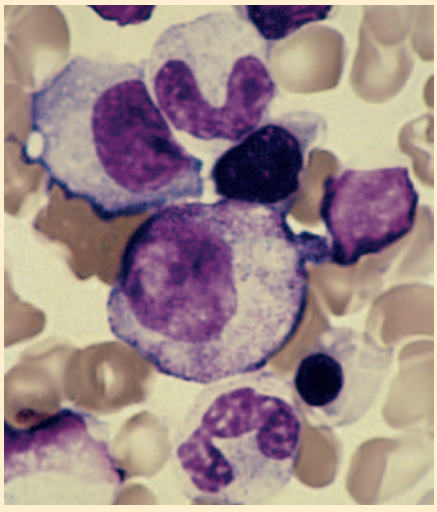
M. Amadori,
B. Begni, L. Milani

ANTI-INFLAMMATORY ACTIVITY OF LOW DOSE IFN-α - *IN VITRO STUDY ON PORCINE LEUKOCYTES*

SUMMARY

Exposure of swine Peripheral Blood Mononuclear Cells (PBMC) to low concentrations of IFN-α causes a strong, dose dependent decrease of CD14 expression. The strongest decrease is at 5 Units ml^{-1} IFN-α, with or without a subsequent exposure of cells to purified lipopolysaccharide (LPS).
The largest reduction of TNF-α accumulation in the medium of swine PBMC and Pulmonary Alveolar Macrophages (PAM), stimulated with bacterial LPS, is caused by a 10-fold lower interferon-α concentration (0.5 Units ml^{-1}). With this treatment, the expression of the TNF-α gene in PAM is also strongly reduced, as opposed to cells reacted with 50 Units ml^{-1} IFN-α. On the contrary, the expression of the IL-1β and IL-6 genes is stimulated at both concentrations. Owing to the above, IFN-α could play an important role in the control of the inflammatory response to bacterial endotoxins in pigs.

KEY WORDS **IFN-α LOW DOSE, INFLAMMATION, IN VITRO STUDY**



From: www.taconichills.k12.ny.us

INTRODUCTION

Pigs in intensive breeding suffer from different diseases in the post-weaning phase, among them the PRRS (porcine reproductive and respiratory syndrome) (1, 2) and the PMWS (postweaning multisystemic wasting syndrome) (3), caused by the PRRS virus and the type 2 porcine Circovirus respectively.
Despite the widespread presence of the viral infection in pig breeding, only sick animals present major inflammatory lesions and systemic dysfunction of the Immune System (4, 5, 6, 7).

In fact, the viral infections mentioned above would have the effect of increasing the inflammatory response of the animals to bacterial lipopolysaccharides (LPS) (8, 9) present in significant concentration in the air of intensive breeding.

Based on these assumptions, we focused our attention on oral treatment with ***low-dose* IFN-α**, which has been shown to be effective in different experimental models of chronic inflammation and autoimmune diseases (10, 11, 12).
In fact, it is apparent from the published results the potential role of IFN-α in the regulation of both the innate and the acquired immune response in many animal species, including pigs (10, 13, 14, 15).
Consequently, an *in vitro* study was conducted on the activity of IFN-α on porcine leucocytes in response to bacterial LPS.

MATERIALS AND METHODS

CELLS

Samples of heparinized venous blood were taken from weaned pigs (average weight 30 kg). These were diluted 1:2 with RPMI 1640 *medium*, supplemented with antibiotic and centrifuged over Histopaque® - 1077 (*Sigma-Aldrich, St Louis, USA*). The peripheral blood mononuclear cells (PBMC) obtained were washed twice with RPMI 1640, counted and cultured at 2 x 10^6 ml^{-1} in RPMI 1640 + 10% of foetal bovine serum (FBS) (*Biochrom KG, Berlin*) in plates for cell culture with 12 wells.
The pulmonary alveolar macrophages (PAM) were taken from weaned pigs euthanized after general anaesthesia by bronchoalveolar lavage.
The cells were cultured as described above (RPMI 1640 + 10% FBS) in plates for cell culture with 12 wells.

IFN-α (U/ml)	% CD14 + PBMC	MCF*
0 (control)	5.2	96
0.05	4.8	100
0.5	2.4**	98
5	0.2**	91
50	4.6	100

TAB. 1

Expression of CD14 in the porcine PBMC treated with IFN-α and LPS.

