CHANGES IN DEOXYRIBONUCLEASE I INHIBITOR IN MOUSE SPLEEN AFTER INJECTION OF A SYNTHETIC POLYANION

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The synthetic polyanion pyran (a copolymer of divinyl ether and maleic anhydride), if injected into mice, raises the antibody titer against sheep's red blood cells and also activity of serum DNase I and splenic inhibitor of DNase I. At the same time, the weight of the spleen increases. The possible role of the DNase I – inhibitor system in mechanisms of the adjuvant action of the synthetic polyanion is discussed.

KEY WORDS: DNase I; mouse spleen; polyanions; pyran.

One of the main problems in immunology is how to control immune reactions in man and animals. An urgent aspect of this problem is the study of mechanisms of action of biologically active substances with a stimulating or inhibiting effect on the immune system. An important role among these substances is ascribed to high-polymer synthetic compounds belonging to the class of polyelectrolytes (polyanions, polycations, and polyampholytes). It is stated in the literature [3] that polyanions and polycations at the cellular level stimulate migration of stem cells and B- and T-lymphocytes, sharply increase the effectiveness of cooperation of T- and B-lymphocytes, and also replace the helper activity of T-cells. Meanwhile, at the subcellular level, the mechanisms of the adjuvant action of synthetic polyelectrolytes have been inadequately studied.

The aim of this investigation was to study the effect of a synthetic polyanion of the pyran copolymer type (copolymers of divinyl ether and maleic anhydride) on intracellular activity of DNase I inhibitor which, according to data in the literature [7], is an actin protein, capable of forming a specific complex with DNase I.

EXPERIMENTAL METHOD

Experiments were carried out on male noninbred and CBA mice weighing 20 g. Pyran copolymer 77-14 (fraction 1), highly saturated with anions, was used. The pyran copolymers were synthesized by V. S. Kutyreva in the department headed by Professor V. S. Étlis (V. A. Kargin Institute of Polymers, Dzerzhinsk).

This substance was injected intraperitoneally in a single dose of 125 mg/kg body weight. The animals were killed at different times after injection of pyran and the spleen was perfused in situ with cold physiological saline and then "gently" homogenized in a Potter-Elvehjem glass homogenizer with Teflon pestle for 120 sec at 1200 rpm. The splenic homogenates thus obtained from control and experimental mice (dilution: w/v = 10) were centrifuged at 150,000 g for 30 min in the MSE Superspeed-65 ultracentrifuge. Activity of DNase I inhibitor was determined in the resulting supernatants. The scheme of the determinations was as follows: bovine DNase I + Mg^{++} + supernatant, incubation for 30 min at 37°C, buffering of the samples to pH 7.0-7.2, followed by addition of DNA. The further course of the analysis was described previously [2]. The difference between the original and residual activity of bovine DNase I served as the index of inhibitor activity. Activity of bovine DNase I was judged from the quantity of nucleoside monophosphates liberated as a result of enzymic hydrolysis of DNA during incubation for 60 min at 37°C. The course of determination of DNase I activity was described by the writers previously [2].

Total protein in the samples was determined by Lowry's method [4, 8]. DNA isolated from calf thymus [1, 6] was used as the substrate for determination of activity of DNase I and its inhibitor.

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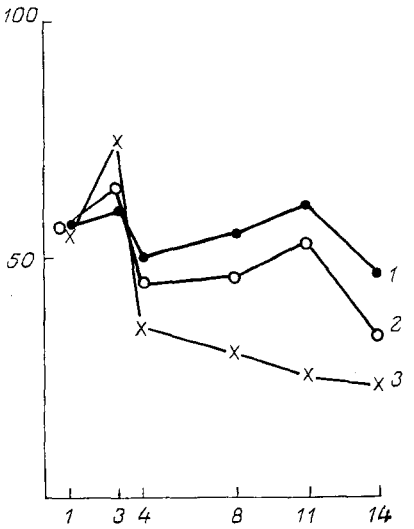

Fig. 1. Residual activity of bovine DNase I after incubation with splenic supernatants of mice of control and experimental groups. 1) Normal; 2) after injection of Freund's complete adjuvant; 3) after injection of pyran. Abscissa, days after injection of pyran and Freund's complete adjuvant; ordinate, residual bovine DNase I activity, expressed in % of its initial activity.

