Characteristics of oedema formation induced by N-formyl-methionyl-leucyl-phenylalanine in rabbit skin

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1 The characteristics of N-formyl-methionyl-leucyl-phenylalanine (FMLP)-induced oedema formation were investigated *in vivo* in rabbit skin.

2 FMLP injected intradermally alone induced a small increase in plasma leakage, but marked synergism with prostaglandin E₂ (PGE₂) in producing oedema responses was observed. In the presence of PGE₂, FMLP was equiactive with C5a des Arg and 100–1000 times more active than histamine in terms of permeability-increasing activity. The response to FMLP was not dependent on endogenous histamine release.

3 FMLP-induced responses were of long duration ($t_{1/2} \sim 40\text{--}50$ min) when compared with bradykinin ($t_{1/2} \sim 4\text{--}5$ min).

4 The activity of a range of N-formyl peptides in increasing vascular permeability in skin correlated well with their activity as neutrophil stimulants *in vitro*.

5 Intravenous infusion of zymosan-activated plasma (ZAP) resulted in transient neutropenia and inhibition of oedema formation induced by FMLP and C5a des Arg in the skin. Responses to bradykinin were unaffected by the infusion of ZAP.

6 Intravenous injection of the non-steroidal antiinflammatory drug, ibuprofen, resulted in an inhibition of FMLP-induced, but not histamine-induced, oedema formation. This effect was independent of cyclo-oxygenase inhibition and the drug did not induced neutropenia.

7 Intravenous injection of the microtubule blocking agent colchicine inhibited FMLP-induced oedema formation. Responses to bradykinin were unaffected. When colchicine was administered after intradermal FMLP, subsequent plasma leakage was abolished.

8 The inference that receptors have evolved to bacterial secretions (i.e. FMLP) and products of the interaction of bacterial cell walls with tissue fluid (i.e. C5a des Arg), is consistent with the hypothesis that oedema formation is fundamentally a functional process concerned with regulating microbial lysis and opsonisation in an infected tissue.

Introduction

Peptides such as N-formyl-methionyl-leucyl-phenylalanine (FMLP) are powerful chemotactic substances for neutrophils (Schiffmann *et al.*, 1975; Showell *et al.*, 1976; Marasco *et al.*, 1984). Although originally described as prokaryotic in origin

(Schiffmann *et al.*, 1975), chemotactic N-formyl peptides have also been reported to be released from damaged mitochondria of eukaryotic cells (Carp, 1982). In addition to being chemotactic *in vitro*, FMLP induces leukocyte accumulation following intradermal (i.d.) injection in skin (Issekutz, 1981; Colditz & Movat, 1984). Although weak in inducing oedema when injected alone into rabbit skin, FMLP is potent in inducing oedema in the presence of potentiating doses of prostaglandins (Wedmore & Williams, 1981). In hamster lung, FMLP-induced neutrophil accumulation was observed to be accom-

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panied by a small permeability change which it was suggested might be due to histamine release (Desai *et al.*, 1979). Furthermore, histamine release from human basophil-rich leukocytes was stimulated by N-formyl peptides (Hook *et al.*, 1976), but it is unclear whether endogenous histamine is released in rabbit skin in response to FMLP.

Unlike increased permeability induced by i.d. injection of mediators such as bradykinin and histamine, leakage responses induced by FMLP are dependent on circulating neutrophils. Thus, injection of FMLP induces a rapid interaction (detected within 6 min of injection) between circulating neutrophils and venular endothelium that leads to increased vascular permeability (Wedmore & Williams, 1981). The nature of this interaction remains to be determined, but it results in a protracted increase in permeability ($t_{1/2} \sim 50$ min) when compared with bradykinin and histamine ($t_{1/2} \sim 4$ –5 min) (Wedmore & Williams, 1981). In an attempt to investigate the underlying mechanisms, we used the microtubule blocking agent colchicine to determine whether functionally active neutrophils were required for the full duration of the permeability-increasing action of FMLP.

