combination of α-interferon with ACV marked synergism was observed, for MIC of α-interferon in conjunction with ACV was reduced compared with the value of this parameter for each substance separately, by 443 and 14 times respectively. The value of IECP, namely 0.072, also was evidence of the considerable mutual potentiation of the inhibitory action of ACV and α-interferon on HSV-II reproduction. Thus the 10 combinations of Soviet antiherpetic chemotherapeutic agents and α-interferon investigated showed the value of combined treatment of herpetic infection. With respect to antiherpetic activity, all the combinations tested in this investigation can be arranged in the following order: AC + α-interferon > Ara-A + BVDU + α-interferon > ACV + Ara-A + BVDU + α-interferon > ACV + BVDU + α-interferon > ACV + Ara-A + α-interferon > ACV + BVDU > ACV + Ara-A + BVDU > Ara-A + BVDU > ACV + Ara-A > Ara-A + α-interferon.

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# PEPTIDOGLYCAN ISOLATED FROM *Lactobacillus bulgaricus*: COMPLEMENT-MEDIATED EFFECT ON MATURATION OF PRECURSOR T CELLS


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KEY WORDS: peptidoglycan; complement; differentiation of lymphocytes.


Peptidoglycans (PG) in the cell walls of microorganisms possess immunomodulating activity [1, 11] and can activate the complement system [6]. Fragments of the components of complement formed as a result of activation of the complement system in turn perform the functions of immunoregulators [2, 8]. The immunomodulating action of structural components of the cell walls of microorganisms is realized at the level not only of differentiated lymphocytes, but also of precursors of T and B cells. For example, lipopolysaccharides stimulate T-cell differentiation [10]. The effect of PG isolated from the walls of Gram-positive bacteria on the early stages of T-cell differentiation remains virtually unstudied.

The aim of this investigation was to study the ability of PG from *Lactobacillus bulgaricus* to induce expression of theta-antigen (TAG) on pre-T-lymphocytes of mouse bone marrow and the role of complement as a possible mediator of this induction.

## EXPERIMENTAL METHOD

A bone-marrow suspension from CBA mice was enriched with pre-T-cells on a column with nylon fiber. After concentration the pre-T-lymphocytes ($5 \times 10^6$/ml) were incubated with PG


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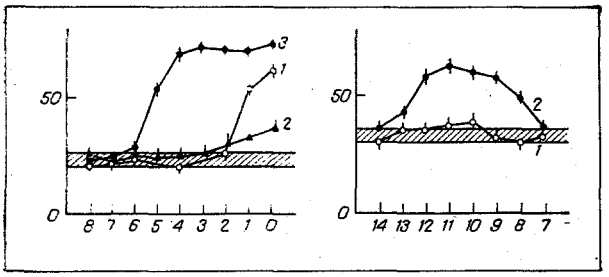


Fig. 1 Fig. 2

Fig. 1. Incubation of bone marrow cells in the presence of homologous serum. Abscissa, log. of PG concentration (in μg/ml); ordinate, percentage of cells expressing TAG. 1) Incubation of cells with PG for 30 min, 2) for 60 min, 3) for 2 h.

Fig. 2. Effect of C3 component and C3a fragment on expression of TAG on pre-T lymphocytes. Abscissa, log. of protein concentration (in M); ordinate, percentage of cells expressing TAG. 1) Incubation of cells with C3 component, 2) incubation of cells with C3a fragment.

at 37°C for 30 min or 1 or 2 h. The presence of TAG was determined by the lymphocytotoxic test, using anti-θ-serum and guinea pig complement, previously absorbed with thymocytes. Results of the test using thymocytes from the same mice served as the positive control. The results were read as the ratio between the number of dying and living cells. The action of each dose of PG was studied no fewer than 8-10 times and compared with the control. The significance of the difference was assessed by the Fisher–Student t test.

