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The role of CD18 in IL-8 induced dermal and synovial inflammation

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1 The intradermal administration of endothelial IL-8 ($IL\text{-}8_{1\text{-}77}$) or monocyte derived IL-8 ($IL\text{-}8_{1\text{-}72}$) to rabbits produced a concentration-dependent increase in plasma extravasation and an accumulation of polymorphonuclear leukocytes (PMNs) when measured over a 3 h time period. When plasma extravasation and PMN accumulation were measured over a 30 min time period no significant increases in PMN accumulation or plasma extravasation were observed in response to IL-8 alone. However, under these conditions, the addition of prostaglandin E_2 (100 pmol) produced a significant potentiation of IL-8-induced plasma extravasation. There was no significant difference between the biological activities of $IL\text{-}8_{1\text{-}77}$ and $IL\text{-}8_{1\text{-}72}$.

2 Plasma extravasation and PMN accumulation induced by IL-8 were inhibited in rabbits pretreated with the monoclonal antibody designated IB4 (1 mg kg^{-1}, i.v.) directed against the common β chain (CD18) of the leukocyte integrins.

3 The intra-articular administration to rabbits of $IL\text{-}8_{1\text{-}77}$ (1 nmol) resulted 24 h later in the appearance of a mixed population of leukocytes (PMNs and mononuclear cells) in synovial lavage fluid. Biochemical analyses revealed the presence of an increased level of sulphated proteoglycans (sPG) and of the metalloproteinase stromelysin. Pretreatment of rabbits with IB4 (3 mg kg^{-1}, i.v.) inhibited the accumulation of PMNs but had no effect on the mononuclear infiltrate nor on the levels of sPG or stromelysin.

4 The intradermal or intra-articular injection of *E. coli*-derived endotoxin induced similar inflammatory changes to those observed with IL-8. The possibility that the biological activities of IL-8 were attributable to minor contamination with endotoxin is unlikely for two reasons. Firstly, biological effects of endotoxin were observed at levels greater than that contained in the IL-8 preparation. Secondly, reduction of the endotoxin content of the IL-8 preparation by a factor of 10 did not produce a concomitant reduction in the observed biological activity of the IL-8.

Keywords: Inflammation; interleukin 8; leukocyte integrins; PMNs; plasma extravasation; cartilage degradation; stromelysin; proteoglycan

Introduction

The local accumulation of polymorphonuclear leukocytes (PMNs) is a characteristic feature of the inflammatory response to both infectious and non-infectious stimuli. This process is mediated by the elaboration of molecules which exhibit chemotactic activity for PMNs. Many compounds, of both humoral and cellular origin, have been identified which have chemotactic activity for PMNs. In the last few years, a novel PMN chemotactic activity, referred to variously as monocyte derived neutrophil chemotactic factor (MDNCF: Yoshimura *et al.*, 1987a, b), monocyte-derived neutrophil-activating peptide (MONAP: Schroder *et al.*, 1987), neutrophil activating factor (NAF: Walz *et al.*, 1987) and interleukin 8 (IL-8: Westwick *et al.*, 1989) has been identified and characterized. Monocytes are not the only source of IL-8, as similar material has also been identified, either functionally or at the gene level, from endothelial cells (Strieter *et al.*, 1988; 1989a; Gimbrone *et al.*, 1989; Schroder & Christophers, 1989), fibroblasts (Strieter *et al.*, 1989b; Van Damme *et al.*, 1989; Schroder *et al.*, 1990), human T-lymphocytes (Maestrelli *et al.*, 1988; Schroder *et al.*, 1988), hepatocytes (Thornton *et al.*, 1990) and epithelial cells (Elner *et al.*, 1990).

IL-8 is generated initially as a precursor of 99 amino acids (Matsushima *et al.*, 1988) which includes a signal peptide of 22 amino acids. Different mature forms of IL-8 have been identified (Yoshimura *et al.*, 1989) which suggests that the precursor of IL-8 undergoes multiple forms of processing. Human peripheral blood monocytes stimulated with lipopolysaccharide (LPS) have been reported to generate either equivalent amounts of the 72 and 77 amino acid peptides (Yoshimura *et al.*, 1989) or predominantly the 72 amino acid variant (Lindley *et al.*, 1988). However, the predominant IL-8 peptide generated by both endothelial cells (Gimbrone *et al.*, 1989) and fibroblasts (Schroder *et al.*, 1990) is the 77 amino acid variant containing an N-terminal pentapeptide extension.

In addition to its PMN chemotactic activity *in vitro*, IL-8 elicits a range of biological activities. These include induction of PMN degranulation (Schroder *et al.*, 1987; Peveri *et al.*, 1988), stimulation of PMN superoxide anion formation (Thelen *et al.*, 1988), elevation of PMN cytosolic free calcium (Thelen *et al.*, 1988), enhancement of PMN-mediated *Candida* killing (Djeu *et al.*, 1990), stimulation of the 5-lipoxygenase enzyme (Schroder, 1989), stimulation of PMN adherence to normal endothelial cells (Carveth *et al.*, 1989), inhibition of PMN adhesion to IL-1-stimulated endothelial cells (Gimbrone *et al.*, 1989), histamine release (White *et al.*, 1989) and chemotactic activity *in vitro* for T-lymphocytes (Larsen *et al.*, 1989).

