PREPARATION AND PROPERTIES OF INTERLEUKIN-1 FROM HUMAN BLOOD MONOCYTES

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Various inducers of interleukin-1 (IL-1) are known, including substances of bacterial and viral origin, components of complement, phagocytosis activators, etc. [3].

In the investigation described below an attempt was made to compare the action of inducers in order to create optimal programs for IL-1 production.

EXPERIMENTAL METHOD

Mononuclear cells were obtained by fractionation of conserved donated blood by Böyum's method. The cells in a concentration of 5×10^6/ml were resuspended in Eagle's medium (Institute of Poliomyelitis and Virus Encephalitis, Moscow) with the addition of 10% fetal calf serum ("Serva"), 200 mM glutamine, and 80 μg/ml gentamicin in plastic Petri dishes or in flat-bottomed micropanels. After incubation for 2 h at 37°C and with 5% CO_2 the cells were washed with culture medium. Cells adherent to plastic were reincubated in the presence of IL-1 inducers. The following inducers were used: prodigiosan (Moscow Pharmaceutical Chemical Preparations Factory), pyrogenal (N. F. Gamaleya Institute of Epidemiology and Microbiology), staphylococcal vaccine (Bacterial Preparations Production Enterprise, Khar'kov), hemolytic streptococcal allergen (Kazan' Research Institute of Epidemiology and Microbiology), saponin ("Calbiochem"), protein A ("Serva"), dextran sulfate ("Calbiochem"), zymosan ("Serva") and lipopolysaccharide (LPS) from *E. coli* B4:0111 ("Calbiochem"). The culture medium was collected after 24 h, centrifuged at 10,000 rpm, and kept at −20°C. IL-1 activity was estimated by its ability to intensify proliferation of thymocytes, stimulated by suboptimal doses of mitogenic lectin [7]. The level of proliferation was determined by measuring incorporation of ^{3}H-thymidine and expressed as stimulation indices, calculated as the ratio between the number of counts per minute (cpm) in samples with the test specimen and the number of counts per minute in samples not containing the specimen. For chromatographic fractionation the culture medium was concentrated by precipitation with ammonium sulfate at 80% saturation. The residue was dissolved in 0.02 M Tris-HCl buffer (pH 7.4) with 0.15 M NaCl and applied to a column with Sephacryl S-200 (2.6 × 90 cm), equilibrated with the same buffer. The column was calibrated beforehand by means of a set of standard marker proteins with mol. wt. of between 13 and 67 kilodaltons. Isoelectric focusing was carried out in a flat layer of "Ultrodex" granulated gel (LKB, Sweden) with 2% of ampholytes (produced jointly by Kazan' University and the All-Union

TABLE 1. Effect of Analgin on Induced IL-1 Production by Human Peripheral Blood Monocytes

IL-1 inducer	Level of stimulation of thymocyte proliferation	
	cpm	index of stimulation
Prodigiosan	132 298±5 671	5.54
Prodigiosan + analgin	206 012±11 874	8.63
Control	23 877±1 135	—
Control + analgin	28 011±1 783	1.17

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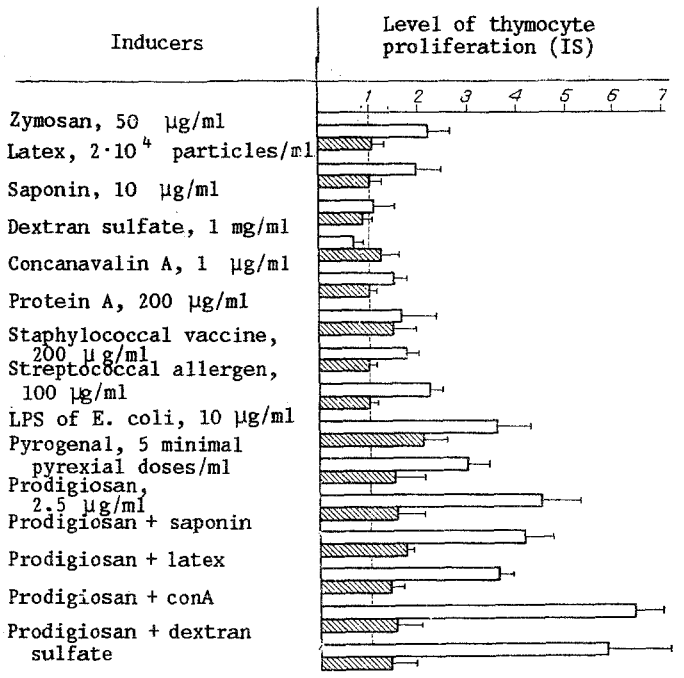

Fig. 1. Parameters of secretory IL-1 production by human peripheral blood monocytes. Unshaded columns — index of stimulation of thymocyte proliferation under the influence of culture medium of stimulated monocytes, used in a dilution of 1:200; shaded columns — inducer control.

Research Institute of Highly Pure Biological Preparations) with a pH range from 4.0 to 9.0, for 16-18 h. The pH of the gel after the end of the focusing process was measured with an "Orion" surface electrolyte (USA).

