Int. J. Exp. Path. (1995), **76**, 331–337

Differential effects of interferon-γ and -β on fatty acid turnover, lipid bilayer fluidity and TNF-α release in murine macrophage J774.2 cells

H. DARMANI*, J.L. HARWOOD† AND S.K. JACKSON*

*Department of *Medical Microbiology, University of Wales College of Medicine, Cardiff CF4 4XN and †Department of Biochemistry, University of Wales College of Cardiff, Cardiff CF1 1ST, UK*

Received for publication 7 October 1994
Accepted for publication 7 June 1995

Summary. The effects of interferon (IFN)-γ and IFN-β on the incorporation of ^{14}C-linoleic acid into J774.2 cell membrane phospholipids were examined. Interferon-γ induced a statistically significant increase in incorporation of ^{14}C-linoleic acid into all the major phospholipid classes. In contrast, IFN-β induced a slightly reduced incorporation of this fatty acid into the phospholipids. Neither IFN-γ nor IFN-β had any effect on the incorporation of the saturated fatty acid ^{14}C-stearic acid into the cellular phospholipids. Interferon-γ had no effect on the metabolism of ^{14}C-linoleic acid in the fibroblast cell line L929. Macrophage membrane fluidity was assessed by spin-label ESR spectroscopy after incubation with either IFN-γ or IFN-β. Interferon-γ significantly increased membrane fluidity whereas IFN-β significantly decreased the fluidity. The findings of this study reveal that IFN-γ might act on the enzymes controlling the labelling of the *sn*2 position of phospholipids (linoleic acid) but not the *sn*1 position (stearic acid), and this increases the polyunsaturated fatty acid content of macrophage membranes. This increase in polyunsaturation is reflected in the increased membrane fluidity. We also conclude that IFN-β and INF-γ have different mechanisms of action on macrophage membrane lipid metabolism.

Keywords: LPS, macrophage, interferons, phospholipids, fatty acid metabolism

Bacterial lipopolysaccharide (LPS), a major constituent of the outer membrane of Gram-negative bacteria, plays an important role in the pathological manifestation of septic shock (Morrison & Ryan 1987). Development of heightened sensitivity to the lethal effects of LPS in some patients still remains unexplained, although animal models have shown that concomitant bacterial, viral or fungal infections greatly increase patient's susceptibility (Suter & Kirsanow 1961). Interferon (IFN)-γ has been identified as one of the key mediators of such infection induced sensitization to LPS (Galanos & Freudenberg 1993). The important role that IFN-γ plays in LPS hypersensitivity is demonstrated by the inability to induce sensitization to LPS in LPS-resistant C57BL/10ScCr mice which has been linked with the impairment of the production of IFN-γ in these mice (Galanos & Freudenberg 1993). Furthermore, recent experiments in mice with a targeted disruption of the

Correspondence: Dr S.K. Jackson, Department of Medical Microbiology, University of Wales College of Medicine, Cardiff, CF4 4XN, UK.

interferon-γ receptor, showed that after BCG injection, these animals were resistant to doses of LPS which were lethal in the receptor-positive wild-type strains (Kamijo *et al.* 1993).

Our recent work suggests that IFN-γ may change LPS sensitivity through altering the lipid metabolism of macrophage cells (Darmani *et al.* 1993). Interferon-γ induces increased unsaturation of the phosphatidylethanolamine (PE) fraction of the membrane phospholipids which, we believe, predisposes to LPS sensitivity (Jackson *et al.* 1992). The increased sensitivity is proposed to be a result of the consequent changes in the biophysical and biochemical properties of the activated cells which lead to enhanced binding of LPS to these activated cells (Darmani *et al.* 1994). Enhanced binding of LPS to macrophage cells has also been demonstrated after increasng unsaturation with exogenously supplied polyunsaturated fatty acids, namely linoleic and arachidonic acids, to mimic changes which may play an intimate role in LPS–macrophage interactions (Darmani *et al.* 1994). Control experiments with a saturated fatty acid showed no increase in LPS–macrophage interaction (Darmani *et al.* 1994).