In the initial experiments of Wedmore & Williams (1981) which investigated the neutrophil dependency of oedema formation induced by FMLP, C5a and leukotriene B₄ (LTB₄) in rabbit skin, nitrogen mustard was administered to rabbits 3 days before the experiment to induce neutropenia. In the current investigation we wished to establish techniques for inducing rapid inhibition of neutrophil-dependent oedema. In other studies, i.v. infusion of chemotactic factors (C5a or FMLP) or activators of the complement system *in vivo* in rabbits resulted in a profound but transient neutropenia (McCall *et al.*, 1974; O'Flaherty *et al.*, 1977). In the experiments described here, we infused zymosan-activated plasma (as a source of C5a des Arg) into rabbits, and measured oedema responses to FMLP before and after this treatment. Additionally, recent reports have indicated that the non-steroidal antiinflammatory compound ibuprofen can inhibit certain aspects of neutrophil function independent of cyclo-oxygenase inhibition (Maderazo *et al.*, 1984; Abramson *et al.*, 1985; Nielsen & Webster, 1987). Furthermore, ibuprofen has been shown to inhibit C5a-induced oedema formation *in vivo* (Rampart & Williams, 1986a). Thus, we also investigated the effects of this drug on oedema formation induced by FMLP. In further experiments we investigated the effects of intravenous colchicine on FMLP-induced oedema. With this drug, we were especially interested to determine whether inhibition could be obtained when it was administered after intradermal injection of FMLP.

Methods

Animals

Male New Zealand White specific pathogen-free rabbits (2.5–3.5 kg) were purchased from Hacking and Churchill, Huntingdon, Cambridgeshire.

Endotoxin determination

The amount of endotoxin in solutions for i.v. administration was determined by use of *Limulus* amoebocyte lysate and a chromogenic substrate (Coatest, Kabivitrum, Stockholm, Sweden). The limit of detection was approximately 0.01 ng endotoxin ml⁻¹.

Measurement of local oedema formation in rabbit skin

Oedema formation was measured as the local accumulation of i.v. injected ¹²⁵I-human serum albumin into skin sites as described (Hellewell & Williams, 1989). Agents under test were freshly prepared in sterile phosphate buffered saline (PBS) from stock solutions and 0.1 ml injected i.d. in six replicates per treatment according to a balanced site injection plan. After 30 min, a 10 ml cardiac blood sample was taken into heparin and the plasma prepared. The animal was then killed and plasma leakage determined as described by Hellewell & Williams (1989).

In the experiments designed to observe the effects of systemic administration of zymosan-activated plasma (ZAP), colchicine or ibuprofen on FMLP-induced oedema formation, the procedure was designed so that each animal served as its own control. Test agents (C5a des Arg + PGE₂, FMLP + PGE₂, bradykinin + PGE₂ or histamine + PGE₂) were injected i.d. after i.v. ¹²⁵I-albumin and Evans blue dye, and plasma protein accumulation was allowed to proceed for 30 min. ZAP, colchicine or ibuprofen was then injected i.v. and 5 min later the remaining skin sites were injected with the same combination of test agents. After a further 30 min, the animals were killed and local oedema formation assessed as described above. ZAP was filtered (0.22 µm, Millex) and infused i.v. at 6 ml min⁻¹ for 30 s, then at 0.5 ml min⁻¹ for the remainder of the experiment. Colchicine and ibuprofen were dissolved in sterile saline at 1 mg ml⁻¹ and 20 mg ml⁻¹ respectively and injected i.v. (1 ml kg⁻¹). Control animals received saline alone (1 ml kg⁻¹).

For total and differential leukocyte counts, samples of peripheral blood were taken from a central ear artery at intervals before and after systemic administration of compounds. Total leukocytes were determined in a Coulter counter (type ZBI) and May-Grunwald-Giemsa stain was used for differential counts on at least 200 cells.

