Biochem. J. (1990) 271, 797–801 (Printed in Great Britain)

A novel neutrophil chemoattractant generated during an inflammatory reaction in the rabbit peritoneal cavity *in vivo*

Purification, partial amino acid sequence and structural relationship to interleukin 8

Beatrice C. BEAUBIEN,*‡ Paul D. COLLINS,* Peter J. JOSE,*§ Nicholas F. TOTTY,† Justin HSUAN,† Michael D. WATERFIELD† and Timothy J. WILLIAMS*

*Department of Applied Pharmacology, National Heart and Lung Institute, Dovehouse Street, London SW3 6LY, and †Ludwig Institute for Cancer Research, Riding House Street, London W1P 8BT, U.K.

An inflammatory reaction was induced *in vivo* by injection of zymosan into the peritoneal cavity of the rabbit. The inflammatory exudate was found to contain oedema-inducing and neutrophil chemoattractant activity when assayed in rabbit skin *in vivo*, using ^{125}I-albumin and ^{111}In-neutrophils. This activity was additional to that of complement fragment C5a, which was removed by an affinity gel. Two chemoattractants were isolated by cation-exchange, gel-filtration and reversed-phase h.p.l.c. One of these, which ran as a single band of 6–8 kDa on SDS/PAGE, was subjected to *N*-terminal sequence analysis without reduction and alkylation of cysteine residues. Positive identification of 28 of the first 31 amino acids revealed a rabbit homologue of interleukin-8 (75% sequence identity with human interleukin-8). The demonstration of interleukin-8 as a major neutrophil chemoattractant in an inflammatory reaction *in vivo* provides the basis for further investigations into the role of this cytokine in the inflammatory process.

INTRODUCTION

Zymosan (yeast cell walls) induces an acute inflammatory response in the skin and peritoneal cavity of rabbits [1–3]. This response is characterized by local microvascular plasma protein leakage and the accumulation of neutrophil leucocytes. The peak rate of plasma protein leakage occurs within 2 h of zymosan injection and is dependent on the presence of circulating neutrophils. The early response is largely due to the extravascular generation of the complement fragment C5a, which induces a rapid adherence of circulating neutrophils to microvascular endothelial cells, followed by neutrophil emigration [1,4,5]. The interaction between neutrophils and endothelial cells gives rise to increased microvascular permeability to plasma proteins [4].

We have recently obtained evidence that another neutrophil chemoattractant is generated later than C5a, i.e. at 4–6 h after intraperitoneal injection of zymosan (P. D. Collins, P. J. Jose & T. J. Williams, unpublished work). Vascular-permeability-increasing activity and neutrophil chemoattractant activity in fluids obtained up to 2 h after intraperitoneal injection of zymosan were abolished by anti-C5a antibodies. However, 4–6 h exudate fluids contained substantial activity, even in the presence of an excess of anti-C5a antibodies, indicating the presence of a neutrophil chemoattractant, or chemoattractants, other than C5a.

The present paper describes the purification and *N*-terminal amino-acid-sequence analysis of this novel activity generated *in vivo*. The purified peptide shows substantial similarity to human interleukin 8 (IL-8), sufficient for it to be considered a rabbit homologue. IL-8 is the name proposed for a cytokine released *in vitro* from a variety of cell types, primarily cells of the monocyte/macrophage lineage, and previously known by a variety of other names which describe its ability to activate neutrophils *in vitro* [6–12]. Human IL-8 induces neutrophil accumulation and neutrophil-dependent oedema formation in the rabbit [13–15].

The present paper provides evidence that IL-8 is generated *in vivo* in an inflammatory reaction characterized by an early phase of extravascular complement activation. Thus these observations suggest that IL-8 could provide a link between the early and later phases of the inflammatory response.