**** Fluorescence Mean channel***

***** Significance compared with the control value (Fisher's exact test, p < 0.01)***

- The porcine PBMC were treated with IFN-α in the listed concentrations. The next day bacterial LPS (1 μg ml^{-1}) were added. The expression of CD14 was detected through flow cytometry on day 4 of the experiment.

IFN-α (U/ml)	% CD14 + PAM	MCF*
0 (control)	11.3	106
0.05	10.2	97
0.5	5.5**	96
5	1.4**	83
50	5.6**	74

TAB. 2

Expression of CD14 in the porcine PAM treated with IFN-α and LPS.

**** Fluorescence Mean channel***

***** Significance compared with the control value (Fisher's exact test, p < 0.01)***

- The porcine PAM were treated with IFN-α in the listed concentrations. The next day bacterial LPS (1 μg ml^{-1}) were added. The expression of CD14 was detected through flow cytometry after 24h.

The samples of pulmonary alveolar macrophages and blood were collected under the responsibility of a veterinarian and the supervision of the regional National Health Service.

INTERFERON-α

Purified human lymphoblastoid IFN-α (*Sigma-Aldrich, St. Louis – USA*) was frozen in aliquots to -80° C and used in the following *in vitro* experiments.
The biological activity was determined through inhibition of the cytopathic effect induced by Vesicular Stomatitis Virus on bovine MDBK cells and human HEP-2 cells (16).
The human IFN-α is active on porcine cells in vitro (17) and in pigs treated orally with low-dose IFN-α (13).

TREATMENT WITH IFN-α AND LPS

Porcine PBMC and PAM, cultured on plates with 12 wells, were supplemented with IFN-α at different concentrations (from 0 to 50 IU ml^{-1}) and incubated at 37°C in a humidified incubator with 5% CO_2. After 16-24 hours, the cells were stimulated with 1μg ml^{-1} of bacterial LPS from *Escherichia coli* 0111:B4 *(Sigma-Aldrich, St. Louis – USA)* or left untreated as control.
Subsequently, the cells were re-incubated at 37°C in the presence of 5% CO_2. The cells then underwent flow cytometry with mAb for the CD14 and RT-PCR for the genes of the principal inflammatory cytokines; their culture *medium* underwent bioanalysis for the presence of TNF-α.
10 U of human lymphoblastoid IFN per kg body weight were administered to 4 pigs aged 30 days, on 15 successive days in lyophilized form [TF 21 (only for veterinarian use - Editor's note) Guna S.p.a., Milan] and mixed with the feed. Five other pigs of the same age and genetic profile were used as controls. Blood samples were taken on day 15 in heparinized test tubes at the end of the oral treatment with IFN-α.
The concentration of IFN-α administered is much lower than that usually found in the serum and the intestinal secretions of pigs affected by viral infections (18).

FLOW CYTOMETRY

The PBMC and the adherent PAM were obtained by treatment with EDTA (0.02% final). These were mixed with the non-adherent cells, washed twice in phosphate-buffered saline solution (PBS, pH 7.4) and suspended in buffer for flow cytometry (PBS, pH 7.4, 2% of FBS submitted to thermal treatment, sodium azide 0,1%).
The cells were analysed using the Bryte-HS flow cytometer *(BIO-RAD)* for CD14 surface expression with monoclonal antibody (MIL-2) (kindly supplied by Dr. Karen Haverson, University of Bristol, U.K.). 10,000 cells were analysed in the selection grid. The percentage of CD14 + cells was obtained through subtraction of the value corresponding to cells marked only with murine anti-IgG to fluorescein.

TRANSFER OF CELLS TREATED WITH IFN-α

The porcine PBMC were cultivated at 2 x 10^6 ml^{-1} in 2 plates with 12 wells (donor and recipient respectively).

Six donor wells were treated in duplicate with **0**, **0,5** and **50 U ml^{-1}** of **IFN-α** respectively.
- After 4 hours of incubation at 37°C, 5% CO_2, the non-adherent cells were removed from 3 wells (one for every treatment with IFN-α) and washed twice in RPMI 1640 with FBS (10%).
2 x 10^5 cells for each IFN-α treatment were then transferred to 3 other recipient wells and cultured at 37°C, 5% CO_2, overnight, together with the control cells of the recipient wells not supplemented with syngeneic cells, nor treated with IFN-α. The next day, all the cells were stimulated for 16 hours with 1 µg ml^{-1} of bacterial LPS at 37°C, 5% CO_2.