In this study we examine the effects of IFN-β on the incorporation of radiolabelled saturated and unsaturated fatty acids, the induction of TNF-α production and the fluidity of the membrane lipid bilayer of the murine macrophage cell line J774.2 and compare them with the effects of the key sensitization mediator IFN-γ.

Materials and methods

Reagents

All reagents were purchased from Sigma Chemical Company Ltd, Poole, UK, unless otherwise stated. ^{14}C-Linoleic and stearic acids (both at 50 μCi/μmol) were supplied by Amersham International Plc, Aylesbury, UK. Interferon-γ was purchased from Genzyme, Kent, UK. Interferon-β and 5-doxyl stearic acid were purchased from Sigma Chemical company.

Cell culture

Cells were cultivated in Dulbecco's modification of Eagle's medium (DMEM) which had been supplemented with 10% fetal calf serum (FCS), 100 units/ml penicillin and 100 μg/ml streptomycin.

The murine (BALB/C) tumour monocyte-macrophage J774.2 (European Collection of Animal Cell Cultures, Salisbury, Wilts., UK), was the cell line used in the course of this investigation. These cells were maintained in DMEM supplemented as described above.

Pretreatment with IFN-γ and INF-β

Exposure of J774.2 cells (1×10^6) to murine recombinant IFN-γ and INF-β (50 U/ml) was carried out for 18 hours in a 37°C, humidified 5% CO_2 incubator. Dose–response experiments (1–1000 U/ml) showed that 50 U/ml of recombinant interferon was optimal in several cellular responses (TNF-α release, LPS binding, linoleic acid incorporation) for the cells used. At the end of the incubation period the cells were dislodged by gentle agitation, washed three times with DMEM and collected by centrifugation at 1000 ***g*** for 5 minutes, and finally resuspended in DMEM.

Preparation of ammonium salts of radiolabelled fatty acids

To aid solubility, the radiolabelled fatty acids were converted into their ammonium salts as follows. The fatty acids were supplied in either ethanol or toluene and as a first stage the solvent was evaporated under a stream of nitrogen. The fatty acids were then suspended in 0.2 ml of 2 M ammonia solution at 60–70°C for 30 minutes. The solution was evaporated to dryness under a stream of nitrogen and the resulting ammonium salt was resuspended in a known volume of growth medium.

Incorporation of ^{14}C-fatty acids

Cells were incubated in the presence of 0.2 μCi of the radiolabelled fatty acids (ammonium salt) with or without LPS, IFN-γ and IFN-β for 18 hours at 37°C in a humidified CO_2 incubator. After the appropriate incubation time, the cells were centrifuged at 1000 ***g*** for 3 minutes, washed in 10 ml PBS and collected by centrifugation at 1000 ***g*** for 3 minutes, and finally resuspended in 1 ml deionized water and sonicated in an ultrasonic water bath at maximum power. Once complete lysis of the cells had been achieved (verified by light microscopy) the cells were processed for phospholipid extraction.

Extraction of phospholipids

The method of Garbus *et al.* (1963) was used. To 1 ml of the lysed cell suspension 3.75 ml chloroform/methanol (1 : 2 v/v) was added, mixed thoroughly and left at room temperature for 30 minutes. Then chloroform (1.25 ml)

and 2 M KCl in 0.5 M KH_2PO_4 buffer, pH 7.4 (1.25 ml) were added and the solution mixed thoroughly again. The chloroform phase containing the extracted phospholipids was dried down in a stream of nitrogen and then subjected to thin layer chromatography.

Thin layer chromatography

The dried phospholipids were dissolved in 30 μl chloroform, spotted onto silica gel G plates (BDH) and chromatographed in a solvent system consisting of chloroform : methanol : acetic acid : water (50 : 30 : 8 : 1, by volume). Phospholipid standards were chromatographed on separate lanes. The resolved radiolabelled phospholipids were located by exposure to iodine vapour, and the appropriate areas scraped off into scintillation vials for radioactivity determination.