MATERIALS AND METHODS

Collection of inflammatory exudates

New Zealand White rabbits (3–5 kg; Froxfield Farm, Froxfield, Hants., U.K.) were anaesthetized with intravenous sodium pentobarbitone (30 mg/kg), and a suspension of zymosan [500 mg in 75 ml of sterile saline (0.9% NaCl)] was injected into the peritoneal cavity via a 16-gauge polyethylene cannula [3]. Polymixin B sulphate (40 μM) was mixed with the zymosan to inhibit the effects of any endotoxin present. After 6 h the animals were killed by an anaesthetic overdose and an incision was made (10 cm) through the ventral surface of the abdomen into the peritoneal cavity. All the available exudate was collected into heparin (10 units/ml), kept cold on ice, and 50 ml of sterile saline was added to wash out the remaining exudate. Samples were centrifuged (2000 *g* for 10 min at 2 °C) to remove the accumulated cells and any remaining zymosan particles. Initial experiments showed the activity in exudate to be stabilized by moderate heating, possibly due to inactivation of proteinases. The supernatant fluid was therefore heated to 60 °C for 30 min, cooled and centrifuged (5000 *g* for 10 min at 2 °C) to remove a small amount of precipitated protein. The peritoneal exudate fluid used for purification consisted of 560 ml of available exudate, collected from 11 rabbits, combined with 480 ml of saline washes.

Abbreviations used: PBS, phosphate-buffered saline (0.15 M-NaCl in 0.01 M-sodium phosphate buffer at pH 5.5 or pH 7.4); TFA, trifluoroacetic acid; RPF, rabbit peritoneal factor; MDNCF, monocyte-derived neutrophil chemotactic factor; LUCT, lung carcinoma-derived chemotaxin; IL-8, interleukin 8; hr, human recombinant.

‡ Present address: Loeb Research Institute, 725 Parkdale Avenue, Ottawa, Canada K1Y 4K9.

§ To whom correspondence should be addressed.

Rabbit skin bioassay for mediators of oedema formation and neutrophil accumulation

Inflammatory activity in the peritoneal exudate, at various stages in the purification, was monitored by its ability to induce oedema formation [16] and neutrophil accumulation [17,18] in rabbit skin. Briefly, rabbits were anaesthetized with sodium pentobarbitone, the dorsal skin was shaved and radiolabelled tracers (^{125}I-albumin and, in some experiments, ^{111}In-neutrophils) were injected intravenously. Test samples, mixed with a potentiating dose of prostaglandin E_2 (3×10^{-10} mol/100 μl per skin site) to facilitate measurement [16] and goat anti-(rabbit C5a) IgG to block responses to any remaining C5a [19], were injected intradermally in triplicate. After 30 min the animals were killed with an anaesthetic overdose. The dorsal skin was removed, and the injection sites were punched out and counted for radioactivity in a multi-well Cobra Auto-Gamma radiation counter with spill-over correction (Packard).

Aliquots of h.p.l.c. fractions used for bioassay (2–10 % of each column fraction) were freeze-dried in the presence of a freezing mixture designed to prevent absorption of biological activity to the tubes; when reconstituted with water, each sample contained 0.1 % BSA in PBS, pH 7.4. Results (mean ± S.E.M.) are expressed as μl of plasma/skin site (oedema formation) and ^{111}In-neutrophil accumulation/skin site.

Removal of C5a using an affinity gel

Guinea-pig anti-(rabbit C5a) antiserum [2] was mixed with an equal volume of Protein A–Sepharose 6BCL at pH 8.0. After washing off unbound material, the bound IgG was covalently attached by use of the homobifunctional agent dimethyl pimelimidate [20]. Peritoneal exudate fluid, prepared as above, was mixed with the anti-C5a gel (15 ml of exudate/ml of gel) for 30 min at room temperature and then centrifuged (370 *g* for 5 min). The supernatant plus a saline wash of the gel was used in subsequent purification steps. By using this procedure, the C5a content of the exudate was reduced from 48.0 to 0.3 nM, as determined by radioimmunoassay [2]. The anti-C5a gel was regenerated for further use by washing with 0.2 M-glycine/HCl buffer, pH 2.5, followed by PBS, pH 7.4.

