Group A Streptococcal Polysaccharide Inhibits the Functional Activity of CD8+ Supressor Cells Stimulated with ConA in a Human Blood Mononuclear Cell Culture

E. V. Gnezditskaya, E. A. Bazanova, V. G. Nesterenko, L. K. Popova, and V. Yu. Sanina

UDC 616.98:579.862.1]-07:616.155.92-078.33

Translated from *Byulleten' Eksperimental'noi Biologii i Meditsiny*, Vol. 115, № 2, pp. 386-389, April, 1993
Original article submitted November 26, 1992

Key Words: *thymus; epidermal antigens; cross-reacting antigens; group A streptococcal polysaccharide; peripheral blood; blood T suppressors; streptococcosis*

A number of data confirm the assumption that group A streptococcal polysaccharide (A-SP), carrying common determinants with thymus epidermal antigens (factors) [1,5], may possess immunomodulating activity and play the role of their functional analog [3,4]. On some thymic lymphocytes receptors for A-SP are found [2] and their regulatory influence on the thymic T_μ- and T_γ-cell level is determined. Correspondingly, when the level of these cells is low it stimulates, and when the level is high it inhibits the expression of the Fc_μ and Fc_γ receptors on thymocytes [4]. It has also been established that in the presence of protein antigen A-SP induces nonspecific cytotoxic reactions in a culture of normal animal spleen cells [3]. The data on the presence on nonspecific T suppressors of the receptor for rhamnose [7], which forms part of the A-SP cross-reacting determinants [1,5,6], suggest an A-SP mediated influence on cytotoxic effectors through the inhibition of nonspecific T-suppressor activity [3].

The aim of the present work was to study the effect of A-SP on the functional activity of T suppressors induced by ConA in a human blood mononuclear cell (MNC) culture.

Research Institute of Transplantology and Artificial Organs; N. F. Gamalei Research Institute of Experimental Medicine, Russian Academy of Medical Sciences, Moscow
(Presented by V.I. Shumakov, Member of the Russian Academy of Medical Sciences)

MATERIALS AND METHODS

A-SP was obtained by formamide extraction from a group A streptococcus culture (strain № 6/49, Prague collection), treated with pepsin [6]. The concentration of A-SP was 30 μg/ml. MNC were obtained from the venous blood of 20 healthy donors aged 25-42, and were isolated on a Ficoll-Paake gradient (Pharmacia). The cells were cultured in RPMI-1640 (Flow Laboratories) with corresponding additives [9] in 96-well panels for cell culturing (Linbro) at 37°C and 5% CO_2. Each well was supplied with 200 μl of a mixture containing 10 lymphocytes per milliliter. The proliferative activity of MNC stimulated with ConA (25 μg/ml, Serva, Germany) or PHA (2-10 μg/ml, Serva, Germany) was determined according to the incorporation of ^3H-thymidine (1 μg per culture) added 6 hours before cell harvesting. The proliferative index was calculated by the formula: $(NI_{mit}) : (NI_s) \times 100\%$, where NI_{mit} is the mean number of pulses per min produced by the radioactive label decay in the mitogen-stimulated culture, and NI_s is the spontaneous proliferation of MNC in the medium. The suppressor activity of

0007-4868/93/0004-0409$12.50

MNC induced by ConA (25 μg/ml) was estimated according to the inhibition of the proliferation of autologous MNC stimulated by PHA (2 μg/ml). MNC prestimulated with ConA (48 hours) were mixed with autologous MNC (1:1) cultured in a medium without mitogen (target cells). At the moment of transfer PHA was added to the cell mixture for 72 hours. The index of proliferation inhibition was calculated by the formula:

