ACTION OF T-ACTIVIN ON IMMUNE INTERFERON PRODUCTION

A. G. Chuchalin, V. A. Babushkina,
V. Ya. Arion, R. D. Aspetov,
T. B. Shaikhinova, A. S. Novokhatskii,
and Yu. A. Baryshkov

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Several biologically active substances which participate in development, formation, and maturation of the immune system have been obtained in recent years from the thymus [1]. It has been shown that these substances can be used to abolish disturbances of the T-component of the immune system [3]. Data indicating the effect of some thymus preparations on interferon production have recently been published [7]. However, this problem has still received only little study. The preparation T-activin, a highly active thymus factor, has been studied in many immunologic tests.

In the present investigation the action of T-activin on synthesis of immune interferon was studied.

EXPERIMENTAL METHOD

Lymphocytes were isolated in a Ficoll–Verografin density gradient from heparinized blood from 8 healthy blood donors and 29 patients with chronic nonspecific lung diseases (CNLD) in the phase of exacerbation of the inflammatory process (the subjects' ages ranged from 20 to 45 years) [6]. Suspensions of a lymphocyte culture were prepared in Eagle's medium containing 3% calf embryonic serum, 300 μg/ml of glutamine, and 0.16 mg/ml of gentamicin, in a concentration of 10^6 cells in 1 ml. Parallel blood samples from donors and patients with CNLD in a volume of 1 ml were centrifuged at 1000 rpm for 10 min to remove the plasma and to wash the blood cells with Hanks' solution. A whole blood culture was prepared by resuspending the residue of blood cells in 1 ml of nutrient medium. The resulting suspensions were poured in a volume of 1 ml into plastic 24-well panels (from "NUPS," and T-activin prepared by the method described previously [2], or levamisole (from Gedeon Richter, Hungary) was added in doses of 10, 1, and 0.1 μg/ml, and the suspensions were stimulated with different doses of staphylococcal enterotoxin A (SEA), generously provided by Professor Yu. V. Ezepchuk (N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR), and with phytohemagglutinin (PHA) from Serva (West Germany). Cultures not containing T-activin and levamisole, but stimulated by mitogens, and also cell suspensions with levamisole and T-activin, but not stimulated by mitogens, were used as the controls. A reference preparation of human P8 leukocytic α-interferon, produced by the N. F. Gamaleya Institute of Epidemiology and Microbiology, Academy of Medical Sciences of the USSR, was used as the standard. The cells were cultured at 37°C in a chamber containing 5% CO_2 for 72 h, after which interferon activity in the supernatant was determined by a micromethod based on inhibition of the cytopathic action of vesicular stomatitis virus (VSV) in diploid cells of human embryonic fibroblasts by the method described previously [6].

The numerical results were subjected to statistical analysis by the Student–Fisher method.

EXPERIMENTAL RESULTS

The effects of different concentrations of T-activin and levamisole on γ-interferon production by peripheral blood lymphocytes of three healthy blood donors, induced by various doses of SEA, are given in Table 1.

N. I. Pirogov Second Moscow Medical Institute. D. I. Ivanovskii Institute of Virology, Academy of Medical Sciences of the USSR, Moscow. (Presented by Academician of the Academy of Medical Sciences of the USSR Yu. M. Lopukhin.) Translated from Byulleten' Éksperimental'noi Biologii i Meditsiny, Vol. 98, No. 7, pp. 76-78, July, 1984. Original article submitted July 15, 1983.

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TABLE 1. Effect of T-Activin and Levamisole on Immune Interferon Production by Blood Lymphocytes from Healthy Subjects Induced by Various Doses of SEA

Dose of SEA, μg/ml	Interferon activity (in units/ml) in lymphocyte culture						
	control	in presence of T-activin, μg/ml			in presence of levamisole, μg/ml		
		0,1	1,0	10,0	0,1	1,0	10,0
10,0	133±26	133±26	160	133±26	133±26	133±26	160
1,0	133±26	106±26	160	133±26	160	160	133,2
0,1	66±13	106±26	106±26	106±26	66±13	106±26	66±13
0,01	33±6,6	66±13	106±26	53±13	33±6	106±26	33±6
0,001	26±6	33±6	133±26	33±6	33±6	106±26	26±6
0,0001	13±3	16±6	33±6	26±6	26±6	53±13	26±5
0	10	10	10	10	10	10	10

TABLE 2. Effect of T-Activin *in Vitro* on Immune Interferon Production in Patients with CNLD