Labelling of cell membranes for spin-label ESR spectroscopy

Control, IFN-γ and IFN-β (18 h at 37°) pretreated cells were washed with PBS and collected by centrifugation at 1000 ***g*** for 5 minutes three times before being finally resuspended in 1 ml of the buffer. The cell suspensions were incubated at room temperature for 20 minutes in the presence of 10 μl of 20 mg/ml doxyl stearic acid (in ethanol) (final ethanol concentration <1%). The spin-labelled cells were washed three times in PBS by centrifugation at 1000 ***g*** for 5 minutes in order to remove any unbound label. Samples for electron spin resonance (ESR) spectroscopy were placed into 1 mm internal diameter tubes and analysed by ESR spectroscopy.

ESR measurements

All spectra were recorded at room temperature on a Varian E104 ESR spectrometer operating at 9.3 GHz at 3300 G field and 10 mW microwave power.

Membrane fluidity was measured using the empirical order parameter, *S*, which was derived from the following equation:

$$S = (A^{\parallel} - A^{\perp})/(A_{zz} - A_{xx})$$

where $A^{\parallel}$ is half the distance between the outer hyperfine lines and $A^{\perp}$ is half the distance of the inner hyperfine splittings. $A_{zz} - A_{xx}$ was taken as 25 G (McConnell & McFarland 1970), and corrections for polarity changes were not used.

The $2A^{\parallel}$ value is used to estimate the freedom of motion of the spin-label and therefore to determine the rigidity of the membrane in which it is located (Hubbell & McConnell 1971). From the equation above it can be seen that an increase in $2A^{\parallel}$ will result in an increase in the order parameter (*S*) and thus reflect an increased rigidity in the membrane lipids.

Determination of TNF-α release

J774.2 cells (1×10^6) were pre-incubated in the presence or absence of IFN-γ or IFN-β (50 U/ml) for 18 hours at 37°C in complete medium. The cells were then washed in PBS and LPS (100 ng/ml) was added and the cells incubated for a further 3 hours at 37°C. Following this incubation, the supernatants were removed, centrifuged briefly in a microfuge and stored at −80°C for TNF assay. The production of TNF-α by J774.2 cells was determined in the culture supernates by an ELISA assay for TNF-α (Factor-Test-X; Genzyme Diagnostics, West Malling, Kent). The assay was performed as per the manufacturer's instructions.

Statistical analyses

Most experiments were performed in triplicate on at least 3 separate occasions. Statistical significance was estimated with Student's *t*-test.

Results

Uptake of ^{14}C-linoleate and ^{14}C-stearate into the phosphatidylcholine (PC), phosphatidylethanolamine (PE), phosphatidylinositol (PI) and the neutral lipid (NL) fractions from macrophages was followed after pretreatment with IFN-γ or IFN-β.

Figure 1a shows that IFN-γ induces a statistically significant increase in the incorporation of ^{14}C-linoleic acid into the PC, PE and NL ($P < 0.05$ compared with control cells) fractions of the macrophages. Interferon-γ induced a 31% increase in uptake of linoleic acid into the PC fraction, 22% increase into the PI fraction, 31% increase into the PE fraction and 51% increase into the NL fraction. These results are in contrast to the effects of IFN-β on uptake of linoleic acid, as shown in Figure 1b. Interferon-β pretreatment of cells results in a slightly decreased incorporation of ^{14}C-linoleic acid into all phospholipids and also into the neutral lipids.

The uptake and incorporation of ^{14}C-stearate, a saturated fatty acid, into lipid fractions from macrophages was followed to see whether the changes previously seen were specific to the polyunsaturated fatty acid linoleate. The results shown in Figure 2 revealed that IFN-γ pretreatment did not significantly increase the

