

Prostaglandin and fatty acid modulation of *Escherichia coli* O157 phagocytosis by human monocytic cells

J. DAVIDSON, A. KERR, K. GUY & D. ROTONDO *Department of Immunology, University of Strathclyde, Glasgow, UK*

SUMMARY

Phagocytosis by human monocytes is an important primary survival mechanism particularly during bacterial infection. However, the processes that control the events and mediators involved in the activation of monocytes and their impact on the phagocytosis of bacteria are poorly understood. The effect of bacterial endotoxin, interleukin-1 β (IL-1 β), fatty acids and prostaglandin E₂ (PGE₂) on the phagocytosis of fluorescein isothiocyanate (FITC)-labelled *Escherichia coli* (O157) by human blood monocytes and U937 cells was studied by flow cytometry. Endotoxin increased the phagocytosis of labelled bacteria by both monocytes and U937 cells. IL-1 β and the polyunsaturated fatty acids; dihomo- γ -linolenic and arachidonic acids also increased the phagocytic activity of both monocytes and U937 cells. In contrast, PGE₂ suppressed phagocytosis in a concentration-dependent manner. The cyclo-oxygenase inhibitor, ketoprofen, further enhanced the increased phagocytic activity in the presence of endotoxin and interleukin-1 (IL-1) indicating suppression by endogenous prostaglandins. This was confirmed by the data which showed that lipopolysaccharide (LPS) and IL-1 increased PGE₂ release and ketoprofen inhibited release. Endotoxin and fatty acids increased IL-1 β release also, whereas PGE₂ inhibited release. The data suggest that phagocytic activity may be linked to changes in IL-1 levels. The data presented in this study also suggest that monocyte phagocytosis in the course of bacterial infection would be altered during pathophysiological events which result in elevation of extracellular fatty acids.

INTRODUCTION

The ability of blood monocytes and macrophages to phagocytose bacteria and subsequently kill them is an important primary survival function of these cells as an immediate first line defence mechanism. Phagocytic uptake of pathogenic bacteria by monocytes or tissue macrophages appears to be the critical first step in the killing of these bacteria. This view is strengthened by the observation that there is an increased morbidity and mortality following infection with organisms that can evade phagocytosis such as some strains of *Streptococcus pneumoniae*.¹ It is not, however, certain whether the pathogenicity of other bacterial strains correlates with the modulation of phagocytic activity. Following phagocytosis the bacteria are lysed within phagolysosomes with the release of a variety of products including bioactive lipids such as arachidonic acid,² although the effect of lipids released during lysis on subsequent phagocytosis is not clearly understood. Phagocytic cells will phagocytose most particulate matter in the course of their scavenger role but the most effective substrates for phagocytosis are particles of microbial origin such as bacteria and fungi. Contact with the bacterial cell

surface or endotoxin (lipopolysaccharide) released from the bacteria activates monocytes and/or macrophages initiating the acute phase immune response. Systemically this results in fever, the major manifestation of the acute phase response, and in localized regions, culminates in inflammatory responses. Both the systemic and localized effects of bacteria are thought to be mediated by the endogenous production of cytokines, primarily interleukin-1 (IL-1). IL-1 has been shown to sequentially stimulate the release of eicosanoids such as prostaglandin E₂ (PGE₂), which is synthesized directly from arachidonic acid liberated via activation of lipases, including phospholipase A₂.^{3,4} The formation of PGE₂ is particularly important in this sequence of activation responses as the progression of the cascade is limited by PGE₂ which is produced at a later stage after a sufficient activation has already occurred. However, the role of PGE₂ in the modulation of the phagocytic activity of monocytic cells is poorly understood, as is the effect of fatty acids especially those that are immediate precursors for prostanoid formation such as arachidonic acid. This is particularly important in the context of phagocytic activity when extracellular levels of fatty acids become elevated such as in diabetes, severe trauma, stress, etc., or when intracellular levels increase following either the activation of lipases or the release of fatty acids from lysed bacteria. We have previously demonstrated that the release of cytokines from human monocytic cells can be altered by a variety of fatty acids including

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Correspondence: Dr J. Davidson, Department of Immunology, University of Strathclyde, Glasgow G4 0NR, UK.

arachidonic acid and PGE₂.⁵ In the present study we investigated the effect of PGE₂ and fatty acids in addition to purified endotoxin and IL-1 β on the phagocytosis of *Escherichia coli* by human blood monocytes and U937 cells.

