British Journal of Pharmacology (1998) 125, 319–326
http://www.stockton-press.co.uk/bjp

Investigation of the functional role played by the chemokine monocyte chemoattractant protein-1 in interleukin-1-induced murine peritonitis

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1 Intraperitoneal (i.p.) injection of murine recombinant IL-1β (mrIL-1β) produced a dose-dependent (0.5–50 ng) and time-related (0.5–2 h) secretion of murine monocyte chemoattractant protein-1 (mMCP-1; 3–4 ng per cavity) in the lavage fluids. MCP-1 mRNA could also be detected in the cell pellets by reverse transcriptase-polymerase chain reaction (RT-PCR).

2 MCP-1 levels were reduced by more than 90% by co-administration of IL-1 receptor antagonist (10 μg) ($n=6$, $P<0.05$). In contrast, an IL-1 mutant with low affinity for IL-1 receptor type I, termed yIL-1$\beta\Delta4$ (50 ng), produced only a modest release of the chemokine. Treatment of mice with dexamethasone (DEX) ($\sim$1 mg kg^{-1} s.c.) reduced mrIL-1β-induced mMCP-1 gene expression (apparent total inhibition) and protein release in the lavage fluids ($\sim$40% reduction; $n=10$; $P<0.05$). Drastic reductions in the numbers of residential macrophages or mast cells did not modify the levels of mMCP-1 recovered in the lavage fluids.

3 Injection of mrIL-1β produced neutrophil accumulation into the peritoneal cavities (maximal at 4 h with $1.42\pm0.15\times10^6$ cells per mouse). Co-injection of a specific polyclonal antibody against mMCP-1 reduced this process by more than 50% ($n=6$; $P<0.05$). In conclusion, we studied the mechanisms leading to the specific release of the CC chemokine mMCP-1 after *in vivo* administration of mrIL-1β.

Keywords: Neutrophils; chemokines; inflammation; dexamethasone

Introduction

Migration of inflammatory cells into an extravascular site requires a series of co-ordinated events which have recently begun to be elucidated (Granger & Kubes, 1994). Besides several cell-cell interaction phenomena, the presence of a chemotactic agent on the endothelium of post-capillary venules is crucial to achieve leucocyte accumulation during the host inflammatory response. The specific leucocyte population elicited into the experimental inflammatory site is determined by the nature of the stimulus employed and the consequent magnitude and specificity of chemotactic factors produced. This assumption is supported by the recent characterization of a family of chemotactic cytokines (chemokines) which exhibit considerable target cell specificity (Baggiolini *et al*., 1994).

Chemokines are small proteins with molecular weight in the range of 8–12 kDa. Four subfamilies of chemokines have been classified, namely: CXC, CC, C, CX_3C chemokines, being defined by the presence of highly conserved cysteine residues (Rollins, 1997). Monocyte chemoattractant protein-1 (MCP-1) is the prototype of the CC family of chemokines which also includes macrophage inflammatory protein-1α (MIP-1α) and regulated on activation normal T expressed and secreted (RANTES). MCP-1 was originally characterized as the product of an immediate early gene (JE gene) induced by platelet-derived growth factor and cloned from murine fibroblasts (Rollins, 1996). Since the initial discovery, both human and mouse MCP-1 (mMCP-1 or JE) have been shown to be activator agents for monocytes *in vitro*, being able to induce intracellular calcium fluxes, F-actin polymerization, adhesion molecule up-regulation as well as chemotaxis (Sozzani *et al*., 1991; Rollins, 1996). However, the ability of MCP-1 to cause monocyte accumulation *in vivo* still remains contradictory, with both positive (Zachariae *et al*., 1990; Fuentes *et al*., 1995; Gunn *et al*., 1997; Ajuebor *et al*., 1998) and negative (DeLisser *et al*., 1994; Ernst *et al*., 1995) reports.

MCP-1 gene and protein is expressed by many cell types upon activation, including macrophages, endothelial and mesanglial cells, as well as vascular smooth muscle, and epithelial cells (Baggiolini *et al*., 1994). In addition, MCP-1 gene expression has been reported in several distinct models of inflammation and injury, e.g. experimental autoimmune encephalomyelitis (Ransohoff *et al*., 1993), inflammatory bowel disease (Grimm *et al*., 1996), experimental pulmonary fibrosis (Zhang *et al*., 1994; Antoniades *et al*., 1992) and acute peritonitis (Ajuebor *et al*., 1998).

A complex network of cytokines and chemokines operates during the different phases of the host inflammatory response and the exact nature of the different relationships have recently begun to be unravelled. For instance, interleukin (IL)-4 is a potent inducer of MCP-1 by murine peritoneal macrophage (Kikuchi *et al*., 1994) and human endothelial cells (Rollins & Pober, 1991), whereas IL-13 increases MCP-1 production by vascular smooth muscle cells (Jordan *et al*., 1997). Similar *in vitro* studies have described IL-1β as a potent inducer of MCP-1 gene expression and protein release from various cells types such as pulmonary alveolar macrophage and type II epithelial cells (Brieland *et al*., 1995; Standiford *et al*., 1991), mesanglial, mesothelial and endothelial cells (Rollins *et al*., 1990; Brown *et al*., 1992; Antony *et al*., 1995).