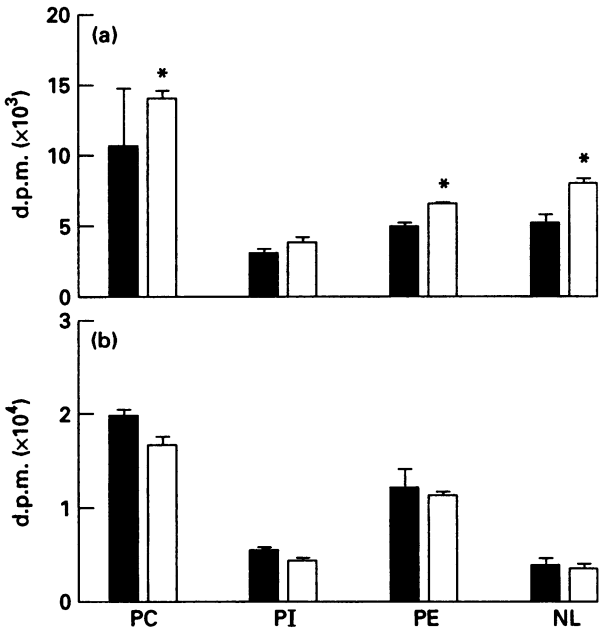

Figure 1. The effects of a, □, IFN-γ and b, □, IFN-β on the uptake of ^{14}C-linoleic acid into the PC, PI, PE and NL fractions of J774.2 cell membrane phospholipids. Cells were incubated with 0.2 μCi of ^{14}C-linoleic acid for 18 hours at 37°C. ■, Control cells. Error bars represent ± standard error of the means from 3 separate experiments. *$P < 0.05$.

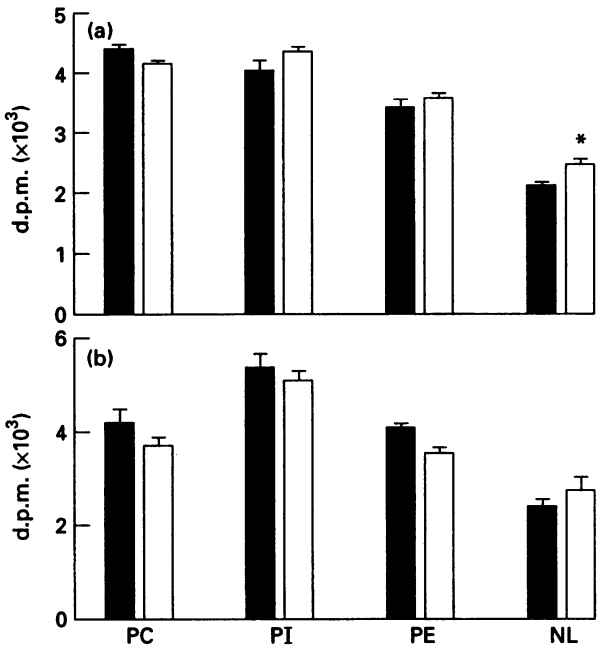

Figure 2. The effects of a, □, IFN-γ and b, □, IFN-β on the uptake of ^{14}C-stearic acid into the PC, PI, PE and NL fractions of J774.2 cell membrane phospholipids. Cells were incubated with 0.2 μCi of ^{14}C-stearic acid for 18 hours at 37°C. ■, Control cells. Error bars represent ± standard error of the means from 3 separate experiments. *$P < 0.05$.

labelling of any phospholipid fraction, although an increase was observed in the labelling of the NL fractions. Thus, the action of IFN-γ was not general for any exogenous fatty acid. Interferon-β pretreatment had little effect on the labelling by ^{14}C-stearate of any fraction either.

Figure 3 shows the uptake and incorporation of linoleic acid into the PC, PI, PE and NL fractions of L929 cells (a murine fibroblast cell line which is a subclone of the parental strain L, derived from normal subcutaneous areolar and adipose tissue). In contrast to the stimulatory effects of IFN-γ on lipid uptake and turnover in the macrophage J774.2 cells, IFN-γ did not affect phospholipid metabolism in the non-macrophage L929 cells.