MATERIALS AND METHODS

Whole blood was kindly supplied by the Glasgow and West of Scotland Blood Transfusion Service, Carlisle, UK. The U937 cell line was obtained from the European Collection of Animal Cell Cultures (ECACC), Porton Down, Salisbury, UK. *E. coli* (O157) was obtained as a kind gift from Dr M. Taravati, Department of Immunology, University of Strathclyde, Glasgow. RPMI-1640, fetal calf serum and Hank's balanced salt solution were obtained from Gibco, Paisley, UK. Lipopolysaccharide (LPS) (*Salmonella abortus equi*), Histopaque, gelatin, fluorescein isothiocyanate-isomer 1 (FITC) and ketoprofen were obtained from Sigma Chemical Co., Poole, UK. Bovine thrombin was obtained from Armour Pharmaceutical Company, E. Sussex, UK. Interleukin-1 β was a kind gift from DuPont, PA. Arachidonic acid, oleic acid and PGE₂ were obtained from Cascade Biochem, Reading, UK.

Cell preparations

All cell preparations were carried out using aseptic techniques and sterile materials in a Class II laminar air flow cabinet.

U937 cells were propagated continuously in RPMI-1640/10% fetal calf serum. Cells were maintained for 18–24 hr in serum-free RPMI-1640 before being harvested.

Monocytes were isolated from human peripheral blood. Blood was mixed in equal volumes with Hank's balanced salt solution without calcium or magnesium (HBSS). Histopaque (10 ml) solution at 22° was placed in sterile 30 ml universal tubes and the plasma-poor diluted blood (12 ml) was placed carefully onto the surface of the Histopaque solution. Tubes were centrifuged at 400 *g* for 90 min at 22°. The layer at the interface of the HBSS/Histopaque was carefully removed and mixed in equal volumes with HBSS in sterile 30 ml universal tubes. Tubes were then centrifuged at 400 *g* for 40 min to produce a cell pellet. The supernatant was removed and the pellet was resuspended in 3 ml of RPMI culture medium (without fetal calf serum). The enriched mononuclear-cell suspension was placed into sterile Petri dishes and incubated at 37°, 5% CO₂ and 100% humidity for 4 hr. The Petri dish was washed gently with HBSS and the surface was then gently scraped with a sterile rubber policeman and the resultant suspension decanted into universal tubes. Fresh HBSS was added and the tubes were centrifuged at 400 *g* for 20 min at 22°. The supernatant was discarded and the cell pellet was resuspended in an appropriate volume of RPMI required for experiments.

The number and viability of both adherent cells (monocytes) and U937 cells was assessed by the exclusion of trypan blue dye in the course of cell counting in a haemocytometer. Both monocytes and U937 cells were adjusted to produce a final concentration of 1×10^6 viable cells/ml and placed in Eppendorf tubes.

Preincubations were carried out with various agents (LPS, IL-1 β , prostaglandins, and fatty acids) prior to incubation with FITC-labelled bacteria. Preincubations, which were carried out over various time courses and concentrations (as

described in the results section), were achieved by adding the agents to the cell suspensions and incubating at 37°, 5% CO₂, 100% humidity. Incubations were carried out in plastic 1.5 ml Eppendorf tubes in a final volume of 200 μ l consisting of 100 μ l cell suspension, 20 μ l of the various agents added in a 10-fold higher concentration than the required final concentration and the remaining volume made up to 200 μ l with RPMI-1640. All agents used were diluted from their stock concentrations to at least 1:100 in RPMI-1640 medium.

Preparation of FITC-labelled *E. coli*

FITC-labelled *E. coli* were prepared by the method described by Gelfand *et al.*⁶ Heat-killed bacteria (4×10^{10} bacteria/ml) were washed 3 times with 0.9% saline and then resuspended in 0.9% saline. The suspension was adjusted so that a 1:10 dilution had an optical density of 2.0 at a wavelength of 540 nm. One volume of this bacterial solution was diluted with 5 volumes of the carbonate/bicarbonate buffer (pH 9.5) consisting of; 0.5 M Na₂CO₃ and 0.5 M NaHCO₃.

Fluorescein 5-isothiocyanate [isomer 1] (FITC) was prepared as a 0.03% solution in the carbonate/bicarbonate buffer (pH 9.5). Two volumes of the FITC solution were then added to the bacteria. This mixture was incubated at 22° in darkness for 2 hr. The bacterial suspensions were washed three times in veronal buffered saline (pH 8.6) containing: 0.1 M sodium diethyl barbiturate, 0.15 mM CaCl₂, 1 mM MgSO₄, 0.1% gelatin and finally resuspended in the same buffer. At this stage the FITC-labelled bacteria were separated into aliquots containing 5×10^9 bacteria per ml and stored at -20°.

Bacteria were opsonized by incubating them with human serum. One volume of the fluorescent-labelled bacterial solution was added to one volume of undiluted human serum and incubated at 37° for 15 min. The bacteria were washed three times in ice-cold veronal buffered saline (pH 8.6 as above) and resuspended in RPMI 1640 at a concentration of 1×10^9 bacteria/ml.

