Br. J. Pharmacol. (1988), **94**, 1143–1148

Evidence that neutrophil accumulation induced by interleukin-1 requires both local protein biosynthesis and neutrophil CD18 antigen expression *in vivo*

[1]M. Rampart & [2]T.J. Williams

Vascular Biology Section, MRC Clinical Research Centre, Watford Road, Harrow, Middlesex HA1 3UJ

1 Mechanisms involved in neutrophil accumulation induced by intradermal injection of interleukin-1 (IL-1) in the rabbit were investigated using intravenously-injected ^{111}In-labelled neutrophils. C5a des Arg, N-formyl-methionyl-leucyl-phenylalanine (FMLP) and leukotriene B_4 (LTB_4) were included for comparison.

2 Local inhibition of protein biosynthesis in the skin using actinomycin-D or cycloheximide blocked ^{111}In-neutrophil accumulation induced by IL-1, but not that induced by the other mediators.

3 Actinomycin-D and cycloheximide had no effect on local plasma protein leakage induced by intradermally-injected C5a des Arg, or that induced by zymosan. ^{111}In-neutrophil accumulation induced by zymosan was, however, partially suppressed.

4 A monoclonal antibody, MoAb 60.3, recognising neutrophil surface CD18 antigen, was preincubated with ^{111}In-neutrophils before intravenous injection. This pretreatment did not affect circulating numbers of radiolabelled cells, but it inhibited their accumulation in response to IL-1, C5a des Arg and the other mediators.

5 The results suggest that neutrophil accumulation induced by IL-1, but not the other mediators, requires local protein biosynthesis, probably in the microvascular endothelium. Neutrophil accumulation to IL-1 and the other mediators appears to require neutrophil surface antigen, CD18. The inflammatory response to zymosan may be mediated by both endogenous C5a des Arg and IL-1.

Introduction

The increased adherence of neutrophils to venular endothelial cells at sites of tissue injury or microbial invasion is a key phase in the acute inflammatory response. Local increased adherence of neutrophils to endothelial cells is stimulated by chemical messengers generated extravascularly. These mediators can arise either from complement activation in extravascular tissue fluid or from activation of cells, such as macrophages. The 73 amino acid complement fragment C5a des Arg, detected in high concentrations in inflammatory exudates (Williams & Jose, 1981; Jose *et al.*, 1983; Forrest *et al.*, 1986), has potent chemotactic properties for neutrophils *in vitro*. C5a des Arg induces neutrophil accumulation and neutrophil-dependent oedema *in vivo* when injected intradermally in the rabbit (Williams & Jose, 1981; Wedmore & Williams, 1981; Movat *et al.*, 1984). N-formyl-methionyl-leucyl-phenylalanine (FMLP) and leukotriene (LTB_4) have similar activities (Higgs *et al.*, 1981; Movat *et al.*, 1984). There is also evidence that cell derived monokines, such as interleukin-1 (IL-1) can trigger the influx of neutrophils into an inflamed area (Goto *et al.*, 1984; Cybulsky *et al.*, 1986; Beck *et al.*, 1986; Granstein *et al.*, 1986; Pettipher *et al.*, 1986).

Using ^{111}In-labelled neutrophils, we have recently shown, in a preliminary study (Williams *et al.*, 1987), that the rate of neutrophil accumulation in response to C5a des Arg in rabbit skin *in vivo* is maximal in the first 30 min and declines with a half-life of approximately 110 min. Other chemoattractants, such as FMLP and LTB_4, showed similar time

[1] Present address: Department of Experimental Pharmacology, University of Antwerp, B-2610 Wilrijk, Belgium.
[2] Author for correspondence.

courses, but with shorter half-lives of 50 and 30 min, respectively. In contrast, human recombinant IL-1 was found to cause neutrophil accumulation that was not detectable in the first 30 min, but peaked at 4 h (α and β forms having identical potency and kinetics). These experiments were carried out in the presence of a locally-injected vasodilator prostaglandin and under these conditions neutrophil accumulation induced by C5a des Arg, FMLP and LTB_4 was associated with a parallel time course of plasma protein leakage. However, little plasma protein leakage was observed with IL-1, even when the prostaglandin was injected at the peak of neutrophil infiltration. Similarly, other workers have shown that IL-1 induces neutrophil accumulation in the rabbit knee joint that is not associated with joint swelling (Pettipher *et al.*, 1986).