The C3a fragments of the C3 components of complement was obtained from serum containing 1 M 6-aminocaproic acid, activated (1 h, 37°C) by zymosan (5 g/liter), by immunoaffinity chromatography on anti-C3a-sepharose prepared by ourselves. Homogeneity of C3a was verified by polyacrylamide gel (PAG) electrophoresis [5], and antigenic specificity was verified by immunoelectrophoresis with commercial anti-C3a-serum (Calbiochem, USA). R3-reagent, consisting of normal human serum deprived of the C3 component, was obtained by the method in [3]. Conversion of the C3 component was assessed by the immunoblotting method [12], using $^{125}$I-labeled monoclonal anti-C3a-immunoglobulins. C3a was determined quantitatively by analysis of autoradiographs on an Ultroscan scanner (LKB, Sweden).

## EXPERIMENTAL RESULTS

The ability of PG to induce expression of TAG was studied in relation to the dose of the compound and the time of incubation with precursor T cells in medium 199 after addition of homologous serum, normal and inactivated at 56°C for 1 h. It was found that in the absence of native serum PG did not affect maturation of the pre-T cells and the level of expression of TAG did not exceed control values over the whole range of concentrations of the compound used. However, when the medium 199 was exchanged for homologous native serum, incubation of the pre-T cells with PG led to an increase in the number of cells expressing TAG on their surface, characteristic of mature T cells (Fig. 1). The appearance of TAG on the cell surface depended on the dose of PG and on the duration of its action. After incubation of the pre-T lymphocytes for 30 min with PG no significant increase was found in the number of cells carrying TAG compared with the control. A higher than the control value of theta-positive cells was observed only after incubation of lymphocytes for 1 h with PG in high doses. The highest number of cells expressing TAG was obtained by lengthening the time of interaction of PG with pre-T cells to 2 h. In this case the number of cells carrying TAG was increased by 40-50% compared with the control when PG was used in doses of $10^{-4}$ to 1 mg/ml.

To prove that the effect of PG on maturation of pre-T cells is realized through the complement system experiments were carried out using heat-inactivated serum. The results showed that in this version of the experiments PG, in any of the doses used, did not act on TAG generation by pre-T lymphocytes. It was thus established that the induction of T-cell maturation is not due to the direct action of PG on the cell, but is mediated through the complement system

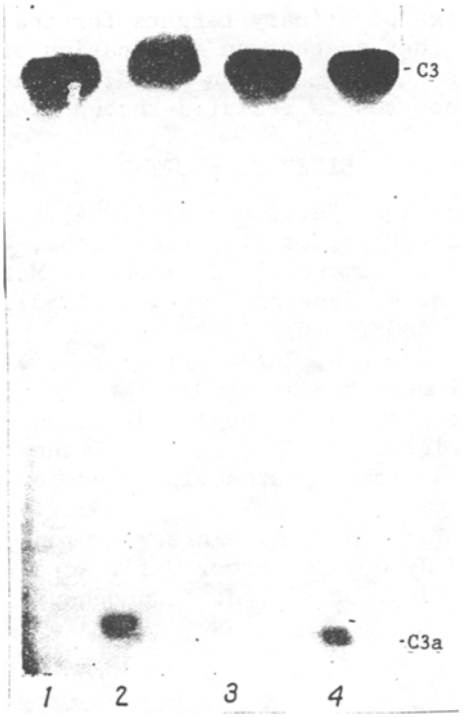


Fig. 3. Immunoblotting of serum samples obtained after incubation. 1) Native serum, 2) with zymosan (4 mg/ml), 3) with peptidoglycan (0.1 mg/ml), 4) with peptidoglycan (4 mg/ml).