Little information is available in the literature on the *in vivo* interrelationship between IL-1β and MCP-1, with the exception of a study which showed that IL-1β induces pulmonary granulomas in glucan-treated rats by modulating

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MCP-1 production (Flory *et al*., 1995). Here, we have characterized the ability of IL-1β to induce CC chemokine gene expression and protein release in the murine peritoneal cavity with particular attention to mMCP-1. Evidence is provided for the identification of the IL-1 receptor involved and the sensitivity of this process to administration of the glucocorticoid hormone, dexamethasone (DEX). A functional role for this activation of the MCP-1 pathway is also provided.

Methods

Animals

Male Swiss Albino mice were purchased from Tuck (Essex, U.K.) and maintained on a standard chow pellet diet with tap water *ad libitum* using a 12 h light/dark cycle. Experimental animals weighed between 28 and 32 g.

Inflammatory models

Murine recombinant IL-1β (mrIL-1β) was given intraperitoneally (i.p.) at doses ranging from 0.5 to 50 ng in 250 μl of sterile saline. At different time-points, animals were euthanized by CO_2 exposure, peritoneal cavities washed with 3 ml of phosphate buffered saline (PBS) containing 3 mM ethylenediaminetetracetic acid sodium salt (EDTA). Aliquots of the lavage fluids were then stained with Turk's solution (0.01% crystal violet in 3% acetic acid) and differential countings performed using a Neubauer hemocytometer and a light microscope (Olympus B061). In a few cases differential cell counts were performed on May-Grunwald-Giemsa stained cytospin preparations as previously described (Teixeira *et al*., 1997). Lavage fluids were then centrifuged at $400 \times g$ for 10 min and supernatants stored at -20°C prior to mMCP-1 evaluation by enzyme-linked immunoadsorbant assay (ELISA; see below). Cell pellets were then treated for RNA extraction and reverse transcriptase polymerase chain reaction (RT-PCR) analysis (see below).

Lipopolysaccharide (LPS) peritonitis was used as a positive control (e.g. for chemokine expression) as reported by (VanOtteren *et al*., 1995). Mice received 30 μg i.p. LPS in 0.3 ml of sterile saline, and peritoneal cavities were washed 3 h later (Ajuebor *et al*., 1998).

Drug modulation of mrIL-1β effects

Drug modulation of cell influx and mMCP-1 production in response to mrIL-1β treatment was investigated using a human recombinant IL-1 receptor antagonist, previously shown to potently inhibit MCP-1 production *in vitro* (Brown *et al*., 1992), and an IL-1β mutant, previously shown to have much lower affinity for the receptor type I, mutant yIL-1$\beta\Delta$4 (Mugridge *et al*., 1995). Doses of IL-1 receptor antagonist ranging from 1 to 10 μg per cavity, and of yIL-1$\beta\Delta$4 between 3–50 ng per mouse were given.

The glucocorticoid hormone DEX was given subcutaneously (s.c.) at the dose of 30 μg per mouse (corresponding approximately to 1 mg kg^{-1}) 1 h before mrIL-1β. The dose of the steroid was based on our previous study using the zymosan peritonitis model (Ajuebor *et al*., 1998).

In all cases peritoneal cavities were washed 2 h later and mMCP-1 contents in the lavage fluids determined. In the case of DEX experiments, cell pellets were also kept and analysed for mMCP-1 mRNA levels by RT-PCR.

In a separate set of experiments the role of endogenous CC chemokines and neutrophil accumulation was investigated. Mice received 100 μg i.p. of specific goat anti-murine MCP-1 polyclonal immunoglobulin G, or the same dose of control goat immunoglobulin G, at time 0 together with 10 ng mrIL-1β. Peritoneal cavities were washed 4 h later and processed for cell influx as described above.

Depletion of specific subsets of peritoneal cells

Peritoneal macrophages were depleted using multilamellar liposomes encapsulating dichloromethylene-bisphosphonate (Cl_2MDP or clodronate) prepared according to a published procedure (Van Rooijen & Sanders, 1994). Briefly, 86 mg phosphatidylcholine and 8 mg cholesterol were dissolved in 10 ml chloroform in a sterile round bottom flask. By vacuum rotary evaporation at 37°C a thin film was formed on the wall of the flask. Cl_2MDP (1.89 g dissolved in 10 ml sterile PBS) was encapsulated into the preparation of phosphatidylcholine + cholesterol by gently shaking for 10 min. The solution was kept for 2 h at room temperature and then sonicated for 3 min at 20°C. Free Cl_2MDP was removed by three centrifugations in a Beckman Ultracentrifuge L-80 Optima at $100{,}000 \times g$ for 30 min at 4°C. Liposomes, layered on top of the supernatants, were collected and washed twice by centrifugation using the same parameters. Pelleted liposome were resuspended in 4 ml sterile PBS. Mice were treated intraperitoneally with 100 μl of liposome preparation for three consecutive days. MrIL-1β (10 ng i.p.) was given 24 h after the last administration, and peritoneal cavites were lavaged 2 h later and handled as described above.