These findings imply that IL-1 has a different mechanism of action *in vivo* and are consistent with the results of recent *in vitro* experiments suggesting that C5a des Arg, like FMLP and LTB_4, may increase adherence by stimulating the increased expression of a glycoprotein complex (Mac1, Mo 1, CD11b/CD18) on the surface of neutrophils (Sanchez-Madrid *et al.*, 1983; Wallis *et al.*, 1986), whereas IL-1 may act by causing the expression of adhesive molecules (E-LAMs) on endothelial cells (Bevilacqua *et al.*, 1985a,b). *In vitro*, the former process involves rapid exocytosis by the neutrophil whereas the latter involves a slow protein biosynthesis-dependent mechanism in the endothelium (Berger *et al.*, 1984; Bevilacqua *et al.*, 1985a,b). Some preliminary evidence that these differences observed *in vitro* may be related to *in vivo* mechanisms has recently been obtained (Williams *et al.*, 1987; McComb *et al.*, 1987). Further, it has been shown that intravenous injection of a monoclonal antibody, MoAb 60.3, that binds to the neutrophil surface CD18 antigen, inhibits neutrophil accumulation induced by intradermal injections of zymosan-activated serum, FMLP and LTB_4 in the rabbit (Arfors *et al.*, 1987). The same antibody has also been shown to inhibit partially adherence of neutrophils to endothelial cells stimulated with IL-1 *in vitro* (Pohlman *et al.*, 1986).

The experiments described here were designed to investigate mechanisms involved in local neutrophil accumulation induced by intradermally-injected IL-1 *in vivo*. Two procedures were employed. Firstly, local protein synthesis was blocked, using cycloheximide and actinomycin-D injected intradermally, to study possible effects on ^{111}In-neutrophil accumulation. Secondly, ^{111}In-neutrophils were pre-incubated with MoAb 60.3, to block surface CD18 antigen, before injection into the circulation. Three chemoattractants C5a des Arg, FMLP and LTB_4 were included for comparison with IL-1.

Methods

Animals

Male, specific pathogen-free New Zealand white rabbits (2.5–3 kg) were purchased from Froxfield Farm, Froxfield, Hampshire.

Preparation of ^{111}In-labelled neutrophils

Rabbits were anaesthetized with Sagatal (30 mg kg^{-1}) and bled out into acid citrate dextrose via a carotid cannula. Rabbit neutrophils (>90% pure) were harvested from 70 ml of citrated blood by a two layer discontinuous (40/60%) Percoll-plasma gradient after initial red cell sedimentation with hydroxyethyl starch (3% final concentration). Neutrophils (approximately 5×10^7 cells in 2 ml) were incubated with ^{111}In Cl_3 (50–200 μCi) chelated to 2-mercaptopyridine-N-oxide (40 μg ml^{-1}) for 15 min at room temperature. The labelled cells were washed twice in 11 ml of autologous citrated plasma and resuspended in 6 ml. The cells were then divided equally for i.v. injection into two recipient rabbits.

Treatment of ^{111}In-neutrophils with MoAb 60.3

Preliminary experiments with isolated neutrophils *in vitro* at the concentration shown above demonstrated that MoAb 60.3 at 88 μg ml^{-1} saturated all the available binding sites, as measured by a two-layer immunofluorescence flow cytometry technique (Wallis *et al.*, 1986).

In the experiments shown in Figure 4 the labelled cells were divided into two 1 ml samples immediately before the first wash. One ml of the monoclonal antibody recognising CD18 antigen, MoAb 60.3, in citrated plasma (MoAb 60.3, 88 μg ml^{-1}) was then added to one sample. An equal volume of citrated plasma was added to the other. After a further 10 min, both samples of cells were then washed twice before injection into test and control recipients.

