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COMPARATIVE ASSESSMENT OF THE PROPERTIES OF MACROPHAGE MIGRATION INHIBITING FACTOR AND OF INTERFERON

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Some functional properties of interferon and of a factor inhibiting migration of macrophages (MMIF), obtained by stimulation of human tonsillar lymphocytes by Newcastle disease virus (NDV) or streptolysin O, were investigated. Both interferon and MMIF were shown to inhibit migration of human tonsillar cells actively, but they differed in their antiviral activity and their sensitivity to heating to 56°C for 30 min. MMIF production reached its maximum later than interferon production. Stimulation of human tonsillar lymphocytes by NDV led to the production of a broader spectrum of mediators of hypersensitivity of delayed type than stimulation by streptolysin O.

KEY WORDS: *cellular immunity; interferon; macrophage migration inhibiting factor.*

Mediators of hypersensitivity of delayed type (HDT) have been studied widely in recent years. The suggestion has been made that some of them have a common chemical structure and functional properties [7, 8].

This paper describes the study of some properties of interferon, obtained by the action of Newcastle disease virus (NDV) on a culture of tonsillar lymphocytes, and a macrophage migration inhibiting factor (MMIF) obtained by treatment of a culture of human tonsillar lymphocytes with streptolysin O. The basis for the investigation was the observation that human tonsillar lymphocytes participate actively in HDT reactions [1, 3, 4, 9, 10].

EXPERIMENTAL METHOD

MMIF were obtained by cultivating human tonsillar cells with streptolysin O. The tonsillar tissue was cut into small pieces with scissors, filtered through Capron, and washed off with medium No. 199 containing 10% heat-inactivated calf serum, 100 units/ml of penicillin, and 60 μg/ml of streptomycin. Viable tonsillar cells (10^7) were grown in 2 ml of this medium containing 0.1 ml of a 5% solution of streptolysin O at 37°C for 12-30 h. The supernatant obtained after culture was dialyzed against physiological saline for 48 h and lyophilized [10].

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TABLE 1. Inhibition of Migration of Tonsillar Cells by MMIF and Interferon (mean index)

Index	MMIF	Interferon		Supernatant after cultivation of cells without antigen
		whole	diluted 1:10	
M	0,57	0,51	0,72	1,30
$\pm m$	0,08	0,12	0,08	0,14
n	9	9	6	9
P	<0,001	<0,001	<0,001	—

TABLE 2. Comparison of Some Properties of Interferon and MMIF Obtained from Tonsillar Cells

Agent tested	Properties			
	thermolability (heating to 56°C for 30 min)	sensitivity to trypsin	antiviral activity	time of maximal production
Interferon	—	+	+	18-24 h
MMIF	+	+	—	Over 24 h

Interferon was obtained from tonsillar cells by treating them with NDV. To obtain the cells, tonsillar tissue was trypsinized in 0.2% trypsin solution for 1 h. The resulting suspension was subsequently washed off with medium No. 199, after which the viability of the cells, as shown by the Trypan Blue test, was 90-95%. A cell suspension containing $5 \cdot 10^6$ cells/ml was used. As inducer of interferon formation, NDV was used in a dose of 1-10 TCD_{50} per cell. Interferon was obtained 24 h after treatment of tonsillar cells with the virus at 37°C. Interferon activity was determined by titration in a primary culture of human embryonic fibroblasts against 100 TCD_{50} of vesicular stomatitis virus. As a rule, the titers of the interferon used were 1:32-1:64. Supernatants obtained by culturing tonsillar cells without NDV or streptolysin O were used as the control.