$$\frac{NI_{mix}PHA - NI_{st}ConA}{NI_{st}PHA - NI_{s}} \times 100\%,$$

where($NI_{mix}PHA$) is the mean number of pulses in the mixture stimulated with PHA, ($NI_{st}ConA$) - in MNC stimulated only with ConA, ($NI_{st}PHA$) - in MNC stimulated only with PHA (targets), and NI_s is the spontaneous proliferation of MNC targets. Along with this, a double staining technique was used for estimating the T-suppressor activity, which allows for a simultaneous determination of the lymphocyte phenotype (monoclonal antibodies to CD4 and CD8 antigens) and their functional activity according to the ability to incorporate MTT (3-[4,5-dimethylthiazol-2-yl]-2,5-diphenyl tetrazolium bromide, Sigma, USA). In the cytoplasm of activated cells under the influence of mitochondrial enzymes MTT disintegrates with the formation of formazan granules. In contrast to ^{3}H-thymidine, the MTT technique demonstrates the functionally active cells [8,9]. Prior to use, MTT was dissolved in phosphate buffer (5 mg/ml), pH 7.2, added to the culture medium (1:10), and incubated with the cells for 30 min. The production of formazan granules was arrested by sodium azide. The MNC mixture was placed on slides pretreated with poly-L-lysine (100 μg/ml). Attached cells were treated with MCA against CD4 and CD8, and with a mixture of these antibodies taken in equal volumes. The number of CD4, CD8, and CD4,8 antigens per 100 lymphocytes was determined, and the number of MTT cells in these subpopulations (MTT+CD4, MTT+CD8, MTT+CD4,8). The absolute numbers of CD4, CD8 and CD4,8 cells were calculated:

CD4,8 = CD4 + CD8 - CD4,8; MTT + CD4,8 = MTT + CD4 + MTT + CD8 - MTT + CD4,8;

CD4 = CD4 - CD4,8; MTT + CD4 = MTT + CD4 - - MTT + CD4,8;

CD8 = CD8 - CD4,8; MTT + CD8 = MTT + CD8 - - MTT + CD4,8.

Activation index of the CD4 (CD8) subpopulation was determined as the ratio of the MTT+CD4 (MTT+CD8) number to the total number of CD4 (CD8) lymphocytes expressed in percent. The statistical analysis was carried out with the aid of Student's t test.

RESULTS

According to the data obtained (Table 1), the proliferation level of MNC cultivated in the presence of A-SP does not differ from the spontaneous level. The number of CD4 and CD8 lymphocytes under the influence of A-SP were 36±1.2 and 23±5.4% and also did not differ from the control. At the same time, the activation index of the CD4 subpopulation under the influence of A-SP increased from 18±7.4 to 45±9.8% ($p<0.05$). Simultaneously, in the presence of A-SP the CD8-lymphocyte activation index dropped to 17±9.4% in comparison with 42±7.6% in the control cultures ($p<0.05$). The experimental results give evidence of the absence of mitogenic effects of A-SP in the concentration used on any MNC subpopulation, and of the lack of influence on the total number of CD4 and CD8 cells, but at the same time A-SP causes a rise of the CD4 activity level along with a simultaneous drop in the level of CD8 lymphocytes.

In the next series of experiments the action of A-SP on the proliferative and functional activity of T suppressors induced with ConA was studied. Table 2 shows that the proliferative index in the presence of A-SP was 9.2±2.36 and did not differ from the control value (ConA stimulation). The total number of CD4 and CD8 lymphocytes in the MNC culture stimulated with mitogen in the presence of A-SP were 28±4.2 and 56±7.2%, respectively, and again did not exceed the controls. At the same time, for the simultaneous addition of mitogen and A-SP to the medium a drop in the activation index of the CD8 lymphocyte subpopulation was observed - from

TABLE 1. Effect of A–SP on Proliferation and Functional Activity of Human Peripheral Blood CD4 and CD8 Lymphocytes

Culturing conditions	Spontaneous proliferation, pulses per min	Proliferation index	Lymphocytes, %		MTT cells in subpopulations, %	
			CD4	CD8	CD4	CD8
MNC medium	796±137.3		36±4.14	20±5.26	18±7.42	42±7.65
A–SP		1.05±0.17	36±5.16	23±5.38	45±9.77	17±8.44

Note: here and in Table 2 the cells were cultured for 48 h at 37°C, 5% CO_2.

TABLE 2. Effect of A–SP on Proliferation and Functional Activity of Human Peripheral Blood CD4 and CD8 Lymphocytes Stimulated with ConA

Culturing conditions	Proliferation index	Lymphocytes, %		MTT cells in subpopulations, %		PHA-induced proliferation inhibition index, %
		CD4	CD8	CD4	CD8	
MNC + ConA	9.2±2.36	28±4.16	56±7.02	69±9.52	71±9.50	38±5.5
ConA + A–SP	10.7±2.98	21±4.19	57±8.52	63±11.2	38±6.27	–26±12.7

71±9.5% (ConA stimulation) to 42±14.2% ($p<0.05$), that is, to the level seen when MNC had been cultured in the medium. The CD4-lymphocyte activation index did not differ from the level in the ConA-stimulated culture (Table 2).