Measurement of neutrophil and plasma albumin accumulation in the skin in vivo

Rabbits were anaesthetized with Sagatal and the fur on their backs was removed with clippers. Neutrophil infiltration and oedema formation in the back skin was measured as the local accumulation of i.v. injected ^{111}In-labelled neutrophils and ^{125}I-human serum albumin (15 μCi, mixed with 2 ml of a 2.5% Evans blue dye solution). The agents under investigation were injected intradermally in 0.1 ml volumes according to a balanced site pattern, each treatment having six replicates. Animals were killed 4 h later by an overdose of Sagatal. The back skin was removed,

the injection sites were excised with a 17 mm diameter punch and the skin samples were counted in a 12 head gamma counter with automatic spill-over and cross-talk correction (LKB Wallac 1260 Multigamma II). Results were expressed in terms of number of ^{111}In-neutrophils per site by dividing skin sample ^{111}In counts by ^{111}In counts per cell in preparations before i.v. injection. Exudate volumes were expressed as μl of plasma by dividing skin sample ^{125}I counts by ^{125}I counts in 1 μl of plasma (Williams & Jose, 1981; Wedmore & Williams, 1981).

Results are presented as mean values ± s.e.mean for the number of rabbits indicated ($n = 4$–7); one datum unit being the mean of six replicates in each rabbit. All data in the text are subtracted for intradermal saline control levels. For statistical analysis of data, the Wilcoxon rank sum test was used. A P value of <0.05 was considered to be statistically significant.

Materials

FMLP, cycloheximide (CHX), actinomycin-D (Act-D), zymosan and 2-mercaptopyridine N-oxide were from Sigma Chemical Co., Poole, Dorset. Evans blue dye was from BDH, Poole, Dorset. Sagatal (pentobarbitone sodium, 60 mg ml^{-1}) was from May and Baker, Dagenham, Essex. Percoll was from Pharmacia Fine Chemicals, Uppsala, Sweden. Hespan (6% hydroxy-ethyl starch in 0.9% NaCl) was from American Hospital Supply, Didcot, Oxfordshire. Steriflex (sterile, pyrogen-free isotonic saline solution) was from The Boots Co., Nottingham. $^{111}InCl_3$ (2 mCi in 0.2 ml sterile, pyrogen-free 0.04 N hydrochloric acid) and ^{125}I-human serum albumin (50 μCi 20 mg^{-1} albumin in 1 ml sterile isotonic saline) were from Amersham International, Amersham, Buckinghamshire.

The following were generous gifts: LTB_4 from Dr S.J. Foster, ICI, Macclesfield; human recombinant interleukin-1α (IL-1α), 1.4×10^{-15} mol unit^{-1} from Dr D. Westmacott, Roche Products Ltd, Welwyn Garden City, Hertfordshire; human recombinant interleukin-1β (IL-1β), 2×10^{-15} mol unit^{-1} from Professor W. Fiers, Dept. Molecular Biology, University of Gent, Gent, Belgium; MoAb 60.3 from Dr J.M. Harlan, University of Washington, Seattle, Washington, U.S.A.

All working solutions were freshly made up each day in sterile, pyrogen-free saline.

Zymosan-activated plasma as a source of C5a des Arg was prepared by incubating heparinised (10 u ml^{-1}) rabbit plasma with zymosan (5 mg ml^{-1}) for 30 min at 37°C. Zymosan was removed by centrifugation (2×10 min, 2500 g) and activated plasma stored in aliquots at −25°C. The C5a des Arg content of activated plasma was measured by radioimmunoassay (Jose *et al.*, 1983).