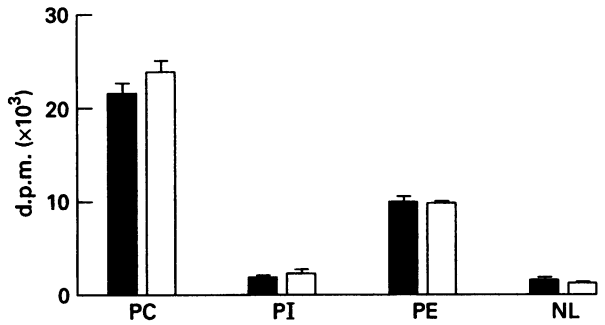

Figure 3. The effect of □, IFN-γ on the uptake of ^{14}C-linoleic acid into the PC, PI, PE and NL fractions of L929 cell membrane phospholipids. Cells were incubated with 0.2 μCi of ^{14}C-linoleic acid for 18 h at 37°C. ■, Control cells. Error bars represent ± standard error of the means of 3 separate experiments.

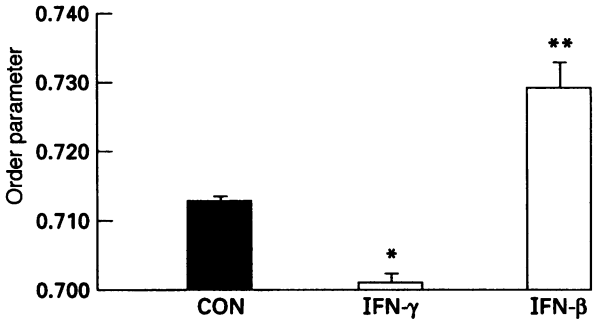

Figure 4. Changes in order parameter (obtained from ESR spectra) for control cells and cells treated with IFN-γ and IFN-β. Cells were labelled with the spin-probe, 5-doxyl stearate, at room temperature for 20 minutes. Error bars represent ± standard error of the means of five separate experiments. *$P < 0.05$, **$P < 0.01$.

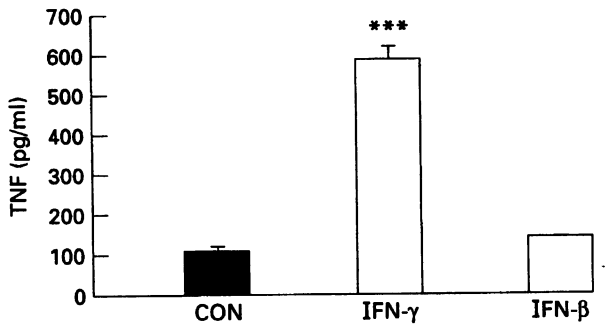

Figure 5. The release of TNF-α from control cells and cells treated with IFN-γ and IFN-β after stimulation with LPS (100 ng/ml). TNF-α levels were determined by mouse TNF-α ELISA. Error bars represent ± standard error of the means from 3 separate experiments. ***$P < 0.001$.

Figure 4 shows the effects of IFN-γ and IFN-β on membrane fluidity. A spin-label, 5-doxyl stearate, was incorporated into the plasma membrane lipid bilayer of intact cells to examine the effects of IFN-γ and IFN-β on the fluidity of the lipid bilayer of these cells. Electron spin resonance was used to measure the freedom of motion of the spin-label probe, thus providing a measure of the membrane rigidity. Experiments with ascorbate reduction of the 5-doxyl stearic acid suggested that, over the time course of the experiment, the spin-label was located predominantly in the plasma membrane (results not shown).

The changes in order parameter, calculated from the measured *A* values in the ESR spectra obtained from control cells and cells treated with IFN-γ and IFN-β, are shown in Figure 4. Interferon-γ pretreated cells labelled with 5-doxyl stearate showed a significant decrease in order parameter ($P < 0.05$ *vs* control cells). This would correlate with a significant change in membrane composition and indicates that the spin-label probe is in a significantly more fluid environment. Thus, the increased freedom of motion of the spin-label within the plasma membrane of IFN-γ treated cells indicates an increase in the effective fluidity of the plasma membrane lipid bilayer. In contrast, IFN-β-pretreated cells labelled with 5-doxyl stearate showed a significant increase in order parameter ($P < 0.01$) indicating that the spin-label probe is in a significantly more rigid environment. The decreased freedom of motion of the spin-label probe within the plasma membrane of IFN-β treated cells indicates a decrease in the effective fluidity of the plasma membrane lipid bilayer.

