of AVP in preparative quantities and the study of its properties are extremely difficult tasks [6, 5], and this restricts the investigation of this most important factor in antiviral immunity. Technically it seems a more promising approach to use messenger RNAs for AVP, as was done in the present investigation. The methods of induction, isolation, and testing of AVP-mRNA developed by the writers [1] enable samples with marked biological activity to be obtained comparatively simply. During translation of AVP-mRNA, the specificity of action characteristic of interferon was not observed [3].

The long-term resistance developed by the cells in response to administration of homologous and, what is particularly important, of heterologous AVP-mRNA may be a new and effective method of nonspecific protection of cells against viruses.

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PROTECTIVE ACTION OF SOME DIPHOSPHONATES AGAINST INJURY TO LYMPHOCYTES BY ANTILYMPHOCYTIC SERUM

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Rabbit antilymphocytic serum and complement were used in quantities causing death of 50% of human lymphocytes isolated in a Ficoll–Verografin (amidotrizoate) density gradient. The experimental samples (0.2 ml of a lymphocyte suspension containing 2.4×10^4–6×10^4 cells) were treated with 10 mM solutions of diphosphonates [disodium salt of hydroxyethylenediphosphonic (HEDP) acid, alkylated HEDP-acid, aminomethyl-HEDP-acid, aminobenzyldiphosphonic and aminoisopropyldiphosphonic acids] in doses of 0.001 to 0.2 ml. A decrease in the number of dead cells was observed after staining with 0.1% trypan blue. The disodium salt of HEDP-acid and alkylated HEDP-acid proved to be most effective and exhibited protective properties in doses as low as 0.01 ml. In a dose of 0.1 ml, all the tested compounds had a marked protective action.

KEY WORDS: lymphocyte; antilymphocytic serum; diphosphonates.

Certain diseases are based on immunologic injury to the outer cell membranes, frequently accompanied by the liberation of biologically active substances of pathogenetic significance. As a rule these reactions depend on complement and require the participation of calcium and magnesium ions. Marcelli and Renoux [5], for instance, showed that the intensity of liberation of histamine from mast cells is reduced in the absence of calcium ions, which are bound by EDTA. More recently work has been published on various substances which have protective properties in relation to cell membranes. In particular, synthetic diphosphonates have been shown to possess this property. These compounds are complex ones which can interfere with calcium and mag- metabolism [1-3].

The object of the present investigation was to study the protective action of a series of disphosphonates against immunologic injury to the outer cell membrane of human lymphocytes by antilymphocytic serum in the presence of complement in vitro.

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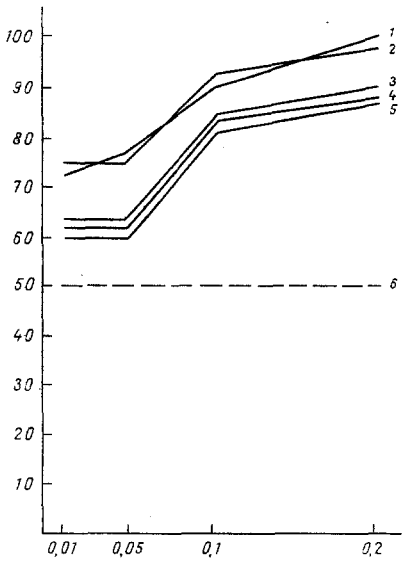

Fig. 1. Effect of dose of diphosphonates on number of living cells. Abscissa, number of living cells (in %); ordinate, dose of diphosphonates (in ml). 1) Alkylated HEPD-acid; 2) disodium salt of HEPD-acid; 3) aminomethyl-HEDP-acid, 4) aminoisopropyldiphosphonic acid; 5) aminobenzyldiphosphonic acid; 6) control.