Peritoneal mast cells were depleted using a well characterized protocol (Das *et al*., 1997). Briefly, mice received a single dose of compound 48/80 (1.2 mg kg^{-1}) 72 h prior to challenge with 10 ng mrIL-1β. Peritoneal cavites were lavaged 2 h later and handled as described above.

Detection of chemokine protein by ELISA

The release of mMCP-1 during IL-1β peritonitis was quantified using a specific ELISA, which shows negligible (<1%) cross-reactivity with several murine cytokines and chemokines and high sensitivity (~detection limit 9 pg ml^{-1}) (data furnished by the manufacturer). Lavage fluids (100 μl) were assayed per sample and compared to a standard curve constructed with 0–2.5 ng ml^{-1} murine MCP-1. Similarly, lavage fluids were tested for the murine chemokines KC (standard curve ranging from 0–1 ng ml^{-1}) and MIP-1α (standard curve up to 300 pg ml^{-1}) and and the cytokine tumor necrosis factor-α (TNF-α; standard curve ranging from 0–1.5 ng ml^{-1}) using commercially available ELISA according to the manufacturer's instructions.

Detection of chemokine mRNA by RT-PCR Analysis

Total RNA was isolated according to manufacturer's protocol and the yield and purity of the RNA was estimated spectrophotometrically at 260 nm and 280 nm of wavelength. Total RNA (3 μg) was used for the generation of cDNA. PCR amplification reactions were then performed on aliquots of the cDNA. For the mMCP-1 target, the primers were 5′-ACT-GAA-GCC-AGC-TCT-CTC-TTC-CTC-3′ and 5′-TTC-CTT-CTT-GGG-GTC-AGC-ACA-GAC-3′ (forward and reverse) which amplified a fragment 274 base pairs in length. For the murine RANTES (mRANTES) target, primers were 5′-GCC-CAC-GTC-AAG-GAG-TAT-TTC-TAC-3′ and 5′-AGG-

ACT-AGA-GCA-AGC-GAT-GAC-AGG-3′ (forward and reverse) and amplified a fragment 205 base pairs in length.

All PCR reactions were performed in a final volume of 25 μl. For mMCP-1, the PCR profile consisted of 1 cycle of denaturation at 94°C for 2 min, followed by 35 cycles of denaturation at 94°C (45 s), annealing at 57°C (45 s) and extension at 72°C (30 s). For mRANTES, an initial denaturation at 94°C for 2 min, followed by 30 cycles of denaturation at 94°C (45 s), annealing at 55°C (45 s) and extension at 72°C (30 s). Amplification products were visualized by ethidium bromide fluorescence in agarose gels. Bands of the expected sizes were obtained. Images were inverted using the Graphic Converter software (version 2.1) running on a Macintosh Performa 6200.

Materials

MrIL-1β and human recombinant IL-1 receptor antagonist were generous gift of Dr R.C. Newton (DuPont-Merck, Glenolden, DE, U.S.A.) and the mutant yIL-1βΔ4 was a gift of Prof L. Parente (University of Palermo, Palermo, Italy). DEX (sodium phosphate salt solution) was from David Ball Laboratories (Warwick, U.K.). Goat anti-mMCP-1 immunoglobulin G as well as the Quantikine™ ELISA kits for murine TNF-α and KC were purchased from R&D Systems (Abingdon, U.K.) whereas the specific murine MCP-1 ELISA Cytoscreen™ was from BioSource International (Canarillo, CA). Non-immune goat, LPS (*Escherichia Coli* serotype 0111:B4) and all other chemicals were from Sigma Chemical Co. (Poole, U.K.). Cl_2MDP (or clodronate) was a generous gift of Boehringer Mannheim (U.K.).

Reagents for the PCR reaction were purchased from the following companies: mMCP-1 and mRANTES primers from OligoExpress Ltd. (Middlesex, U.K.); Trizol™ reagent (lysis buffer for RNA preparation) from Gibco BRL (Paisley, U.K.) and Ready-to-Go™ T-Primed First-strand kit and PCR Beads from Pharmacia Biosystem Europe (St. Albans, U.K.).

Statistics

Data are reported as mean ± s.e.mean of *n* mice per group, and statistical differences were evaluated by one-way analysis of variance once the Bartlett's test confirmed the homogeneity of the variances. *Post hoc* comparisons were made with the test of Bonferroni using Instat™ software (version 2.04) running on a Macintosh Performa 6200. A threshold value of *P* less than 0.05 was taken as significant.

