The results of these investigations thus indicate that after inoculation with different production batches of live measles vaccine, attenuated measles virus penetrates into the brain, as shown by specific fluorescence of its various structures. In turn, this indicates that attenuated measles virus of strain L-16 possesses residual affinity for the cellular and vascular structures of the brain and spinal cord of newborn animals. Meanwhile, the absence of the disease in the newborn animals, in the writers' opinion, is an indication that the vaccinal measles virus is sufficiently well attenuated. However, despite this fact, the presence of some degree of neurotropism suggests that under conditions of modified reactivity of the recipient, the attenuated measles virus could give rise to postvaccinal complications. There is evidence in the literature that measles vaccination [1-3] is accomplished not only by the development of vaccination reactions, but also sometimes by the development of postvaccinal lesions of the CNS.

As regards the pathomorphological changes, they can be regarded as due to the direct action of the attenuated virus on brain structures.

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ABILITY OF THERMO- AND ACID-STABLE SERUM SERINE PROTEASE INHIBITOR TO INHIBIT MITOGEN-STIMULATED TRANSFORMATION OF LYMPHOCYTES

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The ability of a thermostable and acid-stable serine protease inhibitor from rabbit blood serum (TASPI) to inhibit the transformation of human peripheral blood lymphocytes stimulated by phytohemagglutinin (PHA) or concanavalin A (con A) was demonstrated. The degree of inhibition depends on the concentration of the inhibitor and its specific activity. The maximal degree of inhibition was 50-70%. TASPI has no cytotoxicity. Stronger inhibition of transformation is observed if TASPI is added to the culture 24 h after the addition of PHA. Data on the antiprotease activity of human blood serum, either native or inactivated under different conditions, are given. The results suggest that TASPI participates in the control of the biological activity of lymphoid tissue cells.

KEY WORDS: *antiproteases; lymphocyte transformation.*

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The nature and role of the proteases of lymphoid tissue cells, and also of the system of phagocytic monocytes and polymorphs have been the subjects of many recent investigations [4, 5, 7, 11]. It is supposed that the proteases participate in the transformation and proliferation of lymphocytes stimulated by mitogens, antigens, or allogeneic cells, of intercellular cooperation, and in the manifestation of the cytotoxic activity of lymphocytes [6, 10]; their role has also been demonstrated in the activation of certain components of complement and the generation of physiologically active peptides [9].

The problem accordingly arises of the role of natural protease inhibitors (of the blood serum and tissues) in the control of the protease activity of lymphoid tissue cells and of the processes whose development or activity they determine.

In this investigation the ability of a thermostable and acid-stable serine protease inhibitor from rabbit blood serum (TASPI) [2] to lower the intensity of transformation of human peripheral blood lymphocytes, stimulated by phytohemagglutinin (PHA) or by concanavalin A (con A), was studied.

EXPERIMENTAL METHODS

TASPI (12-30 i.u./mg)* was isolated from rabbit blood serum by the method described in [1]. The solution was sterilized by passage through a membrane filter (0.24 μ). The serum antiprotease activity was estimated by determining the hydrolysis of BAEE* by trypsin. To determine the antiprotease activity in native serum, 0.2 ml of serum diluted 1:50 was treated with 1.7 ml of 0.05 M Tris-HCl buffer, pH 8.0, and 0.1 ml of trypsin (from Serva) solution in a concentration of 0.1 mg/ml and 1 ml of $1.5 \cdot 10^{-3}$ M BAEE (from Koch-Light). In order to determine antiprotease activity in sera inactivated at 56°C for 1 h, 0.2 ml of serum diluted twice was taken as the sample.

The increase in optical density was measured on the SF-16 instrument at 253 nm and 25°C for 5 min against a mixture of reagents.

Lymphocytes were isolated from donors' blood by Boyum's method [3] in the modification used to obtain lymphocytes from large volumes of blood. Na_2 EDTA was used as the anticoagulant. Red blood cells were sedimented by the addition of gelatin solution (final concentration 1%). The suspension of leukocytes was centrifuged and the residue resuspended in 5 ml of plasma. The resulting suspension was layered above a gradient formed by Verografin (Triosil) or Hypaque and Ficoll (d = 1.078-1.080). After centrifugation (40 min, 400g) the intermediate layer was collected, washed with medium No. 199 containing 5% inactivated group AB serum (pooled from three donors), and resuspended in the same medium. The resulting cell suspension contained chiefly lymphocytes, under 5% of polymorphs and monocytes, and traces of platelets.

The lymphocytes ($25 \cdot 10^4$ cells/ml) were cultured in centrifuge tubes in medium 199 containing 5% inactivated serum. The lymphocytes were stimulated by PHA (Difco), 12 μg/ml, or by con A (Sigma), 10 μg/ml.

