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EFFECT OF NATURAL AND SYNTHETIC IMMUNOMODULATORS ON THE SYNTHESIS OF INTERFERON BY PERITONEAL CELLS OF MICE

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Summary. - The effect of different natural and synthetic immunomodulators on the spontaneous interferon (IFN) synthesis by freshly isolated resident peritoneal cells of BALB/c, NZB and C3H mice was investigated. Actinomycetal glycolipids isolated from *Curtobacterium betae, Faenia rectivirgula, Rothia dentocariosa* and *Saccharopolyspora hirsuta* at the concentration 1-20 μg/ml were found to potentiate the IFN synthesis by the peritoneal cells of BALB/c mice. Similar results were obtained when dsRNA, LPS of *Shigella sonnei* and lipid A isolated from the LPS were used. The effect of potentiation of the physiological IFN production by the immunomodulators was observed also in the cells of C3H and NZB mice. In contrast, the inhibition of the IFN synthesis was observed when the peritoneal cells of BALB/c and NZB mice were treated with imuthiol at concentration 0.1-10 μg/ml. Thymomodulin (TFX-Polfa) at concentration of 1-100 μg/ml had no effect on the spontaneous IFN production.

Key words: interferon; mice; peritoneal cells; immunomodulators

Introduction

Peritoneal, alveolar and other tissue macrophages have been recognized as the cells which release continuously alfa and beta IFNs in healthy human and animal organisms (Khan *et al.,* 1989; Bocci, 1987). These IFNs synthesized locally, together with several other cytokines, modify the properties of many different cell types and regulate their proliferation, differentiation, maturation and various functions. Some of the cells i.e., peritoneal and alveolar macrophages release small amounts of IFN when transferred to *in vitro* culture conditions (Błach-Olszewska, 1987*a*; Paulesu *et al.,* 1987).

We have found that the synthesis of physiological IFN is under the control of substances which are released by macrophages (Błach-Olszewska, 1986, 1987).

The natural regulators may influence the level of IFN secreted by the cells. If the macrophages released more inhibitor, the level of IFN synthesis may be low or even undetecable. On the other hand, in the absence of the inhibitor the titre of IFN may be high. Peritoneal cells may produce different levels of the IFN regulators and as the result, they may release different amounts of IFN e.g. between ≤ 2–512 units/ml. Such variable IFN levels have been observed in the individual mice deriving from the same colony and having the age of 6–8 weeks. Apart from this, there is a more general difference in the ability of mouse strains to produce IFN. Some of them are good producers of physiological IFN (BALB/c, NZB, DBA/2) whereas several other strains e.g. C3H, AKR, CBA are poor IFN producers (Błach-Olszewska, 1987*a*). It is of interest that the poor producers have high activity of the inhibitors of IFN production and they usually develop high percentage of spontaneous tumours.

In view of the disturbances in the regulation of physiological IFN levels the question arises if it is possible to modulate their release by immunomodulators. We have studied the effects of the substances derived from bacteria, thymus and synthetic compounds on the production of physiological IFN in mice.

Materials and Methods

Isolation of phage dsRNA. The dsRNA was obtained from *Escherichia coli* strain Q13 infected with ambermutant sus_{11} bacteriophage f2. Mutant sus_{11} contained nonpolar ambermutation in the cistron of membrane protein that leads to abundant accumulation of the replicative forms of phage. The routine phenol DNA extraction method was used. Cellular DNA was digested by DNase. Deproteinisation of the fraction resistant to RNase A was performed with chloroform in the presence of 5 % suspension of bentonite and 0.05 % of sodium dodecylsulphate (SDS). Isopropanol was used for fractionation. The process was performed at 5–10 °C and a 2 g/l concentration of nucleic acid was achieved by precipitation with 0.3 mol/l solution of sodium acetate. Sediment of high molecular dsRNA obtained after centrifugation was washed with a mixture of 0.5 volume of isopropanol and one volume 0.3 mol/l sodium acetate. The sediment was dissolved in water, 5 % suspension of bentonite was added and it was dialysed against water for 48 hr. Bentonite was removed by ultracentrifugation at 30 000 g, 90 min. The physicochemical properties of the dsRNA were described (Lozha *et al.*, 1981; Feldmane *et al.*, 1984).