Incubation of monocytic cells with FITC-labelled bacteria

Monocytic cells which had been pre-incubated with the respective agents as described above, were incubated with 4×10^7 FITC-labelled *E. coli* per tube for 30 min at 37° 5% CO₂, 100% humidity.

Following incubation with the bacteria, cell samples were left on ice for 5 min, washed three times in ice-cold phosphate-buffered saline (PBS) and fixed with a final concentration of 1% formaldehyde in PBS. Crystal violet quenching solution was also used to discriminate the fluorescence signal of bacteria adhering to the extracellular surface from that within the cells (phagocytosed). The quenching solution was added to the samples immediately prior to flow cytometric analysis.

Analysis by flow cytometry

Flow cytometry was carried out using a Coulter Epics-Profile II Flow Cytometer (Coulter Electronics, Luton, UK). Aliquots of formaldehyde-fixed cells were passed through the flow cytometer using an excitation wavelength of 488 nm. The fluorescence parameters from single cells were collected using a logarithmic amplifier after selecting the monocytic cells by gating the cell population according to their forward and side scatter properties. 5000 cells were analysed per tube. The fluorescence of each sample was determined and given as the

mean fluorescence channel number (logarithmic value). The fluorescence values of the samples were converted to a linear value, termed relative fluorescence intensity (RFI). RFI was calculated from the mean fluorescence channel (MFC) number as follows:

$$\text{RFI} = \text{Antilog} (\text{MFC} \times 3/256) \quad (1)$$

where 3 is the number of bands of 10 on the log scale and 256 is the number of channels by which this scale is divided.

Data were analysed by Student's unpaired *t*-test, where $P \leq 0.05$ was deemed to be significantly different from the appropriate control.

Interleukin-1 assay

In several experiments the supernatant from cell incubations was collected for estimation of IL-1 β released from cells. Supernatants were diluted with PBS in order to yield a concentration of IL-1 within the range of the standard curve for the assay. IL-1 β was measured by enzyme-linked immunosorbent assay (ELISA) using a kit supplied by Amersham Life Sciences, Amersham, UK. None of the agents used in the study altered the standard curve for IL-1 β .

Prostaglandin E₂ assay

In some experiments the supernatant from cell incubations was collected for estimation of PGE₂. The sample of supernatant was diluted in HBSS to yield a PGE₂ concentration of 0.5–20 pg/100 μ l which was within the range of the standard curve (0.25–25 pg/100 μ l) for the PGE₂ assay. PGE₂ was measured by radioimmunoassay using a kit supplied by New England Nuclear (Hamburg, Germany). The HBSS-diluted sample was used directly in the assay which was carried out exactly as described in the kit. Neither the HBSS, RPMI nor any of the agents used in this study was shown to alter the PGE₂ standard curve.

RESULTS

Uptake of FITC-labelled *E. coli* into monocytes and U937 cells

Initial experiments were carried out to ascertain the optimal number of bacteria the time required to allow sufficient FITC-labelled bacteria to yield a fluorescence signal above the background autofluorescence of cells.

Incubation of either monocytes or U937 cells with FITC-labelled bacteria alone resulted in a time dependent increase in fluorescence associated with internalised bacteria, i.e. in the presence of crystal violet to quench fluorescence on the external surface cells. Experiments were also carried out to assess the potential for release of fluorescent label from bacteria and also the loss of fluorescent label from cells following phagocytosis. FITC-labelled bacteria were incubated in the final culture medium alone for up to 2 hr followed by mild centrifugation and collection of the supernatant. No FITC fluorescence was detected in the supernatant fluid analysed in a fluorimeter, indicating that the bacteria had not released any FITC into the incubation medium. In addition, monocytes and U937 cells which had been preincubated with FITC-labelled bacteria, washed with fresh culture medium and re-incubated for 2 hr did not release any detectable FITC label into the culture medium. This would indicate that the localization of FITC label would accurately reflect the uptake of bacteria into cells

without being complicated by the circulation of free FITC label.

The relative fluorescence intensity (RFI as calculated above) increased in monocytes following incubation with FITC-labelled bacteria for periods up to 30 min. Longer periods of 1 hr and 3 hr did not result in an increase in the RFI for both monocytes and U937 cells. In monocytes the RFI increased from 856 ± 128 after 10 min incubation with FITC-bacteria, 1578 ± 332 (mean \pm SD of $n=3$) after 20 min and to 2334 ± 387 after 30 min. The RFI following 1 hr and 3 hr incubations were 2564 ± 437 and 2498 ± 267 , respectively. Similar results were obtained for U937 cells where the RFI increased from 2122 ± 428 after 10 min to 5056 ± 278 after 30 min. All subsequent experiments were carried out by pre-incubating the cells with the respective agents at varying concentrations or for various periods followed immediately by the addition of FITC-labelled *E. coli* for 30 min in order to prevent degradation, intracellular metabolism and dispersion of the FITC. The fluorescence signal should have been representative of the bacteria taken up and therefore reflect the phagocytic activity of the cells.