The sequelae of IL-8 administration to animals include a series of biological responses consistent with its profile of activity *in vitro*. For example, in rabbits the local intradermal injection of IL-8 induces PMN accumulation and plasma extravasation (Colditz *et al.*, 1989; Rampart *et al.*, 1989; Colditz *et al.*, 1990). Furthermore, the intra-articular injection of IL-8 reslts in PMN accumulation in synovial lavage fluid (Akahoshi *et al.*, 1990). PMN accumulation in tissues,

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in response to locally generated or administered mediators, is preceded by an adhesive interaction between the PMN and the vascular endothelium. This interaction is dependent, at least in part, on the recognition of ligands (counter-receptors) on the endothelial surface (e.g. ICAM-1) by the leukocyte β_2 integrin CD11/CD18 complex on the PMN plasma membrane (Carlos & Harlan, 1990). IL-8 increases the surface expression of the CD11/CD18 complex on PMNs (Detmers *et al.*, 1990) and antibodies directed against the CD11/CD18 complex inhibit the IL-8 stimulated increase in PMN adherence to normal vascular endothelial monolayers (Carveth *et al.*, 1989).

The majority of data available to date, concerning the biological activities of IL-8, *in vivo* and *in vitro*, have been obtained with the 72 amino acid monocyte derived peptide ($IL\text{-}8_{1\text{-}72}$). In order to define further the relative activities of $IL\text{-}8_{1\text{-}72}$ and $IL\text{-}8_{1\text{-}77}$ we compared the effects of the two peptides on dermal PMN accumulation and plasma extravasation in the rabbit. We also examined the ability of $IL\text{-}8_{1\text{-}77}$ to elicit joint inflammation in the rabbit knee. Furthermore, we examined the extent to which IL-8-induced dermal and joint inflammation is dependent on the CD11/CD18 complex by utilising a monoclonal antibody, designated IB4, directed against the common β chain (CD18) of the leukocyte integrins, which effectively blocks the interaction of CD18 with its counter-receptor on endothelial cells (Lundberg & Wright, 1990).

Methods

Animals

Female New Zealand White rabbits (2.5–3.0 kg) were purchased from Hazelton Research Products (Denver, PA, USA). All experimental procedures involving the use of animals were approved by the Merck Institutional Animal Care and Use Committee. Animals were handled and cared for in accordance with guidelines set forth in the National Institutes of Health guide for the care and use of laboratory animals.

Measurement of plasma extravasation and PMN accumulation in rabbit skin

The dorsal hair of female New Zealand White rabbits (2–2.5 kg) was shaved at least 24 h before experimentation. Rabbits were anaesthetized by an intramuscular injection of ketamine HCl (25 mg kg^{-1}) and xylazine (2 mg kg^{-1}) and [^{125}I]-bovine serum albumin (10 μCi) was injected into the marginal ear vein, as a marker for plasma extravasation. Groups of animals were then administered the anti-CD18 monoclonal antibody, IB4 (1 mg kg^{-1}) or an equivalent volume of saline. IL-8 (1–100 pmol in a volume of 50 μl) or saline were injected intradermally into 4 replicate sites in the dorsum 15 min after the injection of the antibody. Three hours later the animals were re-anaesthetized by an intramuscular injection of ketamine HCl (25 mg kg^{-1}) and xylazine (2 mg kg^{-1}), and a blood sample (1 ml) was taken by cardiac puncture. The blood was centrifuged (800 *g*; 3 min; 20°C) to prepare plasma which was aspirated and retained. Animals were then killed with approximately 750 μl Socumb (sodium pentobarbitone, 389 mg ml^{-1} in 40% isopropyl alcohol) and injection sites were excised with a 6 mm biopsy punch. Radioactivity (^{125}I) present in skin samples and cell-free plasma (50 μl) was measured in a gamma counter. By reference to the specific radioactivity of the cell-free plasma, the extent of plasma extravasation was expressed as μl plasma equivalents per 6 mm biopsy. The skin biopsy was then homogenized in 5 ml of 0.5% hexadecyltrimethyl ammonium bromide (HTAB) with a polytron homogenizer. Chloroform (1 ml) was added to the sample, which was then vortexed and centrifuged (1600 *g*; 15 min; 20°C). Four aliquots (50 μl) of the aqueous supernatant were added to wells in a 96 well plate for measurement of myeloperoxidase (MPO) activity, as an index of PMN content. Duplicate wells of the 96 well plate received buffer (KH_2PO_4 44 mM; K_2HPO_4 6 mM; H_2O_2 0.0015%; pH 6.0) alone and duplicate wells received buffer containing MPO substrate (3',3-dimethoxybenzidine dihydrochloride, 1.1 mM). Reactions were allowed to proceed for 15 min at room temperature and MPO activity was measured as the change in absorbance at 450 nm measured in a plate reading spectrophotometer. By reference to a standard curve constructed with known quantities of rabbit PMNs in HTAB, the extent of PMN accumulation in each skin biopsy was estimated.

