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Octreotide regulates CC but not CXC LPS-induced chemokine secretion in rat Kupffer cells

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1 Kupffer cells (KC) and lipopolysaccharide (LPS) interaction is the initial event leading to hepatic inflammation and fibrosis in many types of liver injury. We studied chemokine secretion by KC activated with LPS and the possible effect of the somatostatin analogue octreotide, in the regulation of this process.

2 KC isolated from Sprague–Dawley rats were cultured in the presence of LPS added alone or with different concentrations of octreotide for 24 and 48 h, and chemokine production was assessed in culture supernatants by ELISA. CC chemokine mRNA expression was assessed by semiquantitative RT–PCR.

3 Vehicle-stimulated KC produced a basal amount of CC and CXC chemokines. LPS-stimulated KC secreted significantly increased amounts of IL-8 (GRO/CINC-1) ($P<0.001$), MIP-2 ($P<0.001$), MCP-1 ($P<0.001$), and RANTES ($P<0.01$).

4 Octreotide inhibited LPS-induced secretion of the CC chemokines MCP-1 ($P<0.05$) and RANTES ($P<0.05$), but not the CXC chemokines IL-8 (GRO/CINC-1) and MIP-2, in a concentration-dependent manner. Downregulation of basal and LPS-induced mRNA expression of the CC chemokines was also observed in the presence of octreotide.

5 Pretreatment with phosphatidylinositol 3 (PI3)-kinase inhibitors reduced chemokine production by LPS-treated KC in both the mRNA and protein level. Furthermore, it prevented the octreotide inhibitory effect on LPS-induced chemokine secretion, indicating a possible involvement of the PI3-kinase pathway.

6 In conclusion, these data demonstrate that chemokine secretion by KC can be differentially regulated by octreotide, and suggest that this somatostatin analogue may have immunoregulatory effects on resident liver macrophages.
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Abbreviations: CINC, cytokine-induced neutrophil chemoattractant; GRO, growth-related oncogene; HSC, hepatic stellate cell(s); KC, Kupffer cell(s); IL-8, interleukin-8; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein-1; MIP-2, macrophage inflammatory protein-2; PI3-kinase(s), phosphatidylinositol 3 (PI3)-kinase(s); RANTES, regulated on activation, normal T-cell expressed and secreted

Introduction

The sinusoidal cells of the liver play a critical role in liver homeostasis. Among them, the resident liver macrophages, that are the Kupffer cells (KC), are the first cells to be exposed to infective, immunoreactive, particulate, or toxic (e.g. ethanol) materials absorbed from the gastrointestinal tract. They function as antigen-presenting cells and scavengers of microorganisms, endotoxins, degenerated cells, and immune complexes (Nolan, 1981). They participate in the surveillance of tumour growth (Bayon *et al*., 1996) and regeneration processes in the liver (Fausto *et al*., 1995), and they seem to play a key role in innate immune responses and host defence through the expression and secretion of soluble inflammatory mediators (Winwood & Arthur, 1993).

The ability of KC to eliminate and detoxify endotoxins, such as lipopolysaccharide (LPS), is an important physiological regulatory function. There is accumulating evidence that KC and LPS interaction may be the initiating event leading to hepatotoxicity in a variety of types of liver injury, like endotoxinaemia, alcoholic liver injury, and ischaemia/reperfusion injury (Liu *et al*., 1995; Su, 2002). Therefore, primary KC cultures and their interaction with LPS represent a valid *in vitro* model to explore and modulate certain pathophysiologic mechanisms leading to hepatic injury.

