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Anti-inflammatory activity of IFN-β in carrageenan-induced pleurisy in the mouse

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SUMMARY

The effect of IFN-β on the development of the inflammatory reaction was studied in an experimental animal model, carrageenan-induced pleurisy in the mouse. Intrapleural inoculation of IFN-β at the same time as carrageenan administration inhibited both migration of inflammatory cells and exudate formation in the pleural cavity in a dose-dependent fashion. Similarly, IFN-β decreased the presence of the arachidonate metabolites PGI_2, TXA_2 and PGE_2 (highly active molecules involved in the regulation of the inflammatory reaction) in inflammatory exudates. A marked inhibition of the inflammatory response to carrageenan was also evident when IFN-β was administered several hours after the inflammatory challenge. In contrast, administration of IFN-γ did not modify significantly any of the inflammatory parameters considered.

Keywords interferon inflammation eicosanoids

INTRODUCTION

Interferons (IFN), a group of antiviral proteins produced by several cell types in response to viral challenge or immune stimulation, have been shown to regulate many biological responses. IFN-α and IFN-β and, more efficiently, IFN-γ are in fact potent activators of macrophages (Mϕ) *in vitro*, able to increase their phagocytic and cytotoxic capacities and to modulate their secretory and phenotypic features. Furthermore, IFN appear capable of regulating the production of inflammatory molecules by Mϕ. In particular, IFN-γ could increase the release from Mϕ of interleukin 1 (IL-1; a molecule deeply involved in the onset and development of inflammatory reactions) (Boraschi, Censini & Tagliabue, 1984b); it increased the production of reactive oxygen species (Murray, Spitalny & Nathan, 1985), and either increased or depressed (according to the experimental conditions) the synthesis of arachidonic acid (AA)-derived molecules such as prostaglandin E_2 (PGE_2), prostacyclin (PGI_2) and thromboxane A_2 (TXA_2) (Boraschi *et al.*, 1985). Conversely, IFN-α and IFN-β had a weaker effect, if at all, on IL-1 production by Mϕ (Boraschi *et al.*, 1984b), and generally depressed the production of oxygen metabolites and eicosanoids (Boraschi & Tagliabue, 1984).

On the basis of these observations on the inhibition of inflammatory mediator release by IFN-α and IFN-β, together with earlier reports on the anti-inflammatory activity of IFN inducers *in vivo* (Kapusta & Mendelson, 1967; Koltai & Mecs, 1973; Koltai, Mecs & Kasa, 1981), the present study was designed to assess the possible anti-inflammatory activity of IFN-β *in vivo*, as compared to IFN-γ, in a murine model of inflammation, the carrageenan-induced pleurisy, that is characterized

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by strong Mϕ infiltration and eicosanoid release (Ackerman *et al.*, 1980; Di Rosa, Giroud & Willoughby, 1971).

The results presented here indicate that IFN-β was able to inhibit the development of the inflammatory response to carrageenan in the pleural cavity when administered locally together with, or after, the inflammatory challenge. On the other hand, in agreement with the observations *in vitro*, IFN-γ did not demonstrate any inhibitory activity on the carrageenan-induced inflammation.

MATERIALS AND METHODS

Mice. C3H/HeNCrlBR mice, obtained from Charles River Breeding Laboratories (Calco, Italy), were bred in our animal facilities and housed in air-conditioned rooms on a 12 h light-dark schedule (illumination: 0700 h to 1900 h). Food and water were available *ad libitum*. Male mice between 16 and 24 weeks of age were used in all experiments.

Induction of carrageenan pleurisy. Carrageenan (type V, Sigma Chemical Co., St Louis, MO) was suspended at 1% in pyrogen-free saline, alone or with different concentrations of IFN. Groups of five mice were lightly anesthetized with ether, the chest skin was opened and retracted and 0·2 ml of the carrageenan suspension was injected into the pleural cavity. The skin was then sutured. In another series of experiments, mice received carrageenan in saline at time 0; IFN-β was then injected intrapleurally in 0·2 ml saline at different time intervals (2, 6, 18 or 45 h). Control mice received the same volume of saline alone at the same time intervals.