Induction of joint inflammation

Female NZW rabbits (3.0–3.5 kg) were anaesthetized with an intramuscular injection of ketamine HCl (30 mg kg^{-1}) plus xylazine (3 mg kg^{-1}). Groups of animals were administered either the anti-CD18 monoclonal antibody, IB4 (3 mg kg^{-1}), a dose known to prevent agonist-induced PMN accumulation in skin and synovial cavity for 24 h (data not shown), or an equivalent volume of saline intravenously via the marginal ear vein. After 15 min the rear knees were shaved, swabbed with isopropyl alcohol and injected intra-articularly with IL-8 (1 nmol in a volume of 500 μl) or 500 μl saline into the contralateral knee. The animals were allowed to regain consciousness and 24 h later were killed with approximately 750 μl Socumb. The knee joints were lavaged with 2 ml of sterile PBS containing EDTA (5 mM). Lavage fluids were immediately placed on ice. Samples (100 μl) of lavage fluid were removed for the determination of leukocytes, the remainder was centrifuged (1600 *g*; 15 min; 4°C) and the cell-free supernatant frozen at −20°C pending further biochemical analyses.

Measurement of cell accumulation in synovial lavage fluid

Total and differential cell counts of synovial lavage fluid were determined by light microscopy. Leukocytes were counted in an improved Neubauer counting chamber following staining of cells with gentian violet (0.1% crystal violet, 1% acetic acid in distilled water).

Measurement of sulphated proteoglycans (sPG) in synovial lavage fluid

The concentration of sPG in cell-free synovial lavage fluid was measured by the 1,9-dimethylmethylene blue binding assay (Farndale *et al.*, 1982). Keratan sulphate (Sigma, St. Louis, MO., U.S.A.) was used to generate the standard curve. Data are expressed as the concentration of sulphated PG in $\mu g\ ml^{-1}$ of synovial lavage fluid.

Measurement of stromelysin in synovial lavage fluid

Stromelysin levels in synovial lavage fluid were measured by a double antibody trapping ELISA based on a similar assay for the measurement of human stromelysin (Walakovits *et al.*, 1991). The modification of the assay for measuring rabbit stromelysin requires the use of sheep anti-rabbit prostromeylsin. The assay has a half maximum of detection of ~50 ng ml^{-1} with a log-linear range of 0.5–250 ng ml^{-1}. The assay detects both rabbit prostromelysin and active stromelysin with equivalent sensitivity. Rabbit stromelysin complexed with rabbit tissue inhibitor of metalloproteinase (TIMP) is also recognised by the assay but with approximately 2 fold lower sensitivity than free activated enzyme. The assay does not recognise the closely related metalloproteinase, rabbit collagenase, even at concentrations up to 1 $\mu g\ ml^{-1}$.

Materials

Monocyte and endothelial-derived IL-8 were supplied as freeze dried preparations from Biosource International (Westlake Village, CA, U.S.A.). The material was reconstituted in pyrogen-free water and diluted for use in endotoxin-free bovine serum albumin (1 mg ml^{-1}) in pyrogen-free saline. Endotoxin levels of IL-8 were determined by a colorimetric *Limulus* amoebocyte lysate assay (Whittaker Bioproducts, Walkersville, MD, U.S.A.). Where indicated in the results section, certain IL-8 preparations were passed through a 'Detoxi-Gel' column (Pierce, Rockford, IL, U.S.A.) for removal of endotoxin.

The IB4 monoclonal anti-CD18 antibody was purified by protein A chromatography from serum-free culture medium supernatants of IB4 murine hybridoma cells (Wright *et al.*, 1983) grown in a hollow fibre bioreactor. The antibody was prepared as a stock solution at 1 mg ml^{-1} in PBS (pH 7.2) and had an endotoxin level of <100 pg mg^{-1} protein.

Statistical analyses

Dose-response data were analysed by analysis of variance and other comparisons were made by Student's *t* test as indicated in the text or figure legends.

Results

IL-8 induced dermal inflammation

The intradermal injection of either IL-8_{1-77} or IL-8_{1-72} produced a concentration-dependent increase in plasma extravasation (Figure 1a) and PMN accumulation (Figure 1b). Whilst both peptides induced significant increases in plasma extravasation and PMN accumulation compared with saline-injected skin sites there were no significant differences in response betwen the two peptides at any concentration tested. *In vitro*, IL-8_{1-72} is more active than IL-8_{1-77} in assays of PMN activation (Herbert *et al.*, 1990; Schroder *et al.*, 1990). It is possible that a difference in *in vivo* activity of the two peptides exists but was masked in the experiments described here by instability of the peptides during the 3 h measurement period of PMN accumulation and plasma extravasation. Therefore, an additional series of experiments were performed in the presence and absence of the vasodilator prostaglandin E_2 (PGE_2) which potentiates the activity of permeability increasing agents (Williams & Jose, 1981) to allow the measurement of responses over a 30 min period. Neither IL-8_{1-72} nor IL-8_{1-77} when injected alone into rabbit skin induced increases in plasma extravasation or PMN accumulation (Table 1). The addition of PGE_2 (100 pmol) to either IL-8_{1-72} or IL-8_{1-77} produced a marked increase in plasma extravasation compared with either agent alone but did not significantly increase PMN accumulation. Furthermore, no significant difference existed in the activity of IL-8_{1-72} and IL-8_{1-77} in the presence of PGE_2.