Somatostatin is a phylogenetically ancient, multigene family of peptides with two bioactive products: somatostatin-14 and somatostatin-28 (Reichlin, 1983). In the periphery, somatostatin is secreted in the gastrointestinal tract and pancreas either from nerve endings in the intestinal mucosa and hepatoportal area, or from non-neuronal cells distributed throughout the length of the gastrointestinal tract (McIntosh, 1985; el Salhy *et al*., 1993). Somatostatin inhibits glandular

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secretion, neurotransmission, smooth-muscle contractility, and absorption of nutrients in the GI tract (Reichlin, 1983). Furthermore, it has been suggested that it may have direct immunomodulatory actions, and it is considered to be a local anti-inflammatory factor (van Hagen *et al*., 1999). Specifically, it inhibits the migration of circulating leukocytes to the inflammatory site, suppresses blood vessel permeability, induces vasoconstriction, and inhibits angiogenesis (Karalis *et al*., 1994; Reubi *et al*., 1994). It also suppresses the release of colony-stimulating activity by splenic lymphocytes and inhibits immunoglobulin production by B-lymphocytes (ten Bokum *et al*., 2000).

Octreotide, the first somatostatin analogue introduced for clinical use, has the advantage of a greater elimination half-life than the natural peptide, and therefore there is no need for continuous intravenous infusion of the agent. Octreotide seems to share the inhibitory effects of the natural peptide on leukocyte functions (Niedermuhlbichler & Wiedermann, 1992). Up to date, it has been used in the treatment of secreting pituitary adenomas, metastatic islet-cell and carcinoid tumours, somatostatin receptor-positive neuroendocrine tumours, inoperable hepatocellular carcinoma, acute oesophageal variceal bleeding, and refractory diarrhoea syndromes (Lamberts *et al*., 1996; Kouroumalis *et al*., 1998).

Over the past decade, it has been recognized that extravascular leukocyte accumulation is a multi-step process that requires a series of co-ordinated signals, including the expression of chemokines and their receptors (Sallusto *et al*., 2000). Unlike the classical chemoattractants, chemokines are quite diverse in their target-cell selectivity. For example, the C-X-C family, of which the prototype is interleukin-8 (IL-8), includes members all of which are potent neutrophil chemotactic and activating agents. The C-C family includes monocyte chemoattractant protein-1 (MCP-1), and regulated on activation, normal T-cell expressed and secreted (RANTES), and its members exhibit differential chemotactic activity to monocytes, subpopulations of T-cells, eosinophils or dendritic cells (Rollins, 1997). In the present study, we explored *in vitro* the chemokine secretion by LPS-activated rat KC, as chemokine production has been reported to be involved in liver inflammation and fibrosis, through the recruitment of specific leukocyte populations in hepatic injury (Bone-Larson *et al*., 2000). In view of the increasing use of octreotide in clinical practice and the previously reported immunomodulatory properties of somatostatin and analogues, including cell recruitment (Karalis *et al*., 1994), we additionally investigated the *in vitro* effect of octreotide on basal and LPS-induced production of chemokines from primary rat KC cultures.

Methods

Animals

KC were isolated from pathogen-free male Sprague–Dawley rats over 12 months old (450–600 g). Animals were fed *ad libitum*. All studies were approved by the Veterinary Administration Office of Heraklion, Ministry of Agriculture, and conformed to the National and EU directions for the care and treatment of laboratory animals.

Cell isolation

Cell isolation was performed according to a previously published methodology (Valatas *et al*., 2003). Briefly, the liver tissue was enzymatically digested by perfusion through the portal vein using a combination of 0.2% Pronase and 0.01% Collagenase at $10\,ml\,min^{-1}$, 37°C, in a nonrecirculating fashion. Following a second incubation with 0.03% Pronase and 0.01% DNAse at 37°C for 30 min, the liver homogenate was filtered through a nylon mesh to remove undigested tissue, and the cell suspension was loaded on a double-layer discontinuous Iodixanol gradient of 11.7 and 17.6% Optiprep™, in order to separate sinusoidal cells from viable hepatocytes (1400 g for 17 min at 4°C). KC were further separated from the other sinusoidal cells by centrifugal elutriation. KC collected at flow rates of 45 and $60\,ml\,min^{-1}$ at 2500 r.p.m. (J2-MC, JE-6B-Standard Beckman, Paolo Alto, CA, U.S.A.) were further purified by selective adherence to plastic. This resulted in a cultured population of >95% ED-2-positive, >95% nonspecific esterase activity-positive KC. Viability was more than 98% by Trypan blue exclusion test.