Collection and processing of pleural exudates. The development of pleural inflammation was usually assessed 4, 24, 48 and 72 h after the injection of carrageenan. Mice were killed in an ether-saturated chamber. The chest was opened, the pleural exudate was removed by aspiration, and the cavity was washed with 0·2 ml of pyrogen-free saline containing 20 units/ml of heparin (Liquemin, Roche, Milan, Italy). The exudate and washing fluids were pooled and centrifuged at 500 ***g*** for 10 min at 4°C; the supernatant was decanted and its weight was taken as a measure of fluid exudate volume. The volumes reported were corrected by subtracting the washing volume of 0·2 ml from the total volume observed. Supernatants were routinely stored at −80°C until the eicosanoid determinations were performed.

The cell pellets were suspended in 0·5 ml of pyrogen-free saline containing 20 units/ml of heparin, and total cell counts were performed with a haemocytometer. Identification of exudate cell populations was performed by morphological examination of smears prepared by cytocentrifugation (Cytospin centrifuge, Shandon Southern, Camberley, UK) and stained with Diff-Quik (Harleco, Gibbstown, NJ).

Interferons. Purified mouse fibroblast IFN-β (lot 83001, $5{\cdot}6 \times 10^7$ IU/mg protein) and mock IFN-α/β (lot 81021, <32 IU/mg protein) were obtained from Lee BioMolecular, San Diego, CA. Recombinant murine IFN-γ (r-IFN-γ), produced by *E. coli* after introduction of the mouse IFN-γ gene under trp promoter control (Gray & Goeddel, 1983) and highly purified (>90% pure, $1{\cdot}3 \times 10^7$ IU/mg protein, lot 3209-14), was obtained from Boehringer-Ingelheim through the courtesy of Dr G. R. Adolf and Dr P. Swetly.

IFN were aliquoted in pyrogen-free saline and stored at −80°C for up to six months without appreciable loss of activity, as determined by their protective effect on L929 cells challenged with vescicular stomatitis virus. IFN preparations (2×10^4 IU/ml) were found free of endotoxin by the Limulus Amebocyte Lysate assay (M.A. Bioproducts, Walkersville, MD) with a sensitivity of ≤0·02 EU/ml.

Radioimmunoassay for eicosanoid determination. The presence of AA metabolites in pleural exudates was determined by radio-immunoassay (RIA) with commercially available kits (Du Pont-NEN, Boston, MA). Cross-reactivities of the antiserum to 6-keto-$PGF_{1\alpha}$ (the stable metabolite of PGI_2) with other PGs were: $PGF_{2\alpha}$, 2·6%; PGE_1, 1·9%; TXB_2, 1·4%; PGE_2, 1·1%; $PGF_{1\alpha}$, 0·8%; PGA_1 and PGD_2, 0·2%; PGA_2, 0·04%; AA and other AA metabolites, <0·005%. Cross reactivities for the antiserum anti-TXB_2 (the stable metabolite of TXA_2) were: PGD_2, 3·9%; PGE_2, 0·23%; $PGF_{2\alpha}$, 0·07%; 6-keto-$PGF_{1\alpha}$ and PGE_1, 0·06%; $PGF_{1\alpha}$, 0·02%; PGA_2, 0·007%; AA and other AA

metabolites, <0·005%. Cross-reactivities of the anti-PGE_2 antiserum were: PGE_1, 3·7%; PGA_2 and DHK-PGE_2, 0·4%; $PGF_{1\alpha}$, 0·03%; TXB_2, 0·02%;AA and other AA metabolites, <0·01%.

Statistical analysis. All the parameters measured, i.e. cell numbers, exudate volume and eicosanoid concentrations, are expressed as total amounts per mouse and reported as mean ± s.e.m. of values obtained from separate mice within single experiments, representative of two to nine experiments performed. Statistical significance was assessed by Student's *t*-test for paired and unpaired samples.

RESULTS

Characterization of carrageenan pleurisy in the mouse. Pleural inflammation was induced by local inoculation of 0·2 ml of a 1% suspension of carrageenan. This concentration was selected from dose-response experiments (carrageenan concentrations ranging from 0·01% to 5%) as the minimal dose causing maximal inflammatory response (not shown).