The lymphocytes were cultured for 72 h. To each culture 2 μCi [^{3}H]thymidine (specific activity 6 Ci/mmole) was added 69 h after the beginning of stimulation. After 3 h the cells were transferred to Synpor membrane filters (0.6-0.9 μ) and rinsed successively with 0.9% sodium chloride solution and 5% TCA solution. The radioactivity of the dry samples was measured on a Mark II liquid scintillation counter, using a scintillation fluid based on toluene. The results were expressed in counts per minute (cpm). The time of addition of the TASPI to the culture and its concentration depended on the objects of the particular experiment.

EXPERIMENTAL RESULTS

The intensity of transformation of lymphocytes stimulated by PHA as a function of the concentration and specific activity of TASPI is illustrated in Fig. 1. TASPI with a specific activity of 30 i.u., in a concentration of 100 μg/ml, was found to inhibit the response of the lymphocytes by 50%. A further increase in concentration to 400 μg/ml did not cause any increase in the inhibition of transformation.

*The inhibitory unit (i.u.) corresponds to the quantity of inhibitor which inhibits hydrolysis of 1 μmole of N-benzoyl-L-arginine ethyl ester (BAEE) under standard conditions.

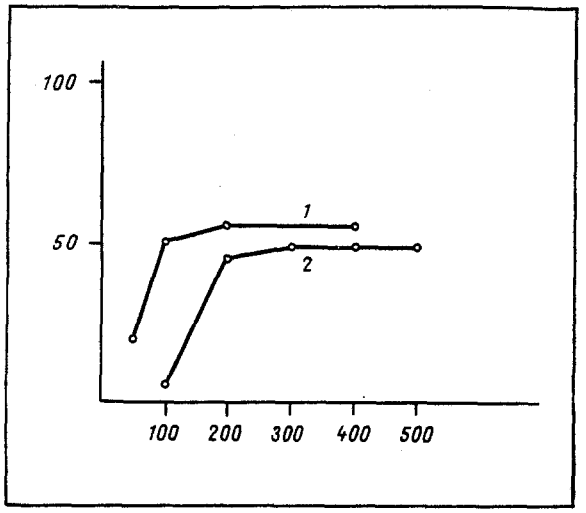

Fig. 1. Degree of inhibition of transformation of lymphocytes stimulated by PHA as a function of concentration and specific activity of TASPI in culture medium. Abscissa, concentration of TASPI (in μg/ml); ordinate, inhibition (in %). 1) TASPI 30 i.u./mg; 2) TASPI 12 i.u./mg.

TABLE 1. Effect of Time of Addition of TASPI to Culture on Intensity of Transformation of PHA-Stimulated Lymphocytes (M±m)

Experimental conditions	Incorporation of [^{3}H] thymidine, cpm	% of inhibition
PHA, 12 μg/ml	47 000±1 500	0
PHA + TASPI, 30 i.u./mg, 100 μg/ml simultaneously	31 000±1 300	34
PHA + TASPI:		
after 24 h	22 700±1 230	52
» 48 h	48 700±1 350	0

Note. Mean results of five determinations shown.

TASPI with a specific activity of 12 i.u. had no inhibitory activity in a concentration of 100 μg/ml, but with an increase in its concentration to 200 μg/ml a statistically significant decrease in the intensity of incorporation of [^{3}H]thymidine into the DNA of the stimulated lymphocytes was observed. The results show that TASPI inhibits transformation of lymphocytes stimulated by PHA. This conclusion was also valid for the case when con A was used as the mitogen. The minimal TASPI concentration at which inhibition was observed depended on the specific activity of the preparations used, showing that the biological effect of TASPI preparations is determined by their antiprotease activity. A fourfold increase in the TASPI concentration compared with the minimal inhibitory concentration did not lead to a greater decrease in the intensity of transformation. It can tentatively be suggested that TASPI controls either the activation of only a particular subpopulation of lymphocytes or the increase in the intensity of transformation of T lymphocytes stimulated by PHA which develops as a result of the simultaneous action of proteases from monocytes or neutrophils and PHA [8]. In the latter case, during the action of TASPI the level of transformation which would take place on activation of "pure" lymphocytes (uncontaminated by other types of cells) by PHA, is preserved. The ability of TASPI to inhibit transformation is not determined by its cytotoxic action on lymphocytes. Incubation of lymphocytes labeled with $Na_2{}^{51}CrO_4$ in the presence of TASPI for 2 h did not increase the elimination of ^{51}Cr compared with the spontaneous level. The absence of cytotoxicity of TASPI was also shown by the results of experiments (Fig. 1)

TABLE 2. Antiprotease Activity of Human Blood Serum, Native and Inactivated under Different Conditions

Conditions of inactivation (temperature, time, pH)	Antiprotease activity, i.u./ml
Native serum	23.1
56°C, 1 h, pH 7.2	2.1
56°C, 1 h, pH 5.0	2.5
56°C, 1 h, pH 7.2; 56°C, 1 h, pH 5.0	2.3

in which a fourfold increase in the TASPI concentration in the culture medium was not accompanied by any increase in the inhibition effect. The results shown in Fig. 1 were obtained in experiments in which the mitogen and TASPI were added to the culture at the same time. When the effect of the time of addition of TASPI to the culture after the beginning of stimulation on its ability to inhibit lymphocyte transformation was studied, stronger inhibition was obtained when the TASPI was added to the culture after 24 h (Table 1).