Glycolipids were prepared from the following bacterial strains: *Curtobacterium betae* ATCC (American Type Culture Collection) 1347, *Faenia rectivirgula* IMRU (Waksman Institute of Microbiology Collection, Rutgers University) 1258, *Rothia dentocariosa* LL-Pba2 (Lechevalier's Collection, Waksman Institute of Microbiology), *Saccharopolyspora hirsuta* K52 (received from M. Goodfellow, The University of Newcastle, United Kingdom). The biomass of the strains was obtained as described by Mordarska (1985–1986). Wet cells were extracted with chloroform–methanol (2:1, v/v) at 30 °C for 12 hrs. The crude lipids were fractionated on a column of HI-Flosil (60–200 mesh) using successively reagent grade chloroform, aceton and methanol. The glycolipid fractions present in the acetone eluates were purified by preparative thin-layer chromatography (TCL) accomplished on plates coated with silica gel PF 254 and the solvent system: chloroform–methanol–water (65:25:4, v/v). The preliminary analysis on the chemical character of the glycolipids indicated that they were diglycosyl diglycerides (Mordarska, 1985–1986). For IFN induction 1 ml of phosphate buffered saline (PBS) was added to 1 mg of pure glycolipid fraction and than sonicated.

Isolation of lipopolysaccharide. Shigella sonnei and lipid A: *Shigella sonnei* phase I strain 9773 derived from Dysentary Reference Laboratory, London, was used. The bacteria were grown at 37 °C in a synthetic minimal medium containing casein peptone (0.5 %) and nicotinic acid (0.00001 %). The cultures were aerated by shaking. After growth for 24 hrs at 37 °C the bacteria were harvested by centrifugation, washed three times with phosphate buffered saline and finally dried in acetone. LPS was isolated from the bacterial dry mass by the fenol - water method (Westphal *et al.*, 1952) and purified by column chromatography on Sepharose 2b (Romanowska, 1970).

Lipid A was prepared by 1 % acetic acid hydrolysis of the LPS at 100 °C for 1.5 hr. The sediment of lipid A was washed three times with water and then dried. Complex lipid A and bovine serum albumin (BSA) was prepared according to Galanos *et al.* (1971).

Sodium diethyldithiocarbamate (DETC, imuthiol) was provided by Merieux. It was dissolved in PBS.

Thymomodulin (TFX) was a product of Thymoorgan-GmbH Pharmazie Co.KG D-3387 Vienenburg, Germany, licenced by Polfa-Jelenia Góra, Poland.

L_{929} *mouse fibroblasts* were used for EMC virus multiplication and IFN titration. They were cultured in Eagle's medium (MEM) supplemented with 10 % of heat inactivated calf serum (C.S.) and antibiotics: penicillin and streptomycin.

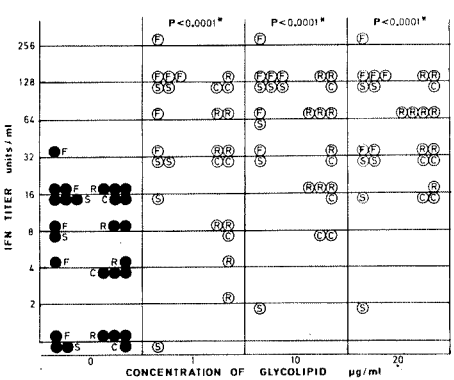
Female BALB/c/Iiw, NZB/Iiw, C3H/Iiw, inbred mice, 8 weeks old were obtained from Animal Breeding Center of this Institute.

Encephalomyocarditis virus (EMCV) was propagated in L_{929} cells and stored at 4 °C. It was used as challenge virus in IFN titration.

IFN production and assay. Mouse resident peritoneal cells were washed off from the peritoneal cavity of mice with 5 ml of MEM supplemented with 10 % heat inactivated calf serum. Cell suspension ($2x10^6$cells/ml) was distributed in volumes of 0.5–1.0 ml into several glass tubes with rubber stoppers and incubated at 26 °C for 24 hrs. The cultures were centrifuged and the supernatants were assayed for IFN activity by inhibition of cytopathic affect caused by EMCV in L_{929} cells cultured in 96-well Falcon microplates. Mouse IFN-β from National Institute of Health, Bethesda, was used as reference standard (G002-904-511) included in all titrations.