The effect of the anti-CD18 monoclonal antibody IB4 (1 mg kg^{-1}, i.v.) on IL-8-induced dermal inflammation was examined. Plasma extravasation (Figure 2a) and PMN accumulation (Figure 2b) induced by either IL-8_{1-72} or IL-8_{1-77} were significantly inhibited in IB4 compared with vehicle-injected control animals.

IL-8-induced joint inflammation

Because no difference in activity between IL-8_{1-72} and IL-8_{1-77} was apparent in studies on dermal inflammation only one of the peptides (IL-8_{1-77}) was used to investigate the effects of IL-8 on joint inflammation in the rabbit knee. The intra-articular injection of IL-8_{1-77} (Figure 3) induced a significant ($P<0.001$) increase in total leukocytes, PMNs and mononuclear leukocytes in synovial lavage fluid compared with the

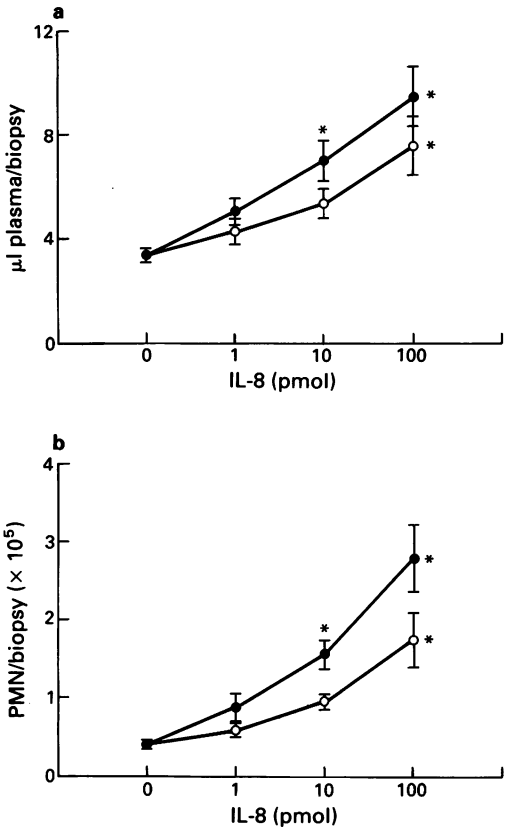

Figure 1 The effects of IL-8_{1-72} (1–100 pmol: ○) and IL-8_{1-77} (1–100 pmol: ●) on dermal plasma extravasation (a) and dermal PMN accumulation (b) were determined. Plasma extravasation was measured 3 h after the intradermal injection of stimuli as the local accumulation of intravenously injected ^{125}I-labelled human serum albumin and PMN accumulation was measured as the tissue content of myeloperoxidase (MPO). MPO levels were converted to numbers of cells from a standard curve constructed from known numbers of rabbit peripheral blood PMNs. Each point represents the mean and vertical lines the standard error for triplicate determinations in 6–12 animals. *$P<0.05$ (ANOVA) compared with saline-injected skin.

Table 1 The effects of IL-8_{1-72} and IL-8_{1-77} on plasma extravasation and PMN accumulation in rabbit skin

Treatment	*Plasma* (µl/site)	*PMNs* (× 10^{-5}/site
Saline	3.6 ± 0.4	0.3 ± 0.1
PGE_2	5.1 ± 0.8	0.3 ± 0.1
IL-8_{1-72}	3.8 ± 0.5	0.4 ± 0.2
IL-8_{1-77}	5.2 ± 0.6	0.5 ± 0.2
IL-8_{1-72} + PGE_2	13.4 ± 1.1*	0.7 ± 0.2
IL-8_{1-77} + PGE_2	12.7 ± 0.5*	0.7 ± 0.2

The effects of IL-8_{1-72} (100 pmol) and IL-8_{1-77} (100 pmol), in the presence and absence of prostaglandin E_2 (PGE_2; 100 pmol), on plasma extravasation and PMN accumulation were measured in rabbit skin 30 min following the intradermal injection of stimuli. Plasma extravasation was measured as the local accumulation of intravenously injectd ^{125}I-labelled bovine serum albumin and PMN accumulation as the myeloperoxidase activity of skin homogenates. Data are presented as the mean ± s.e.mean of duplicate determinations in 3 animals. *$P<0.001$ (Student's *t* test) compared with peptide alone.