Statistical analysis

Data are presented as the mean $\pm$ s.e.mean and have been analysed by Student's paired *t* test for within animal treatments and Student's *t* test for between animal comparison. A *P* value of <0.05 was considered statistically significant. Before calculating percentage inhibition of a response, the background value for i.d. injection of PGE₂ alone was subtracted from the data.

Drugs and materials

Prostaglandin E₂ (PGE₂) arachidonic acid (AA), bradykinin, zymosan, N-formyl-methionyl-leucyl-phenylalanine (FMLP), N-formyl-norleucyl-phenylalanine (FNLP), N-formyl-methionyl-phenylalanine (FMP) and N-formyl-methionine (FM) were purchased from the Sigma Chemical Co., Poole, Dorset; N-formyl-methionyl-methionyl-phenylalanine (FMMP) was from Miles Laboratories, Stoke Poges, Buckinghamshire; histamine and Evans blue dye were from BDH Chemicals, Poole, Dorset; sodium pentobarbitone was from May and Baker, Dagenham, Essex; ¹²⁵I-human serum albumin (50 μ Ci ml⁻¹, 20 mg albumin ml⁻¹) was from Amersham International plc, Amersham, Buckinghamshire; sterile 0.9% saline (Steriflex) was from the Boots Company, Nottingham, Nottinghamshire, U.K.; sterile phosphate buffered saline (PBS) was from the Clinical Research Centre media department. Zymosan-activated plasma (ZAP) was used as a source of C5a des Arg (Hellewell & Williams, 1989).

The following were gifts; purified rabbit C5a, in its physiologically more stable form C5a des Arg, was from Dr P.J. Jose in our laboratory (Williams & Jose, 1981; Jose *et al.*, 1983); N-formyl-methionyl-leucyl-phenylalanyl-phenylalanine (FMLPP) was from Dr R.J. Freer, Medical College of Virginia, Richmond, Virginia, U.S.A.; sodium ibuprofen dihydrate was from Dr I.M. Hunneyball, the Boots Company; mepyramine maleate was from May and Baker Ltd.

Results

Characteristics of FMLP-induced oedema

Figure 1 shows that i.d. injection of FMLP alone induced a small but significant increase in leakage of ¹²⁵I-albumin over a 30 min period when compared with sites injected with PBS. Similarly, injection of histamine alone induced a small increase in ¹²⁵I-albumin leakage. However, as Figure 1 also illus-

Figure 1 Oedema responses in rabbit skin induced by i.d. injection of FMLP and histamine, showing synergism with prostaglandin E₂ (PGE₂). The symbols represent oedema responses to FMLP alone (○); responses to FMLP + PGE₂ (3×10^{-10} mol) (●); responses to histamine alone (△); responses to histamine + PGE₂ (▲). The dashed line shows the control value obtained after i.d. injection of PBS. Data from 6 rabbits, **P* < 0.05 with respect to PBS controls.

trates, when the vasodilator PGE₂ (3×10^{-10} mol) was mixed with either FMLP or histamine before i.d. injection, a potentiation of the response was observed. The potentiation occurs as a result of synergism between increased microvascular permeability, induced by FMLP or histamine, and the increased microvascular blood flow induced by PGE₂. Injection of PGE₂ alone induced little oedema formation. When tested in the presence of PGE₂, FMLP was 100–1000 times more active than histamine in increasing microvascular permeability (Figure 1) although, FMLP was equiactive with purified rabbit C5a des Arg (data not shown).

In some experiments we measured the accumulation of i.v. injected ¹²⁵I-albumin over a 4 h period and found that FMLP elicited responses which were significantly greater than those measured over 30 min. In 6 rabbits, responses to i.d. injection of FMLP (5×10^{-11} mol) were $6 \pm 2 \mu$ l at 30 min and $17 \pm 3 \mu$ l at 4 h (*P* < 0.05; results subtracted for PBS control value). Neither of these responses was affected by the local administration of indomethacin (10^{-8} mol, a dose previously demonstrated to inhibit the cyclo-oxygenase in rabbit skin; Williams & Peck, 1977); corresponding leakage values were $5 \pm 2 \mu$ l and $18 \pm 3 \mu$ l respectively.