EXPERIMENTAL METHOD

The quantity of rabbit antilymphocytic serum (ALS) and complement required to cause death of 50% of human lymphocytes was determined in preliminary experiments.* Native rabbit serum was used as the source of complement: the complement in it was titrated by the method of Campbell et al. [4]. Lymphocytes were isolated from the peripheral blood of children in a Ficoll–Verografin (amidotrizoate) density gradient (density of the liquid 1.077). The number of cells obtained by this method from 5 ml of venous blood varied from 6×10^5 to 1.5×10^6. Solutions (10 mM) of the diphosphonates [disodium salt of hydroxyethylenediphosphonic (HEDP) acid, alkylated (HEDP) acid, aminomethyl-HEDP-acid, aminobenzyldiphosphonic and aminoisopropyldiphosphonic acids] were made up in Tris-HCl buffer, pH 7.5. To samples containing 0.2 ml of lymphocyte suspension (from 2.4×10^4 to 6×10^4 cells) were added previously titrated doses of rabbit ALS and complement, followed by between 0.001 and 0.2 ml of solutions of the diphosphonates. To equalize the volume, Tris-HCl buffer was added to the control series of tubes in volumes corresponding to the doses of diphosphonates. The mixture was incubated for 1 h at room temperature. Films were then prepared after the addition of one drop of 0.1% trypan blue solution to two drops of the lymphocyte suspension, and the number of living and dead cells was counted. Altogether 30 experiments were performed.

EXPERIMENTAL RESULTS

In a dose of 0.001 ml none of the diphosphonates used had any protective action, i.e., the percentage of living cells was not higher than in the control (Fig. 1). In a dose of 0.01 ml the disodium salt of HEDP-acid caused a small increase in the number of living lymphocytes, whereas the other compounds (aminomethyl-HEDP-acid, aminobenzyldiphosphonic and aminoisopropyldiphosphonic acids) were ineffective. A sharp increase in the living lymphocytes (up to 82-94%) was observed by the use of all compounds in a dose of 0.1 ml. Increasing the dose of the diphosphonates to 0.2 ml had a very small positive effect compared with the previous dose.

* The ALS and complements were provided by A. N. Mats, Senior Scientific Assistant at the I. I. Mechnikov Research Institute of Vaccines and Sera.

The compounds tested likewise had no cytotoxic action in concentrations 5 times greater than the maximal concentration used in the experiments. Differences found were statistically significant (by Student's criterion).

It can thus be concluded from these results that diphosphonates have a protective action on the outer cell membrane of lymphocytes against immunologic injury. This fact may be of great importance to the understanding of the mechanisms of development and the treatment of diseases in which the leading pathogenic factor is a disturbance of the integrity of the outer cell membrane on an immunologic basis.

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STATE OF NONSPECIFIC RESISTANCE IN GERMFREE AND *Escherichia coli* CONTAMINATED MINIATURE PIGLETS

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The phagocytic activity of the leukocytes and the serum complement, properdin, and lysozyme levels were studied in germfree miniature piglets and similar animals contaminated with *Escherichia coli* 055 and *E. coli* 083. In the presence of autologous serum and complement phagocytosis of *E. coli* 055 cells was inhibited, but it was considerably intensified under the influence of specific opsonins (antibodies against *E. coli* 055). Lowered levels of complement, properdin, and lysozyme were found in the germfree animals. After peroral monocontamination with *E. coli* the formation of properdin and complement was stimulated the most, and that of lysozyme the least. Antibodies against *E. coli* 055 were not found in the monocontaminated piglets. The highest lysozyme levels were found in the previously germfree animals, which points to the role of other contamination factors than *E. coli* cells in the stimulation of lysozyme. It is concluded that microbial contamination plays an important role in the development of the cellular and humoral factors of resistance.

KEY WORDS: gnotobiotic miniature piglets; phagocytosis; complement; properdin; lysozyme.

With the development of the gnotobiological approach the attention of research workers has been increasingly attracted to the study of the role of the microbial factor in the formation of the immunobiological reactivity of the host. Experiments on germfree animals of various species have yielded new data on the role of the microflora and its individual representatives in the mechanisms of immunogenesis and also of nonspecific resistance of the host to infection. Meanwhile, the role of the microbial factor in the formation of resistance has still been only inadequately studied. Germfree piglets are particularly valuable for the study of the microbial influence on the development of immunobiological reactivity and nonspecific resistance to infection, for because of the nature of the structure of their placenta (of the chorioepithelial type), no globulins of maternal origin can enter the fetuses [9].

Methods of obtaining and rearing germfree miniature piglets were developed and introduced at the Research Laboratory of Experimental Biological Models, Academy of Sciences of the USSR, beginning in 1975.

Research Laboratory of Experimental Biological Models, Academy of Sciences of the USSR, Moscow Region. (Presented by Academician of the Academy of Medical Sciences of the USSR, N. N. Zhukov-Verezhnikov.)

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