BIOANALYSIS OF THE TNF-α

The medium of the cells treated with IFN-α and/or LPS was sampled at different times during each experiment and frozen at -80°C for bio-analysis of the TNF-α.
The same procedure was followed for the PBMC supplemented with syngeneic cells treated with IFN-α.
The analysis was performed as described in Reference 19.
WEHI 164 (ATCC) cells in RPMI 1640 with 10% FBS (3 x 10^4/well) were seeded onto plates with 96 wells in the presence of the serially diluted samples to base log2 and of 1 µg ml^{-1} (final) of *Actinomycin D (Sigma-Aldrich, St. Louis-USA)*. After 18-hour incubation at 37°C, the presence of vital cells was determined with 3-(4,5-dimethylthiazole-2-xl)2,5-diphenyl-tetrazolium bromide (MTT) *(Sigma-Aldrich, St. Louis-USA)*.
- The concentration of TNF-α was determined by comparing the *end-point* of the samples with that of a standard preparation of recombinant pig TNF-α *(Pierce Endogen)*.

SEMIQUANTITATIVE RT-PCR ANALYSIS OF THE PAM

The total RNA was isolated from PAM with triazolium according to the producer's instructions *(Gibco BRL / Life Technologies)*. 3 µg of total RNA *were inversely transcribed with random hexamer primers and Moloney murine leukaemia virus-reverse-transcriptase (MMLV, Gibco BRL)* using standard procedures.
Amplification of the hypoxanthine phosphoribosyltransferase (HPRT) gene was carried out to normalize the cDNA content from the different cell samples.
The amplification conditions were: denaturation at 94°C for 5 min. followed by 25 cycles of 30 seconds each of denaturation at 94°C, 30 seconds at 56°C, 45 seconds at 72°C, followed by 3 more minutes at the same temperature. To complete the PCR reaction on every *target* gene, normalized quantities of cDNA were diluted with PCR buffer (50mM KCl, 10 mM Tris-HCl, 2mM $MgCl_2$) to a final 50 µl, containing 50 pmol of *primer set,* additional dNTPs (0,8 mM of final concentration) and 2 U of Taq DNA polymerase. The cytokine gene expression was studied through co-amplification of the *target* genes and HPRT under the same PCR cycle conditions used for the normalization within the exponential phase of amplification of both genes.
The level of expression of the genes was determined by calculating the ratio of *target* gene to HPRT gene, using IMAGEQUANT software *(Molecular Dynamics, Amersham Biosciences)*. The tests were performed in triplicate for statistical analysis.

The following ***primers*** were used:
– HPRT *forward primer:* 5′ – CCATCACATCGTAGCCCTCT – 3′
– HPRT *reverse primer:* 5′ – TCAAATCCAACAAAGTCTGGC –3′
– IL-1β *forward primer:* 5′ – ATGGCCCCAAAGAGATGAAG – 3′
– IL-1β *reverse primer:* 5′ – TGCACGTTTCAAGGATGATG – 3′
– TNF-α *forward primer:* 5′ – AACCTCAGATAAGCCCGTCG – 3′
– TNF-α *reverse primer:* 5′ – ATGGCAGAGAGGAGGTTGAC – 3′
– IL-6 *forward primer:* 5′ – TGCTCTTCACCTCTCCGGAC – 3′
– IL-6 *reverse primer:* 5′ – CTTTCTGGAGGTAGTCCAGG – 3′.

SUPEROXIDE ANION SECRETION

After incubation for one night with 0 and 0.5 U ml^{-1} of IFN-α, the superoxide anion secretion in the PAM culture *medium* was measured by analysis of the cytochrome C (20) following stimulation with phorbol-12-myristate-13-acetate (PMA).

TRANSFER OF THE SYNGENEIC PBMC TREATED WITH IFNα	LPS TREATMENT*	NO LPS TREATMENT*
0 U/ml	800 pg TNF-α/ml	100 pg TNF-α/ml
0.5 U/ml	100 pg TNF-α/ml	100 pg TNF-α/ml
50 U/ml	100 pg TNF-α/ml	100 pg TNF-α/ml

TAB. 3

Secretion of TNF-α after transfer of the syngeneic PBMC treated with IFN-α.
- The porcine PBMC (4x10^6) were supplemented with 2x10^5 syngeneic cells treated with the listed IFN-α concentrations for 4 hours at 37°C.
The next day, the cells were stimulated with LPS (1 µg ml^{-1}) or kept as untreated control.
The medium was sampled after 24 hours for TNF-α bio-analysis.
*** The results are expressed in pg ml^{-1} after calibration with standard laboratory pig recombinant TNF-α *(Pierce Endogen)*.**

RESULTS

▶ *Down-regulation of CD14 expression*

It emerged from 4 separate experiments

that IFN-α **reduces** the surface expression of CD14 in PBMC (5 pigs) and in PAM (1 pig) dose-dependently.
Peak activity was detected at 5 U ml^{-1}.
It is interesting to note that the activity was unchanged after subsequent addition of LPS.
Two representative experiments are reported in TAB. 1, 2.