Permeability responses to FMLP were independent of histamine release. In 6 rabbits, oedema responses to injection of a mixture of histamine (2.5×10^{-8} mol) + PGE₂ (3×10^{-10} mol) were suppressed by $84 \pm 3\%$ after the local administration of 3×10^{-9} mol mepyramine (*P* < 0.005). The suscepti-

Figure 2 Oedema responses in rabbit skin induced by i.d. injection of a range of N-formyl peptides. All peptides were injected with PGE₂ (3×10^{-10} mol). The dashed line shows the control value obtained after i.d. injection of PBS. Data from 6 replicate injections. For abbreviations, see text.

bility of oedema responses induced by FMLP (2.5×10^{-11} mol) + PGE₂ to inhibition by local administration of mepyramine showed some inter-animal variation; however, in the same group of animals described above, the effect of mepyramine was minimal; $18 \pm 6\%$ suppression ($P > 0.05$).

Figure 2 shows the results of an experiment in which we compared the ability of six formyl-peptides to increase microvascular permeability in rabbit skin. In the presence of PGE₂ (3×10^{-10} mol), the peptide most potent in increasing permeability was FMLPP; significant oedema responses ($P < 0.05$) being obtained with 5×10^{-14} mol. At higher doses, FMLPP showed similar activity to FMLP. The analogues FNLP and FMMP were approximately 10 times less active than FMLP, while FMP was considerably weaker (Figure 2). We were unable to detect any permeability-increasing activity of FM, i.e. the response to i.d. injection of FM + PGE₂ was no different to that obtained with PGE₂ alone.

Effect of intravenous infusion of zymosan-activated plasma

Figure 3 shows the effect of infusion of ZAP on oedema responses to FMLP + PGE₂, C5a des Arg + PGE₂ and bradykinin + PGE₂. Responses to FMLP and C5a des Arg were reduced by 81% and 78% respectively, while the leakage to bradykinin was only partially reduced (20% suppression) (Figure 3). When the same experiment was repeated in 3 rabbits, the inhibitions were $84 \pm 2\%$, $82 \pm 4\%$ and

Figure 3 Effect of i.v. infusion of zymosan-activated plasma (ZAP) on oedema responses in rabbit skin induced by FMLP + prostaglandin E₂ (PGE₂), C5a des Arg + PGE₂ and bradykinin + PGE₂. The open columns represent responses obtained before, and stippled columns represent responses after infusion of ZAP. FMLP and C5a were injected at 5×10^{-11} mol, bradykinin at 10^{-10} mol and PGE₂ at 3×10^{-10} mol. The dashed line shows the control value after i.d. injection of PBS. Data from 6 replicate injections, * $P < 0.05$, compared with control.

$15 \pm 7\%$ for FMLP, C5a des Arg and bradykinin respectively. Thus, infusion of ZAP resulted in a selective suppression of neutrophil-dependent oedema responses. This is probably due to a transient neutropenia and neutrophil sequestration in vascular beds induced by the infusion of ZAP. Circulating white cell numbers ($\times 10^6 \text{ ml}^{-1}$, $n = 3$) before ZAP were 6.6 ± 0.9 , 5 min after initiation of ZAP infusion 3.2 ± 0.6 ($P < 0.05$) and 35 min after ZAP (i.e. the end of the experiment) were 4.6 ± 0.7 ($P < 0.05$). Corresponding neutrophil numbers ($\times 10^6 \text{ ml}^{-1}$) were 2.3 ± 0.4 , 0.1 ± 0.04 ($P < 0.05$) and 0.8 ± 0.3 ($P < 0.05$) respectively.

Suppression of FMLP-induced oedema formation by ibuprofen

The effects of i.v. administration of either saline (1 ml kg^{-1}) or ibuprofen (20 mg kg^{-1}) on oedema responses to FMLP + PGE₂, histamine + PGE₂, FMLP + arachidonic acid (AA) and histamine + AA are shown in Figure 4.