Cation exchange

The C5a-depleted exudate was adjusted to pH 5.5 using 0.15 M-HCl and mixed with CM-Sephadex C25 (50 ml of exudate/ml of gel, equilibrated in PBS, pH 5.5) for 30 min. The gel was allowed to settle overnight at 4 °C. Most proteins (> 90 %) remained in the supernatant, which had no detectable activity in the rabbit skin oedema assay. After a wash step with PBS, pH 5.5, the active material was eluted with 1.5 M-NaCl in 0.075 M-disodium phosphate (pH 8.2, 4 ml/ml of gel). The eluate was diluted 10-fold with water and the pH adjusted to 5.5 with 0.15 M-HCl. This was filtered through a 0.2 μm-pore-size filter and applied to a TSK 535CM h.p.l.c. column (0.75 cm × 15 cm, equilibrated in pH 5.5 PBS) at a flow rate of 1 ml/min. The column was washed at 1 ml/min with pH 5.5 PBS (10 min) and then with 0.1 M-ammonium acetate buffer, pH 5.5 (10 min). No bioactive material was detected in the breakthrough or washes. The column was eluted with a linear gradient of 0.1 M-ammonium acetate, pH 5.5, to 1.0 M-ammonium acetate, pH 7.6 (0.5 ml/min; 36 min). This was followed by a steeper gradient of 1.0–2.0 M-ammonium acetate, pH 7.6. Fractions were collected every 2 min.

Size exclusion

Freeze-dried active material from the cation-exchange h.p.l.c. column was dissolved in 800 μl of 0.1 % trifluoroacetic acid (TFA) and applied (two runs of 400 μl) to a TSK G2000SW h.p.l.c. column (0.75 cm × 60 cm; equilibrated in 0.1 % TFA). This was eluted at 0.8 ml/min, and 1 min fractions were collected.

Reversed phase h.p.l.c.

Freeze-dried active material from the size-exclusion-h.p.l.c. step was subjected to repeated chromatography on a wide-pore [30 nm (300 Å)] Vydac C_{18} column (0.4 cm × 25 cm). Three different gradients of acetonitrile in 0.1 % TFA were run, each at 0.5 ml/min, with 1 min fractions being collected. The optimal gradient, used on the third run, was determined to be from 30 to 50 % acetonitrile over 30 min.

PAGE analysis

Aliquots from all the active fractions were put aside during the course of the purification and analysed by PAGE after the reversed-phase fractionation. The Phast gel system used for this procedure consisted of SDS-containing precast gels (8–25 % acrylamide gradient) in conjunction with SDS buffer strips. Samples were dried, resuspended in 5 μl of sample buffer [0.01 M-Tris/HCl, pH 8.0, containing 2.5 % (w/v) SDS, 5 % (v/v) β-mercaptoethanol and 0.01 % Bromophenol Blue], boiled for 3 min and centrifuged. Gels were silver-stained using a double staining cycle and extended development to obtain a sensitivity of approx. 0.6 ng/0.5 μl per track with the hrMDNCF/IL-8 standard.

Amino acid sequencing

The *N*-terminal sequence was obtained by using an Applied Biosystems model 477A pulse-liquid sequencer with an on-line amino acid phenylthiohydantoin derivative analyser (model 120A, utilizing fast cycle chemistry) [21]. Half the material in fraction 39 from the final reversed-phase h.p.l.c. step was used for sequencing and, because this amount of protein was limiting, no chemical modification of putative cysteine residues was performed before Edman degradation. The *N*-terminal alanine signal of 40 pmol (background subtracted) suggested a sequencer loading of approx. 60–70 pmol of the protein (typical first-step yield, 60 %).

Materials

Zymosan, polymixin B sulphate and prostaglandin E_2 were from Sigma Chemical Co., Poole, Dorset, U.K.; pentobarbitone sodium (Sagatal) was from May and Baker, Dagenham, Essex, U.K.; Venisystems 16-gauge cannulae were from Abbot Ireland, Sligo, Ireland; sterile pyrogen-free saline was from Travenol Laboratories, Thetford, Norfolk, U.K.; heparin was from Paynes and Byrne, Greenford, Middx., U.K.; ^{125}I-human serum albumin and $^{111}InCl_3$ were from Amersham International, Amersham, Bucks., U.K.; dimethyl pimelimidate was from Pierce and Warriner, Chester, U.K.

The Waters h.p.l.c. system was composed of a 600E quaternary gradient pump, a 490E multiple-wavelength detector and an 800E data station. TFA, acetonitrile and water used for h.p.l.c. were of low-absorbance h.p.l.c. grade from BDH, Poole, Dorset, U.K., and Rathburn Chemicals, Walkerburn, Peeblesshire, Scotland, U.K. The wide-pore Vydac reversed-phase column was from H.p.l.c. Technology, Macclesfield, Cheshire, U.K. The TSK H.P.L.C. columns, other gel matrixes, the Phast gel system and materials for SDS/PAGE were from Pharmacia/LKB, Milton Keynes, Bucks., U.K. Silver stain was from Bio-Rad Laboratories, Watford, Herts., U.K.