Figure 5 shows the effects of IFN-γ and IFN-β priming on the release of TNF-α from LPS-stimulated J774.2 cells. Interferon-γ pretreatment induced a significant increase in the release of TNF-α from these macrophage cells ($P < 0.001$ *vs* control cells). This is in contrast to the effects of pretreatment of cells with IFN-β, which did not increase TNF-α production.

Discussion

We have previously reported that IFN-γ may exert its effects on macrophage cells through increasing the polyunsaturation of the fatty acyl side-chains of membrane phospholipids (Jackson *et al.* 1992) which may be important in the interaction of macrophage-like cells with endotoxin (Darmani *et al.* 1994). We have shown in this study that, unlike IFN-γ, which specifically increases the uptake of the polyunsaturated fatty acid linoleic acid into membrane phospholipids, IFN-β pretreatment induced slightly decreased incorporation of this fatty acid into all phospholipids.

The results of the spin-label ESR spectroscopy revealed that in contrast to IFN-γ, IFN-β induces a significant decrease in membrane lipid bilayer fluidity of J774.2 cells. Changes in membrane fluidity may result from altered lipid composition of cell membranes or altered protein composition or both. Interferon-β has been reported to induce an increase in the saturated fatty acid content of cellular lipids of mouse sarcoma S-180 cells (Chandrabose & Cuatrecasas 1981), which was associated with decreased membrane fluidity. In our experiments there was a decreased incorporation of linoleate into macrophage membranes, but this was not statistically significant. We did not measure total lipid composition and therefore it is possible that IFN-β had effects on the fatty acid composition of macrophages or altered protein expression which could contribute to the altered membrane fluidity.

Interferon-γ can upregulate a variety of immune functions in the immunocompromised host, yet it also induces harmful effects by sensitizing immunocompetent animals to subclinical doses of endotoxin (Williams *et al.* 1992). Although the interferons share many biological functions, IFN-γ is a much stronger activator of monocyte and macrophage cells. Differences between IFN-γ and IFN-β have been reported in their action to modulate macrophage early gene expression (Hamilton *et al.* 1989). Interferon-γ and IFN-β act through the generation of overlapping but non-identical mechanisms to stimulate the expression of LPS inducible genes in murine peritoneal macrophages (Hamilton *et al.* 1989). Furthermore, IFN-γ has been reported to inhibit the LPS induced desensitization of a human monocyte line, an effect which was not mediated by IFN-α or IFN-β (Haas *et al.* 1990).

Interferon-γ has been identified as a mediator of

endotoxin hypersensitivity in *Propionibacterium acnes* induced LPS hypersensitivity in mice (Katschinski *et al.* 1992) and human monocytes have been shown to express heightened responses to bacterial LPS (Hayes & Zoon 1993) as a result of IFN-γ priming. Furthermore, IFN-γ has been reported to be a key element in the complex process whereby BCG infection leads to sensitization to endotoxin (Kamijo *et al.* 1993). BCG infection was found to be lethal in mice with a targeted disruption of the IFN-γ receptor gene and this clearly illustrates the critical role of IFN-γ in the ability of normal mice to contain infection with the BCG strain of *M. bovis* (Kamijo *et al.* 1993). We believe that, at least in part, IFN-γ primes macrophages to be hyper-responsive to triggering agents such as LPS through changes in the phospholipid fatty acid turnover and composition. Interferon-γ increased the metabolism of ^{14}C-linoleic acid which may be metabolized via the lipoxygenase pathway to form hydroxylinoleic acids. Leukotrienes are thought to play a very important role in controlling TNF production (Schade 1986). There is now increasing evidence that hydroxylinoleic acid derivatives are also of great importance in this regard (Schade *et al.* 1992). We have shown in this study that TNF-α production is increased to a much greater degree by IFN-γ than by IFN-β pretreatment.