The effect of LPS and IL-1 β on the uptake of FITC-labelled *E. coli* into monocytes and U937 cells

The uptake of FITC-labelled bacteria into monocytic cells was estimated following preincubation with LPS (*Salmonella abortus equi*) or IL-1 β . U937 cells were preincubated with varying concentrations of LPS between 0.1 and 100 μ g/ml for 3 hr before the addition of FITC-labelled bacteria for 30 min. No significant effect was observed with the lowest concentration of LPS used but a concentration-dependent increase in the RFI was observed with concentrations of LPS between 1 μ g/ml and 100 μ g/ml. A similar concentration-dependent increase in the uptake of FITC-labelled bacteria also occurred in monocyte incubations where a significant increase in uptake was observed with 0.1 μ g/ml LPS and a maximal increase was observed between 10 μ g/ml and 100 μ g/ml. The EC₅₀ (effective half-maximal) concentrations for the effect of LPS on U937 cells and monocytes were calculated to be 4.83 μ g/ml and 1.91 μ g/ml, respectively. On this basis human monocytes were in the order of 2.5-fold more sensitive to the actions of LPS.

The time course of LPS action was also studied by preincubating cells for various times with 1 μ g/ml LPS prior to adding FITC-labelled *E. coli*. The uptake of labelled-bacteria increased up to 60 min with less of an increase after 120 min. Preincubation with LPS for 10 min and 30 min did not significantly alter the uptake of FITC-labelled bacteria into U937 cells, however, after 60 min and 120 min there was a greater increase in the RFI in incubations with LPS compared to controls. The time course of uptake of labelled-bacteria into monocytes was similar to that of U937 cells with an increase over a 120-min period except that LPS significantly enhanced the RFI, over that of control incubations ($P < 0.05$), for all time periods studied up to 120 min. No significant effect of LPS was observed in longer monocyte and U937 cell incubations up to 24 hr.

The effect of pre-incubating cells with human recombinant IL-1 β on the uptake of FITC-labelled bacteria was also studied as both monocytes and U937 cells can release IL-1 in response to LPS stimulation. The effect of LPS on IL-1 release is

Table 1. Time course of the effect of IL-1 β on the uptake of FITC-labelled *E. coli* into human monocytes. Monocytes, isolated from human peripheral blood were preincubated in the absence or presence of 0.5 nM human recombinant IL-1 β for various periods followed by the addition of FITC-labelled bacteria for 30 min. Fluorescence taken up into cells was analysed by flow cytometry. Values are the means of $n=3 \pm$ SD and are representative of at least 3 separate experiments (* $P < 0.05$)

Treatment/time (min)	10	30	60
Control	4938 \pm 792	4037 \pm 1411	3240 \pm 1478
IL-1 β	55 887 \pm 1213*	14 761 \pm 1921*	8439 \pm 602*

described later. IL-1 β was used at a concentration (500 pM) which we have previously shown to stimulate a maximal release of PGE $_2$ from monocytes where the EC $_{50}$ concentration was 120 pM.⁷ IL-1 β increased the RFI of monocytes preincubated with IL-1 β for 10 min. Preincubations with IL-1 for longer periods resulted in lesser increases in the uptake of labelled-bacteria (Table 1). Preincubation with IL-1 for 30 min increased the RFI to 788.1 \pm 78.8 from 53.2 \pm 14.3 in U937 cells. No significant effect of IL-1 on U937 cells was observed with longer incubation times.

The effect of PGE $_2$ and fatty acids on the uptake of FITC-labelled *E. coli* into monocytes and U937 cells

PGE $_2$ inhibited the uptake of labelled-bacteria in a concentration-dependent manner when preincubated with cells. This occurred in both monocytes and U937 cells (Fig. 1). In a series of previous experiments using PGE $_2$ concentrations of 1 μ M, 10 μ M and 50 μ M there was no significant difference between 10 μ M and 50 μ M and the mean value was less with 50 μ M than 10 μ M for both monocytes and U937 cells. Therefore, in subsequent experiments concentrations greater than 10 μ M were not used. The half-maximal inhibitory concentration (IC $_{50}$) of PGE $_2$ was 1.71 μ M for monocytes and 2.48 μ M for U937 cells.