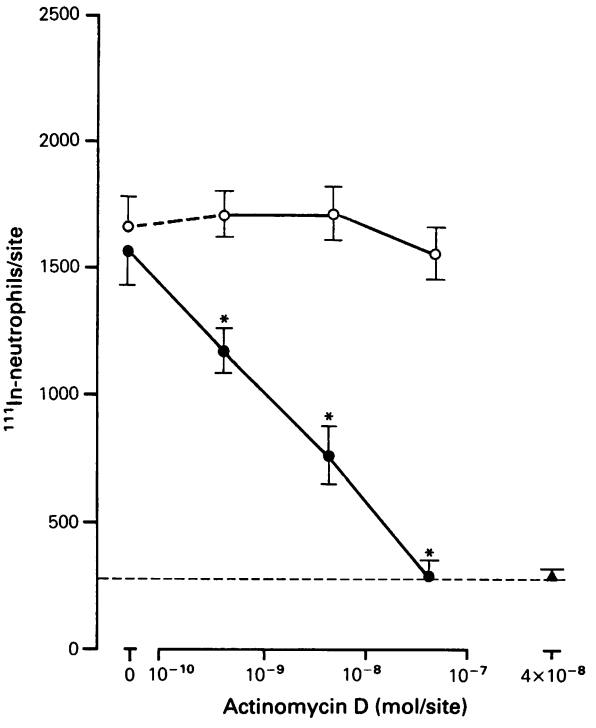

Figure 1 The effect of intradermal injections of increasing amounts of actinomycin-D on neutrophil accumulation induced by interleukin-1α (IL-1α; 10 u/site) (●) in comparison with C5a des Arg (5×10^{-11} mol/site) (○). The actinomycin-D was mixed with the mediators immediately before intradermal injection and neutrophil accumulation was measured over a 4 h period. For experimental details see Methods section. Each point represents the mean for $n = 5$ (IL-1α) or $n = 7$ (C5a des Arg) rabbits; vertical lines indicate s.e.mean. The response to actinomycin-D alone is shown (▲) and the dashed line represents the value for saline controls. *$P < 0.05$.

Results

Co-injection with the intradermal test agents of the RNA synthesis inhibitor, actinomycin-D (Act-D) resulted in a dose-dependent inhibition of ^{111}In-neutrophil accumulation induced by IL-1α (10 u/site). Responses to C5a des Arg (5×10^{-11} mol/site by radioimmunoassay of zymosan-activated plasma) were not affected by Act-D (Figure 1). Similarly, neutrophil accumulation induced by FMLP and LTB_4 was not suppressed by Act-D: FMLP (5×10^{-11} mol/site) alone 1245 ± 100; FMLP with

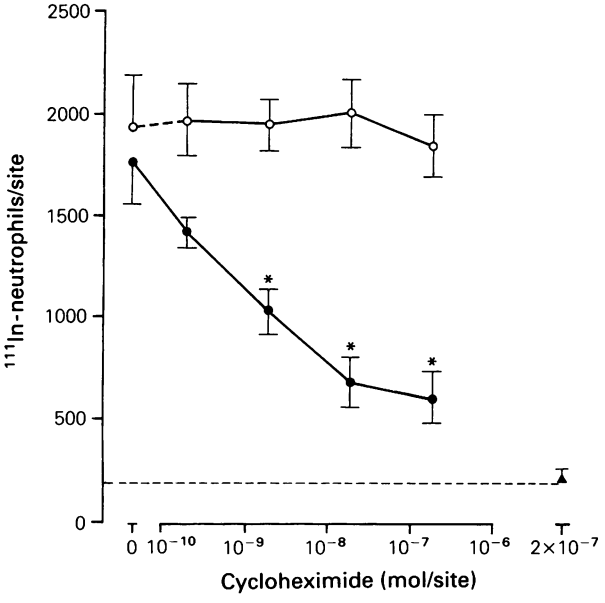

Figure 2 The effect of cycloheximide on neutrophil accumulation induced by interleukin-1α (IL-1α; 10 u/site) (●) in comparison with FMLP (5×10^{-11} mol/site) (○). The procedure was the same as in Figure 1. Each point represents the mean for $n = 5$ rabbits; vertical lines indicate s.e.mean. The response to cycloheximide alone is shown (▲) and the dashed line represents the value for saline controls. $^*P < 0.05$.

Act-D (4×10^{-8} mol/site) 1450 ± 200; LTB_4 (10^{-10} mol/site) alone 785 ± 75; LTB_4 with Act-D (4×10^{-8} mol/site) 705 ± 75. (Results are number of ^{111}In-neutrophils per site and are expressed as mean ± s.e.mean for $n = 6$ sites; all data subtracted for saline backgrounds).