Recently, CD14 has emerged as an important receptor for LPS, particularly when complexed to serum proteins such as lipopolysaccharide binding protein (LBP) (Wright *et al.* 1990). The regulation of CD14 expression is not fully understood although IFN-γ has been reported to up-regulate it on immature macrophage cells and cell lines but down-regulate it on more mature cell lines (Ziegler-Heitbrock & Ulevitch 1993). Little is known about the transcription factors involved in CD14 expression or of its control at the transcriptional or protein levels. However, in addition to control at the gene activation level, it is possible that changes in lipid–protein interactions or activation of membrane proteins through alterations in phospholipid metabolism could also influence CD14 expression. In support of this, IFN-γ was shown to increase macrophage membrane fluidity which might allow greater mobility of membrane receptors. Moreover, we have recently demonstrated enhanced binding of fluoresceine isothiocyanate labelled LPS to IFN-γ, but not to IFN-β, pretreated cells (Darmani *et al.* 1994).

Interferon-γ has been reported to induce a rapid transient activation of phospholipase A_2 (PLA_2) in LAN-5, a human neuroblastoma cell line (Ponzoni *et al.* 1992). A consequence of this was the release of arachidonic acid and the generation of lysophospholipids from membrane phospholipids. The release of fatty acids after cell stimulation is followed by increased acyltransferase activities and this protects the cell membrane from the accumulation of lysophospholipids and free fatty acids (Weltzien 1979; Baba *et al.* 1984). Interferon-γ-induced stimulation of PLA_2 and arachidonic acid metabolism may explain some of the biomolecular mechanisms that mediate signal transduction by IFN-γ (Ponzoni *et al.* 1992). Interferon-γ was previously observed to increase ^{14}C-linoleic acid incorporation into macrophage phospholipids while ^{14}C-arachidonic acid was increased to a lesser extent (Darmani *et al.* 1993). This may be due to the more rapid turnover of arachidonic acid into eicosanoids. Furthermore, our results suggest that IFN-γ can stimulate polyunsaturated fatty acids which are incorporated into the *sn*-2 position of phospholipids, while fatty acids, such as stearic acid, which are esterified predominantly in the *sn*-1 position, were not incorporated. This would indicate that IFN-γ can act specifically to increase the enzymes which deacylate and reacylate the *sn*-2 position.

The results of this study and our previous reports (Darmani *et al.* 1993) indicate that the incorporation of linoleic acid induced by IFN-γ might be expressed only in macrophages or other phagocytic cells. In support of this, IFN-γ failed to alter linoleic acid incorporation into the fibroblast cell line L929. In contrast, IFN-β has been shown to alter phospholipid incorporation in several cell types (Pfeffer *et al.* 1985) but not in macrophages.

The precise mechanism by which endotoxin induces human septic shock remains unknown, but endotoxin is a major mediator of the morbidity and mortality which attends Gram-negative sepsis. The endotoxin molecule has been likened to a 'triggering mediator' which can result in both the release of other directly acting molecules like TNF and in the activation of cellular mechanisms. Further research into the way in which cytokines activate macrophage cells to become hyper-responsive to triggering agents such as endotoxin will enable us to improve the clinical management of patients with this potentially fatal condition.

Acknowledgement

We would like to thank the Medical Research Council (UK) for the award of a grant.

References

Baba A., Tatsuno T. & Iwata H. (1984) Modulation by unsaturated fatty acids of norepinephrine- and adenosine-induced formation of cyclic AMP in brain slices. *J. Neurochem.* **42,** 192–197.

Chandrabose K. & Cuatrecasas P. (1981) Changes in fatty acyl chains of phospholipids induced by interferon in mouse sarcoma S-180 cells. *Biochim. Biophys. Res. Commun.* **98,** 661–668.

Darmani H., Jackson S.K. & Harwood J.L. (1993) Stimulation of phospholipid unsaturated acyl turnover by interferon-γ. *Cellular Immunol.* **152,** 59–71.

Darmani H., Harwood J.L. & Jackson S.K. (1994) Interferon-γ and PUFA increase the binding of LPS to macrophages. *Int. J. Exp. Path.* **75,** 363–368.

Galanos C. & Freudenberg M.A. (1993) Mechanisms of endotoxin shock and endotoxin hypersensitivity. *Immunobiology* **187,** 346–356.