Data in Fig. 1 describe the features of carrageenan-induced pleurisy in the mouse. The inoculation of carrageenan induced an abundant accumulation of exudate fluid, which was already evident after 4 h, peaked at 24–48 h and declined sharply thereafter. The influx of inflammatory cells was characterized by the rapid appearance of polymorphonuclear leukocytes (PMN; 75% of the exudate cells at 4 h), which then rapidly declined in parallel with the appearance of Mϕ. In fact, Mϕ represented the major cell population present in the inflammatory exudate from 24 h after carrageenan inoculation. Parallel with the exudate fluid, the number of exudate cells reached a maximum 48 h after induction of inflammation. All inflammatory parameters were significantly decreased after 72 h. By prolonging the observation period up to 10 days after the inoculation of carrageenan, the inflammatory parameters were found to decline very slowly.

Intrapleural inoculation of saline alone induced a very mild inflammation characterized by an almost undetectable pleural effusion (0·03 ml/mouse, which never increased with time after injection) and by a weak cellular influx (from $1·6 \times 10^6$ cells in the unstimulated pleural cavity to $1·9 \times 10^6$ 4 h after saline inoculation, $2·0 \times 10^6$ after 24 h and $2·8 \times 10^6$ after 48 h).

While the proportions of the various cell types present in the carrageenan-induced exudate were constant from one experiment to another, it was found that absolute values of both maximal cell infiltration and exudate volume (i.e. the extent of inflammatory response to carrageenan) were

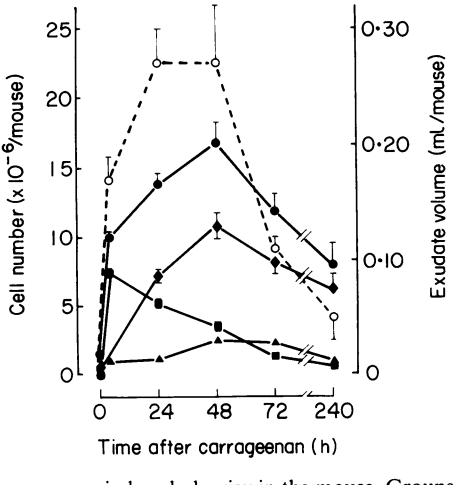

Fig. 1. Characterization of carrageenan-induced pleurisy in the mouse. Groups of five mice were sacrificed at different times after the intrapleural inoculation of 0·2 ml of 1% carrageenan in saline. The inflammatory parameters assessed were: volume of the pleural exudate (○); total number of exudate cells (●); number of Mϕ(♦); of PMN (■); and of lymphocytes (▲).

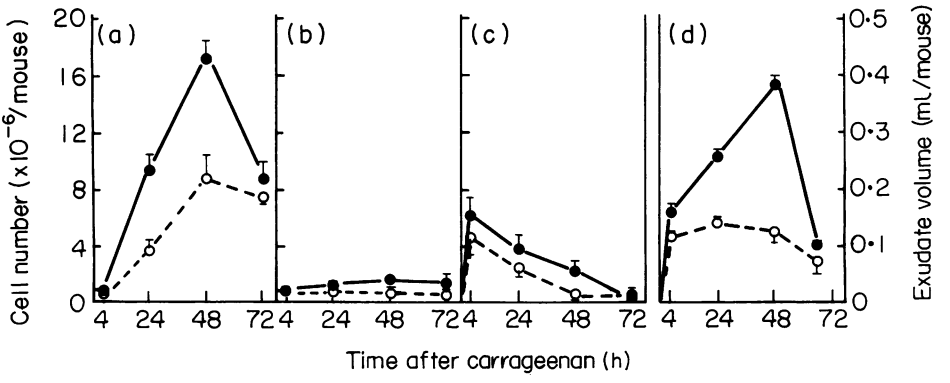

Fig. 2. Effect of IFN-β on the development of carrageenan-induced pleurisy. Mice received an intrapleural inoculation of 0·2 ml of 1% carrageenan in saline alone (●) or containing 2×10^3 IU of IFN-β (○). The inflammatory parameters were assessed after 4, 24, 48 and 72 h. (a) macrophages; (b) lymphocytes; (c) polymorphonuclear leukocytes; (d) pleural exudate.

subject to some variability. This may be due to the well known seasonal variations in the inflammatory response to carrageenan (Labreque, Bélanger & Doré, 1982), as the experiments reported here were not all performed in the same period of the year.