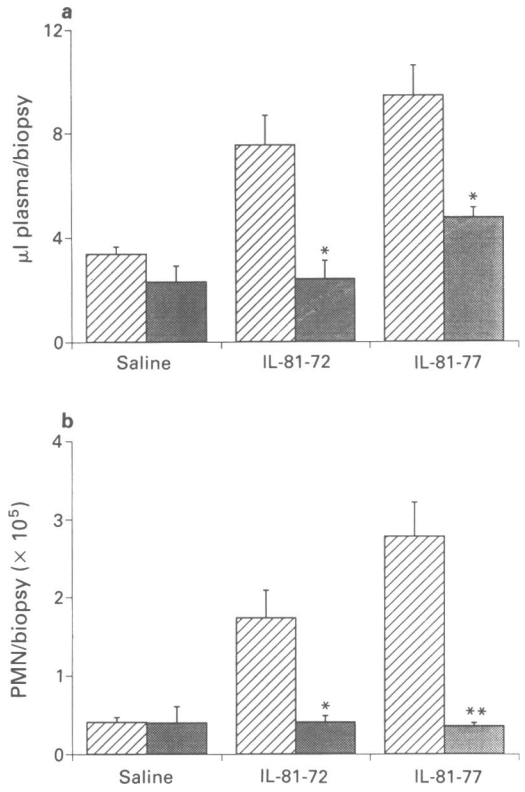

Figure 2 The effects of IB4 (1 mg kg^{-1}, i.v.) on IL-8_{1-72} (100 pmol) and IL-8_{1-77} (100 pmol)-induced dermal plasma extravasation (a) and dermal PMN accumulation (b) were determined. Plasma extravasation was measured 3 h after the intradermal injection of stimuli as the local accumulation of intravenously injected [^{125}I]-labelled human serum albumin and PMN accumulation was measured as the tissue content of myeloperoxidase (MPO). MPO levels were converted to numbers of cells from a standard curve constructed from known numbers of rabbit peripheral blood PMNs. Each column represents the mean and vertical bars the standard error for triplicate determinations in 3–12 animals: control, hatched columns; IB4, stippled columns; $*P<0.05$, $**P<0.01$ (Student's t-test) compared with control animals.

number of cells detected in the contralateral joints injected with saline, which was always below 5×10^3 cells ml^{-1} (data not shown). The pretreatment of rabbits (Figure 3) with IB4 (3 mg kg^{-1}) significantly ($P<0.01$) inhibited the infiltration of total leukocytes and PMNs. Although the number of mononuclear leukocytes was reduced by 54% this difference did not achieve statistical significance.

Biochemical analyses of the synovial lavage fluid revealed a significant ($P<0.0001$) increase in the amount of sPG (Figure 4) and immunoreactive stromelysin ($P<0.05$; Figure 5) detected in synovial lavage fluid from IL-8_{1-77}-injected knees compared with contralateral knees. Neither the increase in proteoglycans nor stromelysin was reduced by pretreatment with IB4.

Endotoxin contamination of IL-8

Examination of the IL-8 preparations with a colorimetric *Limulus* amoebocyte lysate (LAL) assay revealed the presence of low but measurable amounts of endotoxin. The values were 500 and 5 fmol of endotoxin mg^{-1} for IL-8_{1-77} and IL-8_{1-72} respectively. This concentration of endotoxin would

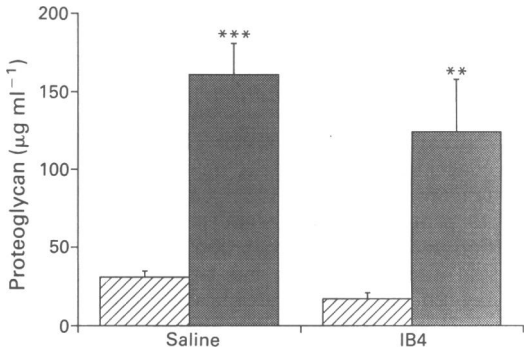

Figure 4 The appearance of sulphated proteoglycans (sPG) in the synovial lavage fluid obtained 24 h after the intra-articular injection of IL-8_{1-77} (1 nmol) was determined in two groups of rabbits treated with either saline or IB4 (3 mg kg^{-1}, i.v.): hatched columns, contralateral; stippled columns, challenge. Sulphated proteoglycans were determined by the dimethylmethylene blue binding assay. Each column represents the mean and vertical bars the standard deviation of 4 animals. $***P<0.0001$; $**P<0.001$ (Student's t test) compared with control animals.

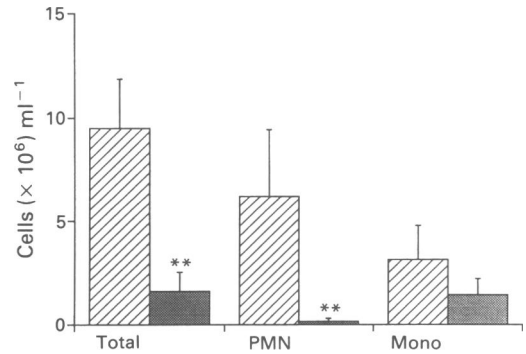

Figure 3 The effect of IL-8_{1-77} (1 nmol) on leukocyte accumulation in the knee joints of rabbits treated with saline (hatched columns) or IB4 (3 mg kg^{-1}, i.v.) (stippled columns). Leukocytes were measured by light microscopy 24 h after the intra-articular injection of IL-8. Each column represents the mean and vertical bars the standard deviation of 4 animals: Mono = mononuclear leukocytes. $**P<0.01$ (Student's t test) compared with control animals.

