Immunology 1987 **62** 149–151

Stimulation of neutrophil respiratory burst and lysosomal enzyme release by human interferon-gamma

I. C. KOWANKO & A. FERRANTE *University Department of Paediatrics and Department of Immunology, Adelaide Children's Hospital, North Adelaide, South Australia*

Accepted for publication 30 April 1987

SUMMARY

Highly purified human interferon-gamma (IFN-γ) induced a respiratory burst and lysosomal enzyme release in human neutrophils. The lymphokine augmented the respiratory burst induced by phorbol myristate acetate (PMA), or *N*-formyl-L-methionyl-L-leucyl-L-phenylanaline (fMLP), and increased the release of specific granule enzymes induced by opsonized zymozan. Viability and locomotion of neutrophils were unaffected by IFN-γ.

We have previously demonstrated that polyclonal activation of human T and B lymphocytes by mitogen or antigen results in the release of cytokines that stimulate the capacity of human neutrophils to kill microorganisms and tumour cells, and stimulate the neutrophil biochemical pathways that are involved in these processes (Ferrante & Mocatta, 1984; Ferrante & Abell, 1986; Ferrante *et al.*, 1987). Interaction between T-cell products and neutrophils may be important in the regulation of inflammation.

Limited studies have been conducted with the lymphokine IFN-γ. Although IFN-γ has been shown to enhance neutrophil antibody-dependent cellular cytotoxicity (ADCC) (Hokland & Berg, 1981; Shalaby *et al.*, 1985; Steinbeck, Roth & Kaeberle, 1986), its effect on the neutrophil oxygen-dependent respiratory burst, lysosomal enzyme release and locomotion is not clear. The purpose of our study was to examine the effects of IFN-γ on these three neutrophil functions.

Figure 1 shows the effects of a range of concentrations of highly purified human leucocyte-derived IFN-γ (Cellular Products Inc., Buffalo, NY; serial 10-fold dilutions of 2×10^4 U/ml stock) on the neutrophil respiratory burst, measured as release of hydrogen peroxide (H_2O_2). Neutrophils were isolated from human blood by the rapid single-step method (Ferrante & Thong, 1982) to $\geqslant 98\%$ purity. The H_2O_2 assay was carried out essentially as previously described using microtitre plates (Paton & Ferrante, 1983). IFN-γ and the stimulant (PMA, or its control) were added simultaneously to the cells with the other reagents for the assay. IFN-γ induced the release of H_2O_2 from neutrophils, and enhanced H_2O_2 production in PMA-stimulated cells in a dose-dependent manner.

The effects of IFN-γ on neutrophil respiratory burst were confirmed with the neutrophil chemiluminescence response. Chemiluminescence of neutrophils (5×10^5) in the presence or absence of 5×10^{-6} M fMLP in a 1-ml reaction mixture containing luminol (0·125 mg/ml) was measured continuously in a luminometer. The mean peak chemiluminescence of 'resting' neutrophils was 0·18 mV, and increased to 0·41 mV in the presence of 2000 U/ml of IFN-γ ($P < 0{\cdot}05$, four experiments). The fMLP-stimulated chemiluminescence was 9·7 mV in the absence and 10·4 mV in the presence of IFN-γ ($P < 0{\cdot}05$).

In contrast to our findings, Steinbeck *et al.* (1986) were unable to show an effect of recombinant bovine IFN-γ on the respiratory burst of bovine neutrophils. In their studies the cells were preincubated with IFN-γ for 2·5 hr. It is possible that a short-lived effect may have been missed because a time-dependent effect was not studied. It is also feasible that the differences between our results and those of Steinbeck *et al.* (1986) may be due to the lack of glycosylation of the recombinant material.

In further studies we examined the effect of IFN-γ on the release of lysosomal enzymes (lysozyme and β-glucuronidase) as previously described (Ferrante *et al.*, 1987) using 10^6 neutrophils with or without opsonized zymozan. The results presented in Table 1 show that lysozyme release was significantly increased in a dose-dependent manner in the presence of IFN-γ, both with and without opsonized zymosan. The effects on degranulation were not due to cell death since the lactate dehydrogenase (LDH) released was the same in either the presence or absence of IFN-γ (Table 1). IFN-γ did not enhance β-glucuronidase release (Table 1). The results suggest that IFN-γ stimulates release selectively from specific granules because lysozyme is present in both azurophilic and specific granules, and β-glucuronidase only in azuriphilic granules.