Effect of IFN-β on carrageenan-induced pleurisy. In order to study the effect of IFN-β on the development of carrageenan-induced pleural inflammation, carrageenan was inoculated either alone or mixed with mouse IFN-β (or with a corresponding dilution of mock IFN-α/β). At different times (4–72 h) after intrapleural injection, mice in the control and IFN-β-treated groups were killed and the inflammatory exudates were evaluated.

As shown in Fig. 2, IFN-β inhibited inflammation, as assessed on both cell migration and exudate formation. The inhibitory effect of IFN-β, which after 4 h was evident only on fluid exudate formation, became clearly apparent on all inflammatory parameters after 24 h, reaching its maximum after 48 h and declining thereafter. The migrating cell type most affected by treatment with IFN-β appeared to be the Mφ, while PMN migration was inhibited to a lesser extent.

IFN-β was also found to inhibit the amount of eicosanoids (i.e. 6-keto-$PGF_{1\alpha}$, TXB_2 and PGE_2) present in the exudates, as reported in Table 1. The inhibition of 6-keto-$PGF_{1\alpha}$, the major

Table 1. Effect of IFN-β on eicosanoids present in carrageenan-induced pleural exudates

Time after carrageenan (h)	IFN-β*	Arachidonate metabolites†		
		6-keto-$PGF_{1\alpha}$	TXB_2	PGE_2
4	–	1,358·0 ± 316·4	207·5 ± 11·3	96·2 ± 19·8
	+	1,233·0 ± 103·4	201·1 ± 6·6	102·8 ± 15·3
24	–	1,103·0 ± 73·9	327·9 ± 96·7	50·7 ± 5·1
	+	460·0 ± 22·4‡	227·5 ± 46·1	49·1 ± 13·4
48	–	1,749·7 ± 260·8	366·9 ± 63·0	119·3 ± 12·1
	+	632·4 ± 145·6‡	143·6 ± 30·9‡	33·8 ± 5·2‡
72	–	1,125·0 ± 106·3	241·7 ± 11·9	76·0 ± 13·8
	+	573·8 ± 199·0‡	158·2 ± 4·2‡	5·7 ± 1·2‡

* IFN-β, 2×10^3 IU/mouse, was injected intrapleurally at the time of carrageenan administration.

† pg/mouse ± s.e.m. of four to five mice/group.

‡ $P < 0{\cdot}01$ vs. control without IFN-β.