Results

Characterization of mrIL-1β induced mMCP-1 production in vivo

Injection of mrIL-1β (0.5–50 ng) into the peritoneal cavity produced a dose-related release of mMCP-1 into the lavage fluids as shown in Figure 1A. More than 80% of the maximal release was observed at the dose of 10 ng IL-1β. The release of MCP-1 was transient, with a sharp increase between 30 min and 2 h post-challenge, where it then reached the maximal values. Murine MCP-1 concentration returned to near basal level by the 4 h time-point (Figure 1B). The mrIL-1β dose of 10 ng and the time-point of 2 h were used in most of the subsequent experiments.

Release of mMCP-1 protein was mirrored by MCP-1 gene activation, as detected by RT-PCR (Figure 1C). MCP-1 gene was not expressed by unstimulated peritoneal cells whereas there was a distinct expression at 1, 2 and 4 h post-mrIL-1β injection. MCP-1 gene expression was no longer detectable at

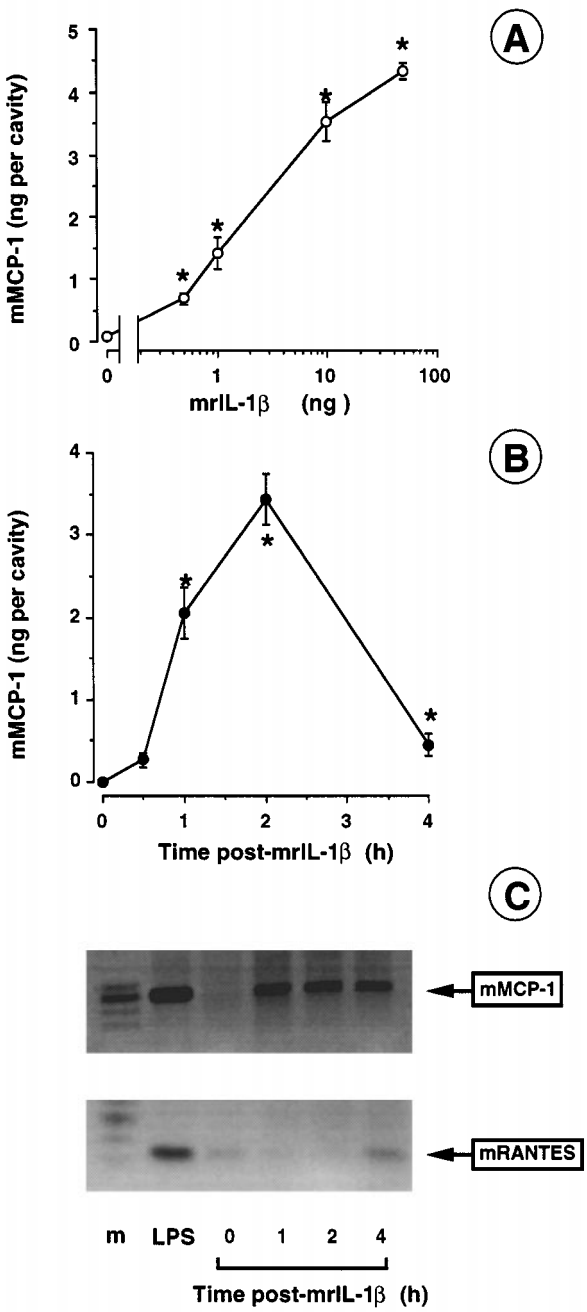

Figure 1 Characterization of mrIL-1β-induced mMCP-1 production *in vivo*. (A) Mice were left untreated or received i.p. the reported doses of mrIL-1β (in 0.25 ml sterile saline). Peritoneal cavities were washed 2 h later. Cell-free supernatants were analysed for mMCP-1 content using a commercially available ELISA. Data are means ± s.e.mean of five to eight animals per group. $^*P<0.05$ vs dose 0 group. (B) Mice were left untreated (time 0) or received 10 ng IL-1β in 0.25 ml sterile saline. Peritoneal cavities were washed at the reported time-points, and cell-free supernatants were analysed for mMCP-1 content using a commercially available ELISA. Data are means ± s.e.mean of six mice per group. $^*P<0.05$ vs time 0 group. (C) PCR products of the peritoneal cell pellets prepared at the reported time-points after injection of 10 ng mrIL-1β. Positive controls were cells prepared 3 h after i.p. injection of LPS (1 mg kg^{-1}). Specific bands for mMCP-1 (274 base pairs) and mRANTES (205 base pairs) were prepared as described in the Methods section. Data were obtained from pooled preparations of mice per group, and are representative of three experiments with similar results. (m) denotes the markers.

8 h (not shown). A band corresponding to mMCP-1 gene product was also obtained in LPS-treated peritoneal cells, used as positive control.

For comparative purposes, gene expression of a distinct CC chemokine was also monitored. Gene activation of mRANTES was modest, and appeared to be delayed such that a clear PCR product was detected only at the 4 h time-point (Figure 1C).

