgG, in order to determine the initial level of R-PS+ thymocytes. In parallel experiments the effect of immunomodulators on R-PS expression was studied. For this purpose, thymocytes were incubated in 0.1 ml theophylline (3mM), adenosine (0.65 mM), or levamisole (24.5 μg/ml) solution and TCM. Thymus lymphocytes incubated for 1 hour at 37°C in medium without the addition of preparations served as a control. The number of R-PS+ cells per 500 lymphocytes was counted in a LYUMAM-3 microscope by simultaneous observation in blue-violet light and phase-contrast. The index of the stimulation effect was calculated as follows: $K=(A-B)/B$, where A is the number of R-PS+ cells in the culture treated with preparation, and B is the number in the untreated culture (control). The effect was evaluated as stimulating ($K>1$) or inhibiting ($K<1$). In addition, the frequency of the stimulating and inhibiting effect of the same preparation on normal and rheumatic thymus lymphocyte cultures was determined. In the preliminary study it was established that normal rabbit antibodies (diluted 1:20) do not react with human thymus lymphocytes. Therefore, the reaction of animal immune serum with R-PS+ cells is specific and is not explained by the immunoglobulin reaction with the thymocyte Fc-receptors. Thymus lymphocytes from children with congenital heart defect were incubated for 3 hours at 37°C (106 cells in 1 ml medium) in order to obtain TCM. The TCM was clarified and stored at -20°C. A-PS was isolated by the formamide method from A-group type 1 streptococci culture, killed by heating and treated with pepsin. The scheme of rabbit immunization with pepsin-pretreated A-group streptococci to produce antibodies against A-PS was described earlier [12].

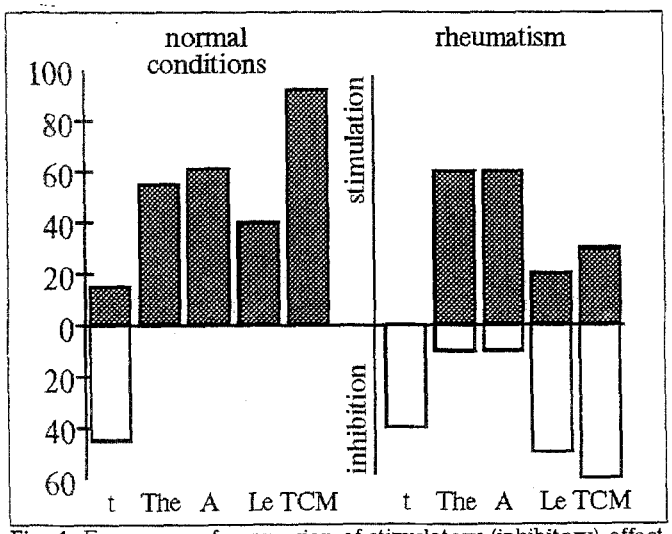

Fig. 1. Frequency of expression of stimulatory (inhibitory) effect of immunomodulators on the induction of R−PS+ cells in cultures of rheumatic and normal thymocytes. Abscissa: immunomodulators; ordinate: percentage of rheumatic and normal lymphocyte cultures in which a stimulatory (inhibitory) effect of immunomodulators was registered. t: thymus lymphocytes were incubated for 1 hour at 37°C without immunomodulators (control); The, A, Le and TCM: addition of theophylline, adenosine, levamisole, and TCM, respectively.

RESULTS

The initial level of R-PS+ cells in the thymus of rheumatic patients was 3.6±1.48% in comparison with 5.6±1.1% in the control (the differences are insignificant).

No significant increase in R-PS cells was observed in the control experiments (1-hour incubation of thymocytes in medium without immunomodulators) in either patients' cells or cells from normal thymus. A reduction of R-PS+ cell number was found with equal frequency in both cases (Fig. 1).

