PRODUCTION OF A FACTOR ENHANCING THE BACTERICIDAL ACTION OF MOUSE MACROPHAGES AND ITS STIMULATION BY INTERFERON

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The authors showed previously [3, 5] that interferon (IFN) preparations have a protective action in staphylococcal infection. The mechanisms of the protective effect of IFN depend on activation of the cellular component of immunity and stimulation of phagocyte function [3, 6]. Even though small doses of IFN were used in the investigations cited, and IFN is known to be excreted from the body rapidly, and even if only a single injection was given, the protective effect of IFN lasted many days; this suggests the existence of other mechanisms inducing the action of IFN. It was therefore interesting to study the possibility of modulation by IFN of the production of splenic lymphocytes of soluble factors enhancing the functional state of phagocytic cells in immunity to infection.

EXPERIMENTAL METHODS

In experiments on CBA and BALB/c mice weighing 18-20 g, murine type I IFN and MFC-IFN, obtained by the method described previously [5] were used. The preparation was injected subcutaneously into the animals in a dose of 10^3 IU (0.2 ml). Inducers of type I IFN, namely Newcastle disease virus (NDV), in a dose of 10^9 PFU/mouse, and the synthetic polyribonucleotide polyI:polyC in a dose of 40 μg/mouse (Sigman, USA) were injected intraperitoneally. The highest titer ($1.5 \cdot 10^3$-$2 \cdot 10^3$ IU/ml) of endogenous IFN in the animals' serum was determined 6-8 h after injection of the inducers. Mouse peritoneal exudate macrophages (MPEM) were obtained by the method in [8]. Mouse splenocytes (from five or six animals) were obtained 1, 3, 4, 5, 7, and 10 days after injection of IFN and its inducers by the usual method, by teasing splenic tissue. The cells ($2 \cdot 10^6$ in 1 ml) were cultured at 37°C for 18-24 h in medium 199 with the addition of 10% fetal calf serum. Preparation of the cells into adherent and nonadherent fractions was carried out on a column packed with nylon wadding [9].

TABLE 1. Effect of Supernatants of Mouse Splenocyte Cultures on Bactericidal Activity of Macrophages according to Results of Nitro-BT Test (M ± m)

Line of Mice	Quantity of reduced formazan per 10^6 cells (in U) in samples of MPEM, treated with								
	culture medium	supernatant of splenocyte from mice receiving MFC-IFN	supernatants of splenocyte cultures from mice receiving IFN (10^3 IV/mouse)						
			1 day	2 days	3 days	4 days	5 days	7 days	10 days
CBA	23±0,84	24±0,84	58±1,68*	56±2,52*	66±2,52*	63±4,6*	37±2,52	36±2,1	34±0,84
	36±2,52	30±2,52	73±0,84*	77±1,26*	90±1,26*	84±0,84*	45±2,52	42±1,26	42±0,84
	11,5±0,84	10±1,26	20±1,68*	22±1,26*	28±1,26*	25±1,68*	—	—	—
BALB/c	38±1,68	38±1,68	52±2,1*	60±1,26*	63,3±0,8*	60±1,26*	34±1,26	32±2,1	29±2,1
	25±0,42	29±0,49	56±1,68*	59±1,26*	66±0,42*	60±0,84*	—	—	—

Legend. Here and in Table 2: *p < 0.001.

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TABLE 2. Effect of Supernatants of Splenocyte Cultures on Antimicrobial Activity of Macrophages (M ± m)

Bacteria	Antimicrobial index in test with MPEM from mice treated with						
	culture medium	supernatant of splenocyte cultures from mice receiving MFC-IFN	supernatant from mice receiving IFN				
			1 day	3 days	5 days	7 days	10 days
Salmonella typhimurium	32±8,2	40±7,5	53±8,4	82±2,7*	47±3,5	45±2,7	42±5,4
Staph. aureus	55±3,36	62±2,8	77±3,08*	94±0,84*	65±3,44	60±2,8	62±3,92

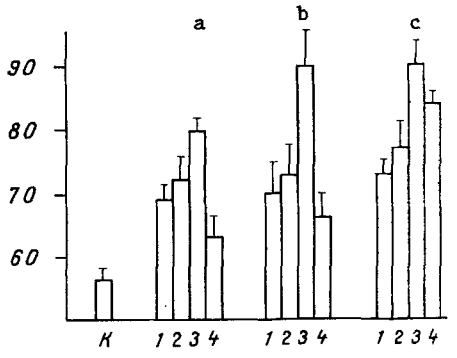

Fig. 1

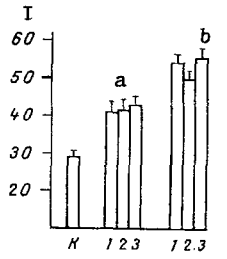

Fig. 2

Fig. 1. Changes in bactericidal activity of MPEM from CBA mice under the influence of a factor obtained after injection of IFN in doses of 10 U/mouse (a), 100 U/mouse (b), and 1000 U/mouse (c). Abscissa, time after injection of IFN into mice (8 days); ordinate, bactericidal activity (in U). K) Control.

