Role of the Specific Determinant of Group A Streptococcus Polysaccharide in Inhibition of T-Suppressor Activity

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Group A streptococcus polysaccharide is found to inhibit the activity of ConA-stimulated T suppressors. A-variant streptococcus polysaccharide, representing an L-rhamnose homopolymer which is identical to the rhamnose carcass of group A streptococcus polysaccharide, does not possess such an effect. The effect of group A streptococcus polysaccharide on the activity of T suppressors is considered to be associated with its determinant including N-acetylglucosamine as an obligatory component.

Key Words: *immunomodulators; T cells; suppressors; group A streptococcus; polysaccharide*

Group A streptococcus polysaccharide (A-PS) represents a low-molecular carbohydrate of the cell wall. It consists of an L-rhamnose polymer and N-acetylglucosamine, which occupies a terminal position in the antigen molecule and is a component of its group-specific determinant [7,8]. Besides the specific determinant, A-PS contains several rhamnose determinants differing in chemical structure, among which determinants common to streptococci of different groups [6] and to staphylococci [1] have been detected. A-PS has been shown to act as an inhibitor of the activity of human peripheral blood T suppressors in different functional systems [4]. This feature varies in different determinants: it may be specific only towards group A streptococci or it may be common to cell-wall carbohydrates of a wide spectrum of microorganisms. Experiments with the A-PS variant (V-PS), whose rhamnose carcass is identical to that of A-PS but contains no N-acetylglucosamine [7,8], may help solve this problem.

Our previous studies demonstrated a correlation between the changed activity of ConA-induced T suppressors and destruction of MTT (3-[4,5-dimethylthiasole-2-yl]-2,5-diphenyltetrazolium bromide, Sigma) in the cytoplasm of lymphocytes labeled with it [4]. The MTT label used with monoclonal antibodies to markers of different T-cell subpopulations helps investigate the effects of different immunomodulators on the functional activity of individual lymphocyte subpopulations by the immunofluorescent method [4,9,10].

This study aimed to elucidate the specific features of the effects of A-PS and V-PS on the activity of the $CD4^+$ and $CD8^+$ lymphocyte subpopulations stimulated with ConA.

MATERIALS AND METHODS

A-PS and V-PS were obtained by formamide extraction from pepsin-treated cultures of group A streptococcus (strain № 6/49) and A variant (strain № 32/18) [8]. The concentration of the preparations was 30 μg/ml. Mononuclear cells (MNC) were isolated from venous blood of 28 healthy donors aged 25 to 42 in a Ficoll-Paque density gradient (Pharmacia). Cell culturing was carried out in RPMI-1640 medium (Flow Lab.) supplemented with 0.3 mg/ml L-glutamine (Labo-Chemic), 2

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mM HEPES buffer (Flow), 100 μg/ml gentamicin, and 10% fetal calf serum (Flow Lab.). Cells were incubated in 96-well Linbro plates for tissue cultures at 37°C in an atmosphere with 5% CO_2. Each well contained 200 μl of initial suspension with 5×10^5 lymphocytes per ml. The proliferative activity of ConA-stimulated (25 μg/ml, Serva) MNC was assessed by incorporation of ^{3}H-thymidine, which was added 6 h before the end of incubation in a dose of 1 μCi per culture. The proliferation index was calculated after the formula: $a/b \times 100\%$, where a is the mean number of counts in three parallel cultures stimulated with mitogen and b is the spontaneous proliferation of MNC in the medium. Before use MTT was dissolved in phosphate buffer, pH 7.2, to attain the concentration of 5 mg/ml, and added to the culture medium in a 1/10 ratio, after which it was incubated with the cells for 30 min. The formation of formazane granules was arrested with sodium azide [11]. The MNC suspension was layered onto slides precoated with poly-L-lysine in a concentration of 100 μg/ml (Serva). Adhered cells were treated with mouse monoclonal antibodies anti-CD4 and anti-CD8 (Becton Dickinson) and with a mixture of these antibodies and an FITC-labeled fraction of rabbit Ig to mouse Ig (N. F. Gamaleya Research Institute of Epidemiology and Microbiology). Cells carrying fluorescent label (*A* - after treatment with anti-CD4, *B* - with anti-CD8, *D* - with a mixture of these monoclonal antibodies) per 100 lymphocytes and the number of MTT$^+$ cells in these subpopulations (*A*MTT$^+$, *B*MTT$^+$, *D*MTT$^+$) were counted. The percentage of CD4$^+$, CD8$^+$, and CD4$^+$8$^+$ cells in the culture was estimated as follows:

$$CD4^+8^+ = A + B - D,$$
$$MTT^+,CD4^+8^+ = AMTT^+ + BMTT^+ - DMTT^+;$$
$$CD4^+ = A - CD4^+8^+,$$
$$MTT^+,CD4^+ = AMTT^+ - MTT^+,CD4^+8^+;$$
$$CD8+ = B - CD4+8+,$$
$$MTT^+,CD8^+ = BMTT^+ - MTT^+,CD4^+8^+.$$

The activation index of the CD4$^+$ subpopulation was determined as the ratio of the MTT$^+$CD4$^+$ count to the total count of CD4$^+$ lymphocytes multiplied by 100%. The activation index of CD8$^+$ lymphocytes in the subpopulation was estimated in the same way. The data were statistically processed using Student's t test. Each group consisted of 7 to 15 donors.