The monounsaturated fatty acid, oleic acid at concen-

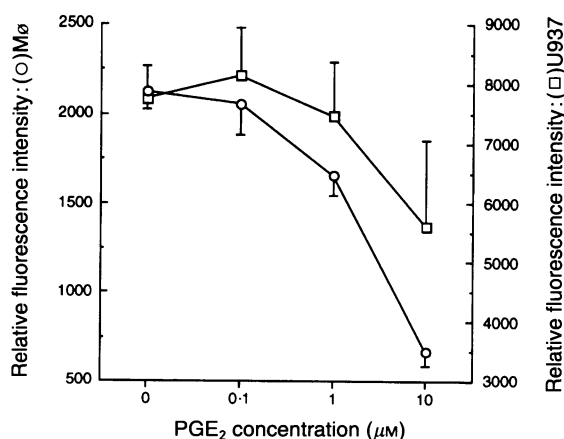


Figure 1. The effect of PGE $_2$ on the uptake of FITC-labelled *E. coli* into monocytic cells. Monocytes, isolated from human peripheral blood (circles), or U937 cells (squares) were preincubated with various concentrations of PGE $_2$ for 2 hr followed by the addition of FITC-labelled bacteria for 30 min. Cells were fixed and fluorescence was analysed by flow cytometry. Values are the means of $n=3 \pm$ SD and are representative of at least three separate experiments.

trations up to 100 μ M did not alter the uptake of FITC-labelled bacteria into either monocytes or U937 cells (Fig. 2). In contrast, the polyunsaturated fatty acid, arachidonic acid, enhanced uptake (Fig. 2). The effects of several fatty acids were directly compared to the effect of arachidonic acid. Palmitic acid and oleic acid did not have any effect whereas a concentration-dependent enhancement was observed with the three-double bond unsaturated C20 fatty acid, dihomo- γ -linolenic acid and a greater enhancement occurred with arachidonic acid (Fig. 3).

The prostaglandin synthesis inhibitor ketoprofen was used to ascertain whether the effects of either LPS, or IL-1 β were modulated by the production of endogenous prostaglandins. Ketoprofen (20 μ M) further enhanced the increased uptake of labelled-bacteria into monocytes incubated with LPS or IL-1 β but did not significantly affect the LPS or IL-1 β -stimulated uptake into U937 cells (Fig. 4).

LPS was shown to increase the release of IL-1 in a concentration-dependent manner in both cell types with an EC $_{50}$ of 0.88 μ g/ml with monocytes and 0.23 μ g/ml with U937 cells. Ketoprofen enhanced the release of IL-1 β from monocytes, both in the absence and presence of LPS, as did arachidonic acid. In contrast PGE $_2$ suppressed release (Fig. 5).

In order to confirm the actions of ketoprofen on prostaglandin production, the release of PGE $_2$ was also measured. In these experiments ketoprofen was added to incubations at the same time as the stimuli. LPS (1 μ g/ml) and IL-1 β increased the release of PGE $_2$ from monocytes to 2.13 \pm 0.12 ng/ml and 1.09 \pm 0.09 ng/ml, respectively, from a control level of 0.45 \pm 0.09 ng/ml. Ketoprofen (20 μ M) suppressed the level of PGE $_2$ in control monocyte incubations to 0.08 \pm 0.02 ng/ml and suppressed the PGE $_2$ level in LPS and IL-1 incubations to 0.91 \pm 0.08 ng/ml and 0.55 \pm 0.09 ng/ml, respectively. The level of PGE $_2$ in U937-cell incubations was below the limit of detection of the assay.

DISCUSSION

The present study clearly demonstrates that the uptake/phagocytosis of *E. coli* into both monocytes and U937 cells can be enhanced in the presence of LPS and IL-1. Furthermore, the increased uptake can be either further enhanced by fatty acids or downregulated by PGE $_2$. These observations strongly indicate an important regulatory serial pathway in the control of phagocytosis during bacterial infection. The phagocytosis of bacteria can thus, potentially be modulated at several points in this pathway either by agents outwith the pathway or at the level of mediators produced within the pathway.