▶ *Reduction of the secretion of TNF-α*

After exposure to the bacterial LPS, the secretion of TNF-α is always reduced (by 2 to 4 times) in PAM treated with IFN-α - in comparison with those untreated - in all 4 experiments.
In comparison with the experiments on the expression of CD14, the greatest decrease in TNF-α in the *medium* was found at a concentration 10 times lower, or at 0.5 U ml^{-1} of IFN-α.
The results of a representative experiment (on 4) are shown in FIG. 1.
In the PBMC, the secretion of TNF-α did not vary significantly in the two different experiments after addition of IFN-α.
Nevertheless, the PBMC drastically reduced the secretion of TNF-α after addition of syngeneic cells treated with IFN-α in a further experiment (TAB. 3).
Moreover, the PBMC of 3 pigs out of 4 treated *in vivo* with IFN-α secreted TNF-α in amounts markedly lower after in vitro stimulation with LPS (< 50 pgs ml^{-1} compared with > 200 pgs ml^{-1} from all 5 control pigs) (t 2.98; $p < 0.05$).

▶ *Expression of cytokine genes*

In three separate RT-PCR experiments on PAM, IFN-α at 0.5 U ml^{-1} significantly reduced the expression of the TNF-α gene after stimulation with bacterial LPS unlike IFN-α at 50 U ml^{-1}.
On the contrary, under the same conditions, the expression of the IL-1β gene was stimulated to a different degree by both the concentrations of IFN-α studied. There were no significant differences related to the expression of the IL-6 gene (FIG. 2).

▶ *Superoxide anion release*

In 2 different experiments, pre-treatment of the PAM with IFN-α (0.5 U ml^{-1}) produced a 3-fold reduction in the release of superoxide anion (OD_{550}), after stimulation with phorbol-12-myristate-13-acetate (PMA).

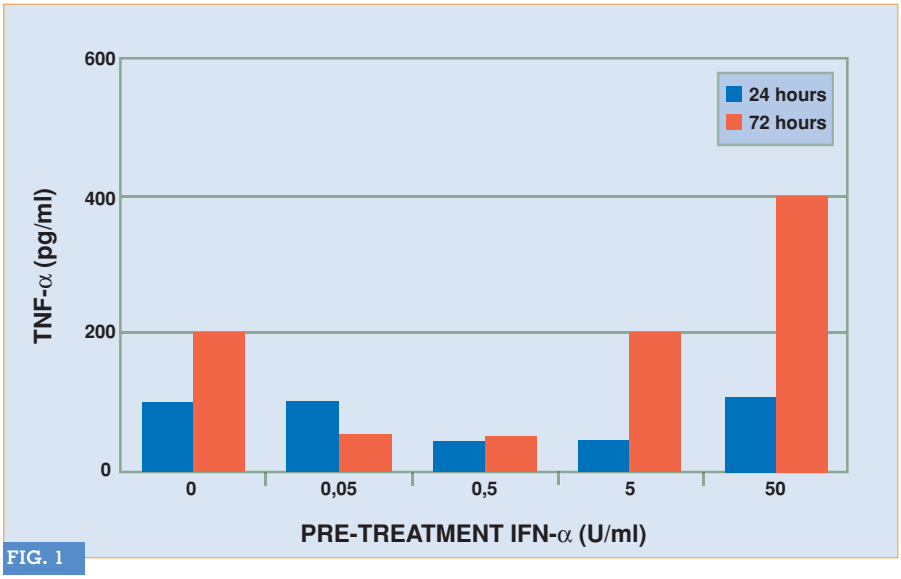


FIG. 1

The porcine PAM was treated with IFN-α at the listed concentrations. The next day bacterial LPS were added (1 μg ml^{-1}). A sample of the *medium* was taken after 24 and 72h for the determination of the TNF-α on WEHI 164 cells. The results are expressed in pg ml^{-1} after calibration with standard laboratory preparation of pig recombinant TNF-α.