Co-injection of IL-1 receptor antagonist significantly attenuated mrIL-1β mediated mMCP-1 release, with more than 80% inhibition at the dose of 10 μg per cavity (Figure 2A). Figure 2B shows that the mutant yIL-1$\beta\Delta$4 was unable to mimick the effect of IL-1β, and at the dose of 50 ng produced a modest release of mMCP-1, comparable to that measured with 1 ng of the parent cytokine (see Figure 1A).

Release of other pro-inflammatory cytokines

For comparative purposes we also measured the protein contents of the CXC chemokine KC and of the pro-inflammatory cytokine TNF-α in the peritoneal lavage fluids. Data in Table 1 show that whereas LPS treatment lead to release of both pro-inflammatory agents, only KC could be found after challenge with mrIL-1β.

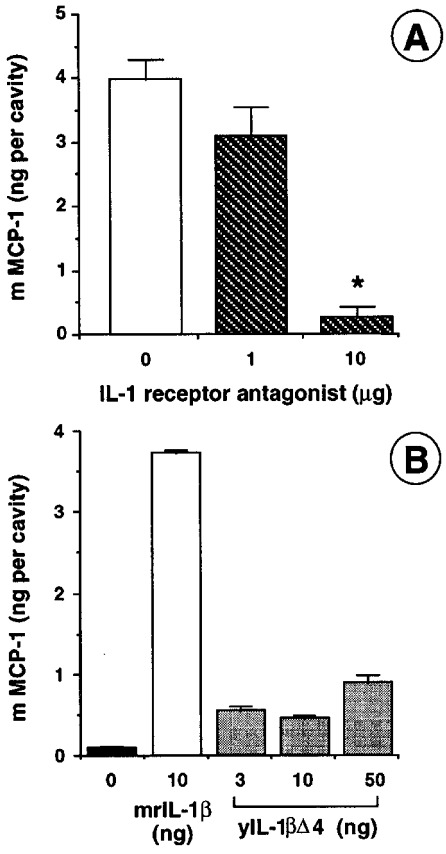

Figure 2 Effect of drugs acting at the IL-1 receptors on mrIL-1β effects. (A) Mice received a combined treatment of 10 ng mrIL-1β with the reported doses of IL-1 receptor antagonist in a final volume of 0.25 ml. Peritoneal cavities were washed 2 h later, and the content of mMCP-1 in the cell-free supernatants was measured by ELISA. Data are means$\pm$s.e.mean of four to six mice per group. *$P<0.05$ vs dose 0. (B) Mice were treated with sterile saline alone (0.25 ml i.p.), containing 10 ng mrIL-1β or the reported doses of yIL-1$\beta\Delta$4. Peritoneal cavitites were washed 2 h later and cell-free lavage fluids analysed for mMCP-1 content by ELISA. Data are means$\pm$s.e.mean of five mice per group.

Effect of DEX on mrIL-1β-induced mMCP-1 gene and protein release

Pretreatment of mice with DEX (1 mg kg^{-1}) produced an inhibitory action with a pronounced effect on the gene (Figure 3B), whereas approximately 40% of reduction was observed on the levels of mMCP-1 protein released into the cavity (Figure 3A).

Investigation on the cellular source of mMCP-1

Table 2 illustrates that more than 90% depletion of peritoneal macrophages prior to challenge with mrIL-1β did not modify the release of mMCP-1 into the lavage fluids. A similar lack of effect was seen when the number of intact mast cells was

Table 1 Comparison between IL-1 and LPS ability to cause release of KC and TNF-α in the mouse peritoneal cavity

I.p. treatment	*Time* (h)	*KC* (ng per cavity)	*TNF-α* (pg per cavity)
Vehicle	3	0	0
LPS	3	31.8$\pm$0.9	282$\pm$18
Vehicle	2	0	0
IL-1β	2	5.2$\pm$0.4	0

Mice were injected with 10 ng i.p. mrIL-1β or with LPS (1 mg kg^{-1} i.p.). Peritoneal cavities were washed either 2 h or 3 h post-challenge, and KC or TNF-α contents in the lavage fluids determined by ELISA (see Methods). Control mice received sterile saline only (0.25 ml). Data are means$\pm$s.e. mean of seven mice per group.