In the present study it was demonstrated that LPS enhanced

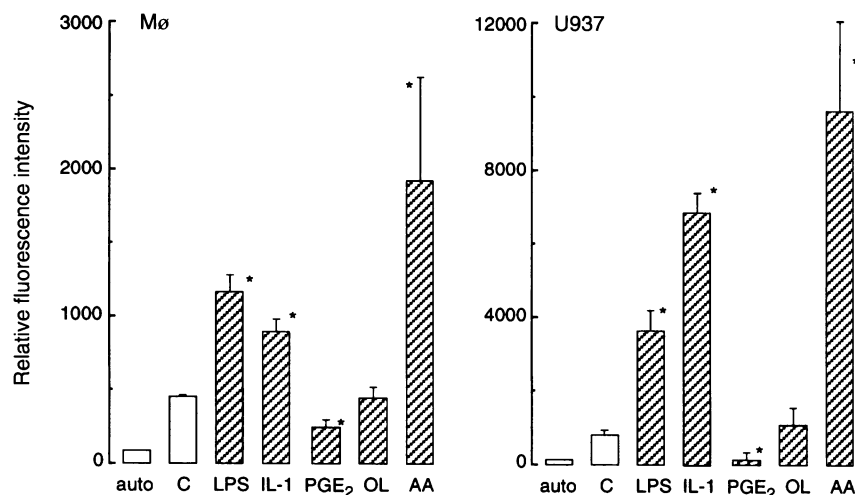


Figure 2. The effect of fatty acids and PGE₂ on the uptake of FITC-labelled *E. coli* into monocytic cells. Monocytes, isolated from human peripheral blood (Mø), or U937 cells were preincubated with either medium alone (C), 1 µg/ml LPS, 0.5 nM human recombinant IL-1β 10 µM PGE₂, 100 µM oleic acid (OL) or 100 µM arachidonic acid for 1 hr followed by the addition of FITC-labelled bacteria for 30 min. Cells were fixed and fluorescence was analysed by flow cytometry. Fluorescence was compared to the autofluorescence of cells incubated alone in the absence of FITC-labelled bacteria (auto). Values are the means of $n=3 \pm$ SD and are representative of at least three separate experiments (* $P < 0.05$).

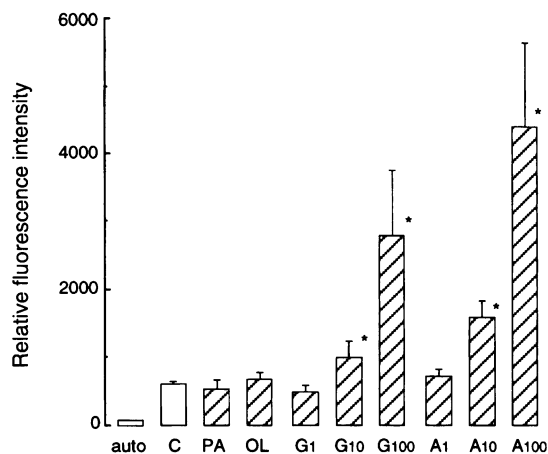


Figure 3. Comparison of the effect of various fatty acids on the uptake of FITC-labelled *E. coli* into monocytic cells. As for Fig. 2 except that cells were preincubated with either control medium alone (C), 100 µM palmitic acid (PA), 100 µM oleic acid (OL), dihomogammalinolenic acid (G) or arachidonic acid (A) for 1 hr (numbers indicate the concentration of fatty acids in µM). FITC-labelled bacteria were added for 30 min and fluorescence taken up into cells was analysed by flow cytometry. Fluorescence was compared to the autofluorescence of cells incubated alone in the absence of FITC-labelled bacteria (auto). Values are the means of $n=3 \pm$ SD and are representative of at least three separate experiments (* $P < 0.05$).

the phagocytosis of *E. coli*. This illustrates at least two important aspects of the mode by which bacteria activate phagocytic processes in human monocytes: (i) the fact that LPS increases uptake suggests that in the course of a bacterial infection, during which bacteria are actively being phagocytosed, the shedding of bacterial cell wall components further facilitates the uptake of bacteria thereby accelerating their own removal; (ii) the nature of the LPS is non-specific and cross-reactive as the LPS used in the present study was derived

from *Salmonella abortus*. It is, however, uncertain whether this cross-reactivity is restricted to coliform bacteria as LPS from other non-coliform bacteria was not studied.

The enhancement of phagocytosis by LPS was shown to occur in the first 2–3 hr of incubation with little or no effect of LPS in longer incubations up to 24 hr. Thus, it would imply that the stimulation of phagocytosis by LPS is restricted to the first few hours of exposure to the bacteria. This may be a result of the stimulation, by LPS, of downstream mediators which may counter its actions by downregulating systems it has initiated.

It is well established that LPS is one of the most potent stimuli for the induction and release of IL-1 from monocytes.⁸ It would also appear that the LPS is derived from bacteria which have shed their cell-wall components, during replication or bacterial lysis.⁸ IL-1 and other cytokine genes are induced following the binding of LPS but it is not certain if intact bacteria, which have not shed cell-wall material, can also lead to the release of IL-1. It is primarily bacterial particles which are phagocytosed but it is not certain whether the phagocytic process *per se* can actually lead to cytokine gene expression and release. Early work on IL-1 revealed that incubation of monocytes with latex particles, presumably devoid of any endotoxin-like activity, can lead to the release of IL-1. As latex particles are routinely used to study the modulation of phagocytosis in monocytes, this would imply that the process of phagocytosis can independently lead to IL-1 release.⁹ Thus, bacteria would be able to induce the expression and release of IL-1 via a combination of phagocytic signalling pathways and, the liberation of LPS. The data obtained in the present study shows that IL-1 can also enhance phagocytic uptake of bacteria indicating that this may be an important role of IL-1 released in the course of phagocytosis. This overall series of events in which the LPS derived from bacteria and the resulting release of IL-1 appear to constitute a positive feedback loop in which the mediators released at earlier stages enhance the actions of