During activation of complement many factors possessing biological activity of their own are formed. One of them is factor C3a, formed as a result of conversion of the C3 component of complement, and known in the literature as an immunomodulator [8, 9]. This state of affairs led to the hypothesis that C3a may play a role in T-lymphocyte differentiation. Two series of experiments were undertaken: in series I the ability of PG to activate complement with the formation of C3a was studied; in series II the effect of C3a was studied on maturation of pre-T lymphocytes. In the experiments of series I PG was incubated with normal pooled donated blood serum in doses of 0.1 and 4 mg/ml at 37°C for 1 h. One of the control samples did not contain PG (negative control), whereas the other (positive control) made use of zymosan, an activator of the alternative path of complement, in a dose of 4 mg/ml. Conversion of the C3 component of complement was determined by the immunoblotting method in 50 µl of each sample of serum. Analysis of the autoradiographs showed that PG activates complement, as is shown by the dose-dependent formation of the C3a fragment (Fig. 3), with mol. wt. of 9200 daltons. The percentage degradation of the C3 component of complement when PG was used in doses of 0.1 and 4 mg/ml, to judge by C3a formation, amounted to 7 and 25%, respectively, which is comparable with C3 conversion under the influence of zymosan in a dose of 4 mg/ml (30% degradation of C3). To determine the effect of C3a on the TAG expression on mouse bone marrow pre-T cells, this same polypeptide, obtained in homogeneous form (as shown by the results of PAG electrophoresis under denaturing conditions and of immunoelectrophoresis with anti-C3a serum), was used. Analysis of the species specificity of C3 showed that the addition of mouse serum to the R3-reagent completely restored the hemolytic activity of complement. These data confirmed information in the literature on the minimal species differences in the biological action of the components of the complement [7] of man and laboratory animals.

Incubation of pre-T cells for 2 h in the presence of the C3 component or its C3a fragment in doses of $10^{-7}$ to $10^{-14}$ M showed (Fig. 2) that the C3 component of complement does not affect TAG expression on pre-T lymphocytes, whereas the C3a fragment causes an increase in the number of cells carrying TAG by 25-30% compared with the control ($p < 0.01$) in doses of $10^{-12}$ to $10^{-18}$ M. Incidentally, when the maximal dose of C3a ($10^{-7}$ M) was used, there was no increase in TAG expression. This effect is often observed during the action of biologically active peptides and it is evidently linked with screening of the receptors by high concentrations of ligand.

Some workers state that types of primary targets for the action of PG can be limited, and as the most probable target they suggest an examination of macrophages [4]. The results given in this paper are evidence that the action of PG, isolated from *L. bulgaricus*, on TAG expression in developing T lymphocytes is mediated through the complement system.

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# DISTURBANCE OF CARBOHYDRATE METABOLISM IN EXPERIMENTAL SECRETORY DIARRHEA INDUCED BY CHOLERA TOXIN


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KEY WORDS: secretion of mucin; cholera toxin; carbohydrate metabolism; mucous membrane of the small intestine.


Injection of cholera toxin (CT) into the lumen of the small intestine induces intensive secretion of electrolytes and water, mediated through elevation of the cAMP level in the mucous membrane [7]. It has also been shown that cholerogen and agents increasing the intracellular cAMP concentration potentiate the Na-dependent transport of glucose or its unmetabolized analogs, both in vivo and in vesicles of enterocyte apical membranes [14]. This is facilitated by the fact that the mucous membrane of the small intestine has well marked ability not only to transport and metabolize glucose in the intestinal lumen, but also to recirculate sugars [1]. One pathway of carbohydrate utilization in the mucous membrane of the small intestine is the synthesis of the glycoproteins of mucin [5, 16], whose secretion is sharply intensified under the influence of CT [9].

On the basis of these data it can be postulated that cholera enterotoxin causes increased utilization of glucose in the mucous membrane of the small intestine on account of the use of an intermediate glycolysis product (fructose-6-phosphate) for synthesis of glucosamine — one of the important components of mucin. We previously reported that secretory diarrhea developing in the rabbit small intestine under the influence of CT is accompanied by stimulation of gluconeogenesis in the liver [2]. It is possible that the increased consumption of glucose by the small intestine is compensated by increased glucose production by the liver. The aim of this investigation was to test the above hypothesis.

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