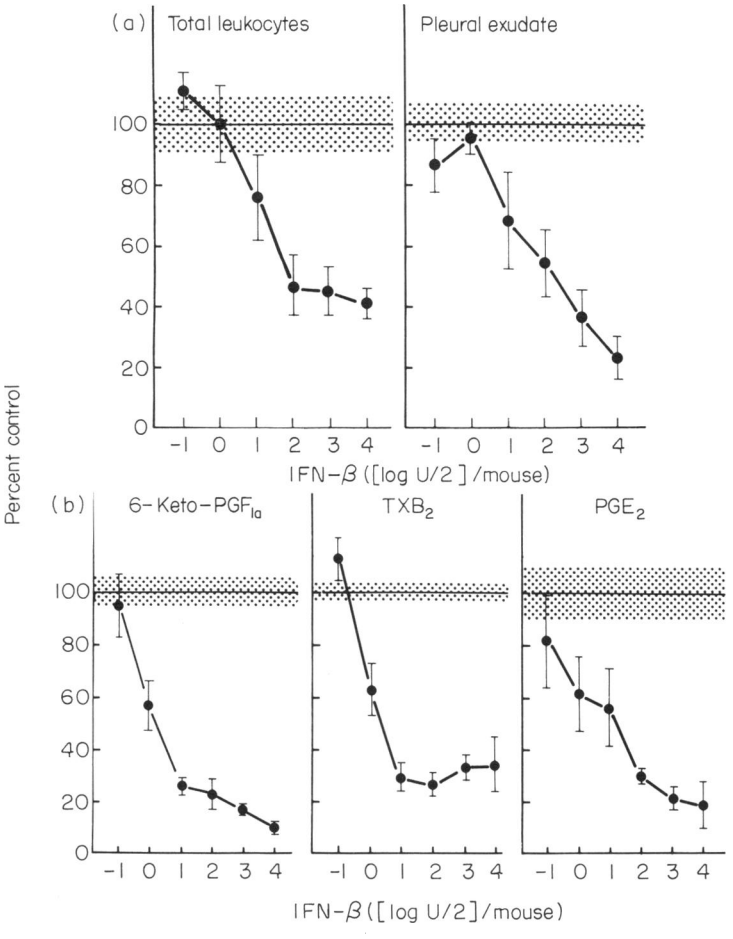

Fig. 3. Dose-dependency of the effect of IFN-β on the carrageenan-induced pleurisy. Mice received an intrapleural injection of 0·2 ml 1% carrageenan alone or mixed with different doses of IFN-β. The inflammatory parameters were measured after 48 h. (a) effect of IFN-β on cell migration and exudate formation. (b) effect of IFN-β on eicosanoid release into the pleural cavity. Shaded areas represent the mean $\pm$s.e.m. of control values in the absence of IFN-β.

arachidonate metabolite present, was already evident 24 h after inoculation of carrageenan, whereas inhibition of TXB_2 and PGE_2 occurred only after 48 h, i.e. at the peak of the inflammatory reaction. No inhibition of any of the inflammatory parameters was observed in mice treated with mock IFN-α/β (not shown).

Data reported in Fig. 3 show that IFN-β exerted its inhibitory activity on the pleural inflammatory reaction in a dose-dependent fashion. In the inhibition of both cell migration and exudate formation, IFN-β was active at doses of 200 IU/mouse or more (Fig. 3a). However, the inhibitory effect of IFN-β on the amount of eicosanoids in the same exudates was much more pronounced. In fact, as shown in Fig. 3b, IFN-β inhibited the presence of eicosanoids in the exudate by 40–50% at doses as low as 2 IU/mouse, and maximally at doses of 20 IU/mouse (6-keto-$PGF_{1\alpha}$ and TXB_2) or 200 IU/mouse (PGE_2).

IFN-β inhibited the carrageenan-induced pleural inflammation not only when inoculated together with the inflammatory agent, but also when administered at later times. In fact, the results reported in Fig. 4a show that IFN-β was able to inhibit Mϕ migration in pleural cavities challenged with carrageenan, also when injected 2, 6, 18 or 45 h after the onset of inflammation. Parallel with

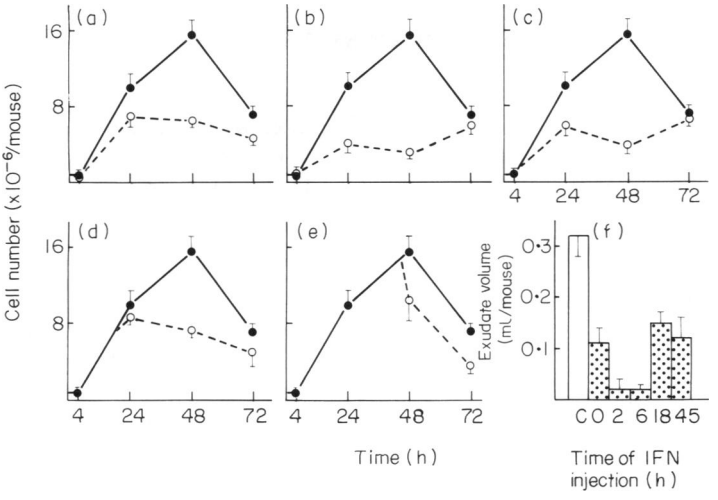

Fig. 4. Anti-inflammatory activity of IFN-β administered at different times after carrageenan. Mφ migration into the pleural cavity was measured in control mice (●) and in mice receiving 2×10^3 IU of IFN-β (○) at different times after carrageenan: (a) 0 h; (b) 2 h; (c) 6 h; (d) 18 h; (e) 45 h; (f) pleural exudates of mice inoculated with 2×10^3 IU of IFN-β at different times after administration of carrageenan. Exudates measured at 48 h. Inoculation of saline alone instead of IFN-β did not modify the development of the carrageenan-induced inflammation (not shown). Open bar: control mice.