Fig. 2. Modulation of bactericidal activity of macrophages of CBA mice by supernatant of splenocytes activated in vivo by IFN and its inducers. 1) IFN (10^3 IU/mouse); 2) polyI-polyC; 3) NDV. a) 1 Day; b) 3 days. Remainder of legend as to Fig. 1.

The resulting supernatants of lymphocyte cultures were tested for the presence of macrophage activating factor in two ways. The nitroblue tetrazolium reduction test (nitro-BT test) was performed by the method in [2]. The results were expressed in optical density units (U)/10^6 cells. Each test was duplicated in five or six flasks. Antimicrobial activity of MPEM against Staphylococcus aureus (museum strain 209) and Salmonella typhimurium (museum strain 79) was determined [1] in 96-well flat-bottomed microplates (Nunc, Denmark). For this purpose syngeneic MPEM (10^6 cells/ml) were introduced into the wells in a volume of 0.05 ml. The plates were kept for 2 h at 37°C to allow adhesion of the cells, after which the test supernatants were added in a volume of 0.05 ml, and incubation of the mixture continued at 37°C for 24 h. At the end of this time the cell monolayer was washed and fresh culture medium added. Bacteria were added to each well in a volume of 0.05 ml (ratio of macrophages to bacteria 1:1). Combined culture of macrophages and bacteria continued at 37°C for 1 h. The number of viable bacteria in the wells was determined after lysis of the macrophages with a 0.25% solution of sodium dodecyl sulfate (final concentration 0.05%). The antimicrobial activity of the macrophages was calculated by the formula given previously [1] and expressed as the antimicrobial index. Preliminary treatment of MPEM with supernatants of splenocyte cultures was carried out for 24 h at 37°C regardless of which test was used.

The results were subjected to statistical analysis by Strelkov's method [7].

EXPERIMENTAL RESULTS

MPEM treated with supernatants of splenocyte cultures from mice receiving IFN in a dose of 10^3 IU showed increased ability to reduce formazan compared with control samples, in which

the MPEM were treated with supernatants of splenocyte cultures from mice receiving MFC-IFN or culture medium (Table 1). Superntants of splenocyte cultures obtained from animals 3 days after injection of IFN were found to have the greatest activating power. Similar results also were obtained in the direct antimicrobial test against bacteria (Table 2). Incidentally, activated MPEM digested bacteria such as Staph. aureus and S. typhimurium sufficiently effectively, evidence of the nonspecific character of phagocyte activation.

Exogenous type I IFN thus induces the production by splenocytes of a factor enhancing the bactericidal activity of macrophages. A particular feature of this phenomenon is that this activity is also induced when IFN is injected in a dose as low as 10 IU. However, the maximal effect was observed when IFN was injected in a dose of 10^3 IU, when maximal production of the factor was maintained for 3-4 days (Fig. 1).

During the investigation of splenocytes, fractionated on a column with nylon wadding, as producers of this activity it was found that the leading role in the secretion of this factor is played by the population of nonadherent cells, consisting mainly of T lymphocytes. However, maximal production of the factor requires the presence of the fraction of adherent cells. Thus on the 3rd day after injection of IFN, supernatants of the splenocyte cultures increased the bactericidal activity of macrophages by 2.4 times (as shown by the results of the nitro-BT test), whereas nonadherent cells gave an increase of 2.1 times and adherent cells an increase of 1.4 times ($p < 0.001$).

A study of the physicochemical characteristics of the factor showed that exposure to a temperature of 56°C for 30 min and adjustment of the pH of the medium to 2.0 did not affect its activity. Treatment with trypsin (100 μg/ml) at 37°C for 1 h, and also heating to 80°C for 30 min led to its complete inactivation. In all probability the factor enhancing the bactericidal activity of macrophages is a polypeptide. The differences in the physicochemical properties indicate that the induced factor is neither γ-IFN nor macrophage-activating factor, which also are known to enhance the bactericidal activity of macrophages [4].

The inducing effect of exogenous IFN on production of a factor enhancing the bactericidal activity of macrophages by mouse splenocytes, described above, suggests that a similar phenomenon takes place in the body during synthesis of endogenous IFN. This is confirmed by results obtained when inducers of type I IFN were injected into mice. For instance on the 3rd day after injection of NDV or polyI:polyC into mice, supernatants of cultures of their splenocytes, just as after injection of IFN, had an activating effect on macrophages (Fig. 2).

The results are thus evidence that a mechanism inducing activation of macrophages by IFN may exist, so that not only may our knowledge of the pleiotropic effects of IFN be broadened, but immunocorrective therapy may also be undertaken more effectively.

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