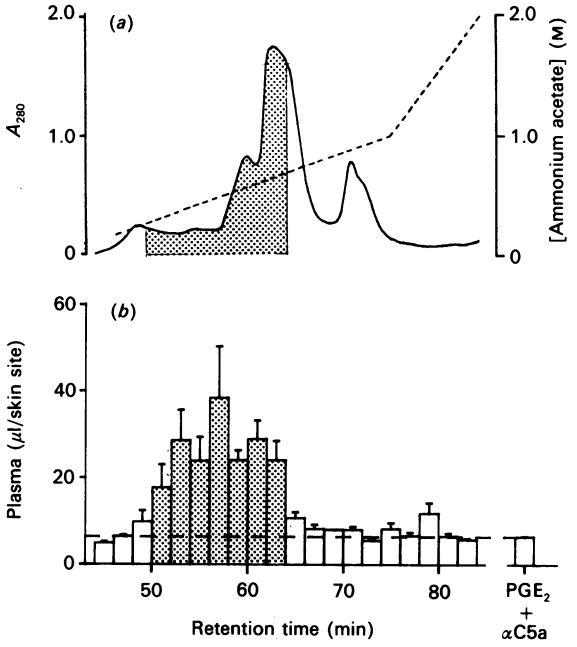

Fig. 1. Cation-exchange h.p.l.c. of inflammatory mediators

Rabbit peritoneal inflammatory exudate, depleted of C5a and eluted from the soft-gel cation-exchanger, was diluted and applied to a TSK 535CM column at pH 5.5. After washing, the column was eluted with linear gradients of ammonium acetate from 0.1 M, pH 5.5, to 1.0 M, pH 7.6, and then to 2.0 M, pH 7.6. (*a*) Ammonium acetate gradients and A_{280}. (*b*) 3% of each fraction was freeze-dried and assayed for oedema-forming activity in the rabbit skin bioassay. The broken line represents the weak response to prostaglandin E_2 (PGE_2) and anti-C5a antibody (αC5a), which were routinely added to each sample to facilitate measurement of the novel activity (see the Materials and methods section). In both (*a*) and (*b*) the shaded region indicates active fractions which were pooled for sizing h.p.l.c.

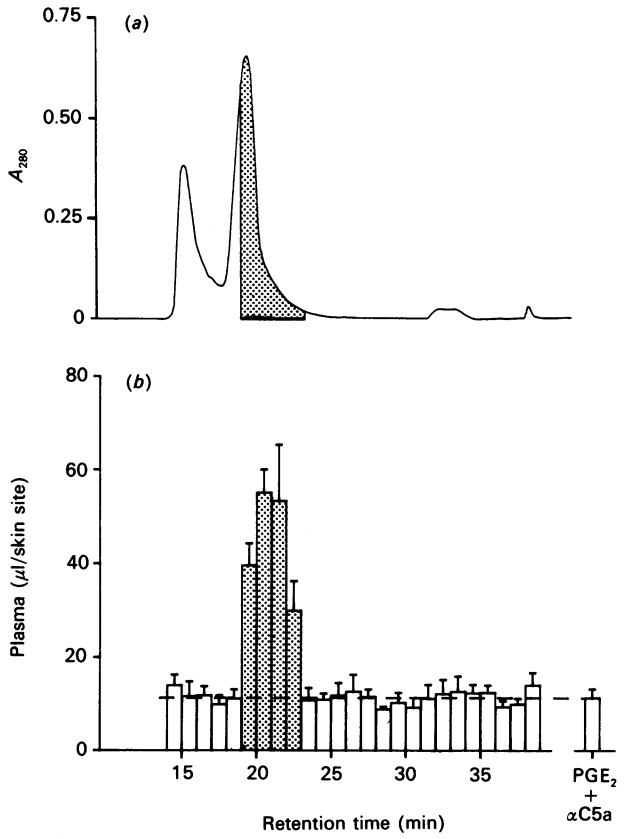

Fig. 2. Sizing h.p.l.c. of inflammatory mediators

Fractions from the cation-exchange h.p.l.c. column were pooled, freeze-dried and applied to a TSK G2000 SW sizing column in 0.1% TFA. (*a*) A_{280}; (*b*) 2.5% of each fraction was freeze-dried and assayed for oedema-forming activity. The broken line shows the weak response to prostaglandin E_2 (PGE_2) and anti-C5a antibody (αC5a). In both (*a*) and (*b*) the shaded region indicates active fractions which were pooled for reversed-phase h.p.l.c.