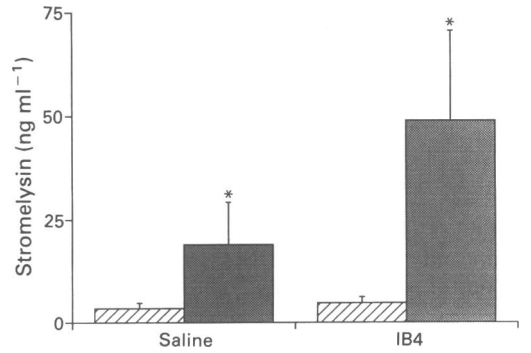

Figure 5 The appearance of stromelysin in the synovial lavage fluid obtained 24 h after the intra-articular injection of IL-8_{1-77} (1 nmol) was determined in two groups of rabbits treated with either saline or IB4 (3 mg kg^{-1}; i.v.): hatched columns, contralateral; stippled columns, challenge. Stromelysin was determined by a specific enzyme linked immunoassay. Each column represents the mean and vertical bars the standard deviation of 4 animals. $*P<0.05$ (Student's t test) compared with control animals.

have resulted in the injection of 5 fmol of endotoxin into the knee joints and a maximum of 0.5 fmol of endotoxin injected intradermally with IL-8_{1-77} or 5 amol of endotoxin with IL-8_{1-72}. Because of the pro-inflamamtory activity of endotoxin, we assessed the potential contribution of contaminant endotoxin to the inflammatory responses evoked by IL-8.

The intradermal injection of authentic *E. coli*-derived endotoxin produced a dose-related increase in dermal PMN accumulation and plasma extravasation (Figure 6). However, the maximum amount of endotoxin which would have been injected intradermally with IL-8_{1-77} (0.5 fmol) would not be sufficient to account for the inflammatory potential of IL-8 in the skin. Similar experiments were performed in the joint (Figure 7) where it can be seen that at levels of endotoxin equivalent to those injected into the joint with IL-8_{1-77} (5 fmol), endotoxin produces little PMN accumulation but did cause substantial release of sPG. With respect to PMN accumulation, it is unlikely that endotoxin contamination is contributing to the biological effect of IL-8. However, a contribution to the release of sPG by endotoxin could not be ruled out. To investigate further the effect of endotoxin, we passed the IL-8_{1-77} solution over a 'Detoxi-Gel' column to reduce the endotoxin content. This resulted in a reduction of endotoxin from 500 fmol mg^{-1} to 50 fmol mg^{-1} but did not result in a loss of IL-8_{1-77} peptide, determined by an IL-8 binding assay to human PMN membranes (data not shown). From our dose-response data for endotoxin in the knee joint we would predict that a 10 fold reduction in the endotoxin content would abolish the ability of the IL-8_{1-77} preparation to release proteoglycans from cartilage, were endotoxin to be the biologically active principal. However the 'low endotoxin' IL-8_{1-77} was as effective as the 'high endotoxin' IL-8_{1-77} in producing PMN accumulation, release of sPG and stromelysin (Table 2).

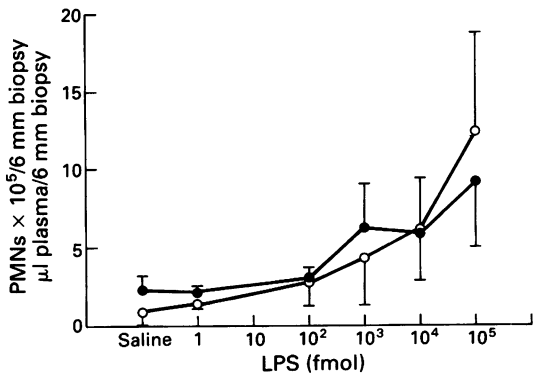

Figure 6 The pro-inflammatory activity of *E. coli*-derived endotoxin injected intradermally in rabbits was determined. Plasma extravasation (●) was measured 3 h after the intradermal injection of stimuli as the local accumulation of intravenously injected ^{125}I-labelled human serum albumin. PMN accumulation (○) was measured as the tissue content of myeloperoxidase (MPO). MPO levels were converted to numbers of cells from a standard curve constructed from known numbers of rabbit peripheral blood PMNs. Each point represents the mean and vertical bars the standard deviation of 5 animals.