Fig. 1

Influence of actinomycetal glycolipids on the physiological production of interferon by peritoneal cells of BALB/c mice Suspension of peritoneal cells isolated from individual mice (2×10^6 cells/ml) was distributed to several tubes and treated with glycolipids of *Faenia rectivirgula* (Ⓕ), *Saccharopolyspora hirsuta* (Ⓢ), *Rothia dentocariosa* (Ⓡ) and *Curtobacterium betae* (Ⓒ). Each experiment included nontreated control cells (●F, ●R, ●S, ●C). After 24 hrs of incubation at 26 °C the media were assayed for IFN activity. * according to median test



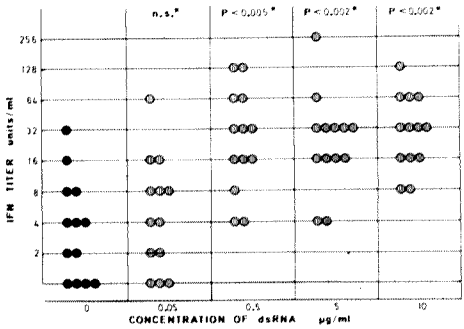


Fig. 2
Effect of dsRNA on the synthesis of physiological interferon by peritoneal cells isolated from BALB/c mice
○ - cells treated with dsRNA, ● - control cells, *according to median test. For other details see Fig. 1.

Results

We followed the effects of natural and synthetic immunomodulators on physiological IFN production by freshly isolated, resident peritoneal macrophages in BALB/c mice. We found that BALB/c mice are the most stable and best producers of this type of IFN of all mouse strains so far tested (Błach-Olszewska, 1987*a*). We have also determined the optimal conditions for the physiological IFN production which may not be the same as for IFN induction (Błach-Olszewska, 1979). In all present experiments we used unseparated fresh peritoneal cells suspended in culture medium at a concentration of 2×10^6 cells/ml. The immunomodulators were added soon after preparation of cells and they were cultured at 26 °C for 24 hrs.

Fig. 1 shows the results of action of 4 different actinomycetal glycolipids on IFN production. The preparations were found to enhance the physiological

Table 1. Influence of immunomodulators of bacterial and viral origin on physiological interferon production by peritoneal cells of BALB/c mice

Immunomodulator	Effective concentrations* µg/ml	Potentiation effect**
Actinomycetal glycolipids	1 - 20	4 - 32 times
LPS *Shigella sonnei*	1 - 10	2 - 16 times
Lipid A	1 - 10	2 - 8 times
dsRNA	0.5 - 10	4 - 32 times

*The concentrations of immunomodulators which cause potentiation of the spontaneous IFN production; **The titres of IFN in cells treated with immunomodulators were compared with IFN levels in control nontreated cells.

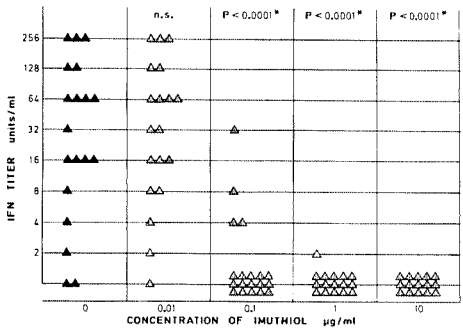


Fig. 3
Effect of imuthiol on physiological IFN production by peritoneal cells of BALB/c mice
Δ - cells treated with imuthiol, ▲ - control nontreated cells. For other details see Fig. 1.

IFN production by mouse peritoneal cells. Enhancement of the IFN synthesis by peritoneal cells was also observed when dsRNA at concentration 0.5–10 µg/ml was added (Fig. 2). The same results were obtained when either LPS or lipid A from *Shigella sonnei* were given to the cells (data not shown). All results are summarized in Table 1. The effective concentrations of immunomodulators which potentiated IFN production by 2–32 fold were 1–20 µg/ml; there was no strict dose dependence within this limits. It is worth pointing out that the activities of the different preparations were comparable. In contrast, thymomodulin (TFX) had no significant effect on the physiological IFN production when added to the peritoneal cells at concentrations of 1–100 µg/ml (Table 2). We have shown that titres of IFN in the cells obtained from individual mice treated with TFX varied in each experiment and in each mice and there was no dose-dependence within the range of 1–100 µg/ml.