RESULTS

Purification

Inflammatory exudate obtained 6 h after the intraperitoneal injection of zymosan was depleted of C5a by use of an affinity gel. The remaining neutrophil chemoattractant and oedema-forming activity was found to bind to the cation-exchanger, CM-Sephadex, at pH 5.0 and 5.5, but not at pH 6.0 and above (at an iso-osmotic salt concentration; results not shown). The cation-exchange step at pH 5.5 facilitated the subsequent h.p.l.c. steps by removing a substantial (> 90%) amount of protein. The cation-exchange h.p.l.c. step produced a broad peak of bioactivity (Fig. 1).

The active fractions from the ion-exchange step were pooled, freeze-dried and subjected to the size-exclusion h.p.l.c. step in 0.1% TFA. A single peak of oedema-forming activity, corresponding to approx. 9 kDa, was observed (Fig. 2).

Active fractions from the sizing step were pooled and freeze-dried for subsequent application to the C_{18} column. Identifying the optimal gradient involved an iterative process. Initially the column was run from 5 to 100% acetonitrile in 0.1% TFA over 50 min. Only a single peak of activity was found, at approx. 50% acetonitrile (results not shown). These fractions were pooled, freeze-dried and applied to the second reversed-phase step; the bioassay profile suggested two peaks of activity, which were not clearly separated (results not shown). The active fractions were again pooled and freeze-dried for application to the third and final reversed-phase step, which incorporated a gradient of 30–50% acetonitrile over 30 min. These conditions separated two peaks of activity in both the oedema-forming and the neutrophil accumulation assays (Fig. 3).

PAGE analysis

The extent of purification was monitored by SDS/PAGE, using 8–25% gels under reducing conditions, followed by silver staining. Analysis of individual fractions from the last C_{18} purification step (Fig. 4) revealed a single band of 6–8 kDa in fractions 39 and 40, corresponding to a protein slightly larger than the hrMDNCF/IL-8 standard. Fraction 34, which was a discrete peak of bioactivity, had at least three bands, none of which corresponded to the hrMDNCF/IL-8 standard.

Sequencing

On the evidence of PAGE and bioassay analysis, fraction 39 from the final reversed-phase step was subjected to *N*-terminal amino-acid-sequence analysis. Only one sequence was detected, and sequencing proceeded to residue 31, with three of these residues remaining unidentified [Fig. 5; rabbit peritoneal factor (RPF)]. Sequence similarity with a family of neutrophil-activating human cytokines (now known as IL-8) is also given in Fig. 5.