The effects of IFN-γ on neutrophil locomotion were also examined. In these experiments neutrophils were pretreated for 1 hr with a range of concentrations of IFN-γ (1–7500 U/10^6 cells or medium only). After incubation the cells were allowed to migrate under agarose in the presence or absence of a chemotac-

Correspondence: Dr I. C. Kowanko, University Dept. of Paediatrics and Dept. of Immunology, Adelaide Children's Hospital, North Adelaide, South Australia 5006.

tic gradient generated with 10^{-9} M fMLP (Ferrante *et al.*, 1987). The random migration distances of IFN-γ-treated neutrophils were 80–120% of control in six experiments, with no consistent dose–response pattern. Similarly, migration of neutrophils in a chemotactic gradient was unaffected by IFN-γ treatment. IFN-γ was neither chemotactic nor chemokinetic for neutrophils.

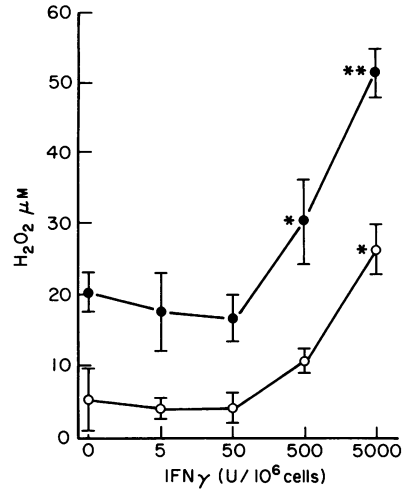

Figure 1. Effect of IFN-γ on neutrophil H_2O_2 release. Neutrophils (2×10^5 in 20 μl) were incubated with IFN-γ dilutions or control (50 μl) in the presence (closed circles) or absence (open circles) of 2×10^{-7} M PMA in microtitre wells with 10 μg horseradish peroxidase and 35 μg phenol red in 200 μl final volume of colourless Hanks' balanced salt solution. H_2O_2 release in 1 hr was measured as change in absorbance at 620 nm and expressed as concentration (μM) evolved relative to a standard curve set up with known amounts of H_2O_2. The mean and SD of seven experiments is shown. (* = $P < 0.05$; ** = $P < 0.01$.)

Table 1. Effect of IFN-γ on neutrophil enzyme release

IFN-γ (U/10^6 cells)		Lysozyme	β-glucuronidase	LDH
3000	Base	21·8±2·6	5·5±1·8	8·1±1·6
	Zym	40·8±6·4	15·9±8·3	8·4±3·0
300	Base	9·1±3·8	5·7±3·0	8·3±1·8
	Zym	26·4±8·0	14·4±6·4	8·6±2·6
30	Base	5·1±0·8	4·7±1·5	8·1±2·3
	Zym	23·2±5·4	12·9±6·3	6·7±0·9
3	Base	5·7±0·4	3·4±1·1	—
	Zym	20·4±4·1	17·7±5·7	—
0	Base	5·1±1·9**	4·7±0·9	6·2±0·6
	Zym	21·8±4·6*	13·6±6·5	7·0±1·4

Neutrophils (10^6 in 50 μl) were incubated for 1 hr with IFN-γ dilutions or control (150 μl) in the presence (Zym) or absence (Base) of 500 μg opsonized zymozan. Cell-free supernatants (from a 1-ml reaction mixture) were analysed for lysozyme, β-glucuronidase and LDH, and expressed as percentage of the total (triton-disrupted cells). The results are expressed as mean ± SD of three or four experiments.
(* = $P < 0.05$; ** = $P < 0.01$.)

In our studies IFN-γ had no effect on neutrophil migration. However, Seow & Thong (1986) showed increased adherence after brief exposure to recombinant IFN-γ. The difference in effects on adherence and migration is not surprising since these appear to be under independent control (Ferrante *et al.*, 1986). In contrast to our findings, Steinbeck *et al.* (1986) demonstrated migration inhibition with recombinant bovine IFN-γ. In these studies the pre-exposure of neutrophils to IFN-γ for 2·5 hr may have resulted in the release of other cytokines, either from contaminating mononuclear leucocytes (MNL) (Vadas *et al.*, 1984) or from neutrophils, which may be responsible for the migration inhibition properties.