Mice were immunized with sheep's red blood cells (SRBC) by a single intraperitoneal injection. A 5% suspension of SRBC was used for immunization in a dose of 0.1 ml/10 g body weight. The antibody titer in the animals' serum was determined by the direct hemagglutination test in the usual way.

EXPERIMENTAL RESULTS

Spleens of mice for determination of intracellular activity of DNase I inhibitor were taken on the 1st, 3rd, 4th, 8th, 11th, and 14th days after injection of pyran. As Fig. 1 shows, the intracellular activity of the inhibitor in the spleen of the experimental group of animals was significantly higher than the control as early as on the 3rd or 4th day after injection of pyran and remained at a high level until the 14th day of the experiment. The weight of the spleen in the mice of this group increased until the 4th day of the experiment and also on subsequent days, to reach 500-600 mg by the 11th-14th day (normally 100-130 mg).

The increase in inhibitor activity in the spleen cells was accompanied by activation of serum DNase I, the activity of which on the 4th day after injection of pyran was twice as high as the control. This parallel between the increase in activity of the splenic inhibitor and of serum DNase I could indicate a direct functional link between these two proteins in the body.

The study of the adjuvant properties of pyran showed that on the 8th day after immunization with SRBC in the mice of the experimental group (intraperitoneal injection of pyran) there was a 60-fold increase in the antibody titer against SRBC compared with the control.

The results showing an increase in activity of splenic DNase I inhibitor activity, activation of serum DNase, and an increase in the antibody titer against SRBC under the influence of pyran can thus be taken to indicate both the ability of this compound to stimulate the animal's immune system and the possible participation of the DNase I–inhibitor system in the mechanisms of the immune response.

It is stated in the literature [7] that DNase I inhibitor contained in the serum and cells of several organs, is an actin protein. Meanwhile, in the modern view, actin proteins can stimulate cell processes such as phagocytosis, cytokinesis, and so on [9]. The increase in the activity of DNase I inhibitor in the mouse spleen after a single injection of pyran may indicate the possible immunogenic function of this protein in the animal.

Polyanions and polycations are stated [3] to bring about a sharp increase in production of antibody-forming cells in mixed culture of T- and B-cells in vitro, but the immune response in cultures of B-cells also was increased. It is therefore better to speak of two levels of action of these substances: an increase in cooperation between T- and B-lymphocytes and replacement of the helper activity of T-cells [4]. In the modern view, the helper function of T-lymphocytes can be reduced to transmission of the antigen in appropriate immunogenic form to B-lymphocytes [5]. This transmission may perhaps take place through the intervention of macrophages (specific signal). Another, nonspecific signal, also originating from T-lymphocytes, initiates reproduction and differentiation of B-lymphocytes and plasma cells – antibody producers. The possibility cannot be ruled out that one link in the complex chain of interaction between immunocompetent cells may be DNase I inhibitor which, as was mentioned above, belongs to the class of actin proteins. It can be tentatively suggested that in this case it performs an important role in proliferation of spleen cells, possibly B-cells. The other component – inhibitor of the nuclease system – of serum DNase I may serve as a unique "triggering signal" for the activation of splenic inhibitor. There is no doubt that this hypothetical mechanism of the adjuvant action of the synthetic pyran polyanion is purely conjectural and requires further experimental confirmation in vitro and in vivo. Nevertheless, the suggested mechanism of action of pyran may be an important stage in the elucidation of the molecular-biological processes lying at the basis of the adjuvant action of synthetic high-polymer compounds.

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