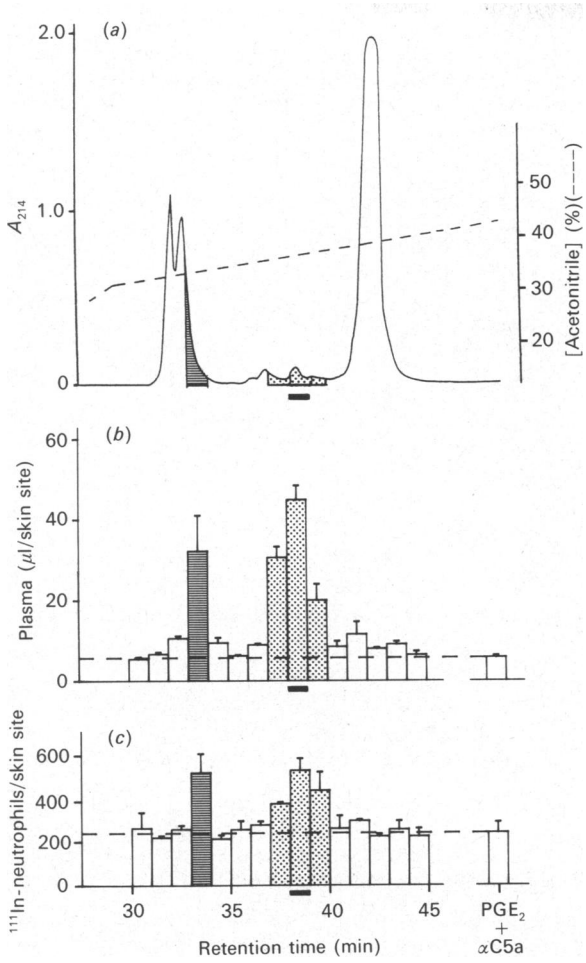

Fig. 3. Reversed-phase h.p.l.c. of inflammatory mediators

Fractions from the sizing step were pooled and applied to a Vydac 30 nm (300 Å) C_{18} reversed-phase column in 0.1% TFA and eluted with gradients of acetonitrile in 0.1% TFA. The Figure shows results of the third and final gradient. (*a*) Acetonitrile gradient and A_{214}; (*b* and *c*) 10% of each fraction was freeze-dried and assayed for oedema-forming (*b*) and neutrophil chemoattractant (*c*) activities in the rabbit skin bioassay *in vivo*. The broken lines show the weak response to prostaglandin E_2 (PGE_2) and anti-C5a antibody (αC5a) which were routinely added to each sample before bioassay. The shaded areas (■,▨) indicate two discrete peaks of bioactive fractions. Fraction 39 (indicated by the bar) was subjected to *N*-terminal amino acid sequencing.

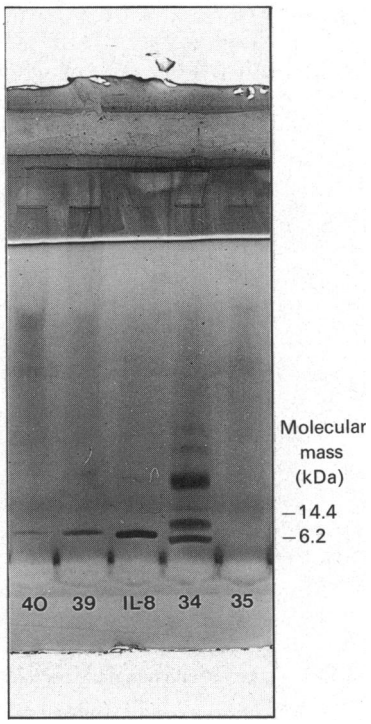

Fig. 4. SDS/PAGE analysis of mediators separated by reversed-phase h.p.l.c.

Fractions 34, 35, 39 and 40 from the final reversed-phase h.p.l.c. gradient (Fig. 3) were run under reducing conditions on an 8–25% acrylamide gradient gel. The gel was silver-stained. Standards used were low-molecular-mass markers (indicated by the bars) and hrMDNCF/IL-8 (25 ng/0.5 μl; approx. 40 times the limit of detection). Fraction 39 was used for *N*-terminal amino acid sequencing.

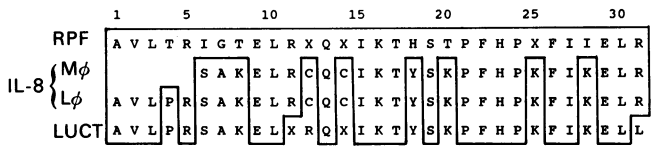

Fig. 5. *N*-Terminal amino acid sequence of the RPF compared with human IL-8 and LUCT

The RPF sequence was obtained from fraction 39 of the final reversed-phase h.p.l.c. gradient. RPF and (human) LUCT [26] were not reduced and alkylated before sequencing. Human IL-8 sometimes lacks the AVLPR sequence at the *N*-terminus (as shown), depending on the cellular source of this cytokine (Mφ, macrophages; Lφ, lymphocytes). Boxes indicate identity of the rabbit and human sequences.

DISCUSSION

The present paper describes the generation *in vivo*, purification and *N*-terminal characterization of a rabbit neutrophil chemoattractant and oedema-inducing protein that shows 75% sequence identity with human IL-8. Recently human IL-8 has been shown to stimulate neutrophil accumulation and oedema formation in the rabbit [13–15]. A characteristic shared by IL-8 and related sequences is the conservation of two disulphide bonds [22,23]. These are formed from a pair of cysteine residues consistently positioned near the *N*-terminus which both link to cysteine positions in the latter half of the molecule.

