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Short Communication

Interferon inducibility in mice treated with *Corynebacterium parvum*

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Summary

Mice were given single intraperitoneal (i.p.) injections of *Corynebacterium parvum*, followed, after different time intervals, by i.p. injections of the interferon inducers polyinosinic–polycytidylic acid (poly-I:poly-C), 10-carboxymethyl-9-acridanone (CMA) or herpes simplex virus. With all three inducers production of interferon in the peritoneal cavity was enhanced in *C. parvum*-pretreated mice. Production of circulating interferon in *C. parvum*-pretreated mice was enhanced with CMA and depressed with poly-I:poly-C as inducers. This modulation of the interferon response was prominent for at least 10 weeks after *C. parvum* injection and then gradually reverted. The increased local interferon production seemed to be caused by macrophages still activated several weeks after treatment with *C. parvum*.

interferon; *Corynebacterium parvum*

Introduction

Corynebacterium parvum is one of the most potent immunomodulating agents in mice [15]. Some humoral and cellular immune reactivities are stimulated while others are suppressed [7,15]. Activation of the lymphoreticular system reaches its maximum

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in the second week and returns to control levels around 24 days after treatment [7]. Injection of *C. parvum* also results in altered activities of nonspecific defence mechanisms, e.g. of natural killer cells [16] or macrophages [7]. Macrophages exhibit increased phagocytic activity with peak levels in the second week after treatment. *C. anaerobium*, a bacterium closely related to *C. parvum*, has been described to induce interferon (IFN) in vivo [23]. Human peripheral leucocytes [9,21] and murine spleen cells [10] produce IFN after in vitro stimulation with *C. parvum*. It has further been shown that the IFN response to viral inducers in the serum is suppressed within the first 2 weeks after treatment with *C. acnes* [5]. Here we demonstrate a long-lasting modulation of the IFN response to inducers of IFN-α,β in *C. parvum*-pretreated mice, certain types of interferon responses being depressed, whereas others are increased.

Experimental

Male DBA/2J mice were obtained from Gl. Bomholtgard Ltd. (8680 Ry, Denmark). At the beginning of the experiments the animals were 5–6 weeks old. They were injected intraperitoneally (i.p.) with 700 μg *C. parvum* (CN 6134, Burroughs Wellcome, Beckenham, U.K.) or saline. At different intervals (from 1 week to 5 months) IFN inducers were injected i.p. using the following doses per mouse: 100 μg of poly-I:poly-C (Serva, Heidelberg, F.R.G.), 10 mg of 10-carboxymethyl-9-acridanone (CMA, a kind gift of Dr. M.J. Kramer, Hoffman-LaRoche, Nutley, NJ) [13] or 5×10^6 plaque-forming units (PFU) of herpes simplex virus (HSV) type 1, prepared as described [25]. Controls were injected with saline. In each experiment at least three *C. parvum*-pretreated and three control mice were tested individually. Mice were bled by cardiac puncture. The peritoneal fluid was collected by washing the peritoneal cavity with 0.5 ml medium (RPMI 1640, supplemented with 2.5% fetal calf serum and antibiotics). Cell free samples were stored at −70°C until tested for their IFN activities.

Peritoneal exudate cells (PEC) were collected by washing the peritoneal cavity with Hanks' balanced salt solution. PEC of individual mice were adjusted to a concentration of 1×10^6/ml in medium and cultured for 18 h in the presence of 25 μg/ml poly-I:poly-C. Controls consisted of unstimulated cultures. Cell-free supernatants were tested for IFN content. Staining of PEC for nonspecific esterase was performed according to the technique of Koski et al. [12]. PEC (1×10^6/ml) were cultured for 18 h and the content of prostaglandin E (PGE) in the supernatants was determined as previously described [6].

To determine IFN titers, test samples were serially diluted by threefold steps in medium and put into the wells of microtiter plates (3040F, Falcon, Oxnard, CA) containing monolayers of L929 cells. A laboratory standard of IFN-α,β prepared by comparison with the NIH standard G-002-904511 was included in each assay. The cells were washed after 18–20 h of incubation and infected with an appropriate dilution of vesicular stomatitis virus. The medium was discarded 48 h later, the cells were fixed with 4% formaldehyde and stained with 0.5% crystal violet. The IFN activities were determined by estimation of the cytopathic effect and are given in international units (IU) per ml. This IFN test system was fully sensitive to the action of

IFN-γ [14]. The antiviral activities in the serum and in the peritoneal fluid were characterized as previously described [19] and fulfilled the criteria of murine IFN-α,β.