DISCUSSION

The antiviral, immunostimulant and antiproliferative activity of IFN-α and the crucial importance of the concentration and *timing* of administration have been known for some time (10, 21, 22).
It is interesting to note that, in contrast to the antiviral activity, the dose-response curves of IFN-α are usually *bell-shaped* in the functions of modulation of the immune system:
over a limited concentration *range*, the observed biological activities decrease or even reach opposite values (e.g.: stimulation *vs.* suppression of the primary antibody response to sheep erythrocytes in the mouse) (23).
In particular, **effective stimulation of the immune system** at *low or intermediate concentrations* is apparent from the usual dose-response curves of IFN-α, according to its hormone-like nature (24) and the type of *in vivo* action (autocrine / paracrine).
This assumption is fully confirmed by the results obtained by the oral treatments with IFN-α in low concentrations in different models of infectious and autoimmune disease. The signal emitted by IFN-α and transmitted by an effective amplification system is induced by very **low oral doses** (1-10 IU/kg body weight) and it disappears after administration of higher daily doses (11).
Higher doses induce a Th1 pro-inflammatory response (25), while parenteral injection of very high doses can trigger an inflammatory and febrile response in humans and in animals (26).
With regard to pigs, the PRRS and PMWS models are useful for evaluating the possible role of IFN-α in the control of the inflammatory response.
In fact, the passage from viral infection to illness is linked to the reduction of secretion of endogenous IFN-α (27) and a major release of IL-10 (27, 28).
In this context, the role of IFN-α, as a regulator of the inflammatory response, could be very important since the PRRS virus substantially increases the susceptibility of pigs to bacterial LPS (8). Moreover, a sequence of DNA of type 2 porcine Circovirus (associated with

PMWS) inhibits the transcription of the IFN-α genes *in vitro* (29).

It is worthy of note that oral administration of IFN-α to weaned pigs can reduce the losses (ill, emaciated, dead pigs) in breeding affected by PRRS and/or PMWS (30).

The assumed role of IFN-α as **a modulator of the inflammatory response is in agreement with the results of this study**; particularly, the CD14 down-regulation (the cellular receptor for the LPS/LPS-binding complex) suggests that the sensitivity to the bacterial endotoxins can be profoundly modified by the IFN-α.

Decreased secretion of TNF-α was also found if porcine PAM is exposed to low concentrations of IFN-α before the LPS treatment.

Confirmation of the regulatory action of the cells treated with IFN-α is very important: these can "*command*" the recipient syngeneic cells to a *down-regulation* of the TNF-α response to bacterial LPS.

The TNF-α response is also significantly reduced in the PBMC of pigs treated orally with IFN-α: this emphasizes the marked *in vivo* amplification of the regulating signal. We also observed excellent concordance between the IFN-α concentration and the TNF-α response both in terms of protein and of the gene expression in PAM treated with LPS (FIG. 1, 2).

The concordance between RT-PCR and the TNF-α bioanalysis should not be taken for granted because the control mechanisms are different for transcriptional, translational and post-translational regulation.

For example, the availability of the conversion enzyme of TNF-α in the cells is fundamental in the production of the active cytokine with 157 amino acids (31).

The efficiency of the adaptive immune response is not threatened in the presence of low concentrations of IFN-α as is apparent from the expression of the IL1-β and IL-6 genes. Moreover, the increased IL1-β gene expression is probably not harmful thanks to potent systemic post-translation control