We showed that IFN-γ modified neutrophil activities at concentrations of about 500–5000 U/10^6 cells/ml. These concentrations are physiologically relevant because they are similar to those measured in serum and synovial fluid of rheumatoid arthritis patients (Cesario *et al.*, 1983) and produced by MNL *in vitro* (Vervliet & Shandene, 1985). Indeed, higher concentrations would be expected in the micro-environment of interacting lymphocytes and neutrophils. It is interesting that other interferon species, IFN-β (Ferrante & Rencis, 1984) and IFN-α (Jarstrandt & Einhorn, 1983; Farr *et al.*, 1983) stimulated the respiratory burst of neutrophils at similar concentrations, although studies with IFN-α have produced conflicting results. Lower concentrations of IFN-γ have been reported to activate macrophages (Nathan *et al.*, 1983) and neutrophil ADCC (Steinbeck *et al.*, 1986). However, it is possible that these effects were in part due to other cytokines produced in response to IFN-γ during the long preincubation and assay times.

Our results clearly show that IFN-γ directly stimulates neutrophil respiratory burst and degranulation, but this phenomenon has not been adequately studied in macrophages. An increase in macrophage superoxide production in response to IFN-γ in the absence of other stimuli was observed by Cassatella *et al.* (1985). It has also been demonstrated that IFN-γ directly induces activation of protein kinase *C* (Becton, Adams & Hamilton, 1985), interleukin-1 secretion (Tweardy *et al.*, 1986) and 1,25-dihydroxy vitamin D3 production (Koeffler *et al.*, 1985) in macrophages.

The IFN-γ used in our studies was provided as a highly purified preparation produced by purified T lymphocytes stimulated with phytohaemagglutinin and purified by adsorption onto and elution from silica gel, and sequential chromatography of the eluate to remove contaminating proteins including interleukin-2 and B-cell growth factor. It is unlikely that other contaminant cytokines were responsible for the effects observed by us because of differences in their known effects on neutrophils. For example, interleukin-1 stimulates the release of azurophilic granule enzymes in the presence of a stimulus (A. Ferrante, M. Nandoskar, A. Walz, D. H. B. Goh and I. Kowanko, manuscript submitted for publication). Granulocyte–macrophage colony-stimulating factor and tumour necrosis factors (α and β) all have neutrophil-migration inhibition properties (Weisbart *et al.*, 1985; A. Ferrante, M. Nandoskar, A. Walz, D. H. B. Goh and I. Kowanko, manuscript submitted for publication; A. Ferrante, M. Nandoskar, D. H. Goh, B. E. J. Bates & L. J. Beard, manuscript submitted for publication). Since polymixin B at concentrations sufficient to abolish the biological activity of bacterial lipopolysaccharide (LPS) failed to prevent the IFN-γ-induced H_2O_2 release by neutrophils, it is unlikely that LPS is responsible for the activity.

This study, to our knowledge, is the first report showing that IFN-γ rapidly stimulates the neutrophil oxygen-dependent respiratory burst and specific granule enzyme release. This is one means by which T lymphocytes could modulate neutrophil function.

ACKNOWLEDGMENTS

This work was supported by grants from the Channel 10 Children's Medical Research Foundation, the National Health and Medical Research Council of Australia and the Arthritis Foundation of Australia (SA). We thank P. Harvey and M. Nandoskar for excellent technical assistance, and Dr E. J. Bates for helpful discussion.

REFERENCES

BECTON D.L., ADAMS D.O. & HAMILTON T.A. (1985) Characterization of protein kinase C activity in interferon gamma treated murine peritoneal macrophages. *J. Cell. Physiol.* **125,** 485.

CASSATELLA M.A., DELLA BIANCA V., BERTON G. & ROSSI F. (1985) Activation by gamma interferon of human macrophage capability to produce toxic oxygen molecules is accompanied by decreased Km of the superoxide-generating NADPH oxidase. *Biochem. biophys. Res. Commun.* **132,** 908.

CESARIO T.C., ANDREWS B.S., MARTIN D.A., JASON M., TREADWELL T., FRIOU G. & TILLES J.G. (1983) Interferon in synovial fluid and serum of patients with rheumatic disease. *J. Rheumatol.* **10,** 6470.