Garbus J., De Luca H.F., Loomans M.E. & Strong F.M. (1963) The rapid incorporation of phosphate into mitochondrial lipids. *J. Biol. Chem.* **238,** 59–63.

Haas J.G., Meyer N., Riethmuller G. & Ziegler-Heitbrock H.W.L. (1990). Inhibition of LPS-induced *in vitro* desensitisation by interferon-γ. *Eur. J. Immunol.* **20,** 1181–1184.

Hamilton T.A., Bredon N., Yoshihiro O. & Tannenbaum C.S. (1989) IFN-γ and IFN-β independently stimulate the expression of lipopolysaccharide-inducible genes in murine peritoneal macrophages. *J. Immunol.* **142**(7), 2325–2331.

Hayes M.P. & Zoon K.C. (1993) Priming of human monocytes for enhanced lipopolysaccharide response expression of alpha interferon, interferon regulatory factors, and tumor necrosis factor. *Infection and Immunity.* **61**(8), 3222–3227.

Hubbell W.L. & McConnell H.M. (1971) Molecular motion in spin-labelled phospholipids and membranes. *J. Am. Chem. Soc.* **93,** 314–326.

Jackson S.K., Darmani H., Stark J.M. & Harwood J.L. (1992) Interferon-γ increases macrophage phospholipid poly-unsaturation: A mechanism of endotoxin sensitivity. *Int. J. Exp. Path.* **73,** 783–791.

Kamijo R., Le J., Shapiro D., Havell E.A., Huang S., Aguet M., Bosland M., & Vilcek J. (1993) Mice that lack the interferon-γ receptor have profoundly altered responses to infection with BCG and subsequent challenge with lipopolysaccharide. *J. Exp. Med.* **178,** 1435–1440.

Katschinski T., Galanos C., Coumbos A. & Freudenberg M.A. (1992) Gamma interferon mediates *Propionibacterium acnes*-induced hypersensitivity to lipopolysaccharide in mice. *Infection and Immunity.* **60,** 1994–2001.

McConnell H.M. & McFarland B.G. (1970) Physics and chemistry of spin-labels. *Quart. Rev. Biophys.* **3,** 91–136.

Morrison D.C. & Ryan J.L. (1987) Endotoxins and disease mechanisms. *Annu. Rev. Med.* **38,** 417–432.

Pfeffer L.M., Kwok B.C.P., Landsberger F.R. & Tamm I. (1985) Interferon stimulates cholesterol and phosphatidylcholine synthesis but inhibits cholesterol ester synthesis in HeLa-S3 cells. *Proc. Natl Acad. Sci. USA* **82,** 2417–2421.

Ponzoni M., Montaldo P.G. & Cornagliaferraris P. (1992) Stimulation of receptor-coupled phospholipase A2 by interferon-gamma.

Schade U.F. (1986) Involvement of lipoxygenase in the activation of mouse macrophages by endotoxin. *Biochem. Biophys. Res. Commun.* **138,** 842–849.

Schade U.F., Engel R., Wolter D. & Jacobs D. (1992) *Abstracts of the 2nd Conference of the international Endotoxin Society,* Vienna, Austria. abstr. 21.

Suter E. & Kirsanow E.M. (1961) Hyperreactivity to endotoxin in mice infected with mycobacteria. Induction of and elicitation of the reactions. *Immunology* **4,** 354–365.

Weltzien H.V. (1979) Cytosolic and membrane perturbing properties of lysophosphatidylcholine. *Biochem. Biophys. Acta* **559,** 259–287.

Williams J.G., Jurkovitch G.J., Hahnel G.B. & Maier R.V. (1992) Macrophage priming by interferon gamma: a selective process with potentially harmful effects. *J Leuk. Biol.* **52,** 579–584.

Wright S.D., Ramos R.A., Tobias P.S., Ulevitch R.J., Mathison J.C. (1990) CD14, a receptor for complexes of lipopolysaccharide (LPS) and LPS binding protein. *Science* **249,** 1431–1433.

Ziegler-Heitbrock H.W.L. & Ulevitch R.J. (1993) CD14: Cell surface receptor and differentiation marker. *Immunol. Today* **14,** 121–125.