Analogous results were obtained when A-SP was added to MNC preincubated with ConA. In MNC washed after mitogen stimulation and cultured for 48 h the CD8-lymphocyte activation index was 81±11.3%. The addition of A-SP to the cells resulted in its decrease to 42±14.2% ($p<0.05$), as had been observed in the previous experiments. These data indicate that in ConA-stimulated MNC only one modulating effect of A-SP is expressed - its ability to decrease the number of activated CD8 lymphocytes. This effect of A-SP does not result from its cooperative interaction with the mitogen.

To find out to what extent the effect of A-SP correlates with the decrease of functional activity of T suppressors stimulated with ConA, the ability of these cells to inhibit autologous MNC proliferation stimulated with PHA was studied. According to the data obtained, the addition of cells preincubated with ConA yielded a PHA-induced MNC proliferation inhibition index at the level of 38±5.4%. In contrast, cells cultured with ConA and A-SP did not affect the proliferative process induced by PHA, or they even stimulated the process. In these experiments the proliferation inhibition index was 26±12.7% (Table 2). The difference is statistically significant ($p<0.05$). From the data obtained it follows that the drop of the CD8-lymphocyte activation index observed in the presence of A-SP correlates well with the functional test results. In this model system (ConA induction of T suppressors) the incorporation of MTT reflects the decrease of T-suppressor functional activity under the influence of A-SP.

Thus, this study presents the first data indicating the ability of A-SP to inhibit the functional activity of T suppressors. Its effect is expressed both for the intact CD8 lymphocytes present in the peripheral blood and for ConA-induced T suppressors. A-SP does not influence CD4 and CD8 antigen expression on the surface of the peripheral blood lymphocytes, does not have independent mitogenic activity, and does not alter the proliferation induced by ConA. Therefore, unlike with most bacterial components, the target for the A-SP effect is not the cell proliferation but the cascade of cell interactions associated with their differentiation process. A-SP perhaps has an immunomodulating activity not only on the T-suppressor function, but also on function of the T helpers/inducers. This is indicated by the fact that A-SP causes not only a drop in the CD8-lymphocyte activation index, but its reciprocal rise in the CD4-cell subpopulation. The mechanism of the A-SP effect on the T suppressors, as well as the role of determinants, common with thymus epidermal antigens (factors), in the realization of the immunomodulationg activity of A-SP should be the subject of future studies.

The inhibition of the suppressor element of the immune system with an exogeneous inhibitor such as A-SP may lead to tolerance failure and to the synthesis of autoantibodies to various tissue antigens of the hostorganism, as well as to activation of macrophagal elements, natural killers, and K cells; that is, it can cause multiple immunoregulatory disturbances. All this may promote the development of the autoimmune process in diseases etiologically associated with streptococcosis.

REFERENCES

1. E. A. Bazanova, E. V. Gnezditskaya, N. A. Borodiyuk, and I. M. Lyampert, *Zh. Mikrobiol.*, № 1, 80-84 (1990).
2. E. V. Gnezditskaya, V. P. Bukhova, E. A. Bazanova, and L. A. Malkina, *Byull. Eksp. Biol.*, **106**, № 10, 467-469 (1988).
3. E. V. Gnezditskaya, E. A. Bazanova, I. M. Lyampert, and L. V. Beletskaya, *Ibid.*, **109**, № 2, 167-169 (1990).
4. E. V. Gnezditskaya, V. P. Bukhova, E. A. Bazanova, and L. A. Malkina, *Immunologiya*, № 4, 68-71 (1990).
5. I. M. Lyampert, E. I. Drobyshevskaya, E. V. Ryzhikova, *et al.*, *Itogi Nauki i Tekhn., Ser. Immunologiya* [in Russian], Moscow (1988), Vol. 22, pp. 43-67.
6. J. E. Coligan, W. C. Schnute, and N. J. Kindr, *J. Immunol.*, **114**, 1654-1658 (1978).
7. C. Kieda and M. Mausigny, *Second Intern. Workshop Human Leucocyte Different Antigens*, New York (1986), Vol. 1, pp. 531-535.
8. T. Mosmann, *J. Immunol. Meth.*, **65**, 55-61 (1983).
9. U. Schauer, J. Krolikowski, and C. H. L. Rieger, *Ibid.*, **116**, 221-227 (1989).