The antiviral activity of both MMIF and interferon was determined with respect to suppression of reproduction of vesicular stomatitis virus, adenovirus, and parainfluenza virus. The activity of these mediators in inhibiting cell migration was determined in the capillary test of inhibition of migration of tonsillar cells (the suspension contained migrating cells) by a modified method [2, 3, 5]. The preparations were introduced into 0.5-ml chambers in the following doses: MMIF, 0.05 ml of a 1% aqueous solution of the lyophilized preparation; interferon, 0.05 ml of the whole preparation or the same volume of the preparation diluted 1:10. Supernatant obtained by culturing tonsillar cells without inducers was used in the control. The index of inhibition of migration was determined planimetrically by projecting the zones of cell migration on photographic film; the pieces of film corresponding to the migration zones were then cut out and weighed. The ratio between the weight of the experimental sample and that of the control was taken as the index of inhibition of migration. Part of the supernatant was treated with trypsin at 37°C for 1 h in the proportion of 100 μg trypsin, in a volume of 0.1 ml to 1 ml of supernatant. The trypsin was then inactivated by the addition of 100 μg (0.1 ml) of soy trypsin inhibitor to 1 ml supernatant. Some of the supernatants were heated to 56°C for 30 min before testing.

EXPERIMENTAL RESULTS

It will be clear from the results in Table 1 that the substances used inhibited migration of tonsillar cells from capillary tubes actively compared with the control; interferon diluted 1:10 was least active. It could be postulated either that NDV induces the production of MMIF by tonsillar lymphocytes besides producing interferon or that the inhibitory action was due to interferon. Some results reflecting the properties of interferon and MMIF obtained from supernatants of human tonsillar cell cultures are given in Table 2. The interferon possessed some degree of thermolability; it was completely inactivated by trypsin; and it inhibited reproduction of vesicular stomatitis virus, adenovirus, and parainfluenza virus. Under the influence of NDV, the tonsillar cells did not begin to form interferon until 6 h after addition of the virus.

MMIF obtained by treatment of tonsillar lymphocytes with streptolysin O had no antiviral activity, was thermostable at 56°C for 30 min, was sensitive also to the action of trypsin; and its production reached a maximum a little later than that of interferon.

The results as regards the inhibitory effect of MMIF are in agreement with those of other workers [10] who observed active production of this mediator by human tonsillar and splenic lymphocytes in the presence of streptococcal antigens. The inhibitory activity of interferon may be connected with the fact that interferon, as a high-molecular-weight compound, is heterogeneous; and part of it is considered [6, 8] to consist of a protein with the functional characteristics of MMIF.

The results of the present investigation are evidence of the important role of the inducer in the formation of mediators of HDT. On stimulation of interferon formation not only antiviral factor, but also other mediators and, in particular, MMIF, are formed in the tonsillar cells under the influence of the inducer virus. Stimulation of the tonsillar lymphocytes by streptolysin O leading to MMIF formation does not cause the production of antiviral factor, i.e., the inducer virus is a stimulator with a broader spectrum of action, and this must be taken into account when the ability of lymphocytes to produce mediators of HDT is studied.

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EFFECT OF STIMULATION AND INHIBITION OF ERYTHROPOIESIS ON ANTIBODY PRODUCTION AND MIGRATION OF B CELLS FROM THE BONE MARROW AND SPLEEN IN MICE

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The effect of stimulation and inhibition of erythropoiesis on the production of antibody-forming cells (AFC) in the spleen and on the migration of B cells from the bone marrow into the spleen was studied in CBA mice. Stimulation of erythropoiesis was shown to increase the number of AFC in the spleen and migration of B cells from the bone marrow into the spleen sharply 1 and 4 days after blood loss. Inhibition of erythropoiesis led to a very small increase in the number of AFC in the spleen 4 and 7 days after transfusion of syngeneic red cells and inhibited migration of B cells from the bone marrow into the spleen. The possible mechanisms of the effect of stimulation and inhibition of erythropoiesis on antibody formation are discussed.

KEY WORDS: *antibody production; migration of B lymphocytes; blood loss; hypertransfusion.*

It is stated in the literature that stimulation of erythropoiesis is responsible for the increase in the survival rate of animals after subtotal irradiation [2] and activates processes such as the formation of endogenous splenic colonies of hematopoietic cells by polypotent stem cells [9] and the migration of hematopoietic stem cells from the bone marrow into the spleen [10]. Stimulation of erythropoiesis thus leads to considerable changes in the population of hematopoietic stem cells, which are precursors of the mature cells of

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