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## **Recombinant Porcine Interferon-Gamma Activates In Vitro Porcine Adherent Mononuclear Cells to Produce Interleukin 1**

B. CHARLEY, SOPHIE LAVERNE and LAURENCE LAVENANT

*INRA, Station de Virologie et d'Immunologie Moléculaires, 78350 Jouy-en-Josas (France)*

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### **ABSTRACT**

Charley, B., Laverne, S. and Lavenant, L., 1990. Recombinant porcine interferon-gamma activates in vitro porcine adherent mononuclear cells to produce interleukin 1. *Vet. Immunol. Immunopathol.*, 25: 117-124.

The effect of recombinant porcine interferon-gamma (rPoIFN $\gamma$ ) on in vitro production of interleukin 1 (IL-1) by porcine blood monocytes was studied. Three-day-old cultures of porcine adherent blood mononuclear cells were treated by doses of rPoIFN $\gamma$  for 3 or 6 days before lipopolysaccharide (LPS)-induction. While rPoIFN $\gamma$  alone had no effect, a combined treatment by rPoIFN $\gamma$  and LPS enhanced the IL-1 secretory potential of adherent mononuclear cells and, to a lesser extent, the level of cell-associated IL-1. The IL-1 activity was neutralized by anti porcine IL-1 $\alpha$  and  $\beta$  antisera. These results demonstrate that rPoIFN $\gamma$  has immunomodulatory effects in vitro on porcine monokine production.

### **INTRODUCTION**

Interferon- $\gamma$  (IFN $\gamma$ ) is a lymphokine produced by activated T lymphocytes. In addition to antiviral activity IFN $\gamma$  has various immunomodulatory effects on different cell types, including T-, B- and NK cells as well as neutrophils and macrophages (reviewed by Welsh, 1984; Trinchieri and Perussia, 1985; Virelizier and Arenzana-Seisdedos, 1985; Russell and Pace, 1987). Expression of DNA-encoded IFN $\gamma$  has recently enabled the evaluation of some of the biological activities of IFN $\gamma$  in domestic animals. Thus, recombinant bovine IFN $\gamma$  (rBoIFN $\gamma$ ) was shown to inhibit in vitro the replication of several viruses (Czarniecki et al., 1986), and to modulate various neutrophil and lymphocyte functions, both in vitro and in vivo (Bielefeldt Ohmann and Babiuk, 1986).

In the porcine species, the gene coding for IFN $\gamma$  has been cloned by Genentech Inc. (U.S.A.) and *E. coli*-derived rPoIFN $\gamma$  was produced. It was previously shown that this molecule exerted antiviral effects in vitro against vesicular

stomatitis virus, the coronavirus transmissible gastroenteritis virus and African swine fever virus, and shared antigenic determinants with bovine but not human IFN $\gamma$  (Charley et al., 1988; Esparza et al., 1988). However, little is known about the immunomodulatory properties of rPoIFN $\gamma$ . In this report, we present results of a study on porcine monocyte activation *in vitro* by rPoIFN $\gamma$ . The functional criterion of monocyte activation analyzed was the production of interleukin 1 (IL-1) by aged monocyte cultures treated with IFN $\gamma$  and subsequently induced by endotoxin, as described for human IFN $\gamma$  by Arenzana-Seisdedos et al. (1985). They showed that aged monocyte cultures do not respond well to lipopolysaccharide stimulation and thus provide a sensitive model to use when evaluating monocyte activation by immunomodulating substances, such as IFN $\gamma$ . Additionally, the use of aged monocyte cultures provides assurance that endotoxin contamination does not yield confusing results in monocyte activation (Arenzana-Seisdedos et al., 1985). We report that rPoIFN $\gamma$  has enhancing effects on the IL-1 secretory potential of aged cultures of porcine blood adherent mononuclear cells.

## MATERIALS AND METHODS

### *Cells and media*

Porcine peripheral blood mononuclear cells (PBMC) were obtained from heparinized blood by density gradient centrifugation (Lymphoprep, density 1.077; Nyegaard, Oslo, Norway). Blood samples were collected from 2- to 4-month-old, conventionally reared animals. Mouse thymocytes were prepared from 3-week-old BALB/cJ mice maintained in our facilities. Cells were cultured in RPMI 1640 medium (Gibco, Paisley, Scotland) supplemented with 10% fetal bovine serum, antibiotics,  $5 \times 10^{-5}$  M 2-mercaptoethanol and  $10^{-3}$  M pyruvate.