Cell culture

The cells were seeded on six-well plates at a density of $3 \times 10^6\,well^{-1}$, and cultured in Dulbecco's modified Eagle medium (DMEM, GibcoBRL) supplemented with $100\,U\,ml^{-1}$ penicillin-streptomycin (GibcoBRL) and 10% FCS (GibcoBRL). Cells were viable after 14 days in culture (Valatas *et al*., 2003). For experiments, cultured cells were washed 24 h after isolation and cultured in fresh medium without foetal calf serum 4 h before stimulation. Growth-arrested cells were treated with the appropriate concentrations of stimuli in medium and incubated for 24 or 48 h. Viability was greater than 95% after 24 and 48 h in culture. The supernatants were collected and stored at −70°C until measured.

Experimental protocol for chemokine evaluation

In order to evaluate LPS- and octreotide-mediated secretion of chemokines, cultured KC were incubated with LPS ($1\,\mu g\,ml^{-1}$) or octreotide (1 and $100\,ng\,ml^{-1}$) for 24 and 48 h. KC stimulation experiments were carried out at serum-free conditions as the availability of serum is a limiting factor only at relatively low concentrations of LPS (Su *et al*., 2002). We have previously shown that isolated KC in culture respond to the presence of 0.1–10 $\mu g\,ml^{-1}$ of LPS by increasing TNFα ($P<0.01$) and NOx ($P<0.01$) production in a concentration-dependent manner (Valatas *et al*., 2003). Based on our previously reported data, we chose an LPS concentration ($1\,\mu g\,ml^{-1}$) known to induce an adequate immune response, in order to explore the production of chemokines from LPS-stimulated KC.

For the evaluation of the effect of octreotide on LPS-stimulated KC, cells cultured in the presence of $1\,\mu g\,ml^{-1}$ of LPS were incubated with various concentrations of octreotide from 0.001 to $100\,ng\,ml^{-1}$ for 24 and 48 h. Furthermore, in order to evaluate the possible involvement of the phosphatidylinositol (PtdIns)-(3) kinase in the intracellular signalling events, following stimulation with octreotide or LPS, the cultured cells were pretreated with different concentrations of the PtdIns-(3) kinase inhibitor Wortmannin (10, 100, 300 nM)

for 15 min, and then 1 $\mu g\,ml^{-1}$ LPS or a combination of LPS (1 $\mu g\,ml^{-1}$) and octreotide (0.1 $ng\,ml^{-1}$) were added for 24 h. Secretion of IL-8, that is, growth-related oncogene/cytokine-induced neutrophil chemoattractant (GRO/CINC-1), MCP-1, macrophage inflammatory protein-2 (MIP-2), and RANTES by stimulated KC, were measured in cell culture supernatants with commercially available solid-phase ELISA assays. All assays were performed according to the manufacturer's instructions.

RT–PCR

Growth-arrested KC in culture were stimulated with vehicle, LPS (1 $\mu g\,ml^{-1}$), octreotide (0.1 $ng\,ml^{-1}$), or the combination of octreotide (0.1 $ng\,ml^{-1}$) and LPS (1 $\mu g\,ml^{-1}$) for 24 h. Furthermore, in order to evaluate the possible involvement of the phosphatidylinositol (PtdIns)-(3) kinase in the intracellular signalling events, following stimulation with octreotide or LPS, cultured cells were pretreated with different concentrations of the PtdIns-(3) kinase inhibitor LY294002 (1, 10, 30 μM) for 15 min, and then 1 $\mu g\,ml^{-1}$ LPS or a combination of LPS (1 $\mu g\,ml^{-1}$) and octreotide (0.1 $ng\,ml^{-1}$) were added for 24 h. Total RNA was then extracted from 3×10^6 KC into TRIzol® (Life Technologies Ltd, U.K.), as described by the manufacturers. Messenger RNA expression level of MCP-1 and RANTES was measured by multiplex semiquantitative RT–PCR. Ribosomal 18S RNA was used as an internal control in all PCR reactions. mRNA (100 ng) was denatured at 70C for 10 min in the presence of 5 μM oligo $(dT)_{12-18}$ primer. It was then reverse transcribed in a 10 μl volume with Superscript II (Gibco), 1 × RT buffer, 1 mM deoxyribonucleotide triphosphates (dNTPs), 5 mM DDT, and 2.5 U μl^{-1} RNAsin (Promega) at 42°C for 60 min. Aliquots (1 μl) of cDNA were PCR amplified in a 25 μl reaction, containing 1 × PCR buffer and 2 mM $MgCl_2$ 0.2 mM dNTPs, 0.5 mM sense and antisense primers, and 0.4 U High Fidelity Expand polymerase (Roche). The sequences of the oligonucleotides were as follows:

MCP-1 forward:	5′-CCTGTTGTTCACAGTTGCTGCC-3′
MCP-1 reverse:	5′-TCTACAGAAGTGCTTGAGGTGG TTG-3′
RANTES forward:	5′-CGTGAAGGAGTATTTTTACACCA GC-3′
RANTES reverse:	5′-CTTGAACCCACTTCTTCTCTGGG-3′
18S forward:	5′-GAGGTGAAATTCTTGGACCGG-3′
18S reverse:	5′-CGAACCTCCGACTTTCGTTCT-3′

The size of each amplicon was established in 396 bp for MCP-1, 110 bp for RANTES, and 93 bp for 18s. The PCR mixture (25 μl total volume) for MCP-1 and 18S consisted of primers for MCP-1 (250 nM each) and 18S (500 nM each), and the conditions for amplification were: 5 min 94°C; 25 cycles of 30 s 94°C, 30 s 63°C, for primers MCP-1/18S annealing, 30 s 72°C, followed by an extension for 7 min at 72°C. The PCR mixture (25 μl total volume) for RANTES and 18S consisted of primers for RANTES (500 nM each) and 18S (250 nM each), and the conditions for amplification were: 5 min 94°C; 30 cycles of 30 s 94°C, 30 s 62°C, for primers RANTES/18S annealing, 30 s 72°C, followed by an extension for 7 min at 72°C. PCR products were resolved by electrophoresis on 2% agarose gels and visualized by ethidium bromide staining. In order to control for genomic contamination, an identical parallel PCR reaction (RT-negative) was performed for each sample containing starting material, which had not been reverse transcribed (Jordan *et al*., 1999). Each set of primers was tested with at least three different RNA samples treated independently. The integrated density of the bands was used as a quantitative parameter, and was calculated by digital image analysis (Scion image). The ratio of the integrated density of each gene divided by that of 18S was used to quantify the results.

Materials

Enzymes for tissue digestion, Pronase, DNAse, and Collagenase B were from Boehringer-Mannheim, Mannheim, Germany. Optiprep™ was purchased from Nycomed-Pharma, Oslo, Norway. LPS (from *Escherichia coli* 026:B6; phenol extract), Wortmannin (*Penicillium funiculosum*), and LY294002 were purchased from Sigma-Aldrich, Steinheim, Germany. Octreotide (Sandostatin 0.1 $mg\,ml^{-1}$) was from Novartis, Basel, Switzerland. All cell culture reagents and plastics were from Gibco BRL and Nalge Nunc International, U.K., respectively. ELISA kits for rat MCP-1, MIP-2, and RANTES were from Biosource, Nivelles, Belgium, and for rat IL-8 (GRO/CINC-1) was from Immuno-Biological Laboratories, Gunma, Japan. TRIzol® was from Life Technologies Ltd, U.K. Oligo $(dT)_{12-18}$ primer, Superscript II, RT buffers, and dNTPs were purchased from Gibco BRL. RNAsin was from Promega Corp., Southampton, U.K. PCR buffers, dNTPs, and expand polymerase were purchased from Roche Molecular Biochemicals, Lewes, Sussex, U.K.

Statistical analysis

For the multiple incubation times experiments, the two-ways ANOVA was used for the analysis of differences between treatment groups and incubation periods. In case of significant differences in variances between groups, further analysis was performed using the Student's *t*-test for unpaired data. For the single incubation time experiments, the one-way ANOVA was used, followed by the least-significance *post hoc* tests. The values represent mean ± s.e.m. of different cell isolation experiments. Statistical significance was established at $P<0.05$.