RESULTS

In the first series of experiments donor MNC were cultured for 48 h at 37°C in parallel in media with and without A-PS or V-PS. The number of counts recorded during degradation of incorporated radioactive thymidine in cultures to which the polysaccharides were added did not differ from the level of spontaneous proliferation. The index of proliferation connected with the presence of A-PS in the medium was 1.05±0.17, while for V-PS it was 0.88±0.21. The results of these experiments indicate that in the dose used the polysaccharides exert no mitogenic effect on any of the subpopulations of human blood MNC.

During MNC culturing in medium with ConA, ConA and A-PS, or ConA and V-PS (Table 1) the proliferation index was, respectively, 9.2±2.36, 10.7±2.98, and 7.0±0.41 (the differences are unreliable). The percentage of CD8$^+$ lymphocytes in the control was 20±5.26, and it increased to 56±7.2% under the influence of ConA ($p\leq0.05$). Polysaccharides virtually did not influence this process. The share of CD8$^+$ in the medium with ConA and A-PS was 57±8.52%, and in the medium with ConA and V-PS it was 54±10.12%. Hence, the addition of both A-PS and V-PS to an MNC culture in parallel with ConA did not influence the proliferation on the whole and, specifically, the proliferation of the CD8$^+$ lymphocyte subpopulation, or the expression of CD8$^+$ antigen.

In the culture stimulated with ConA the activation index in the subpopulation of lymphocytes with CD4$^+$ and CD8$^+$ was increased in comparison with the control from 18±7.42 to 69±9.52 and from 42±7.65 to 71±9.50%, respectively. At the same time, the addition of A-PS caused a rise of the activation index only in the CD4$^+$ subpopulation, whereas in the CD8$^+$ subpopulation the index was unchanged. In contrast, V-PS did not influence either proliferation or activation of T cells. The activation indexes of both lymphocyte subpopulations, CD4$^+$ and CD8$^+$, did not differ from those in cultures stimulated with ConA alone (Table 1).

Previously a correlation between inhibition of suppressor activity and lowering of the activation index in a subpopolation of lymphocytes with the CD8$^+$ phenotype was revealed for the ConA system [4]. These data indicate that A-PS can inhibit the process of differentiation of CD8$^+$ suppressors proliferating under the effect of mitogen. Our findings indicate that V-PS cannot exert such an effect. Bearing in mind the structural peculiarities of V-PS, we may conclude that the effect of A-PS on the activity of CD8$^+$ lymphocytes is due to its determinant including β-N-acetylglucosamine which is absent in V-PS [6]. It should be emphasized that during ConA stimulation neither A-PS nor V-

TABLE 1. Comparison of the Effects of A–PS and V–PS on the Proliferation and Functional Activity of $CD4^+$ and $CD8^+$ Lymphocytes of Human Peripheral Blood during ConA Stimulation

Conditions of MNC culturing	Spontaneous proliferation, cpm	Proliferation index	Percentage of lymphocytes with phenotype		Index of subpopulation activation	
			$CD4^+$	$CD8^+$	$CD4^+$	$CD8^+$
Medium	796±137.3		36±4.14	20±5.26	18±7.42	42±7.65
ConA		9.2±2.36	28±4.16	56±7.20	69±9.52	71±9.50
ConA + A–PS		10.7±2.98	21±4.19	57±8.52	63±11.20	38±6.27
ConA + V–PS		7.0±0.41	26±5.72	54±10.12	63±12.27	54±16.40

PS affects the process of activation of $CD4^+$ lymphocytes in human peripheral blood.

Studies of the specificity of monoclonal antibodies have shown that N-acetylglucosamine is present in several epitopes of A-PS differing in chemical structure [3]. The simplest in composition is the determinant common for A-PS and L-PS. It includes N-acetylglucosamine and does not contain rhamnose residues (DT-1) [2,3,7]. Two other determinants include, besides N-acetylglucosamine, rhamnose. The length of the rhamnose site and specific features of junction of rhamnose residues underlie the chemical differences between these determinants [3]. In one of these determinants, DT-2, rhamnose residues are connected by an α1-2 bond, in another (DT-3) by both α1-2 and α1-3 bonds. Neither DT-2 nor DT-3 is specific for group A streptococci, because not only A-PS, but V-PS as well inhibit the reaction of monoclonal antibodies with A-PS in enzyme immunoassay [3]. Thus, we have to admit that the structure of the specific A-PS determinant including N-acetylglucosamine is not quite clear up to the present time. Evidently, it contains rhamnose, like DT-2 and DT-3, but in contrast to them most likely includes only one rhamnose residue. Hence, the comparison of the immunomodulating properties of A-PS and V-PS was insufficient to answer the question about the specificity of depression of the functional activity of A-PS T suppressors. The structure of the determinant specific for A-PS is also in need of further research.

The results of this study permit us to regard A-PS as one of the principal bacterial factors contributing to immunoregulatory disorders associated with group A streptococcus infection. A study of its properties may eventually help unravel the pathogenetic mechanisms of streptococcal diseases.

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