In the first set of experiments, DBA/2 mice were given i.p. injections of different inducers of IFN-α,β 4 weeks after intraperitoneal administration of *C. parvum*. Table 1 shows that CMA induced about 5 times more IFN both in the peritoneal fluid and in the serum of *C. parvum*-treated mice. With poly-I:poly-C considerably increased IFN activities were seen in the peritoneal fluid whereas in the serum IFN levels were markedly depressed. DBA/2 mice are 'low responders' to HSV [25], and it was interesting to note considerable production of IFN in the peritoneal fluid when the mice were injected previously with *C. parvum*.

Experiments were done to determine duration after inoculation with *C. parvum* of local enhancement and systemic suppression of the IFN response to poly-I:poly-C. Groups of three mice were given *C. parvum* i.p. and challenged with poly-I:poly-C i.p. at different time intervals from 1 to 18 weeks. Appropriate controls were included. Fig. 1 shows results summarized from four experiments. The enhancing effect of *C. parvum* was most pronounced within the first 10 weeks of pretreatment ($P < 10^{-5}$) and then gradually declined.

Table 2 documents the characteristics of PEC and of supernatants of cultured PEC 4 weeks after i.p. injection of *C. parvum* or saline. After in vitro stimulation with poly-I:poly-C (25 μg/ml) PEC from *C. parvum*-pretreated mice produced distinctly higher amounts of IFN than PEC of control mice, suggesting that the enhanced local IFN response resided in the local cell population. The number of PEC recoverable from *C. parvum*-treated mice was slightly increased over that of control mice, but this increase was insufficient to account for the greatly enhanced local IFN response. The total number of cells staining for nonspecific esterase was within control range, while the percentage of highly staining cells was increased about 3-fold in *C. parvum*-pretreated mice. Supernatants of PEC of *C. parvum*-pretreated mice cultured for 18 h showed increased production of PGE.

Immunomodulating bacteria such as *C. anaerobium* [23] or *Bordetella pertussis* [2] induce IFN. They also cause an altered reactivity to inducers of IFN-α,β or IFN-γ. Serum activities of IFN-α,β upon injection of viral inducers are suppressed, while those upon injection of endotoxin are enhanced [5,24]. Recently it has been described that IFN-γ production by spleen cells from *Listeria monocytogenes*-immunized mice is enhanced upon stimulation with T cell mitogens [8] and that high levels of circulating IFN-γ were found after injection of T cell mitogens in mice pretreated with *Propionibacterium acnes* [17]. These altered patterns of IFN production have been observed when the inducers are injected within the period of maximal stimulation of the lymphoreticular system by immunomodulating agents.

Our results on the induction of IFN-α,β in the circulation after prestimulation with *C. parvum* confirm these observations, although, depending on the inducer, enhancement or suppression of serum IFN levels was observed. Three further phenomena, however, are evident from our study: (1) the local IFN response was enhanced upon stimulation with three different types of inducers of IFN-α,β; (2) upon injection with poly-I:poly-C a dissociation between a suppressed systemic and an enhanced local IFN production was observed; (3) the *C. parvum*-pretreated host retained ability to modulate IFN production for a prolonged period.

TABLE 1

Modulation of IFN activities in the peritoneal fluid and in the serum of *C. parvum*-pretreated mice

Inducer[a]	Dose/mouse	Interferon activity (IU/ml)			
		Peritoneal fluid[b]		Serum[b]	
		C. parvum-pretreated	Control	*C. parvum*-pretreated	Control
Poly-I:poly-C	100 μg	6833[c] ± 236	528 ± 90	112 ± 49	3083 ± 118
CMA	10 mg	41000 ± 4546	11000 ± 1855	21566 ± 1742	3733 ± 410
HSV	5×10^6 PFU	363 ± 155	14 ± 6	19 ± 5	8 ± 5

[a] Inducers were injected i.p. 28 days after pretreatment with *C. parvum* (700 μg/mouse i.p.) or saline.
[b] Peritoneal fluids and sera were recovered 5 h after injection of the inducers.
[c] Each value represents the mean ± S.D. of three individually tested mice.