Group of subjects	Interferon activity (in units/ml) in cultures		
	without T-activin	with T-activin	*P*
Healthy blood donors (n = 5)	41,6±9,6	115,2±12,8	<0,05
Patients with CNLD: group 1 (*n*=8)	6,0±1,3	62,0±16,6	<0,05
group 2 (*n*=21)	39,2±5,9	85,1±9,5	<0,01

The results showed that if the production of γ-interferon was comparatively high neither T-activin nor levamisole affected the degree of interferon production and its titers were the same as in control samples untreated with immunomodulators. The stimulating action of T-activin and levamisole was observed on induction with low doses of SEA (0.01, 0.001, and 0.0001 μg/ml). For both T-activin and levamisole a dose of 1 μg/ml was found to be the optimal stimulating dose for γ-interferon biosynthesis. Under these circumstances interferon formation was increased by twice or more ($P < 0.05$). The data showed that neither T-activin nor levamisole is an inducer of γ-interferon.

The same stimulating action of T-activin and levamisole on γ-interferon production was observed when PHA was used as mitogen.

The effect of T-activin and levamisole on immune interferon biosynthesis in whole blood culture was studied in several experiments. γ-Interferon activity in whole-blood culture was found to be appreciably lower than in cultures of lymphocytes. However, the pattern of the stimulating action of T-activin and levamisole on interferon production was found to be similar to that obtained in lymphocyte culture: On induction of a whole-blood culture by SEA in doses of 0.01 and 0.001 μg/ml, T-activin and levamisole, added in a dose of 1 μg/ml, caused γ-interferon production to be increased by twice or more ($P < 0.05$). In this way the technique of investigation of γ-interferon production in order to determine the functional properties of lymphocytes could be considerably simplified.

γ-Interferon production was studied in lymphocyte cultures and whole-blood cultures from 29 patients with CNLD. In both cases the results were similar, and accordingly the results of the study of immune interferon production in whole-blood culture on stimulation with the mitogen SEA in a dose of 0.001 μg/ml are given in Table 2.

Depending on the ability of the lymphocytes to produce immune interferon, two groups of patients with CNLD were distinguished: group 1) patients in whom interferon production was reflected in low figures (from total areactivity up to 8 units/ml, on average 6.0 ± 1.3 units/ml); group 2) patients whose lymphocytes produced γ-interferon in relatively high titers (39.2 ± 5.9 units/ml), approximately the same as the interferon-producing ability of normal human lymphocytes (41 ± 9.6 units/ml). Addition of T-activin to lymphocyte cultures from patients of group 1 considerably enhanced the process of interferon formation (more than tenfold). The

effect of T-activin also was observed in lymphocyte cultures obtained from the patients of group 2. In one case the stimulating effect of T-activin was the same as in the control (healthy blood donors).

The results show that T-activin increases γ-interferon production by normal human lymphocytes, stimulated by low doses of SEA, whereas if high concentration of mitogens are used this effect is not observed. Similar results were obtained on the addition of preparation TP-1 from calf thymus [7] to a culture of human lymphocytes stimulated by PHA. T-Activin evidently exhibits a stimulating action on interferon production if the immune response of the lymphocytes is weak. This effect was observed to a marked degree in 8 of the 29 patients with CNLD in whom the initial interferon-producing activity of the lymphocytes was low.

In the present experiments addition of T-activin or levamisole to a lymphocyte culture in the absence of mitogen did not induce γ-interferon synthesis. These observations indicate that T-activin is not an interferonogen, but does affect the process of interferon biosynthesis.

Correlation between the immune status of the individual and the degree of PHA-induced γ-interferon production by lymphocytes was recently found in children: Low interferon-producing ability of the lymphocytes was found in children suffering from frequent acute respiratory virus infections [5]. In the present experiments γ-interferon biosynthesis was much lower in 8 of the 29 patients with CNLD than in the control.

The results of the tests on cultures of lymphocytes isolated in a Ficoll–Verografin density gradient were similar to those obtained in whole blood culture. Consequently the technique of investigation of γ-interferon production to determine the functional properties of T-lymphocytes can be considerably simplified.

In experiments *in vitro* T-activin stimulated activity of the T-component of immunity in patients with CNLD characterized by a weak immune response of their lymphocytes. This may provide the basis for administration of T-activin to these patients with a view to correcting the immunodeficiency. Potentiation of endogenous interferon production by T-activin may probably also lead to elimination of the pathogenic viral-bacterial flora from the patient.

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