The protein biosynthesis inhibitor, cycloheximide, yielded results very similar to those obtained with Act-D. There was a dose-dependent reduction of ^{111}In-neutrophil accumulation induced by IL-1α and no effect on responses to FMLP (Figure 2). Cycloheximide did not affect neutrophil accumulation induced by C5a des Arg and LTB_4: C5a des Arg (5×10^{-11} mol/site) alone 1090 ± 95; C5a des Arg with cycloheximide (2×10^{-7} mol/site) 950 ± 130; LTB_4 (10^{-10} mol/site) alone 820 ± 70; LTB_4 with cycloheximide (2×10^{-7} mol/site) 765 ± 95.

Act-D and cycloheximide also dose-dependently suppressed neutrophil accumulation induced by IL-1β (10 u/site). Using IL-1β as the stimulus, the effect of the inhibitors given at different times was investigated. Act-D (4×10^{-8} mol/site) and cycloheximide (2×10^{-7} mol/site) were injected intradermally at 4, 2 and 1 h before intradermal injection of IL-1β (10 u/site), co-injected, or injected 1 h afterwards. With co-injection there was suppression of neutrophil accumulation using both inhibitors i.e. Act-D 94 ± 5% inhibition ($n = 4$ rabbits, $P < 0.05$), cycloheximide 75 ± 7% inhibition ($n = 4$ rabbits, $P < 0.05$). There was no significant suppression with the pre- or post-injection of inhibitors. Responses to FMLP and C5a des Arg were measured in the same animals; no significant suppression was seen at any time point.

Simultaneous estimation of plasma protein leakage (measured as the 4 h local accumulation of intravenously injected ^{125}I-human serum albumin) showed that responses to intradermal C5a des Arg (as well as those to FMLP and LTB_4; results not included) were not affected by either cycloheximide or Act-D: C5a des Arg (5×10^{-11} mol/site) alone 39 ± 1 μl; C5a des Arg with Act-D (4×10^{-8} mol/site) 35 ± 1 μl; and C5a des Arg with cycloheximide (2×10^{-7} mol/site) 33 ± 4 μl. The very small oedematous response to IL-1α was almost abolished by both agents: IL-1α (10 u/site) alone 13 ± 1 μl; IL-1α with Act-D (4×10^{-8} mol/site) 3 ± 1 μl ($P < 0.05$); and IL-1α with cycloheximide (2×10^{-7} mol/site) 2 ± 1 μl ($P < 0.05$). (Results are μl plasma per site and are mean ± s.e.mean for $n = 5$ rabbits; all data subtracted for saline backgrounds.)

Figure 3 shows the effect of Act-D and cycloheximide on the release and/or action of endogenous inflammatory mediators induced by zymosan in the skin. Zymosan-induced oedema formation and neu-

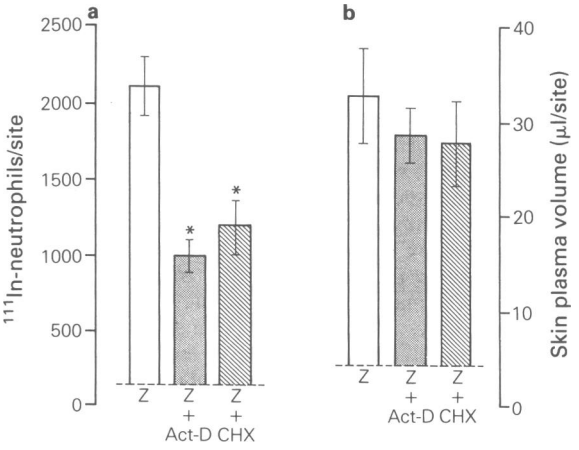

Figure 3 The effect of actinomycin-D (Act-D, 4×10^{-8} mol/site) and cycloheximide (CHX, 2×10^{-7} mol/site) on neutrophil accumulation (a) and plasma protein leakage (b) induced by intradermally injected zymosan (Z, 100 μg/site). For experimental details, see Methods section. Each column represents the mean of results from $n = 6$ rabbits and vertical lines indicate s.e.mean. The dashed lines show the values for saline controls. $^*P < 0.05$.