Figure 4a shows that administration of saline had no significant effect on oedema responses to all combinations of agents tested (i.e. responses over 35–65 min were the same as those measured 0–65 min). In contrast, Figure 4b shows that systemic treatment with ibuprofen resulted in significant suppression of leakage responses to FMLP and histamine when they were potentiated by AA, due to inhibition of cyclo-oxygenase and reduction of vasodilator prosta-

Figure 4 Oedema responses in rabbit skin induced by i.d. injection of mixtures of FMLP + prostaglandin E₂ (PGE₂), histamine (Hist) + PGE₂, FMLP + arachidonic acid (AA) and histamine + AA to show the cyclo-oxygenase-dependent and independent action of ibuprofen. In (a) responses before (○) and after (●) i.v. saline (1 ml kg⁻¹) and in (b) responses before (○) and after (●) i.v. ibuprofen (20 mg kg⁻¹). When injected with PGE₂ (3×10^{-10} mol), FMLP and histamine were tested at the doses shown. When injected with AA (3×10^{-9} mol), FMLP and histamine were injected at their top doses, i.e. 5×10^{-11} mol and 10^{-8} mol respectively. The dashed line shows the control value obtained after i.d. injection of PBS. Data from 6 rabbits, * $P < 0.05$, ** $P < 0.01$, compared with controls.

glandin production. However, when leakage responses to FMLP and histamine were potentiated by the simultaneous injection of PGE₂ to override cyclo-oxygenase inhibition, only the oedema induced by FMLP + PGE₂ was significantly suppressed by the systemic administration of ibuprofen. Responses to histamine + PGE₂ were unaffected by ibuprofen treatment.

The level of endotoxin in the ibuprofen solution (20 mg ml⁻¹) was 0.8 ng ml⁻¹ which we considered to be an insufficient amount to account for the observed effects of the drug on oedema responses to FMLP + PGE₂ (Haslett *et al.*, 1987). Total leukocyte numbers before, 5 and 35 min after ibuprofen were 5.6 ± 1.4 , 4.9 ± 1.3 and $5.5 \pm 1.3 \times 10^6$ cells ml⁻¹, respectively. Neutrophil counts at the same time were 2.0 ± 0.5 , 1.9 ± 0.4 and $2.1 \pm 0.5 \times 10^6$ cells ml⁻¹ respectively ($n = 6$).

Effect of colchicine on FMLP-induced oedema formation

In earlier experiments designed to investigate the neutrophil dependency of oedema formation induced by FMLP, C5a and LTB₄, nitrogen mustard was administered to rabbits 3 days before the experiment (Wedmore & Williams, 1981). In the current investigation we wished to establish a technique for inducing rapid inhibition of neutrophil-dependent oedema.

Figure 5 shows the effect of i.v. administration of colchicine (1 mg kg⁻¹) on oedema formation induced by mixture of FMLP + PGE₂ and bradykinin + PGE₂ in rabbit skin. Internally-controlled experiments were performed in 4 rabbits as described in the previous section. Responses to FMLP + PGE₂ were virtually abolished in the presence of colchicine,

Figure 5 Oedema responses in rabbit skin induced by i.d. injection of mixtures of FMLP + prostaglandin E_2 (PGE_2) and bradykinin + PGE_2 to show the selective effect of i.v. colchicine. The symbols represent: responses before colchicine ($\circ$); responses after i.v. colchicine (1 mg kg^{-1}) ($\bullet$), FMLP and bradykinin were injected at the doses shown mixed with PGE_2 (3×10^{-10} mol). The dashed line shows the control value obtained after i.d. injection of PBS. Data from 4 rabbits. * $P < 0.05$, ** $P < 0.02$ compared with controls.

while those to bradykinin + PGE_2 were unaffected. The amount of endotoxin in the colchicine solution (1 mg ml^{-1}) was 0.05 ng ml^{-1} . In the control animals which received 1 ml kg^{-1} saline i.v., oedema responses to FMLP + PGE_2 and bradykinin + PGE_2 were unaffected, the results (not shown) being similar to those shown in Figure 4a.