Following the effect of imuthiol we found unexpectedly that it was very toxic

Table 2. Production of physiological interferon by peritoneal cells of BALB/c mice treated with thymomodulin (TFX)

TFX concentration µg/ml	Interferon level units/ml									
	Exp.: 1	2	3	4	5	6	7	8	9	10
0	32	256	64	32	16	8	16	256	16	64
1	128	256	64	16	32	4	32	128	16	64
10	128	128	128	16	32	4	32	128	16	64
20	128	128	64	16	32	4	16	32	16	64
50	128	128	64	16	64	4	32	32	32	32
100	32	8	64	8	32	8	16	32	32	32

For details see Fig. 1

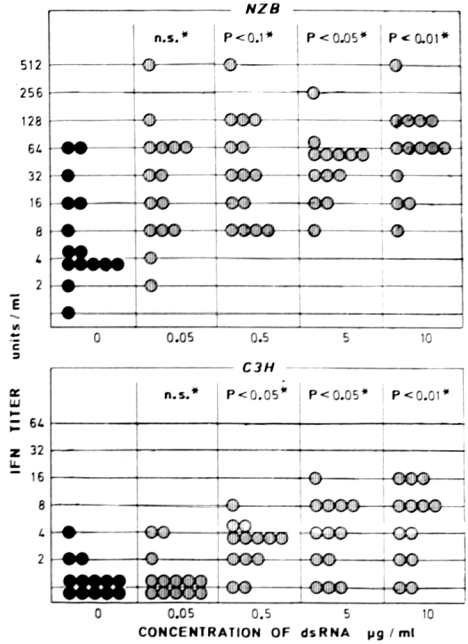


Fig. 4
Potentiation of the physiological IFN production by dsRNA in peritoneal cells of NZB and C3H mice
○ - cells treated with dsRNA, ● - control nontreated cells. For other details see Fig. 1.

for L_{929} cells. The minimal non-toxic concentration of imuthiol for L_{929} cells was approximately 0.1 μg/ml. However, imuthiol at concentration of 1-100 μg/ml was not toxic for peritoneal cells of BALB/c or NZB mice. Because of its high toxicity for L_{929} cells which were used for IFN titration, all materials had to be dialysed 3-4 times against PBS at constant stirring. Imuthiol inhibited the physiological IFN production by the peritoneal cells of BALB/c mice at concentrations of $\geqq 0.1\mu$g/ml (Fig. 3).

Because strains of mice significantly differ in production of the physiological IFN we compared the effects of dsRNA and imuthiol in NZB and C3H mice.

Fig. 5
Inhibitory effect of imuthiol on interferon production by peritoneal cells of NZB mice
△ - cells treated with imuthiol, ▲ - control cells. For other details see Fig. 1.

We have found that dsRNA potentiate the IFN production in both NZB and C3H mouse peritoneal cells (Fig. 4). As in the case of BALB/c mice, imuthiol inhibited the IFN synthesis in NZB mice, but at a higher concentration of ≥ 1 μ g/ml (Fig. 5).

Discussion

Our consistent observation is that different immunomodulators: bacterial glycolipids, phage dsRNA, LPS or lipid A potentiated spontaneous IFN production in peritoneal cells irrespective of mouse strain. In contrast, the low molecular immunomodulator - imuthiol inhibited IFN synthesis whereas thymomodulin had no effect. In addition, dsRNA or imuthiol may also regulate IFN synthesis in C3H and NZB mice which apparently have defects in their IFN regulation system (Błach-Olszewska, 1987*a*).

According to Skurkovich *et al.* (1987) IFN may intensify autoimmune reactions. During the development of disease an acid-labile-IFN-like substance is produced (Borecký *et al.*, 1990). We also have observed high spontaneous production of IFN by peritoneal cells in very young NZB mice which by aging developed haemolytic anemia and other symptoms of autoimmune disease. Inhibition of the IFN production imuthiol may slow down the progress of disease.

According to our results thymomodulin did not influence physiological IFN production. It can be explained in terms of thymomodulin action on T cells but not on macrophages, which are responsible for α/β-IFN production (Skotnicki, 1989). We do not know whether this regulation of IFN synthesis by immunomodulators *in vitro* is equally efficient *in vivo*. However, one may suggest that control of the physiological IFN synthesis by a proper immunomodulator is possible and may be therapeutically useful.

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