N-Terminal sequencing gave only one amino acid per cycle, thus supporting the PAGE evidence of purity. Of the first 31 residues, 28 were positively identified. No chemical modification of putative cysteine residues was performed before Edman degradation, and the cysteine phenylthiohydantoin derivative is not usually recovered in sufficient yield to be identified. This may account for the complete absence of any signal at residues 12 and 14, since cysteine is strongly conserved at these positions in the IL-8 family of cytokines.

Heterogeneity of the peritoneal inflammatory activity was observed during purification. We do not yet know whether the two peaks of activity seen on reversed-phase h.p.l.c. represent two structurally unrelated proteins or variants of the same protein. Heterogeneity at the *N*-terminus is a feature of the IL-8 family. The rabbit homologue of IL-8 identified in the present study has a five-residue sequence (AVLTR) at its *N*-terminus which is similar to the AVLPR encoded in the human MDNCF/IL-8 genome, but is absent from the mature protein

which predominates in mononuclear-phagocyte culture supernatants [24–27]. However, as Fig. 5 shows, this AVLPR pentapeptide is found in human IL-8 released after stimulation of lymphocytes [28] and endothelial cells [29]. AVLPR is also found in a very similar chemotactic protein (LUCT) which is released constitutively by a human lung giant-cell-carcinoma cell line [30].

The present study has identified a protein of the IL-8 family in an '*in vivo*' model of inflammation. Factors with neutrophil chemoattractant properties *in vivo* have been detected in endotoxin-induced inflammatory exudates generated in the rat peritoneal and rabbit pleural cavities [31,32]. The rabbit factor is unlikely to be IL-8, since it was devoid of chemotactic activity *in vitro* and had a molecular mass of 45 kDa [32]. Although the rat factor was not purified, its activities *in vivo* and *in vitro* [31] resemble that of IL-8. We used polymixin B sulphate to negate the effects of any endotoxin contamination of the inflammatory stimulus. Thus we conclude that the IL-8 generation in our experiments was in response to the zymosan particles, perhaps after opsonization, rather than to endotoxin.

It will be important to identify which cells are responsible for the release of the rabbit peritoneal factor. IL-8 production, or the expression of mRNA, has been demonstrated *in vitro* in stimulated monocytes, macrophages, lymphocytes, endothelial cells and fibroblasts. The work of Cunha & Ferreira [31] in the rat suggests that tissue macrophages were the source of their peritoneal neutrophil chemoattractant. However, the presence of the AVLTR pentapeptide at the *N*-terminus of the rabbit peritoneal factor suggests that the source might be cells other than the macrophage, since, in man, lymphocytes [28] and endothelial cells [29], but apparently not macrophages [24], secrete IL-8 containing a similar pentapeptide (AVLPR) at the *N*-terminus.

The purification and identification of a rabbit homologue of IL-8 generated *in vivo* provides the basis for further investigations of the generation, action and cellular source of IL-8 in inflammatory reactions.

This work was supported by the National Asthma Campaign, the Wellcome Trust and Pfizer Central Research, Groton, CT, U.S.A. We thank Miss J. M. Perkins, Dr. O. Cromwell and Dr. C. Corrigan for help with the SDS/PAGE analysis, and Dr. K. Matsushima and Dr. J. J. Oppenheim for generously providing hrMDNCF/IL-8. P. D. C. is a Roche Ph. D. student.