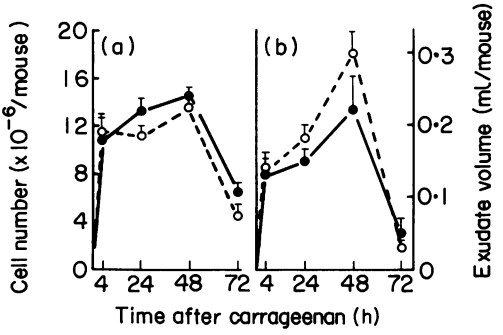

Fig. 5. Lack of effect of recombinant IFN-γ on the development of carrageenan-induced pleurisy. Mice received 0·2 ml 1% carrageenan alone (●) or together with 2×10^3 IU of r-IFN-γ (○). The inflammatory parameters were measured after 4, 24, 48 and 72 h. (a) Total leukocytes; (b) pleural exudate.

the effect on cell infiltration (Fig. 4a and data not shown), the delayed administration of IFN-β also caused a significant inhibition of fluid exudate formation (Fig. 4b). It is of interest that IFN-β given 2 or 6 h after carrageenan had a significantly higher capacity to inhibit the inflammatory process than when administered together with the inflammatory stimulus.

Effect of r-IFN-γ on carrageenan-induced pleurisy. In order to compare the activity of IFN-β and IFN-γ on the development of a nonimmune inflammation like carrageenan pleurisy, experiments were performed in which r-IFN-γ was administered intrapleurally together with carrageenan.

As shown in the representative experiment reported in Fig. 5, r-IFN-γ was unable to induce any significant modification of the development of pleural inflammation. In addition to its lack of effect on carrageenan-induced cell infiltration and exudate formation (Fig. 5), r-IFN-γ was also unable to affect the amount of eicosanoids (6-keto $PGF_{1\alpha}$, TXB_2 and PGE_2) in the pleural exudate (data not shown).

DISCUSSION

Mononuclear phagocytes play a central role in the inflammatory reaction, and have been reported to release many inflammatory mediators *in vitro* in response to various stimuli. In fact, it is a common belief that Mϕ are a major (if not the major) source of PGE_2 and other AA-derived eicosanoids (highly active molecules involved in the modulation of inflammation) at the inflammatory sites (Morley, 1981). In addition, activated Mϕ can produce large amounts of inflammatory oxygen reactive species (Badwey & Karnovsky, 1980), thus also participating through this mechanism in the inflammatory reaction.

Interferons, molecules endowed with multiple biological activities in addition to antiviral capacity, are potent modulators of Mϕ functions *in vitro*, including the Mϕ capacity to release inflammatory molecules. Indeed, IFN-α and IFN-β were found to be able to depress the capacity of murine peritoneal Mϕ to produce the oxygen metabolites superoxide anion and hydrogen peroxide and to release prostaglandins (Boraschi & Tagliabue, 1984).

This observation would thus suggest the possibility of using IFN *in vivo* as anti-inflammatory agents in pathological situations where Mϕ and prostaglandins are chiefly involved. Indeed, the choice of the appropriate situation appears to be essential, since IFN can have opposite effects on cells other than Mϕ. In fact, IFN or IFN inducers can stimulate prostaglandin production in synovial cells and other fibroblasts *in vitro* (Yaron *et al.*, 1977; Fitzpatrick & Stringfellow, 1980; Fuse, Mahmud & Kuwata, 1982), and can induce acute synovitis when inoculated intra-articularly *in vivo* (Yaron *et al.*, 1979). IFN can also exert different activities on different types of Mϕ, inhibiting eicosanoid production in murine peritoneal and pleural Mϕ (Sestini, Tagliabue & Boraschi, 1984), as well as in human blood monocytes (Dore-Duffy, Perry & Kuo, 1983), but increasing PGE_2 release in mouse alveolar Mϕ (Sestini *et al.*, 1984).