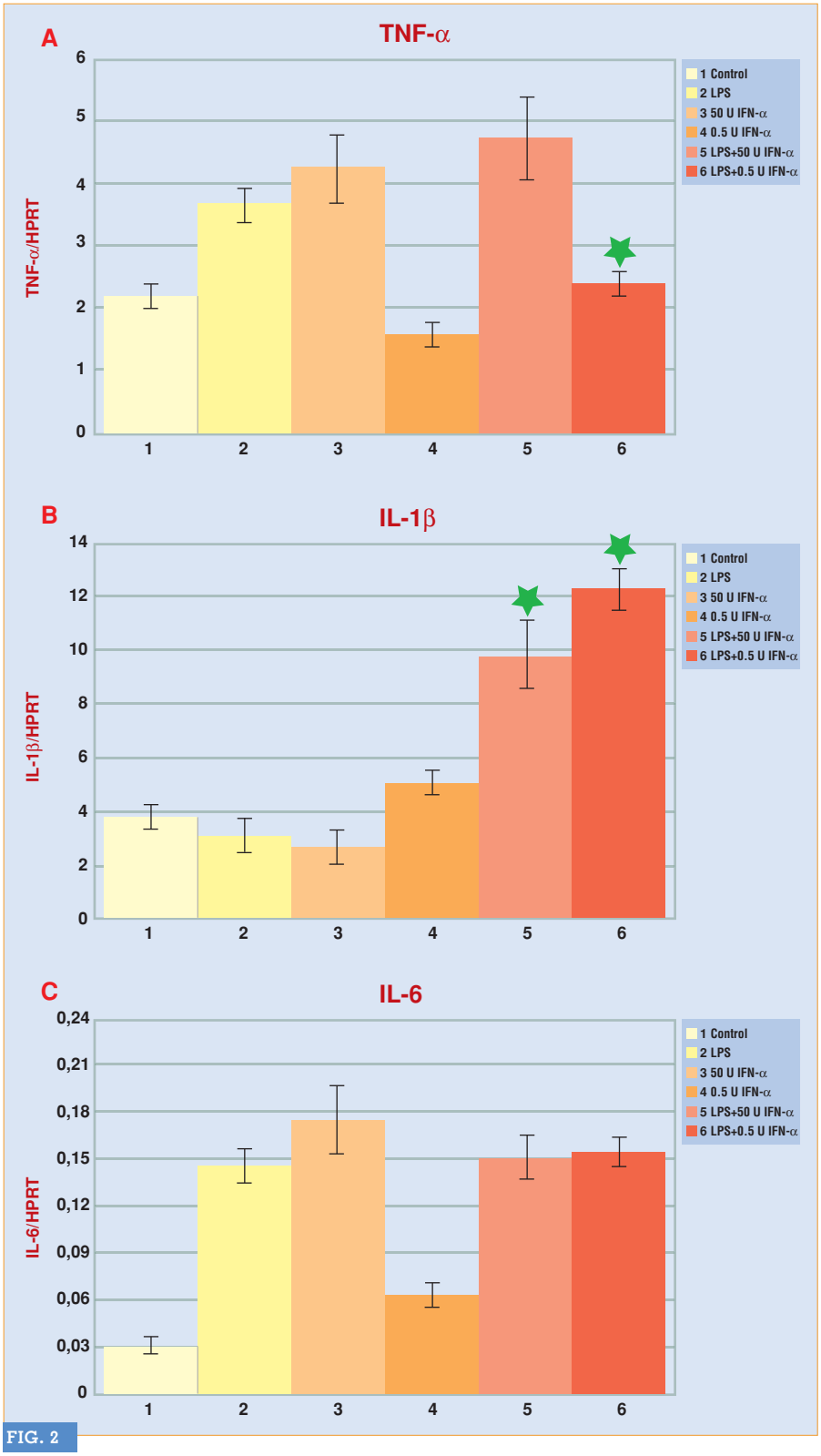


FIG. 2

Effect of IFN-α on TNF-α, IL-1β and IL-6 gene expression.
The PAM were treated in duplicate for one night with IFN-α at 0, 0.5 and 50 U ml^{-1}.
The following day, the cells were stimulated with bacterial LPS or left unstimulated as controls. RNA was extracted and semiquantitative RT-PCR was carried out, as described in "Materials and Methods." The data is expressed as the ratio between target and housekeeping gene (HPRT) based on the densitometric examination of the products of the RT-PCR amplification.
- The tests were performed in triplicate and underwent statistical analysis (variance analysis and Newman-Keuls post test). ★ indicate a significant difference ($p<0.01$) from the values observed in PAM exclusively treated with LPS.

systems (mainly the *decoy* receptor and the IL-1 receptor antagonist) (32).
Since there are no equal control systems for TNF-α, its gene and the cytokine itself could be the primary significant and immediate objectives of *low-titre* IFN-α for the control of the acute inflammatory response.
Moreover, **the rapid down-regulation of CD14 at low concentrations of IFN-α is quite interesting**: the intracellular amplification signal induced by the LPS/LPS binding complex can be reduced and the release of free CD14 can cause a significant *scavenger* action on the bacterial LPS (33).
- In the context of the defence reaction, the IFN-α might have evolved as a homeostatic control factor. As such, it would be activated following pro-inflammatory stressors such as, for instance, the premature weaning and transport of pigs (34, 35) and calves (Amadori M.; *results not published*).