As shown in Fig. 1, the stimulatory effect of theophylline and adenosine on R-PS expression was observed with similar frequency in the cultures of rheumatic and normal thymocytes. However, the stimulatory effect of these immunomodulators was quantitatively weaker on rheumatic thymocytes (Fig. 2). For instance, the level of R-PS+ cells in rheumatic thymocytes in-

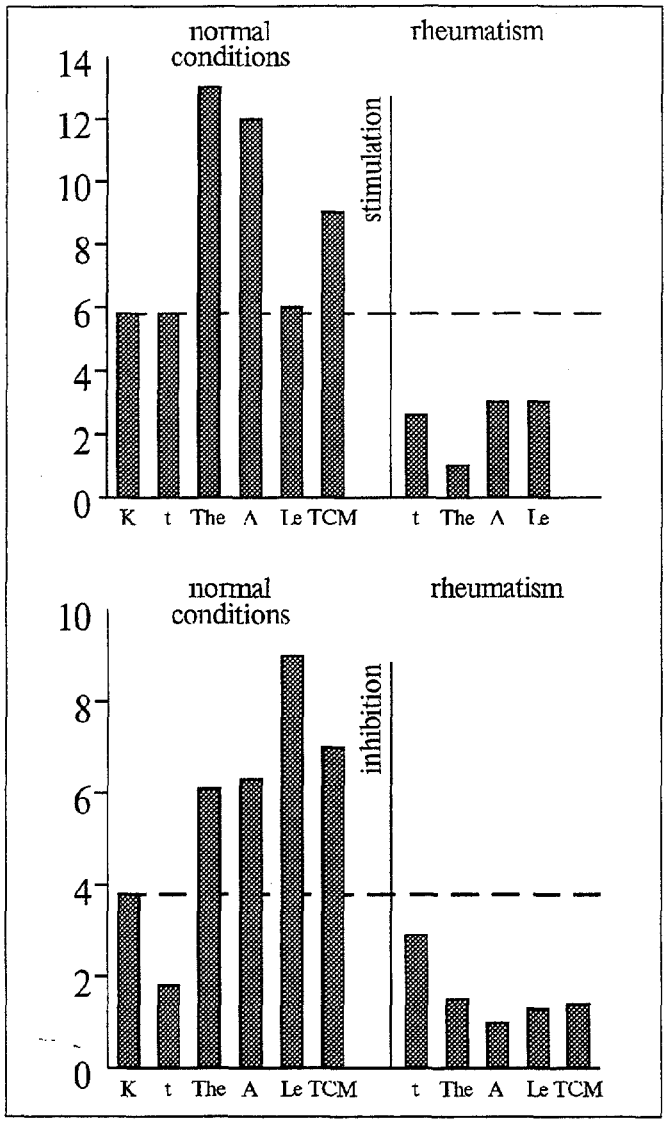

Fig. 2. Quantitative evaluation of stimulatory (inhibitory) effect of immunomodulators on the expression of A–PS receptors by rheumatic and normal thymocytes. Abscissa: immunomodulators; ordinate: percentage of A–PS receptor–bearing cells (R–PS+ cells). Designations: initial level of thymic R–PS+ cells (K); level of R–PS+ cells in control cultures (t), following treatment with theophylline (The), adenosine (A), levamisole (Le), and TCM.

creased to 6.0±0.10% and to 6.1±0.83% after theophylline and adenosine treatment, respectively, and was significantly different from the control ($p<0.01$), but not from the initial level. The frequency of R-PS+ cells in the cultures of normal thymocytes increased to 12.25±2.0% and 11.5±1.63% after theophylline and adenosine treatment, respectively, and significantly exceeded both the control ($p<0.001$) and the mean initial level ($p<0.01$). The frequencies of theophylline-induced R-PS+ cells in the cultures of rheumatic and normal thymocytes differ significantly ($p<0.01$); the same is true for adenosine-induced R-PS+ cells.

The inhibitory effect of theophylline and adenosine on R-PS+ cells in the cultures of rheumatic and normal thymocytes was observed with equal frequency (Fig. 1) and also did not differ quantitatively (Fig. 2).