EXPERIMENTAL RESULTS

The results show that most inducers led to dose-dependent production of IL-1 by monocytes. Figure 1 shows the results of comparative assessment of the action of the inducers used in doses (according to the results of the preliminary experiments) causing maximal IL-1 production. The greatest ability to induce IL-1 production by human blood monocytes was exhibited by preparations of USSR origin: prodigiosan and pyrogenal, and also LPS from *E. coli*, nowadays used in the majority of published schemes for IL-1 preparation [4]. Nominations of different inducers with prodigiosan used in the optimal dose did not cause a significant increase in IL-1 production. Several inducers and their combinations (Fig. 1) themselves were capable of stimulating thymocyte proliferation, and this interfered with IL-1 assay by biological methods. Moreover, the true IL-1 concentration could be masked by the presence of substances with an action opposite to that of IL-1 in the supernatants, for example, prostaglandin E2 (PGE_2), which also is synthesized by activated monocytes [7]. Table 1 shows that stimulation of monocytes by the optimal dose of prodigiosan (2.5 μg/ml) with simultaneous addition of 100 μg/ml of analgin, an inhibitor of prostaglandin synthetase, to the medium led to an increase in the comitogenic activity of IL-1. On the other hand, PGE_2 can compete with IL-1 by lowering the level of thymocyte proliferation, and on the other hand, it can depress expression of IL-1 genes in the course of activation of the monocytes [5, 8] or can have both these effects. Precise determination of the IL-1 concentration requires the use of additional methods of analysis, immunochemical for example. We also found that of 50 healthy blood donors tested, 38.4% showed spontaneous IL-1 production. Similar data are given by Cannon [2], who observed spontaneous appearance of IL-1 after severe physical exertion or after ovulation.

As a result of this study of inducers, we chose for obtaining IL-1 a combination of prodigiosan and concanavalin A, which possessed the highest IL-1-inducing capacity. IL-1 from culture fluid of monocytes stimulated by the above-mentioned inducers was partially purified and characterized biochemically. On gel-filtration on a column with Sephacryl S-200 activity of IL-1 was found in the region of relative molecular weights of 18-20 kilodaltons (Fig. 2). On analysis of fractions from this region by isoelectric focusing, two main forms of IL-1 were

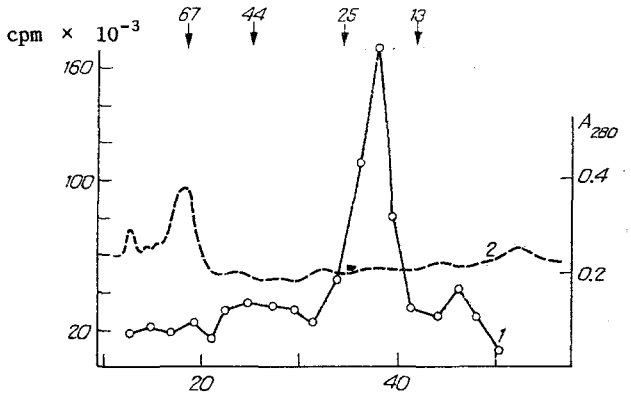

Fig. 2

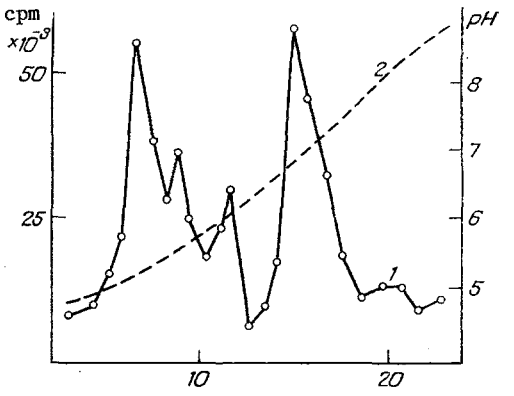

Fig. 3

Fig. 2. Gel-filtration of culture medium of stimulated monocytes on Sephacryl S-200. 1) Comitogenic activity (cpm); 2) elution profile for detection at wavelength of 278 nm.

Fig. 3. Isoelectric focusing of biologically active fractions after gel filtration. 1) Comitogenic activity (cpm); 2) pH in fractions.

discovered, with pI of 6.8 and 5.2-5.4, as well as a minor form with pI of 6.0 (Fig. 3), the existence of which has been described for human and animal IL-1 [1, 6, 7]. It is considered that human IL-1 exists chiefly in the form with pI = 6.8, whereas the relative content of the other forms in IL-1 preparations is significantly lower [6]. In the present case production of two forms of IL-1 with pI of 5.2-5.4 and with pI of 6.8 was found to be about equal, possibly due to the particular feature of the action of the inducers used. Biologically active material obtained as a result of stimulation of human blood monocytes by prodigiosan and concanavalin A, and purified chromatographically on Sephacryl S-200, had a dose-dependent comitogenic action on proliferation of the monocytes, and also did not maintain growth of IL-2-dependent murine T-cell line CTLL, and did not stimulate proliferation of 5-day human lymphoblasts, induced by concanavalin A, i.e., it did not contain IL-2 in its composition. The preparation thus obtained and partially purified can be used as a standard for determination of the biological activity of IL-1.

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