### *Interferon*

Recombinant porcine IFN $\gamma$  (lot no. 4648-58) was kindly provided by Ciba Geigy Ltd. (Basel, Switzerland); specific activity was 5 to  $10 \times 10^6$  antiviral units/mg.

### *Isolation of adherent PBMC*

Porcine PBMC ( $10^7$  cells/ml) in RPMI 1640 complete medium (10% FBS), supplemented with 10% autologous plasma, were plated in round-bottomed microtiter plates (Nunc, Roskild, Denmark) at 0.1 ml/well. Cultures were incubated for 3 days in a 95% air-5% CO $_2$  humidified atmosphere at 37°C. After this initial incubation the cultures were washed three times with warm medium to remove non-adherent cells. The remaining cells consisted of  $74 \pm 2.1\%$  esterase-positive cells, therefore considered as being monocytes, while the other cells were non further characterized lymphocytes.

*Cell incubation with IFN $\gamma$  and IL-1 induction*

Adherent PBMC cultures were incubated in a 95% air-5% CO $_2$  humidified atmosphere at 37°C for 3 days in control medium or in various concentrations of rPoIFN $\gamma$  (0.1 ml/well). At the end of this incubation period, the medium was carefully removed, discarded and replaced by RPMI 1640 complete medium supplemented with 1  $\mu$ g/ml of indomethacin (Sigma, St. Louis, U.S.A.), to avoid possible interference with prostaglandin production, and 20  $\mu$ g/ml of *E. coli* lipopolysaccharide (no. 0111 B4 from Sigma) (Cavaillon et al., 1989). Following an overnight incubation at 37°C, supernatants were harvested and stored at -20°C. Cell lysates were obtained, after adding 50  $\mu$ l of medium per well, by freezing and thawing three times.

*IL-1 assay*

IL-1 activity was tested by the ability of supernatants and cell lysates to induce the proliferation of mouse thymocytes stimulated with submitogenic doses of phytohemagglutinin (PHA), as described (Charley et al., 1983; Arzenana-Seisdedos et al., 1985; Cavaillon et al., 1989). Serial log $_2$  dilutions of 0.1 ml samples were performed, in duplicate, in flat-bottom microtiter plates (Falcon, Oxnard, U.S.A.), in a final volume of 0.1 ml of RPMI 1640 complete medium. Mouse thymocytes (10 $^7$  cells/ml) in RPMI 1640 complete medium, supplemented with 10  $\mu$ g/ml PHA-P (Difco, Detroit, U.S.A.) were added to each well. Cultures were incubated for 3 days at 37°C and pulsed overnight with 37 kBq/well of tritiated thymidine (CEA, Saclay, France). The incorporated radioactivity was collected on filter paper by an automated cell harvester. Interleukin 1 activity was expressed as cpm of tritiated thymidine incorporation.

*Neutralization of porcine IL-1 activity by specific antisera*

In order to assign the biological activity in the samples tested on mouse thymocytes to IL-1, seroneutralization assays were performed on positive supernatants with rabbit antisera directed against the two distinct forms of porcine IL-1. These antisera are able to specifically neutralize porcine IL-1 biological activities, such as cartilage resorption and thymocyte activation (Saklatvala et al., 1985 a,b). Anti-IL-1 $\alpha$  and - $\beta$ , kindly provided by J. Saklatvala (Cambridge, Great Britain), were added at the non toxic dilution of 1/640, to a 1.2 diluted test sample; normal rabbit serum was used as a control. These sera did not exert inhibitory effects on mouse thymocytes alone (Table 1). IL-1 activity was assayed as previously described.

## RESULTS

When porcine adherent PBMC were cultured for 6 days in control medium before LPS induction, no IL-1 activity was detectable in supernatants (Fig.