These results suggest that the action of TASPI is directed toward inhibition of the activity of the proteases which participate in the late stages of lymphocyte activation. It will be noted that the action of TASPI was studied in medium containing 5% inactivated human blood serum, which also has a certain level of antiprotease activity. This is evidently due to the presence of thermostable and acid-stable protease inhibitor in the composition of the serum. This conclusion is based on the results of the study of antiprotease activity of human blood serum inactivated under different conditions (Table 2). The antiprotease activity of inactivated blood serum was 10% of the activity of native serum, and was due mainly to an inhibitor with similar properties to TASPI, for heating the serum at pH 5.0 did not cause any further decrease in the antiprotease activity of the serum inactivated at pH 7.2. The presence of this inhibitor could account for the low intensity of transformation of lymphocytes stimulated by mitogens in medium containing high concentrations of inactivated serum (20-30%) compared with the level of transformation in medium containing 5% serum, and also the higher level of transformation in the presence of inactivated than of native serum. The ability of the same preparation of TASPI to inhibit transformation of lymphocytes isolated from the blood of different donors varied (between 40 and 80% of inhibition), possibly depending on the number of polymorphs and monocytes and the activity of their proteases, which is not constant in different samples of lymphocytes.

The possible interaction of TASPI with these proteases (data on this interaction were obtained by the writers recently) prevents the establishment of the actual TASPI concentration that is effective in controlling the intensity of transformation of PHA-stimulated lymphocytes.

The results relating to the ability of TASPI to lower the intensity of transformation of stimulated lymphocytes have an immediate bearing on the problem of the role of antiproteases in the regulation of biological activity of lymphoid tissue cells. Whereas the activity of lymphocytes in the blood can be controlled by protease inhibitors of high molecular weight, such as α_2-macroglobulin and α_1-antitrypsin, the importance of thermostable and acid-stable inhibitors of the blood serum serine proteases with a molecular weight of 22,000 may increase significantly in the tissues.

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STIMULATION AND SUPPRESSION OF CONTACT DERMATITIS IN MICE BY LOW-MOLECULAR-WEIGHT THYMUS HUMORAL FACTOR

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The effect of a low-molecular-weight lymphocytosis-stimulating substance (LSS) from the thymus on the development of contact sensitivity to picryl chloride was investigated in mice. Small doses of LSS were found to potentiate, whereas large doses suppressed this type of delayed hypersensitivity. Contact sensitivity can be transferred passively by means of lymph node and spleen cells isolated on the 6th day after immunization. The experiments showed that mice receiving large doses of LSS contain cells which suppress the passive transfer of contact sensitivity by immune cells. This suppression was absent after treatment of the cells with θ-antiserum and complement. It is concluded that the suppressor cells influence the effector phase of contact sensitivity.

KEY WORDS: *lymphocytosis-stimulating substance from the thymus; contact sensitivity; adoptive immunity; suppressor lymphocytes.*

It was shown previously that the lymphocytosis-stimulating substance (LSS) of the thymus can potentiate or depress reactions of the delayed hypersensitivity type. This action of the preparation depends on the dose used: A small dose of LSS stimulates, whereas a large dose inhibits this type of immunologic response [2, 3]. Since blockade is observed in the first case, and some increase in the strength of antigen-induced suppression of DNA synthesis in the spleen in the second case [3], it has been suggested that the dose-dependent effect of LSS on the development of hypersensitivity of delayed type (HDT) is connected with certain changes in the generation of suppressor T lymphocytes.

The object of this investigation was to examine this problem.

EXPERIMENTAL METHODS

Experiments were carried out on CBA and A/I mice aged 3 months. LSS was isolated from calf thymus by the method described previously [1].

Contact allergic dermatitis, induced in mice by picryl chloride [4], was used as the model of HDT. The mice were sensitized by a single application of 0.1 ml of a 5% solution of picryl chloride in ethanol to the abdominal region. LSS was injected intraperitoneally in a dose of 0.1 or 1 mg per mouse on the day after sensitization, next day, and 5 days after sensitization. Control animals received an injection of physiological saline at the same time. On the 6th day after application of picryl chloride, a 1% solution of the allergen was applied to the ear of some animals and the inflammatory reaction was read 24 h later, paying attention to thickening of the concha auriculae. Its thickness was measured by means of a micrometer with electromechanical transducer.

In most of the mice the provocation skin test was not carried out. They were used in the experiments to study passive transfer of contact sensitivity. The mice were decapitated on the 6th day after sensitization. A cell suspension was prepared from the spleen and lymph nodes (cervical and axillary) in Eagle's medium. A mixture of lymphocytes, in a dose of 40·

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