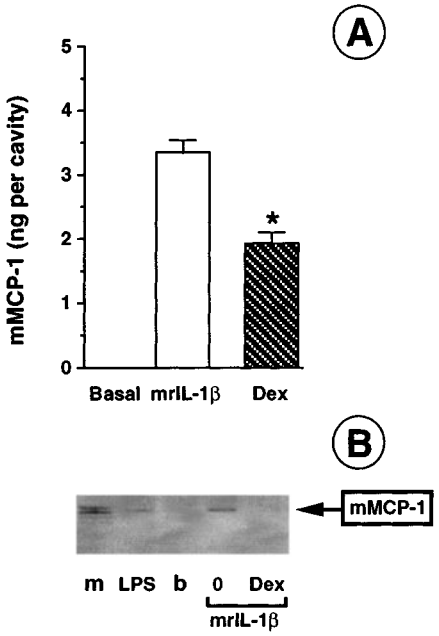

Figure 3 Effect of DEX on mrIL-1β-induced mMCP-1 production and gene expression. (A) Mice were left untreated or received DEX (1 mg kg^{-1} s.c.) 1 h prior to i.p. injection of mrIL-1β (10 ng). Peritoneal cavitites were washed 2 h later and cell-free lavage fluids analysed for mMCP-1 content by ELISA. Data are means$\pm$s.e.mean of ten mice per group. *$P<0.05$ vs mrIL-1β group. (B) PCR products of the peritoneal cell pellets prepared as in A. Positive controls were cells prepared 3 h after i.p. injection of LPS (1 mg kg^{-1}). Specific bands for mMCP-1 are the right position (274 base pairs). Data are representative of two experiments with similar results. (m) denotes the markers and (b) basal.

Table 2 Effect of peritoneal mast cell or macrophage depletion on mMCP-1 release after mrIL-1β injection

Intraperitoneal pre-treatment	*Macrophages* (10^6 per cavity)	*Mast cell* (10^3 per cavity)	*mMCP-1* (ng per cavity)
Vehicle	4.32±1.0	n.d.	2.66±0.50
Liposome	0.35±0.23	n.d.	3.03±0.25
Vehicle	n.d.	7.2±1.5	1.96±0.60
Compound 48/80	n.d.	0.40±0.04	1.56±0.48

Mice were subjected to a treatment with multilamellar liposomes encapsulated with Cl_2 MDP (100 μl i.p. for 3 days, and used 24 h after the last injection) or with compound 48/80 (1.2 mg kg^{-1} i.p., −72 h) prior to administration of mrIL-1β (10 ng i.p.). Peritoneal cavities were washed 2 h later, the content of mMCP-1 in the lavage fluids quantified by ELISA (see Methods). Data are means±s.e.mean of 12 mice per group. n.d., not determined.

greatly reduced by a pretreatment with the specific secretagogue compound 48/80 (Table 2).

A functional role for mrIL-1β-induced mMCP-1

Intraperitoneal injection of mrIL-1β (10 ng) produced a time-dependent accumulation of polymorphonuclear leucocytes (PMN), with a maximal rate of influx between 1 and 4 h post-challenge with the cytokine (almost 4×10^5 cells h^{-1}). The peak of the response was at 4 h, with similarly high number of PMNs being counted in the 8 h and 24 h lavage fluids (Figure 4A). This PMN population consisted essentially of neutrophils (>99%), since no significant eosinophil influx could be seen as assessed from cytospin preparations. No significant changes in monocyte and lymphocyte numbers were seen after mrIL-1β injection, at least over the time course used in these experiments (data not shown).

Co-administration of a polyclonal antibody raised against mMCP-1 significantly inhibited neutrophil infiltration caused by mrIL-1β (Figure 4B). An inhibition of more than 50% was calculated in comparison to the group treated with non-immune goat immunoglobulin G.

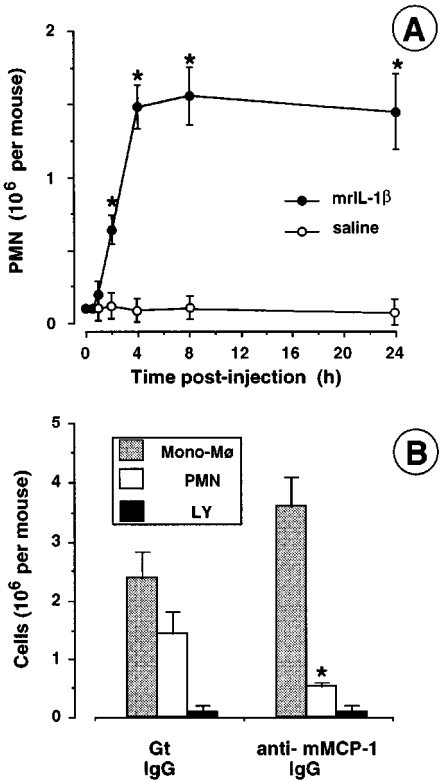

Figure 4 Effect of anti-mMCP-1 IgG on mrIL-1β-induced neutrophil accumulation. (A) Mice were killed by CO_2 exposure at the reported times after mrIL-1β injection (10 ng i.p.) or saline (250μl i.p.). Peritoneal cavities were washed with PBS+EDTA. Neutrophil (PMN) influx was determined after differential cell counting in Turk's solution. Data are means±s.e.mean of six mice per group. *$P<0.05$ vs saline. (B) Mice received 10 ng in 250 μl saline i.p. of mrIL-1β together with 100 μg of non-immune goat (Gt) or anti-mMCP-1 IgG. Peritoneal cavities were washed 4 h later, and the number of monocyte and macrophages (Mono-Mø), neutrophils (PMN) and lymphocytes (LY) determined following differential cell countings in Turk's solution. Data are means±s.e.mean of five to six mice per group. *$P<0.05$ vs respective control IgG.