Results

Chemokine secretion in response to LPS

The production of IL-8 (GRO/CINC-1), MIP-2, MCP-1, and RANTES were measured in supernatants of vehicle- and LPS-stimulated rat KC cultures. Growth-arrested KC when stimulated with vehicle produced a basal amount of chemokines (Figure 1a–d). The basal mean values observed on 48 h experiments are $886 \pm 14\,pg\,ml^{-1}$ for IL-8 (GRO/CINC-1), $1175 \pm 117\,pg\,ml^{-1}$ for MIP-2, $197 \pm 60\,pg\,ml^{-1}$ for MCP-1, and $88 \pm 65\,pg\,ml^{-1}$ for RANTES.

In the presence of 1 $\mu g\,ml^{-1}$ of LPS, cultured KC secreted increased amounts of IL-8 (GRO/CINC-1) from 0.88 ± 0.01 to $19.9 \pm 1.6\,ng\,ml^{-1}$ (F^1_{13}: 87.568, $P<0.001$), MIP-2 from 1175 ± 117 to $5791 \pm 136\,pg\,ml^{-1}$ (F^1_{22}: 926.90, $P<0.001$),

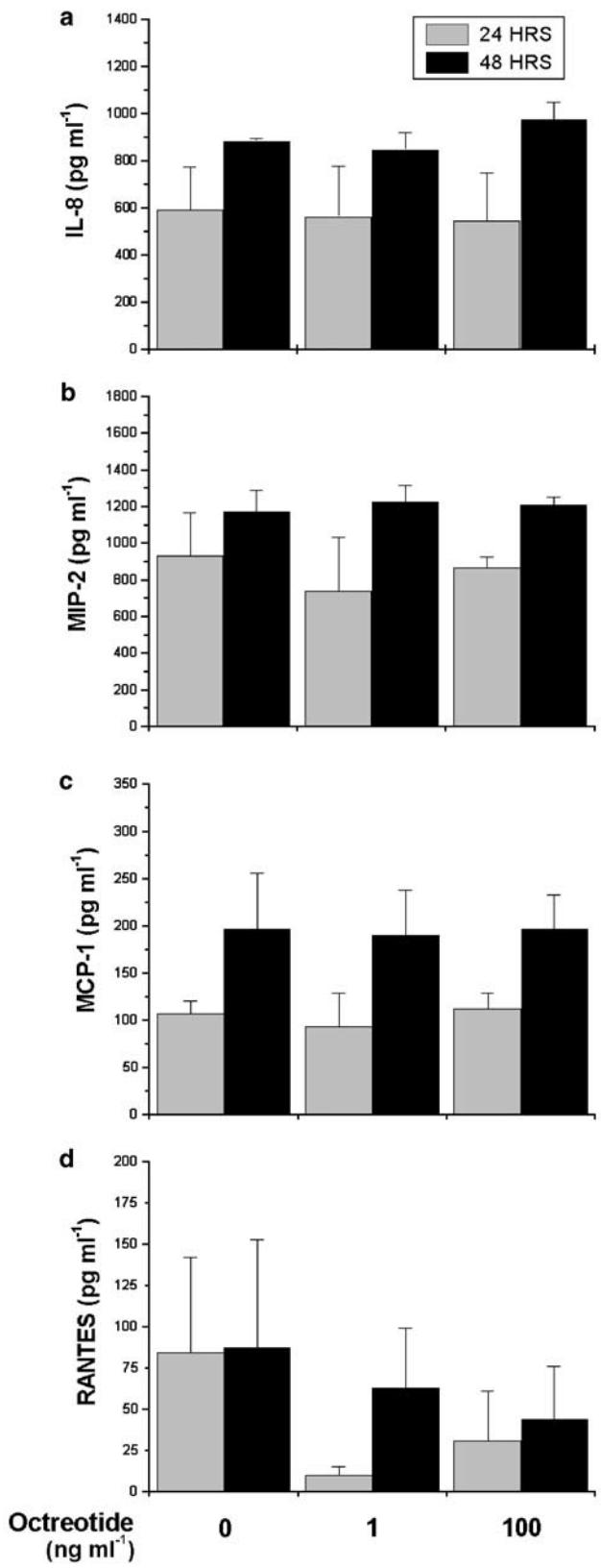

Figure 1 Octreotide has no effect on 'basal' CXC and CC chemokine secretion by KC. KC cultures were treated with vehicle and different concentrations of octreotide, as described in 'Experimental protocol for chemokine evaluation'. Chemokines were measured in culture supernatants by ELISA. (a) IL-8 (GRO/CINC-1), (b) MIP-2, (c) MCP-1, (d) RANTES. Values represent means ± s.e.m. of three experiments.

MCP-1 from 197 ± 60 to $2269 \pm 73\,\mathrm{pg\,ml^{-1}}$ (F^1_{22}: 260.929, $P<0.001$) and RANTES from 88 ± 65 to $506 \pm 134\,\mathrm{pg\,ml^{-1}}$ (F^1_{18}: 14.810, $P<0.01$) (Figures 2–4). Secreted chemokines accumulated in cell culture supernatant in the case of IL-8 (GRO/CINC-1) (F^1_{13}: 5.063, $P<0.05$) and MCP-1 (F^1_{22}: 45.407, $P<0.001$), resulting in greater values measured in 48 h compared to 24 h experiments (Figures 2a and 3), whereas MIP-2 (F^1_{22}: 1.802, $P>0.05$) and RANTES (F^1_{18}: 0.706, $P>0.05$) concentrations remained constant despite increasing incubation times (Figures 2b and 4).

Chemokine secretion in response to octreotide