REFERENCES

1. Williams, T. J. & Jose, P. J. (1981) J. Exp. Med. **153**, 136–153
2. Jose, P. J., Forrest, M. J. & Williams, T. J. (1983) J. Exp. Med. **158**, 2177–2182
3. Forrest, M. J., Jose, P. J. & Williams, T. J. (1986) Br. J. Pharmacol. **89**, 719–730
4. Wedmore, C. V. & Williams, T. J. (1981) Nature (London) **289**, 646–650
5. Williams, T. J., Jose, P. J., Forrest, M. J., Wedmore, C. V. & Clough, G. F. (1984) in White Blood Cell Mechanics: Basic Science and Clinical Aspects (Kroc Foundation Series) (Meiselman, H. J., Lichtman, M. A. & LaCelle, P. L., eds.), pp. 195–208, Alan R. Liss, New York
6. Kownatzki, E., Kapp, A. & Uhrich, S. (1986) Clin. Exp. Immunol. **64**, 214–222
7. Schröder, J.-M., Mrowietz, U., Morita, E. & Christophers, E. (1987) J. Immunol. **139**, 3474–3483
8. Walz, A., Peveri, P., Aschauer, H. & Baggiolini, M. (1987) Biochem. Biophys. Res. Commun. **149**, 755–761
9. Yoshimura, T., Matsushima, K., Tanaka, S., Robinson, E. A., Appella, E., Oppenheim, J. J. & Leonard, E. J. (1987) Proc. Natl. Acad. Sci. U.S.A. **84**, 9233–9237
10. Van Damme, J., Van Beeumen, J., Opdenakker, G. & Billiau, A. (1988) J. Exp. Med. **167**, 1364–1376
11. Larsen, C. G., Anderson, A. O., Appella, E., Oppenheim, J. J. & Matsushima, K. (1989) Science **243**, 1464–1466
12. Westwick, J., Li, S. W. & Camp, R. D. (1989) Immunol. Today **5**, 146–147
13. Colditz, I., Zwahlen, R., Dewald, B. & Baggiolini, M. (1989) Am. J. Pathol. **134**, 755–760
14. Foster, S. J., Aked, D. M., Schröder, J. M. & Christophers, E. (1989) Immunology **67**, 181–183
15. Rampart, M., Van Damme, J., Zonnekyn, L. & Herman, A. G. (1989) Am. J. Pathol. **135**, 1–5
16. Williams, T. J. (1979) Br. J. Pharmacol. **65**, 517–524
17. Rampart, M. & Williams, T. J. (1988) Br. J. Pharmacol. **94**, 1143–1148
18. Nourshargh, S., Rampart, M., Hellewell, P. G., Jose, P. J., Harlan, J. M., Edwards, A. J. & Williams, T. J. (1989) J. Immunol. **142**, 3193–3198
19. Jose, P. J. & Williams, T. J. (1987) Complement **4**, 175
20. Schneider, C., Newman, R. A., Sutherland, D. R., Asser, U. & Greaves, M. F. (1982) J. Biol. Chem. **257**, 10766–10769
21. Hewick, R. M., Hunkapiller, M. W., Hood, L. E. & Dreyer, W. J. (1981) J. Biol. Chem. **256**, 7990–7997
22. Baggiolini, M., Walz, A. & Kunkel, S. L. (1989) J. Clin. Invest. **84**, 1045–1049
23. Wolpe, S. D. & Cerami, A. (1989) FASEB J. **3**, 2565–2573
24. Matsushima, K., Morishita, K., Yoshimura, T., Lavu, S., Kobayashi, Y., Lew, W., Appella, E., Kung, H. F., Leonard, E. J. & Oppenheim, J. J. (1988) J. Exp. Med. **167**, 1883–1893
25. Lindley, I., Aschauer, H., Seifert, J.-M., Lam, C., Brunowsky, W., Kownatzki, E., Thelen, M., Peveri, P., Dewald, B., Von Tscharner, V., Walz, A. & Baggiolini, M. (1988) Proc. Natl. Acad. Sci. U.S.A. **85**, 9199–9203
26. Van Damme, J., Van Beeumen, J., Conings, R., Decock, B. & Billiau, A. (1989) Eur. J. Biochem. **181**, 337–344
27. Yoshimura, T., Robinson, E. A., Appella, E., Matsushima, K., Showalter, S. D., Skeel, A. & Leonard, E. J. (1989) Mol. Immunol. **26**, 87–93
28. Gregory, H., Young, J., Schröder, J.-M., Mrowietz, U., & Christophers, E. (1988) Biochem. Biophys. Res. Commun. **151**, 883–890
29. Gimbrone, M. A., Obin, M. S., Brock, A. F., Luis, E. A., Hass, P. E., Hebert, C. A., Yip, Y. K., Leung, D. W., Lowe, D. G., Kohr, W. J., Darbonne, W. C., Bechtol, K. B. & Baker, J. B. (1989) Science **240**, 1601–1603
30. Suzuki, K., Miyasaka, H., Ota, H., Yamakawa, Y., Tagawa, M., Kuramoto, A. & Mizuno, S. (1989) J. Exp. Med. **169**, 1895–1901
31. Cunha, F. Q. & Ferreira, S. H. (1986) Eur. J. Pharmacol. **129**, 65–76
32. Issekutz, A. C., Megyeri, P. & Issekutz, T. B. (1987) Lab. Invest. **56**, 49–59

Received 27 April 1990/19 June 1990; accepted 27 June 1990