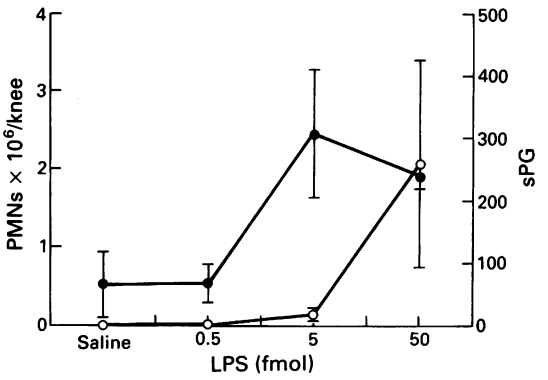

Figure 7 The pro-inflammatory activity of *E. coli*-derived endotoxin injected intra-articularly in rabbits was determined. PMNs (○) were measured by light microscopy 24 h after the intra-articular injection of IL-8_{1-77}. Sulphated proteoglycans (●) were determined by the dimethylmethylene blue binding assay. Each point represents the mean and vertical bars the standard deviation of 5 animals.

Discussion

Several mature forms of the recently described cytokine, interleukin-8, have been described (Lindley *et al.*, 1988; Yoshimura *et al.*, 1989; Schroder *et al.*, 1990). Monocytes have been reported to produce either predominantly the 72 amino acid (IL-8_{1-72}) peptide (Lindley *et al.*, 1988) or equivalent amounts of both peptides (Yoshimura *et al.*, 1989) whereas fibroblasts (Schroder *et al.*, 1990) and endothelial cells (Gimbrone *et al.*, 1989) generate the 77 amino acid (IL-8_{1-77}) variant. Both forms of IL-8 are reported to induce the degranulation of cytochalasin B-treated human PMNs with IL-8_{1-72} being the more potent form (Hebert *et al.*, 1990; Schroder *et al.*, 1990).

In contrast to the documented greater activity of IL-8_{1-72} compared with IL-8_{1-77} *in vitro*, we found the two peptides to have equivalent activity as mediators of plasma extravasation and PMN accumulation in rabbit skin. These data are in agreement with those of Hechtman *et al.* (1991) who found that both peptides were equiactive at inducing dermal PMN accumulation following their intradermal administration to rabbits and were also equiactive as inhibitors of agonist-

Table 2 The effects of IL-8_{1-77} on joint inflammation before (high endotoxin) and after (low endotoxin) passage through a 'Detoxi Gel' column

Treatment	*PMNs* ($\times 10^{-6}$ ml^{-1})	*sPG* (µg ml^{-1})	*Stromelysin* (ng ml^{-1})
Saline	0.003 ± 0.007	25 ± 13	4.6 ± 3.6
IL-8_{1-77} (High endotoxin)	6.2 ± 3.2***	161 ± 40***	19.1 ± 21*
IL-8_{1-77} (Low endotoxin)	4.2 ± 3.5***	144 ± 61**	62.8 ± 39*

IL-8_{1-77} was passed through a column of 'Detoxi Gel' to reduce the endotoxin concentration from 500 fmol mg^{-1} to 50 fmol mg^{-1}. The IL-8_{1-77} preparations (1 nmol in 500 ml) or saline were injected into the knees of rabbits. The animals were killed 24 h later and measurements of PMN accumulation, stromelysin and sulphated proteoglycans (sPG) performed on synovial lavage fluid. Values are mean ± s.d. of 4 animals.

*$P<0.05$; **$P<0.001$; ***$P<0.0001$ vs saline. Differences between high and low endotoxin all non-significant, Student's *t* test.

induced dermal PMN accumulation when the peptides were administered intravenously. It is conceivable that a difference in activity does exist but that this is masked by differential metabolism of the peptides. For example, it has been clearly demonstrated that IL-8_{1-77} is rapidly converted to IL-8_{1-72} by the addition of thrombin (Hebert *et al.*, 1990). In experiments where we measured dermal inflammation over a 30 min as opposed to 3 h period, we did not detect a difference in activity between the peptides. However, without knowing the stability of the peptides *in vivo* under the experimental conditions reported here it is impossible to conclude absolutely that the two IL-8 variants have equivalent activity.

The ability of vasodilator prostaglandins to potentiate plasma extravasation (Williams & Jose, 1981; Wedmore & Williams, 1981) and PMN accumulation (Issekutz, 1981) induced by a range of inflammatory mediators is well established. Our data demonstrating a potentiation of IL-8-induced plasma extravasation by PGE_2 confirm those previously reported by Rampart *et al.* (1989). However, whilst PMN accumulation was marginally greater in the presence of PGE_2, this was not a statistically significant finding and contrasts with the data of Rampart *et al.* (1989). A difference in methods of quantitation of tissue PMNs (enzymatic activity of skin homogenates versus accumulation of radiolabelled leukocytes) may account for this discrepancy.