Circulating leukocyte counts before, 10 and 40 min after colchicine were 5.9 ± 0.9 , 4.8 ± 0.6 and $3.8 \pm 0.6 \times 10^6\text{ cells ml}^{-1}$. Neutrophil counts at the same time points were 2.8 ± 0.8 , 1.2 ± 0.3 ($P < 0.05$) and 0.7 ± 0.2 ($P < 0.05$) $\times 10^6\text{ cells ml}^{-1}$ respectively. Thus, injection of colchicine resulted in a slow decline in both total number of leukocytes and the number of neutrophils. The mechanism for this is unknown but may relate to sequestration in vascular beds such as the lung (Flick *et al.*, 1981).

One of the characteristic features of plasma leakage induced by the neutrophil chemoattractants is the long duration of action of the responses when compared with directly-acting mediators such as bradykinin and histamine (Wedmore & Williams, 1981). The duration of the permeability-increasing activity of bradykinin and histamine is approx. 4 min and that of FMLP is approx. 50 min. The mechanism for this protracted response is unknown; indeed the mechanism by which the rapid interaction between neutrophils and venular endothelium induced by extravascular chemoattractant leads to

Figure 6 Effect of colchicine on the duration of action of (a) bradykinin and (b) FMLP in rabbit skin. FMLP (5×10^{-10} mol) and bradykinin (10^{-10} mol) were injected i.d. at various intervals before an i.v. injection of ^{125}I -albumin and Evans blue dye and either saline (1 ml kg^{-1}) or colchicine (1 mg kg^{-1}) at $t = 0$. PGE_2 (3×10^{-10} mol) was injected into all skin sites and oedema formation assessed after 30 min. In (a) responses to bradykinin and in (b) responses to FMLP in saline- ($\circ$) and colchicine-treated rabbits ($\bullet$). The dashed line shows the control value obtained after i.d. injection of PBS. Data from 6 rabbits, * $P < 0.05$ with respect to saline-treated rabbits.

increased plasma leakage also remains to be established.

We were interested to determine whether colchicine could inhibit FMLP-induced oedema if administered after FMLP injections. Figure 6 shows the effect of colchicine administration (1 mg kg^{-1}) on the duration of action of bradykinin and FMLP in rabbit skin. Colchicine or saline was injected i.v. at the times indicated after i.d. injection of bradykinin and FMLP. Animals were also pretreated with mepyramine (3 mg kg^{-1} i.v.) to block the action of any endogenous histamine that may have been released by FMLP. Saline (1 ml kg^{-1}) or colchicine (1 mg kg^{-1}) was injected i.v. together with ^{125}I -albumin and Evans blue dye and responses measured over 30 min. A potentiating dose of PGE_2 was injected into all sites at the beginning of the measurement period. As shown in Figure 6a, colchicine had no effect on the duration of action of bradykinin, the response half-life in controls being 5 ± 1 min, compared with 4 ± 1 min in the colchicine group. In contrast, the long duration of action of FMLP (response half-life of 36 ± 3 min) was signifi-

cantly reduced at all but one time point in the presence of colchicine (Figure 6b).

Discussion

Oedema formation in response to intradermal injection of FMLP was difficult to detect unless a vasodilator prostaglandin (PGE_2) was present, and in the presence of PGE_2 , FMLP showed similar permeability-increasing activity to rabbit C5a des Arg. The response to FMLP was independent of endogenous histamine release and FMLP was 100–1000 times more potent than histamine. Oedema formation was dependent on circulating neutrophils (Figure 3) and the activity of a range of N-formyl peptides in increasing vascular permeability correlated well with their activity as neutrophil chemoattractants and inducers of lysosomal enzyme secretion *in vitro* (Showell *et al.*, 1976; Freer *et al.*, 1982).