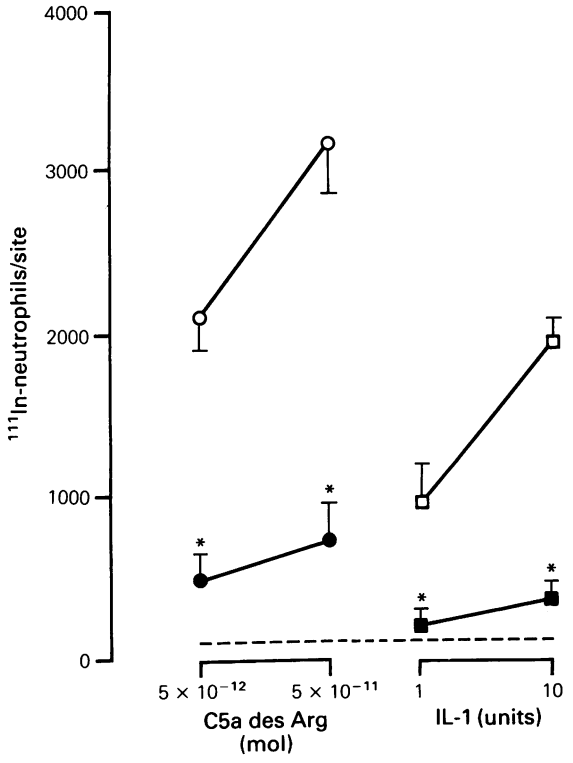

Figure 4 The effect of a monoclonal antibody recognising neutrophil surface CD18 antigen, MoAb 60.3, upon neutrophil accumulation induced by C5a des Arg and interleukin-1α (IL-1α) at the doses shown per site. The antibody was incubated with the ^{111}In-neutrophils *in vitro*, before intravenous injection. (○, □) Responses of control cells; (●, ■) responses of antibody-treated cells. For experimental details see Methods section. Each point represents the mean for $n = 4$ rabbits; vertical lines indicate s.e.mean. The dashed line shows the value for saline controls. $^*P < 0.05$.

trophil accumulation were over a 4 h period. Act-D and cycloheximide, co-injected with zymosan into the skin, had no effect on plasma leakage (b), but significantly suppressed accumulation of ^{111}In-neutrophils by 56% and 47%, respectively (a).

Figure 4 shows the effect of pretreatment of ^{111}In-neutrophils with MoAb 60.3 before intravenous injection. Neutrophils from a single donor were radiolabelled and divided into two aliquots: one incubated with antibody and the other without, as control. Both aliquots were washed and then injected intravenously into a test and control recipient. Neutrophil accumulation was then measured in response to zymosan-activated plasma and IL-1α over four hours. Antibody pretreatment produced a marked inhibition of neutrophil accumulation: inhibition (high and low doses of mediators, respectively) was 85% and 88% for IL-1α and 81% and 82% for zymosan-activated plasma. There was no significant difference in the numbers of circulating labelled neutrophils between test and control rabbits.

Discussion

These results show that neutrophil accumulation and neutrophil-dependent oedema induced by intradermally-injected C5a des Arg, FMLP and LTB_4 are unaffected by local inhibition of protein biosynthesis. In contrast, the slower neutrophil accumulation induced by IL-1 (α and β) is suppressed by inhibition of local protein biosynthesis. Local oedema induced by IL-1α, although very weak, was also suppressed in these experiments.

These observations are consistent with those *in vitro* showing slow, protein synthesis-dependent effects of IL-1 on endothelial cell adherence (involving E-LAM expression) and fast, protein synthesis-independent effects on neutrophil adherence (involving CD11/CD18 antigen complex expression) induced by the other mediators. The results support the general hypothesis that IL-1 acts predominantly on the microvascular endothelium to induce neutrophil attachment *in vivo*, whereas the other mediators act predominantly on neutrophils within the vessel lumen.