Discussion

In this study we have investigated the relationship between *in vivo* administration of IL-1β and activation of the mMCP-1 pathway, finding that mMCP-1 gene expression is followed by protein synthesis and release in the peritoneal lavage fluid. In addition, we provide evidence for a role of endogenous mMCP-1 in the cellular influx elicited by IL-1β.

I.p. injection of mrIL-1β produced a dose-dependent and time-related release of mMCP-1 in the lavage fluids. The amounts of mMCP-1 obtained were significantly lower than those we measured when we used a stronger inflammogen such as zymosan (Ajuebor *et al*., 1998). Nonetheless, the release of mMCP-1 was reproducible and receptor mediated. Co-administration of the specific IL-1 receptor antagonist strongly inhibited mrIL-1β response. The fact that a large excess of IL-1 receptor antagonist was required to block the effect of the cytokine is not a novel finding in *in vivo* experimental systems (Perretti *et al*., 1993; McIntyre *et al*., 1991), and it is related to the presence of a large number of spare receptors for the cytokine (Dinarello, 1991). The efficacy of the IL-1 receptor antagonist does not give an insight into which IL-1 receptor, either type I or type II, is mediating this property of the cytokine. We then tested the effect of the mutant protein yIL-1$\beta\Delta4$. This mutant lacks the first four amino acids of the mature form of IL-1β but it is still able to bind IL-1 receptor type II with the same affinity as the wild-type; however, its binding affinity to IL-1 receptor type I is several orders of magnitude lower (Mugridge *et al*., 1995). The failure of yIL-1$\beta\Delta4$ to induce a significant release of mMCP-1 strongly indicates that this property of the cytokine is mediated by interaction with the type I receptors.

An apparent discrepancy was seen in the time-courses of mMCP-1 mRNA presence (still high at 4 h post-IL-1β) and that of secreted mMCP-1 protein (which peaked at 2 h but was almost back to basal levels by the 4 h time-point). This data suggests that some inhibitory mechanism(s) may be operating

in vivo to block translation of the mMCP-1 mRNA or to impede secretion of the mature protein into the cavity. In line with this hypothesis is the fact that IL-1β-induced MCP-1 protein secretion *in vitro* (such as from endothelial cells) is sustained for a much longer time (Rollins *et al*., 1990; Standiford *et al*., 1991; Antony *et al*., 1995). Potential candidates responsible for this bell-shaped secretion of mMCP-1 could be endogenous corticosterone, or more probably interleukin-10 or transforming growth factor-β (Kitamura, 1997), all shown to suppress MCP-1 synthesis in different experimental conditions.

This experimental system was further validated by assessing the effect of DEX. As expected (Mukaida *et al*., 1991), treatment of mice with this glucocorticoid hormone effectively suppressed mMCP-1 gene activation and protein secretion in response to mrIL-1β. This observation can be coupled to recent ones where DEX treatment was effective in reducing mMCP-1 mRNA and protein release in stronger inflammatory conditions such as those obtained following i.p. injection of zymosan (Ajuebor *et al*., 1998) or intratracheal application of LPS (Yi *et al*., 1996).

IL-1β effect on mMCP-1 release was somehow selective and not the result of a generalized activation within the mouse peritoneal cavity. In contrast to LPS, mrIL-1β injection produced release of the CXC chemokine KC but not that of the multipotent cytokine TNF-α. In addition, mrIL-1β activated mMCP-1 gene as assessed by PCR. Using the latter technique we could confirm the selectivity of mrIL-1β action. Although PCR analyses are at the best semi-quantitative, it was clear that mRANTES gene was modestly expressed only at the 4 h time-point.

Next, we addressed the question of which cell type could be activated by mrIL-1β to produce mMCP-1. Since a specific MCP-1 mRNA band was detected by PCR in the peritoneal cell pellet, we hypothesized that either resident macrophages (which represent 80–90% of total cells) or mast cells (which constitute 2–4% of the total peritoneal cells) could be responsible for this. To test this hypothesis, we chose to monitor mMCP-1 protein levels by ELISA, which is more quantitative than the PCR analysis. Substantial macrophage depletion (more than 90% reduction in macrophage numbers) was obtained according to a published procedure which entails the use of neutral liposomes. The liposomes are phagocytosed by the resident macrophage and once in the intracellular compartment release the cytotoxic drug (Van Rooijen & Sanders, 1994). However, no changes were seen in the amounts of mMCP-1 recovered from the peritoneal cavities treated with mrIL-1β in control and macrophage-depleted animals. Similarly, depletion of resident mast cell contents by causing a selective degranulation with compound 48/80 3 days prior to experimentation is a procedure successfully used to investigate the role played by this cell type in the initiation of the inflammatory response (Diaz *et al*., 1996; Das *et al*., 1997). This procedure did not modify mMCP-1 levels after injection of mrIL-1β. Lymphocytes are also found in the peritoneal cavity. However, in preliminary experiments where cell-associated mMCP-1 was measured by flow cytometry we could not detect any positive staining (not shown), strongly confirming that also in our experimental conditions this cell type is unable to synthesize this chemokine.