FARR B., GWALTNEY J.M., HAYDEN F.G. & MANDELL G.P. (1983) Human polymorphonuclear neutrophil functions are unaffected by human interferon-α_2. *Infect. Immun.* **42,** 1195.

FERRANTE A. & ABELL T.J. (1986) Conditioned medium from stimulated mononuclear leukocytes augments human neutrophil-mediated killing of a virulent *Acanthamoeba* sp. *Infect. Immun.* **51,** 607.

FERRANTE A. & MOCATTA T.J. (1984) Human neutrophils require activation by mononuclear leukocyte conditioned medium to kill the pathogenic free-living amoeba, *Naegleria fowleri. Clin. exp. Immunol.* **56,** 559.

FERRANTE A., NANDOSKAR M., BATES E.J. & GOH D.H.B. (1987) *Staphylococcus aureus*-stimulated human mononuclear leucocytes-conditioned medium augments the basal and stimuli-induced neutrophil respiratory burst and degranulation. *Immunology,* **60,** 431.

FERRANTE A. & RENCIS V.O. (1984) Enhancement of base hexose-monophosphate shunt activity of human polymorphonuclear leucocytes by human β-interferon. *Immunol. Lett.* **8,** 215.

FERRANTE A., ROWAN-KELLY B., SEOW W.K. & THONG Y.H. (1986) Depression of human polymorphonuclear leucocyte function by antimalarial drugs. *Immunology,* **58,** 125.

FERRANTE A. & THONG Y.H. (1982) Separation of mononuclear and polymorphonuclear leucocytes from human blood by the one-step hypaque-ficoll method is dependent on blood column height. *J. immunol. Meth.* **48,** 81.

HOKLAND P. & BERG K. (1981) Interferon enhances the antibody-dependent cellular cytotoxicity (ADCC) of human polymorphonuclear leukocytes. *J. Immunol.* **127,** 1585.

JARSTRANDT C. & EINHORN S. (1983) Effect of interferon on human neutrophilic granules. *Cancer Immunol. Immunother.* **16,** 123.

KOEFFLER H.P., REICHEL H., BISHOP J.E. & NORMAN A.W. (1985) Gamma-interferon stimulates production of 1.25-dihydroxy vitamin D3 by normal human macrophages. *Biochem. biophys. Res. Commun.* **127,** 596.

NATHAN C.F., MURRAY H.W., WIEBE M.E. & RUBIN B.Y. (1983) Identification of interferon-γ as the lymphokine that activates human macrophage oxidative metabolism and antimicrobial activity. *J. exp. Med.* **158,** 670.

PATON J.C. & FERRANTE A. (1983) Inhibition of human polymorphonuclear leucocyte respiratory burst, bactericidal activity, and migration by pneumolysin. *Infect. Immun.* **41,** 1212.

SEOW W.H. & THONG Y.H. (1986) Augmentation of human polymorphonuclear leukocyte adherence by interferon. *Int. Arch. Allergy appl. Immunol.* **79,** 305.

SHALABY M.R., AGGARWAL B.B., RINDERKNECHT E., SVEDERSKY L.P. & PALLADINO M.A. (1985) Activation of human polymorphonuclear neutrophil functions by interferon-γ and tumour necrosis factors. *J. Immunol.* **135,** 2069.

STEINBECK M.J., ROTH J.A. & KAEBERLE M.L. (1986) Activation of bovine neutrophils by recombinant interferon-γ. *Cell. Immunol.* **98,** 137.

TWEARDY D.J., FUJIWARA H., SCILLIAN J.J. & ELLNER J.J. (1986) Concurrent enhancement of monocyte immunoregulatory properties and effector functions by recombinant interferon-gamma. *Cell. Immunol.* **100,** 34.

VADAS M.A., NICOLA N., LOPEZ A.F., METCALF D., JOHNSON G. & PEREIRA A. (1984) Mononuclear cell-mediated enhancement of granulocyte function in man. *J. Immunol.* **133,** 202.

VERVLIET G. & SHANDENE L. (1985) *In vitro* correction of the interleukin-2 and interferon-gamma defect in multiple sclerosis. *Clin. exp. Immunol.* **61,** 556.

WEISBART R.H., GOLDE D.W., CLARK S.C., WONG G.G. & GASSON J.C. (1985) Human granulocyte–macrophage colony-stimulating factor is a neutrophil activator. *Nature (Lond.),* **314,** 361.