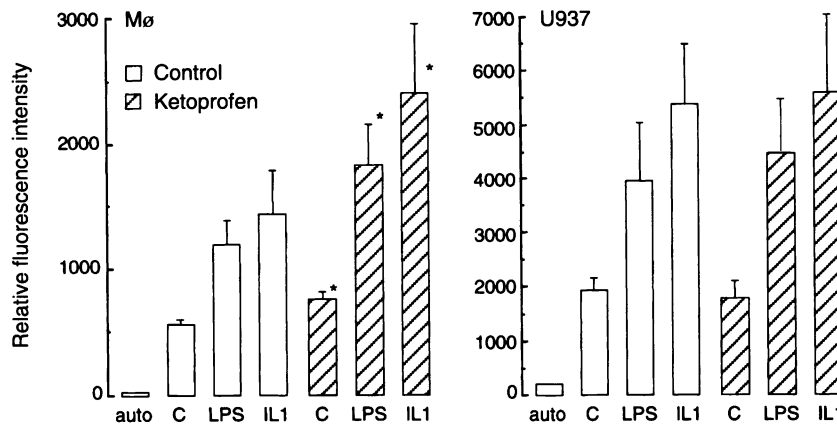


Figure 4. The effect of the cyclooxygenase inhibitor, ketoprofen, on the uptake of FITC-labelled *E. coli* into monocytic cells. As for Fig. 3 except that cells were preincubated with either control medium alone (C), 1 $\mu\text{g/ml}$ LPS or 0.5 nM human recombinant IL-1 β either in the absence (light shaded columns) or presence of 20 μM ketoprofen (dark shaded columns) for 1 hr. FITC-labelled bacteria were added for 30 min and fluorescence taken up into cells was analysed by flow cytometry. Fluorescence was compared to the autofluorescence of cells incubated alone in the absence of FITC-labelled bacteria (auto). Values are the means of $n=3 \pm \text{SD}$ and are representative of at least three separate experiments (* $P < 0.05$).

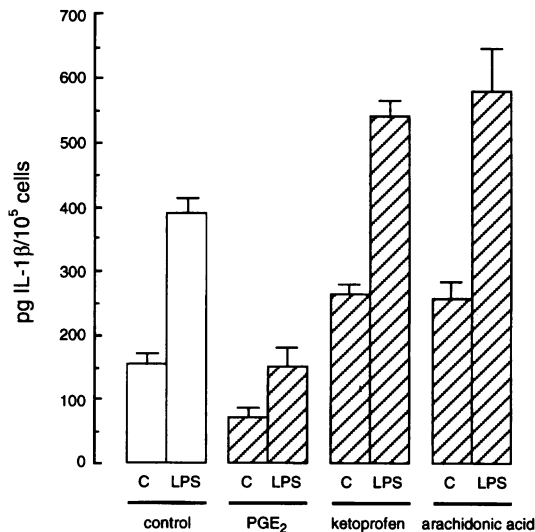


Figure 5. The effect of PGE₂, arachidonic acid and ketoprofen on the release of IL-1 β from monocytes. Monocytes, isolated from human peripheral blood, were incubated either alone in control medium (C) or with 1 $\mu\text{g/ml}$ LPS in the absence (control) or presence of either PGE₂ (10 μM), ketoprofen (20 μM) or arachidonic acid (100 μM) for 18 hr. IL-1 β levels in the supernatants were measured by ELISA. All treatments were significantly different ($P < 0.05$) from the respective control incubation (either C or LPS incubated without any other agents, i.e. first two columns).

mediators produced later in the cascade. This process of the amplification of phagocytosis is analogous to that whereby IL-1 release is itself amplified. LPS induces IL-1 production and the released IL-1 induces further IL-1 release¹⁰⁻¹². It has also been shown in previous studies that IL-1 released in the process of phagocytosis can further upregulate Fc-receptor mediated uptake by monocytes.¹⁵