The stimulatory effect of levamisole on R-PS expression was observed in 38±14% cultures of normal thymocytes and in 20±13,3% of rheumatic thymocyte cultures (the difference is insignificant). In rheumatic patients levamisole increased the R-PS+ cell level to 8.5±0.9%, which significantly exceeded both the control ($p<0.001$) and the initial level ($p<0.01$). The level of R-PS+ cells in the cultures of normal thymocytes treated with levamisole was 5.6±1.2%, i.e., it did not differ from the initial one. These values for normal and rheumatic thymocytes differ significantly ($p<0.05$). The inhibitory effect of levamisole on R-PS expression was exhibited with equal frequency and strength in the cultures of both rheumatic and normal thymocytes (Figs. 1, 2).

As shown in Fig.1, the stimulatory effect of TCM on rheumatic thymocytes could be observed much less frequently than in the case of normal thymocytes (30±13% cases versus 91±8.1%, $p<0.05$). However, in the positive cases the quantitative aspect (i.e., number of induced R-PS cells) was equal in both rheumatic and normal thymocyte cultures (Fig. 2). At the same time, TCM with increased frequency produced an inhibitory effect on R-PS expression in samples of rheumatic thymocytes as compared to normal ones (Fig. 1). Thus, an inhibitory effect of TCM was observed in 55±17% cases of rheumatic thymocyte cultures and in no cases of normal cultures. The level of R-PS+ cells dropped under the influence of TCM to 0.8±0.13% (Fig. 2). This value significantly differed from the control index ($p<0.05$), but not from the initial level of R-PS cells in the thymus of rheumatic patients.

Thus, rheumatism is characterized by an altered response of precursors of R-PS+ thymocytes (in which the expression of this receptor can be induced by immunomodulators) to theophylline, adenosine and TCM. According to the data obtained, thymocytes from rheumatic patients did not differ from normal ones regarding the frequency with which theophylline and adenosine had a stimulatory effect; however, the level of R-PS+ cell induction in rheumatic thymocytes was only half that observed in the case of normal thymocytes. The marked reduction observed in the frequency of a stimulatory effect of TCM means that the thymus R-PS+ cell precursors of rheumatic patients are unable to express R-PS under the influence of TCM-derived factor. At the same time, in the cases of a stimulatory effect of levamisole, the number of R-PS+ cells in rheumatic thymocytes was comparable to that induced by adenosine, theophylline, and TCM in normal thymocytes. It thus follows that the decrease in theo-

phylline- and adenosine-responsive precursors is not related to their quantitative deficit in the thymus of rheumatic patients, but is presumably due to changes in cell differentiation. As a result, the precursors do not reach the stage of responsiveness to TCM, and only some of them (twice as few as in the normal thymus) acquire the property of R-PS expression following induction with theophylline and adenosine. An altered response to TCM is also retained at the level of R-PS+ cells since, unlike in the normal thymus, they may lose these receptors following TCM treatment. As TCM is a complex preparation containing factors released by different thymocyte subpopulations, a change in response to it provides evidence of alterations in the interactions between the R-PS+ cells and their precursors with other thymocyte subpopulations in rheumatism. Moreover, the different pattern of rheumatic thymocyte reactions to TCM as compared to theophylline and adenosine implies different mechanisms of action of these immunomodulators and TCM.

Thus, in rheumatism we can see not only thymus cell damage, but also deep-seated alterations of the properties of R-PS-lymphocytes. Interesting in this connection is the finding [14] that the peripheral blood T-suppressors exhibit receptors of rhamnose, which is known to be a constituent of A-PS cross-reactive determinants [3]. It is plausible to assume that R-PS+ thymocytes are precursors of this subpopulation and that the rheumatism-associated disruption of the suppressor link of immunity takes place at the level of the thymus. A study of the properties of the peripheral blood R-PS+ lymphocytes and of the conditions under which their responsiveness to theophylline and adenosine is normalized can be used in the evaluation of the immune status of patients suffering from rheumatism and assessment of the efficacy of immunocorrective preparations.

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