These opposite effects of IFN on the ability of different cells to release inflammatory molecules may indeed conciliate the contradictory results previously published, claiming both inflammatory (De Somer, Edy & Billiau, 1977;Yaron *et al.*, 1979) and anti-inflammatory properties of IFN and IFN inducers *in vivo* (Kapusta & Mendelson, 1967; Koltai & Mecs, 1973; Koltai *et al.*, 1981; Nethersell *et al.*, 1984).

The model of acute inflammation adopted for the study presented here, carrageenan pleurisy, was chosen for its well-known dependence upon eicosanoid synthesis and for the major presence of Mϕ in the inflammatory infiltrate (Di Rosa *et al.*, 1971; Ackerman *et al.*, 1980), i.e. the appropriate situation to test the hypothesis drawn from the results obtained *in vitro*. This model, designed and widely studied in the rat, has been adapted to the mouse to allow a correct therapeutic use of murine IFN.

The results reported indicate that IFN-β is able to reduce the carrageenan-induced pleurisy both when administered together with the inflammatory agent and also when inoculated after the onset of inflammation.

An assessment of the possible targets of IFN-β action in this model of acute inflammation is quite complex. IFN-β was able to diminish the exudate volume, and this inhibition may be a consequence of the reduced release at the inflammatory site of PGE_2 and PGI_2, molecules responsible for increased vascular permeability during inflammation (Williams, 1979). IFN-β treatment was also able to reduce TXA_2 formation in the inflamed pleural cavities, but the role played by this mediator in inflammation is still somewhat controversial (Tissot *et al.*, 1984). As far as cell infiltration is concerned, the migration of both Mϕ and PMN was inhibited by IFN-β. This decrease of leukocyte infiltration in the pleural cavity would imply the ability of IFN-β to act either directly as an inhibitor of cell migration or, more probably, by inhibiting the release of chemotactic factors from the pleural cells. It has been reported that IFN-β, by reducing the release of arachidonic acid from Mϕ stimulated *in vitro*, could affect the production of both cyclooxygenase and lipoxygenase metabolites (Boraschi *et al.*, 1984a). In this light, lipoxygenase-derived leukotriene B_4, which can be released by both PMN and Mϕ and is a potent chemotactic factor (Ford-Hutchinson *et al.*, 1980), could be inhibited by IFN-β treatment *in vivo*. Another molecule possibly involved in the inflammatory reaction to carrageenan is IL-1, a factor mainly produced by

Mφ, that has been reported both to stimulate the release of eicosanoids and other inflammatory molecules (Dinarello, 1984) and to act as a chemoattractant for PMN and Mφ (Luger *et al.*, 1983). Experiments are in progress in our laboratory to assess the role of IL-1 and LTB_4 in carrageenan-induced pleurisy.

It is important to note that a significant anti-inflammatory effect could be achieved with a single local inoculation of low concentrations of IFN-β (with inhibition of all inflammatory parameters already evident with 200 IU/mouse of IFN-β; Fig. 3a). The potential therapeutic value of IFN-β is further stressed by the finding that the anti-inflammatory effect was potently expressed even when IFN-β was inoculated several hours after the onset of the inflammatory reaction.

In contrast to IFN-α and IFN-β, IFN-γ did not show any anti-inflammatory activity when used *in vitro* to modulate Mφ functions. In fact, according to the different experimental conditions adopted, IFN-γ either increased or left unchanged the ability of Mφ to produce inflammatory molecules such as oxygen reactive species and IL-1 (Murray, Spitalny & Nathan, 1985; Boraschi *et al.*, 1984b), and it either increased or depressed prostaglandin production in response to distinct stimuli (Boraschi *et al.*, 1985). Thus, the intrapleural inoculation of r-IFN-γ together with carrageenan was not expected to yield any anti-inflammatory effect. Indeed, the administration of r-IFN-γ proved unable to modify the development of the inflammatory response in a significant fashion, indicating an apparent lack of involvement of IFN-γ in the regulation of Mφ functions during a nonimmune inflammation.

In conclusion, the data reported here indicate a significant anti-inflammatory activity of IFN-β in a localized nonimmune inflammation characterized by a marked Mφ presence and eicosanoid involvement. These data may thus indicate a potential therapeutic use of IFN-β in particular inflammatory conditions and suggest the possibility that locally produced IFN (Bocci, 1985) may have a role in the modulation of inflammatory reactions *in vivo*.

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