Following the activation of monocytes by LPS, cytokines such as IL-1 are released which in turn activate lipases that release fatty acids, primarily arachidonic acid. These are subsequently converted to eicosanoids, especially prostagland-

ins. The present study showed that arachidonic acid and its less saturated precursor, dihomo- γ -linolenic acid (DGLA), also enhanced the uptake of bacteria into monocytes in a similar manner to both LPS and IL-1. In a previous study we characterized the effect of fatty acids on LPS-stimulated release of cytokines, especially IL-1, from monocytic cells. The study showed that IL-1 production was increased by polyunsaturated fatty acids, such as arachidonic acid and DGLA whereas mono-unsaturated fatty acids including oleic acid or the saturated fatty acid, palmitic acid did not alter production. A similar pattern of effect on phagocytosis of *E. coli* was observed in the present study. This enhancement of phagocytosis by fatty acids is further evidence to support the view that each stage in the series of events involved in monocyte activation reinforces the actions of the previous step. This would also appear to be the case for the control of phagocytic activity of monocytes. The ability of fatty acids to influence phagocytic activity is an important consideration for the uptake of bacteria under circumstances in which the levels of fatty acids, either extracellular or intracellular, become elevated. This could occur in a variety of situations such as trauma, stress or in response to a high level of dietary intake where extracellular/blood levels of fatty acids can increase dramatically. Similarly, intracellular levels of fatty acids increase following the activation of lipases such as phospholipase A₂ which is activated in monocytes by IL-1 and in the context of bacterial uptake the resulting lysis of bacterial components releases fatty acids. The study of Thore *et al.*² showed that following lysis, arachidonic acid originating from the bacteria can be liberated within cells. The effects of arachidonic acid and DGLA in the present study are consistent with the observations of Lennartz and Brown¹⁶ who showed that both exogenous and endogenously released arachidonic acid and DGLA enhance the phagocytosis of both yeast and immunoglobulin G (IgG)-coated erythrocytes (EIgG). Furthermore, the authors concluded that arachidonic acid is essential for phagocytosis of EIgG but not for the uptake of yeast and that both of these pathways were independent of the conversion of fatty acids to their respective prostaglandins.

In the general control of immune cell activation the magnitude of end-responses appear to be limited by the production of prostaglandins, especially PGE₂.¹³ This has been demonstrated to be the case for the control of monocyte-derived cytokines. The present study shows that phagocytosis can also be downregulated by PGE₂. PGE₂ is derived directly from the oxidation of arachidonic acid which was shown to enhance phagocytosis. The downregulation of phagocytosis occurred in response to exogenously added PGE₂ and obviously not PGE₂ derived from fatty acids of endogenous origin. However, the cyclo-oxygenase inhibitor, ketoprofen, increased bacterial uptake implying that endogenous prostaglandins were being produced and that they were exerting an inhibitory action on phagocytosis. The ability of ketoprofen to inhibit PGE₂ production was confirmed by measuring the release of PGE₂ from monocytes, under identical incubation conditions, where it was found that PGE₂ levels in the presence of ketoprofen were much lower than in the absence of ketoprofen. This was observed in control incubations as well as in the presence of either LPS or IL-1 β . This would confirm the view that endogenously produced prostaglandins are actively involved in downregulating phagocytic activity, especially the enhanced activity stimulated by both LPS and IL-1.

It is uncertain if the effects of either LPS, fatty acids or PGE₂ on phagocytosis are direct actions or are mediated indirectly via the modulation of IL-1 as the central regulatory point. All of the above have been clearly shown to modulate IL-1 release in our previous study.⁵ In addition, in this study LPS and polyunsaturated fatty acids increased IL-1 release and phagocytosis. Similarly, PGE₂ decreased both IL-1 β release and phagocytic activity. This would appear to infer that the regulation of phagocytosis is inextricably linked to the modulation of IL-1 levels and that agents which modify levels of IL-1 would also influence phagocytic activity.

The present study demonstrates that phagocytosis of *E. coli* by U937 cells and the modulation of this response is qualitatively similar to that of normal human monocytes. This indicates that U937 cells strongly retain this important monocyte characteristic and may prove useful in understanding the molecular mechanisms which control this process. The major difference between U937 cells and monocytes appears to be related to prostaglandin biosynthesis. U937 cells were shown to respond to PGE₂ by downregulating phagocytic activity in a concentration-dependent manner. This is consistent with our previous studies where we have shown that U937 cells express high-affinity PGE₂ receptors which are linked to functional signal transduction systems including adenylate cyclase.¹⁴ However, no response to ketoprofen was observed in U937 cells whereas ketoprofen clearly enhanced the phagocytic activity of monocytes. This suggests that endogenous prostaglandin production is not involved in U937 cells but can suppress monocyte activity. It is not certain whether U937 cells produce PGE₂ but in the present study no PGE₂ was detected. These cells could be useful in determining individual steps involved in the control of monocyte functions without the potential complication of endogenous prostanoid production.

This study has provided data which strongly suggests that monocytes possess an important regulatory mechanism which can either initially amplify phagocytic activity but can limit phagocytic processes in the latter stages of activation.

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