Intravenous infusion of ZAP was found to be an effective method of inducing transient neutropenia in rabbits and this resulted in selective inhibition of oedema formation induced by FMLP and C5a des Arg (neutrophil-dependent), but not that induced by bradykinin (neutrophil-independent). It was necessary to infuse ZAP for the duration of the experiment to maintain the neutropenia, although even under these conditions there was some return of cells to the circulation within 30 min. Upon cessation of ZAP infusion, we observed a transient neutrophilia and responses to C5a des Arg and FMLP were restored (data not shown). We also attempted analogous experiments infusing FMLP *i.v.*, and while this resulted in a major fall in circulating neutrophil counts as found by O'Flaherty *et al.* (1977), there was also a fall in systemic blood pressure which resulted in significant inhibition of responses to not only FMLP and C5a des Arg, but also to bradykinin. The mechanism for this is unclear, but falls in systemic blood pressure have previously been shown to result in a generalized suppression of oedema responses to both neutrophil-dependent and neutrophil-independent mediators (Rampart & Williams, 1986b).

Ibuprofen administered *i.v.* to rabbits resulted in a selective reduction of oedema responses induced by FMLP + PGE_2 (neutrophil-dependent), but not oedema induced by histamine + PGE_2 (neutrophil-independent). Systemic ibuprofen did not reduce circulating neutrophil counts and the results suggest that the drug can interfere with the interaction between circulating neutrophils and venular endothelial cells, which is the initial step in oedema formation induced by FMLP. Certainly, ibuprofen can

inhibit several aspects of neutrophil function *in vitro* including adherence to surfaces (Nielsen & Webster, 1987). One intriguing possibility is that ibuprofen inhibits the FMLP-induced increased expression of the CD11/CD18 adhesion glycoproteins on the neutrophil (Wallis *et al.*, 1986); however, this remains to be investigated. We have found that *i.v.* ibuprofen selectively inhibits neutrophil-dependent oedema formation in rat skin (Hellewell *et al.*, unpublished) and the drug also inhibits oedema formation induced by C5a des Arg in the rabbit by a cyclo-oxygenase-independent method (Rampart & Williams, 1986a). Whatever the mechanism of action of ibuprofen, it may relate to the observations that ibuprofen suppresses experimentally-induced myocardial infarction, a reaction characterized by neutrophil accumulation (Kirlin *et al.*, 1982; Flynn *et al.*, 1984).

The experiments with colchicine suggest that oedema formation induced by FMLP can be virtually abolished by this compound. The mechanism for this inhibition is unknown but is presumably related to suppression of neutrophil function. That it is not related to an inhibition of endothelial function is indicated by the observation that oedema responses to bradykinin were unaffected. However, at a higher dose of colchicine (3 mg kg^{-1}), we found that while responses to FMLP remained inhibited, those to bradykinin were enhanced, perhaps indicating an effect of the drug at this dose on the endothelium (data not shown). Colchicine is known to interfere with several aspects of neutrophil function *in vitro* such as chemotaxis and chemokinesis (Malech *et al.*, 1977; Valerius, 1978), and aggregation (Lackie, 1974; Cunningham & Smith, 1982). *In vivo* in the rat, colchicine inhibits neutrophil migration into inflammatory lesions (Higgs *et al.*, 1983; Simmons *et al.*, 1983) and suppresses oedema formation in the Arthus reaction (Chang, 1975). We found that intravenous colchicine abolished subsequent plasma protein leakage when administered at different times after intradermal FMLP injection. This suggests that continuing interactions (whatever these may be) between neutrophils and endothelial cells are necessary for the protracted plasma protein leakage induced by this chemoattractant.

The results of this study are consistent with the concept that receptors have evolved to bacterial secretions (i.e. FMLP) and also to products of the interaction of bacterial cell walls with tissue fluid (i.e. C5a des Arg). These observations support the hypothesis that oedema formation is fundamentally a functional process concerned with regulating microbial lysis and opsonisation in an infected tissue.

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