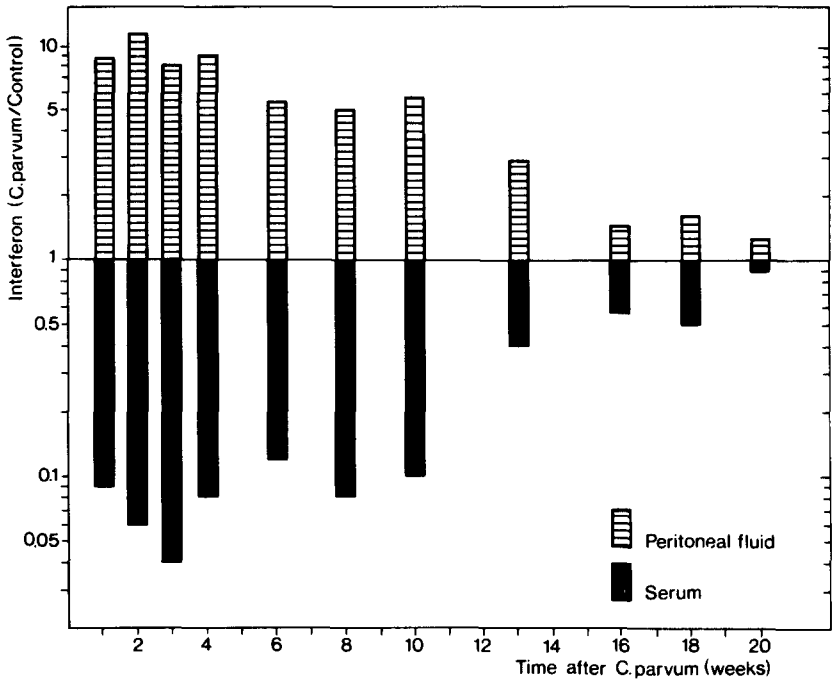

Fig. 1. Ratio of poly-I:poly-C-induced IFN activities measured in the peritoneal fluids or in the sera of *C. parvum*-pretreated and control mice. Results are shown in relation to the time after treatment with *C. parvum* (700 μg/mouse i.p.). Peritoneal fluids and sera were recovered 5 h after i.p. injection of 100 μg poly-I:poly-C. Each value represents the median value of the ratios of 3 to 4 experiments, each experimental group consisting of at least 3 individually tested mice. The modulation of the poly-I:poly-C-induced IFN induction within the first 10 weeks of pretreatment was found to be highly significant (Wilcoxon signed rank test followed by the Hodges–Lehmann estimate [7]: $P < 10^{-5}$. For details of the statistical analysis see ref. 4).

TABLE 2

Characteristics of PEC and of supernatants of cultured PEC 4 weeks after i.p. injection of *C. parvum* or saline

Characteristics	*C. parvum*	Saline
Total number of PEC/mouse[a]	1.9 ± 0.1	1.6 ± 0.1
% macrophages[b]	57 ± 9	48 ± 7
% highly esterase-positive macrophages[b]	32 ± 5	9 ± 5
IFN (IU/ml) after poly-I:poly-C[c]	128 ± 14	7 ± 4
PGE (ng/ml)[d]	8.9 ± 0.3	1.5 ± 0.1

[a] Mean ± S.D. of three experiments each consisting of three mice.

[b] Macrophages were characterized by stains for nonspecific esterase. Two hundred cells per mouse were counted. Given are the mean ± S.D. of four mice.

[c] PEC (1×10^6/ml) were cultured for 18 h in the presence of poly-I:poly-C (25 μg/ml) and the supernatants tested for IFN activities (IU/ml). Mean ± S.D. of three individual mice. Supernatants of unstimulated PEC did not contain antiviral activity.

[d] Levels of PGE (ng/ml) were determined in the supernatants of PEC (1×10^6/ml) after 18 h of culture. Mean ± S.D. of triplicate determinations of two mice.

In regard to observations (1) and (2) we may conclude that measuring serum IFN levels does not reveal the complete pattern of the host's capacity to produce IFN. We have shown that the increased local levels of IFN induced by poly-I:poly-C in *C. parvum*-pretreated mice correlated with increased protection against infection with HSV [20] suggesting that increased local IFN activities may be of greater importance than circulating levels for defence mechanisms at the local site. Furthermore, in *C. parvum*-pretreated mice, lower and therefore less toxic doses of inducers are sufficient to elicit the same IFN activity as in control animals.

The in vivo enhancement of local IFN levels after *C. parvum*-pretreatment was correlated with enhanced in vitro responses of PEC to the inducers indicating that the enhancement phenomenon resided in the local cell population. Previously it has been shown that macrophages are the producer cells of IFN after stimulation with poly-I:poly-C [3]. Our results show that 4 weeks after *C. parvum* the number of activated macrophages was still increased, as documented by the increased number of macrophages exhibiting elevated levels of nonspecific esterase and increased synthesis of PGE. Prolonged activation of macrophages after treatment with *C. parvum* has also been documented by means of the increased cytostatic activity of PEC against tumor cells [1].

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