To confirm this, the sensitivity of porcine leukocytes to bacterial endotoxins can be reduced by the administration of low concentrations of IFN-α.

This last fact has undoubtedly a prognostic value for the in vivo situation. In case of viral and bacterial infections, high concentrations of IFN-α can initially sustain the inflammatory response; on the contrary, low concentrations of IFN-α would reduce it in the subsequent phases of decline of the innate and acquired immune response, as well as during the reported physiological IFN-α response (36).

Therefore, besides the well-known functions of induction / regulation of the innate immune system and of antiviral action, IFN-α can also have a wider and more general role of homeostatic regulation as part of the inflammatory response. ■

ACKNOWLEDGEMENTS

The authors thank Dr. P. Candotti and Dr. S. Rota Nodari for the help given in the oral treatment of the pigs with IFN-α; A. Cristiano, G. Passador and C. Mantovani for their valuable technical help. - Further thanks go to the Ministry of Health, Rome (Project IZSLER 02/03) for the partial financing of the experiment.

References

1) Baron T., Albina E. et Al. – Report on the first outbreaks of the porcine reproductive and respiratory syndrome (PRRS) in France. Diagnosis and viral isolation. Ann. Rech. Vet., **1992**. 23: 161-166.
2) Done S.H., Paton D.J. – Porcine reproductive and respiratory syndrome: clinical disease, pathology and immunosuppression. Vet. Rec., **1995**. 136 : 32-35.
3) Allan G.M., Ellis J.A . – Porcine circoviruses: a review. J. Vet. Diagn. Invest., **2000**.12: 3-14.
4) Gilpin D.F., McCullough K. et Al. – *In vitro* studies on the infection and replication of porcine circovirus type 2 in cells of the porcine immune system. Vet. Immunol. Immunophatol., **2003**. 94: 149-161.
5) Darwich L., Segales J., Mateu E. – Pathogenesis of postweaning multisystemic wasting syndrome caused by Porcine circovirus 2: an immune riddle. Arch. Virol., **2003**. 149: 857-874.
6) Krakowka S., Ellis J.A. et Al. – Immunologic features of porcine circovirus type 2 infection. Viral Immunol., **2002**. 15 : 567-582.
7) Mandrioli L., Sarli G. et Al. – Apoptosis and proliferative acivity in lymph node reaction in postweaning multisystemic wasting syndrome (PMWS). Vet. Immunol. Immunophatol., **2004**. 97: 25-37.
8) Van Gucht S., Van Reeth K., Pensaert M. – Interaction between porcine reproductive-respiratory syndrome virus and bacterial endotoxin in the lungs of pigs: potentiation of cytokine production and respiratory disease. J. Clin. Microbiol., **2003**. 41: 960-966.
9) Labarque G., Van Reeth K. et Al. – Porcine reproductive-respiratory syndrome virus infection predisposes pigs for respiratory signs upon exposure to bacterial lipopolysaccharide. Vet. Microbiol., **2002**. 88: 1-12.
10) Tompkins W.A. – Immunomodulation and Therapeutic Effects of the Oral Use of Interferon-α: Mechanism of Action. J. Interferon Cytokine Res., **1999**. 19: 817-828.
11) Rollwagen F.M., Baqar S. – Oral cytokine administration. Immunol. Today, **1996**. 17: 548-550.
12) Bocci V. – Is interferon effective after oral administration? The state of the art. J. Biol. Regul. Homeost. Agents, **1990**. 4: 81-83.
13) Cummins J.M., Mock R.E. et Al. – Oral treatment of transmissible gastroenteritis with natural human interferon alpha: a field study. Vet. Immunol. Immunopathol., **1995**. 45: 355-360.
14) Cummins J.M., Gawthrop J. et Al. – The effect of low dose oral human interferon alpha therapy on diarrhea in veal calves. Arch. Immunol. Ther. Exp., **1993**. 41: 199-203.
15) Cummins J.M., Hutcheson D.P. et Al. – Oral therapy with human interferon alpha in calves experimentally injected with infectious bovine rhinotracheitis virus. Arch. Immunol. Ther. Exp., **1993**. 41: 193-197.