We have previously presented evidence that the inflammatory response to locally-injected zymosan involves the generation of C5a and C5a des Arg in extravascular tissue fluid (Jose *et al.*, 1983; Forrest *et al.*, 1986). Interestingly, a further component of this reaction is suggested by the experiments presented here. The response to intradermally-injected zymosan involves marked oedema formation. C5a des Arg is potent in causing oedema, especially in the presence of prostaglandin E_2 (PGE_2), but IL-1 is weak in this respect. However, neutrophil accumulation induced by zymosan was partially suppressed by Act-D and cycloheximide whereas oedema formation was unaffected. This suggests that the response to zymosan has a component of neutrophil accumulation and neutrophil-dependent oedema mediated by C5a des Arg, and a further component of neutrophil accumulation mediated by IL-1 or a mediator acting by a similar mechanism.

We were interested to observe that pretreatment of ^{111}In-neutrophils *in vitro* with an antibody binding to surface CD18 antigen suppressed neutrophil accumulation in response to intradermally-injected IL-1 *in vivo*. This would suggest that E-LAMs expressed on microvascular endothelial cells in response to IL-1 adhere to basally-expressed CD11b/CD18 on neutrophils *in vivo*, a possibility

indicated by previous *in vitro* experiments (Pohlman *et al.*, 1986). We were surprised that pretreatment of neutrophils with the anitbody also effectively suppressed their accumulation in response to C5a des Arg. This is the subject of a further study with a range of chemoattractants (S. Nourshargh *et al.*, unpublished). It might have been expected that new expression of CD11b/CD18 on the neutrophil surface in response to the chemoattractant in the skin would have over-ridden the blocking effect of the antibody on basal expression. There are several possibilities to explain this, one of which is that chemoattractants induce a conformational change in surface CD11b/CD18 which is more important for adherence than increased expression.

We are most grateful to Dr John Harlan for the gift of MoAb 60.3 and for helpful discussions. We thank the Medical Research Council, the British Heart Foundation and Pfizer Central Research, Groton, Connecticut, U.S.A., for generously supporting this project.

References

ARFORS, K-E., LUNDBERG, C., LINDBOM, L., LUNDBERG, K., BEATTY, P.G. & J.M. HARLAN (1987). A monoclonal antibody to the membrane glycoprotein complex CD18 inhibits polymorphonuclear leukocyte accumulation and plasma leakage in vivo. *Blood*, **69**, 338–340.

BECK, G., HABICHT, C.S., BENACH, J.L. & MILLER, F. (1986). Interleukin-1: a common endogenous mediator of inflammation and the local Schwartzman reaction. *J. Immunol.*, **136**, 3025–3031.

BERGER, M., O'SHEA, J., CROSS, A.S., FOLKS, T.M., CHUSED, T.M., BROWN, E.J. & FRANK, M.M. (1984). Human neutrophils increase expression of C3bi as well as C3b receptors upon activation. *J. Clin. Invest.*, **74**, 1566–1571.

BEVILACQUA, M.P., POBER, J.S., WHEELER, M.E., COTRAN, R.S. & GIMBRONE, M.A. (1985a). Interleukin-1 activation of vascular endothelium: Effects on procoagulant activity and leukocyte adhesion. *Am. J. Pathol.*, **121**, 394–403.

BEVILACQUA, M.P., POBER, J.S., WHEELER, M.E., COTRAN, R.S. & GIMBRONE, M.A. (1985b). Interleukin-1 acts on cultured human vascular endothelium to increase the adhesion of polymorphonuclear leukocytes, monocytes and related leukocyte cell lines. *J. Clin. Invest.*, **76**, 2003–2011.

CYBULSKY, M.I., COLDITZ, I.G. & MOVAT, H.Z. (1986). The role of interleukin-1 in neutrophil leukocyte emigration induced by endotoxin. *Am. J. Pathol.*, **124**, 367–372.

FORREST, M.J., JOSE, P.J. & WILLIAMS, T.J. (1986). Kinetics of the generation and action of chemical mediators in zymosan-induced inflammation of the rabbit peritoneal cavity. *Br. J. Pharmacol.*, **89**, 719–730.