The ability of IL-8 to induce PMN accumulation in the rabbit knee joint has been described previously in abstract form (Akahoshi *et al.*, 1990). We have confirmed and extended this report by showing that IL-8_{1-77} induces the accumulation of mononuclear leukocytes, and the appearance of sulphated proteoglycans and of stromelysin in the synovial lavage fluid. The accumulation of mononuclear leukocytes in response to IL-8 has not previously been reported. Following the intradermal injection of IL-8, the leukocytic infiltrate determined by light microscopy is reported to be exclusively PMNs with no evidence for monocytes, eosinophils, basophils or lymphocytes (Colditz *et al.*, 1989). Our observation of a mononuclear leukocyte infiltrate, in synovial lavage fluid in response to IL-8_{1-77}, may be explained on the basis of a different anatomical site, the use of IL-8_{1-77} in contrast to IL-8_{1-72} or perhaps the use of a 24 h response in the knee compared with 4 h in the skin.

The mechanism by which IL-8 induces monocyte accumulation is not clear. A direct chemotactic action for monocytes is unlikely considering that monocytes do not exhibit chemotaxis for IL-8 *in vitro* (Yoshimura *et al.*, 1987b). Specific receptors for IL-8 have been demonstrated on PMNs (Besemer *et al.*, 1989; Matsushima & Oppenheim, 1989; Samanta *et al.*, 1989; Grob *et al.*, 1990; Leonard *et al.*, 1990), whilst the evidence for receptors on monocytes is less convincing. Specific binding of IL-8 to peripheral blood monocytes has been reported (Besemer *et al.*, 1989; Matsushima & Oppenheim, 1989; Grob *et al.*, 1990; Leonard *et al.*, 1990). However, the calculated number of receptors per monocyte is approximately one tenth of that on PMNs and may well be attributed to contamination of monocyte preparations with PMNs. An alternative explanation for the accumulation of monocytes *in vivo* in response to IL-8_{1-77} may be an indirect action consequent upon the evoked release of biologically active autacoids from accumulating PMNs or from tissue cells. For example, IL-8 can stimulate PMN 5-lipoxygenase (Schroder, 1989) which, in the presence of arachidonic acid, will result in the formation of leukotriene B_4 and monohydroxyeicosatetraenoic acids with chemotactic activity for monocytes (Bray, 1983).

In comparison to control animals, the synovial lavage fluids from IL-8_{1-77} injected rabbits contained an increased concentration of proteoglycan, indicating that proteoglycan loss from articular cartilage (Pettipher *et al.*, 1989) has taken place in response to IL-8. Cartilage degradation induced by IL-8 stimulated neutrophils *in vitro* has recently been described (Elford & Cooper, 1991). In these experiments the coculture of ^{35}S-labelled cartilage with PMNs and human IL-8 caused a rapid loss of radioactivity from the cartilage. This phenomenon was believed to be mediated via PMN degranulation and was not a direct effect of IL-8 on the cartilage. It is unlikely that the loss of sPG from cartilage observed *in vivo* following the intra-articular injection of IL-8 is mediated via PMN degranulation. This contention is made on the basis that pre-treatment of rabbits with IB4 inhibited IL-8-induced PMN accumulation in synovial lavage fluid, but had no effect on the levels of sPG. Although a role for PMNs in mediating cartilage degradation induced by IL-8 *in vivo* is unlikely, the mechanism by which IL-8 induces cartilage degradation is not clear. The presence of elevated levels of the metalloproteinase stromelysin, an enzyme with the capacity to degrade cartilage (Okada *et al.*, 1986), suggests that the release of sPG is via this enzyme. However, the cellular source of stromelysin and the mechanisms whereby IL-8 stimulates the production of stromelysin, are not known.

The contribution of contaminating endotoxin to the biological effects of IL-8 appears to be minimal. In studies on the dermal effects of IL-8 the amounts of the cytokine injected, and hence of endotoxin, were low and clearly were not sufficient to account for the observed cellular accumulation and plasma extravasation. With respect to studies in the knee joint, larger amounts of IL-8 were injected and the amount of endotoxin in preliminary studies was sufficient to produce release of sPG but not to induce PMN accumulation. Our observation that reducing the amount of endotoxin present in the IL-8_{1-77} by a factor of 10 had no effect on the pro-inflammatory properties of IL-8_{1-77} argues strongly against endotoxin being the biologically active principal. However, we cannot rule out completely a contribution from the endotoxin. We have used an *E. coli* derived endotoxin as an indicator of the biological activity for an endotoxin contaminant of unknown aetiology. It is possible that the endotoxin in our IL-8 preparation is derived from an organism other than *E. coli* or even an alternative strain of the bacterium and may exhibit greater, or lesser, biological activity. Furthermore we cannot exclude the possibility of a synergy between IL-8 and low doses of endotoxin which accounts for the biological phenomena observed.

The results of this study demonstrate that the distinct forms of the recently described cytokine, IL-8, exhibit a similar spectrum of biological activity. We also document novel activities for IL-8 in terms of monocyte accumulation, generation of stromelysin and cartilage degradation. The extent to which IL-8 may contribute to the pathogenesis of inflammatory disease clearly warrants further investigation, particularly in the light of recent reports demonstrating the presence of biologically relevant levels of IL-8 in synovial fluid from patients with rheumatoid arthritis (Akahoshi *et al.*, 1990; Brennan *et al.*, 1990).

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