1a) nor in cell lysates (Fig. 1b). In contrast, 3-day-old cultures of adherent PBMC, when treated for a further 3 days with rPoIFN $\gamma$  (doses ranging from 0.01 to 10  $\mu\text{g}/\text{ml}$ ) before LPS, secreted high levels of IL-1 in their supernatants (Fig. 1a). A similar dose-effect of rPoIFN $\gamma$  on the IL-1 activity associated with the cell lysates was observed but the IL-1 yield was lower than in supernatants (Fig. 1b). A seroneutralization test indicated that the mitogenic activity present in rPoIFN $\gamma$ -treated PBMC supernatants was largely neutralized by anti-porcine IL-1 $\alpha$  and, to a lesser extent, by anti-porcine IL-1 $\beta$  specific antisera (Table 1). Additionally, IL-2 titrations performed on IL-2-dependent mouse T-cell lines by C. Leclerc (Institut Pasteur, Paris) indicated that the mitogenic activity was not related to IL-2 (data not shown). In the absence of LPS induction, no IL-1 activity was detected in the supernatants of rPoIFN $\gamma$ -treated adherent PBMC (Fig. 2). A kinetic study indicated that a higher IL-1 yield

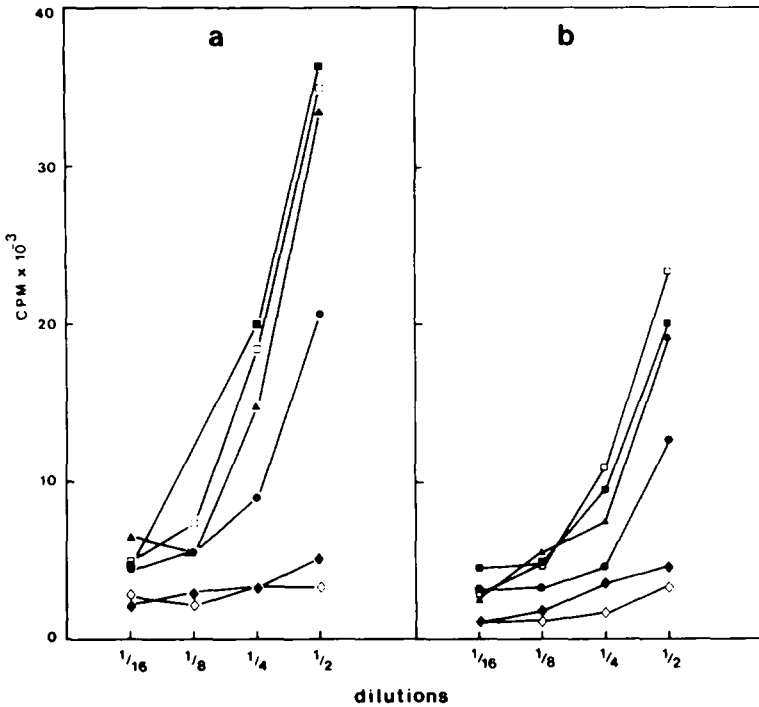


Fig. 1. Dose-effect of rPoIFN $\gamma$  on the IL-1 production in LPS-induced adherent PBMC cultures. Three-day-old PBMC cultures were treated for an additional 3 days with IFN $\gamma$  before LPS induction. rPoIFN $\gamma$  dosage were 10  $\mu\text{g}/\text{ml}$  (■), 1  $\mu\text{g}/\text{ml}$  (▲), 0.1  $\mu\text{g}/\text{ml}$  (□), and 0.01  $\mu\text{g}/\text{ml}$  (●). Controls included PBMC treated with LPS alone (◆) or with control medium (◇). The yield of IL-1 is expressed as cpm of incorporated tritiated thymidine in the thymocyte bioassay. (a) IL-1 production in PBMC supernatants. Data are representative of six experiments. (b) IL-1 production in PBMC cell lysates.

TABLE 1

Inhibition of IL-1 activity in rPoIFN $\gamma$ -treated PBMC supernatants, by rabbit anti-porcine IL-1 $\alpha$  and  $\beta$  specific antisera

Antiserum	IL-1		Background
	cpm	% inhibition	
None	4385	0	556
Normal rabbit serum	3680	16	640
Anti-porcine IL-1 $\alpha$	978	78	667
Anti-porcine IL-1 $\beta$	2948	33	719

A positive PBMC supernatant was assayed, at a 1/2 final dilution, in a thymocyte bioassay, in the presence of 1/640 diluted rabbit sera (see Material and Methods).