GOTO, K., NAKAMURA, S., GOTO, F. & YOSHINAGA, M. (1984). Generation of an interleukin-1-like lymphocyte-stimulating factor at inflammatory sites: correlation with the infiltration of polymorphonuclear leukocytes. *Br. J. Exp. Path.*, **65**, 521–532.

GRANSTEIN, R.D., MARGOLIS, R., MIZEL, S.B. & SANDER, D.N. (1986). In vivo inflammatory activity of epidermal cell-derived thymocyte activating factor and recombinant interleukin-1 in the mouse. *J. Clin. Invest.*, **77**, 1020–1027.

HIGGS, G.A., SALMON, J.A. & SPAYNE, J.A. (1981). The inflammatory effects of hydroperoxy and hydroxy acid products of arachidonate lipoxygenase in rabbit skin. *Br. J. Pharmacol.*, **74**, 429–433.

JOSE, P.J., FORREST, M.J. & WILLIAMS, T.J. (1983). Detection of the complement fragment C5a in inflammatory exudates from the rabbit peritoneal cavity using radioimmunoassay. *J. Exp. Med.*, **158**, 2177–2182.

McCOMB, D.J., CYBULSKY, M.I. & MOVAT, H.Z. (1987). PMN emigration: protein synthesis dependent and independent mechanisms. *Fed. Proc.*, **46**, 1390 (Abstr.).

MOVAT, H.Z., RETTL, C., BURROWES, C.E. & JOHNSTON, M.G. (1984). The in vivo effect of leukotriene B_4 on polymorphonuclear leukocytes and the microcirculation. Comparison with activated complement (C5a des Arg) and enhancement by prostaglandin E_2. *Am. J. Pathol.*, **115**, 233–245.

PETTIPHER, E.R., HIGGS, G.A. & HENDERSON, B. (1986). Interleukin-1 induces leukocyte infiltration and cartilage proteoglycan degradation in the synovial joint. *Proc. Natl. Acad. Sci. U.S.A.*, **83**, 8749–8753.

POHLMAN, T.H., STANNESS, K.A., BEATTY, P.G., OCHS, H.D. & HARLAN, J.M. (1986). An endothelial cell surface factor(s) induced in vitro by lipopolysaccharide, interleukin-1, and tumor necrosis factor α increases neutrophil adherence by a CDw18-dependent mechanism. *J. Immunol.*, **136**, 4548–4553.

SANCHEZ-MADRID, F., NAGY, J.A., ROBBINS, E., SIMON, P. & SPRINGER, T.A. (1983). A human leukocyte differentiation antigen family with distinct α-subunits and a common β-subunit: the lymphocyte function-associated antigen (LFA-1), the C3bi complement receptor (OKM1/Mac1) and the p15095 molecule. *J. Exp. Med.*, **158**, 1785–1803.

WALLIS, W.J., HICKSTEIN, D.D., SCHWARTZ, B.R., JUNE, C.H., OCHS, H.D., BEATTY, P.G., KEBLANOFF, S.J. & HARLAN, J.M. (1986). Monoclonal antibody-defined functional epitopes on the adhesion-promoting glycoprotein complex (CDw18) of human neutrophils. *Blood*, **67**, 1007–1013.

WEDMORE, C.V. & WILLIAMS, T.J. (1981). Control of vascular permeability by polymorphonuclear leukocytes in inflammation. *Nature*, **289**, 646–650.

WILLIAMS, T.J. & JOSE, P.J. (1981). Mediation of increased vascular permeability after complement activation: Histamine-independent action of rabbit C5a. *J. Exp. Med.*, **153**, 136–153.

WILLIAMS, T.J., RAMPART, M., NOURSHARGH, S., HELLEWELL, P.G., BRAIN, S.D. & JOSE, P.J. (1987). Interaction of polymorphonuclear leukocytes and endothelial cells: functional consequences. *Thrombosis & Haemostasis*, **58**, 326 (Abstr.).

(Received January 8, 1988
Revised February 25, 1988
Accepted March 21, 1988)