16) Meager A. – Quantification of interferons by anti-viral assays and their standardization. In: *"Lymphokines and interferons a practical approach"*. Clemens M.J., Morris A.G. et Al., A.J.H. Editors, IRL Press Limited, Oxford, England, **1987**. 129-147.
17) Yasuda K., Fukuoka M. et Al. – Effects of interferon on the steroidogenic functions and proliferation of immature porcine granulose cells in culture. Biol Reprod., **1992**. 47: 931-936.
18) Riffault S. Carrat C. et Al. – Interferon-alpha-producing cells are localized in gut-associated lymphoid tissues in transmissible gastroenteritis virus (TGEV) infected piglets. Vet. Res., **2001**. 32: 71-79.
19) Asai T., Okada M. et Al. – Increased levels of tumor necrosis factor and interleukin 1 in bronchoalveolar lavage fluids form pigs infected with *Mycoplasma hypneumoniae*. Vet. Immunol. Immunopathol., **1993**. 38: 253-260.
20) Du Manoir J.M., Albright B.N. et Al. – Variability of neutrophil and pulmonary alveolar macrophage function in swine. Vet. Immunol. Immunopathol., **2002**. 89: 175-186.
21) Samuel C.E. – Antiviral action of interferon. Clin Microbiol. Rev., **2001**. 14: 778-809.
22) Viscomi G.C. – Structure-activity of type I interferons. Biotherapy, **1997**. 10: 59-86.
23) Nagao Y., Yamashiro K. et Al. – Oral-mucosal administration of IFN-alpha potentiates immune response in mice. J. Interferon Cytokine Res., **1998**. 18: 661-666.
24) Cardoso E., Arzt E. et Al. – Alpha-interferon induces cortisol release by human adrenals in vitro. Int. Arch. Allergy Appl. Immunol., **1990**. 93: 263-266.
25) Belaredelli F., Gresser I. – The neglected role of type I interferon in the T-cell response: implications for its clinical use. Immunol. Today, **1996**. 17: 369-372.
26) Straub O.C. – Studies on the suitability of alpha-hybrid interferon application in cattle. Comp. Immunol. Microbiol. Infect. Dis., **1995**. 18: 239-243.
27) Stevenson L.S., McCullough K. et Al. – Cytokine profiles induced by porcine circovirus type 2 experimental infection in 3-week-old pigs. *Proceedings 7th International Veterinary Immunology Symposium, Quebec City, Canada*, July 25-30 **2004**. 138-139.
28) Suradhat S., Thanawongnuwech R. – Upregulation of interleukin-10 gene expression in the leukocytes of pigs infected with porcine reproductive and respiratory syndrome virus. J. Gen. Virol., **2003**. 84 : 2755-2760.
29) Hasslung F.C., Berg M. et Al. – Identification of a sequence from the genome of porcine circovirus type 2 with an inhibitory effect on IFN-α production by porcine PBMCs. J. Gen. Virol., **2003**. 84 : 2937-2945.
30) Amadori M., Candotti P. et Al. – Somministrazione orale di alfa interferone nel suinetto per il controllo delle sindromi PRSS e PMWS. L'Osservatorio, **2002**. 4: 4-5.
31) Wang H., Czura C.J., Tracey K.J. – Tumor necrosis factor in "The cytokine handbook". Academic Press, London, **2003**. 2: 837-860.
32) Kumar S. – Interleukin 1 family in *"The cytokine handbook"*. Academic Press, London, **2003**. 2: 735-745.
33) Soler-Rodriguez A.M., Zhang H. et Al. – Neutrophil activation by bacterial lipoprotein versus lipolysaccharide: differential requirements for serum and CD14. J. Immunol., **2000**. 164: 2674-2683.

34) Artursson K., Wallgren P., Alm G.V. – Appearance of interferon-α in serum and signs of reduced immune function in pigs after transport and installation in a fattening farm. Vet. Immunol. Immunopathol., **1989**. 23: 345-353.
35) Wrattang E., Wallgren P et Al. – Signs of infections and reduced immune functions at weaning of conventionally reared and specific pathogen free pigs. J. Vet. Med., **1998**. 45: 7-17.
36) Bocci V., Paulesu L. et Al. – The physiologic interferon response. VI. Interferon activity in human plasma after a meal and drinking. Lymphokine Res., **1985**. 4: 151-158.

Author's address

Dr. Massimo Amadori, MD Vet
– Laboratory of Immunoprophylaxis and Animal Comfortwelfare, Zooprophylactic Experimental Institute of Lombardy and Emilia-Romagna
Via A. Bianchi, 9
I – 25124 Brescia