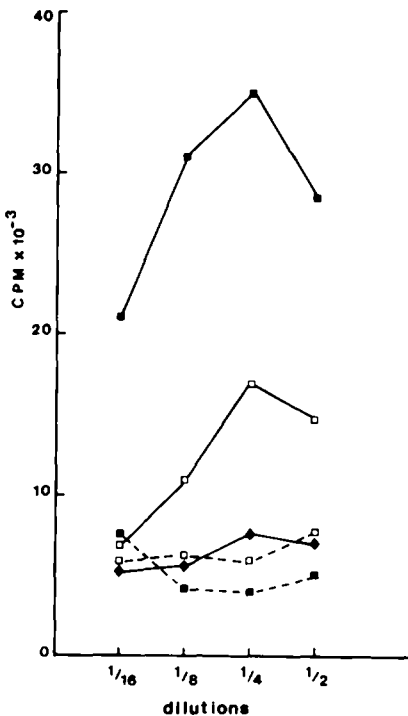


Fig. 2. Effect of rPoIFN $\gamma$  treatment duration on IL-1 production by porcine PBMC. Three-day-old PBMC cultures were treated with rPoIFN $\gamma$  (10  $\mu$ g/ml: —) or with control medium (---) for 3 days (□) or for 6 days (■) before LPS induction. A control was included with PBMC cultures treated for 6 days with rPoIFN but without LPS induction (◆).

was observed when adherent PBMC cultures were treated for 6 days, instead of 3, with IFN $\gamma$  (Fig. 2).

## DISCUSSION

Our data show that rPoIFN $\gamma$  is able to activate porcine adherent mononuclear cells to secrete IL-1 after stimulation with LPS. The experimental protocol used, which follows the description by Arenzana-Seisdedos et al. (1985) for human monocytes, allows the preparation of aged (3 days) cultures which poorly respond to LPS alone (Fig. 1). Thus, 3- to 6-day-old porcine adherent PBMC cultures, containing a large majority (74%) of esterase-positive cells, provide a sensitive assay system to evaluate monocyte activation. A seroneutralization experiment conducted with anti-porcine IL-1 indicated that the mitogenic activity produced by rPoIFN $\gamma$ -treated cells, although lower than that shown in Figs. 1 and 2, could be assigned to IL-1 $\alpha$  and to a lesser extent to IL-1 $\beta$  (Table 1). Since rPoIFN $\gamma$  alone is unable to induce IL-1 secretion (Fig. 2), it appears that rPoIFN $\gamma$  causes a modification to the cells that renders them responsive to LPS. IFN $\gamma$  has been shown, in other animal species, to exert numerous effects on several monocyte-macrophage functions, such as increased expression of membrane receptors and class II molecules, increased phagocytic and antimicrobial activities, or enhanced secretory potential, including cytokine production. In fact, IFN $\gamma$  clearly appears to be one of the macrophage-activating factors (MAF) (reviewed by Trinchieri and Perussia, 1985; Virelizier and Arenzana-Seisdedos, 1985; Russell and Pace, 1987). Using IL-1 secretion as a functional parameter for monocyte activation, we provide experimental evidence that rPoIFN $\gamma$  has MAF properties in the porcine species. The complete state of activation requires a two step procedure, IFN $\gamma$  providing, after a long incubation period (Fig. 2), a first putatively differentiating signal whereas LPS provides the final triggering signal.

The ability of rPoIFN $\gamma$  to increase LPS-induced IL-1 secretion may have important potential implications. IL-1 is known to play a crucial role in the initiation of a specific immune response. It is likely, therefore, that rPoIFN $\gamma$  could modulate *in vivo* the immune responsiveness of treated animals, leading to improved antibody production and/or T-cell functions following vaccination. In addition, since IFN $\gamma$  has been shown to activate microbicidal and tumoricidal functions of rat and bovine alveolar macrophages *in vivo* (Badger et al., 1988; Bielefeldt Ohmann et al., 1987), it will be very important to evaluate the ability of rPoIFN $\gamma$  to activate porcine alveolar macrophages, in order to increase the lung defense mechanisms. Indeed, previous studies have indicated that porcine alveolar macrophages could respond to immunomodulatory compounds such as LPS or muramyl dipeptide (Charley et al., 1983) and recently porcine somatotropin (Edwards et al., 1988). The present report provides initial evidence for the potential immunoenhancing effects of recently available

recombinant PoIFN $\gamma$ . In vivo studies are, therefore, currently undertaken in our laboratory to evaluate the influence of rPoIFN $\gamma$  treatment in immunosuppressed pigs.

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