From this set of experiments we conclude that another cell type(s) such as endothelial cells (Rollins *et al*., 1990) or mesothelial cells (Gimbrone *et al*., 1997; Antony *et al*., 1995) could be responsible for the effect of mrIL-1β. Mesothelial cells may be the best candidate since they have been recently implicated in the production of MCP-1 in related models of inflammation (Gimbrone *et al*., 1997) and can also be present in small numbers in the peritoneal cell pellet. Endothelial cells can be proposed for their known sensitivity to IL-1β, as well as for the predominant presence of IL-1 receptor type I on their surface (Dinarello, 1991). Further studies will clarify this aspect of the IL-1/MCP-1 network.

Finally, a potential function for the mMCP-1 released after challenge with IL-1β was found. Injection of mrIL-1β produced a selective accumulation of granulocytes into the peritoneal cavity, which was not followed by accumulation of monocytes, as already reported after administration into a subcutaneous air-pouch (Perretti & Flower, 1993). Since the profile of cell influx paralleled the release of mMCP-1 (the latter being shifted to the left in the time-course experiments), we postulated that mMCP-1 could contribute at least partly to the observed neutrophil accumulation. Treatment of mice with a specific polyclonal antibody raised against mMCP-1 reduced the accumulation of PMNs by more than 50%. The suggestion that mMCP-1 may contribute to the accumulation of this cell type in acute inflammation is unexpected and it is not substantiated by *in vitro* studies from which it is clear that MCP-1 does not activate neutrophils and is a specific chemoattractant/activator for monocytes (Rollins, 1996). However, *in vivo* situations are more complex and prone to potential interactions between different chemokines and cytokines. In line with this, it has been shown that mMCP-1 injection in the mouse footpad causes cell accumulation, probably secondary to mast cell activation (Alam *et al*., 1994). Similarly, we were unable to observe neutrophil accumulation after mMCP-1 injection into the mouse peritoneal cavity, nonetheless we could demonstrate a functional role for endogenous MCP-1 in the influx not only of monocytes but also in that of granulocytes which is characteristic of the inflammatory response induced by zymosan (Ajuebor *et al*., 1998). This action of endogenous mMCP-1 was not related to an increase in the release of neutrophil chemoattractants, such as TNF-α or KC.

It is difficult to explain how a CC chemokine with no direct effect on neutrophils *in vitro*, may collaborate to recruit this cell type in certain inflammatory conditions. A recent publication has employed TNF-α to produce neutrophil accumulation into 6-day-old murine air-pouches. Similarly to what we have seen in the present study, treatment of mice with an anti-mMCP-1 (or an anti-mMIP-1α or an anti-murine KC polyclonal antibodies) inhibited the migratory action of TNF-α by 40–50% (Tessier *et al*., 1997). It must be said, though, that in contrast to MCP-1, exogenous administration of MIP-1α produces neutrophil influx (see Alam *et al*., 1994, and our unpublished data).

It is possible that CC chemokines, produced in response to administration of IL-1β, TNF-α or zymosan, activate residential macrophages to produce neutrophil chemoattractants other than endogenous KC or TNF-α. However, macrophages are poor target cells for MCP-1 and, for instance, expression of the F4/80 antigen on their cell surface is not altered by incubation with the chemokine (MNA and MP, unpublished data). Finally, murine and human neutrophils bear CCR1 receptors (Power & Wells, 1996) which bind MCP-1 with low affinity (Neote *et al*., 1993). It is therefore possible that endogenous mMCP-1 may activate this receptor (maybe in concert with other chemokines such as MIP-1α, RANTES and MCP-3) and contribute in this manner to the extravasation of blood-derived neutrophils into the tissue site of inflammation described in the present and other studies.

This work was supported by the Joint Research Board of the St. Bart's Trust (grant XLMW) with a PhD studentship to M.N.A. R.J.F. is a Principal Research Fellow of the Wellcome Trust, whereas M.P. is a Post-Doctoral Fellow of the Arthritis and Rheumatism Council. We thank Prof L. Parente (University of Palermo, Italy) for the generous supply of yIL-1βΔ4 and Dr M. Christie (Astra Charnwood, U.K.) for providing the mMCP-1 ELISA and for the helpful discussion. We also acknowledge Dr P. Jose (Imperial College, London) for helpful discussion.

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(Received February 10, 1998
Revised June 12, 1998
Accepted June 18, 1998)