We explored the possible effect of octreotide on basal secretion of chemokines by rat KC. Growth-arrested KC were stimulated with octreotide for 24 and 48 h, and the secreted chemokines were measured in cell culture supernatants and compared to vehicle-stimulated cultures. We observed no effect of octreotide in basal secretion of IL-8 (GRO/CINC-1) (F^2_{12}: 0.0689, $P>0.05$, Figure 1a), MIP-2 (F^2_{18}: 0.0383, $P>0.05$, Figure 1b) and MCP-1 (F^2_{14}: 0.0912, $P>0.05$, Figure 1c) for the concentrations and incubation times tested. In the case of RANTES, octreotide treatment reduced basal RANTES secretion. The maximum inhibitory effect was from 84 ± 58 to $10 \pm 5\,\mathrm{pg\,ml^{-1}}$ RANTES, observed with a concentration of $1\,\mathrm{ng\,ml^{-1}}$ octreotide at 24 h incubation experiments, but the difference did not reach levels of statistical significance (F^2_{17}: 0.545, $P>0.05$, Figure 1d).

Octreotide modulation of LPS-induced chemokine secretion

KC stimulated with LPS were incubated with various concentrations of octreotide for 24 and 48 h. Octreotide stimulation had no effect on LPS-induced CXC chemokine secretion: IL-8 (GRO/CINC-1) (F^5_{39}: 1.645, $P>0.05$, Figure 2a) and MIP-2 (F^5_{50}: 0.925, $P>0.05$, Figure 2b). In contrast, octreotide treatment inhibited LPS-induced secretion of the CC chemokines tested. The observed inhibition was concentration-dependent, and produced a bell-shaped inhibition curve for both MCP-1 (F^5_{50}: 3.050, $P<0.05$, Figure 3c) and RANTES (F^5_{46}: 3.7492, $P<0.01$, Figure 4c). The optimal effect was observed at a concentration of $0.1\,\mathrm{ng\,ml^{-1}}$ of octreotide at 48 h, which reduced MCP-1 production from 2269 ± 73 to $1455 \pm 263\,\mathrm{pg\,ml^{-1}}$ ($35 \pm 12\%$, $P<0.05$, Figure 3) and RANTES production from 506 ± 134 to $172 \pm 46\,\mathrm{pg\,ml^{-1}}$ ($54 \pm 16\%$, $P<0.01$, Figure 4).

A PtdIns 3-kinase inhibitor prevented octreotide inhibition of LPS-induced chemokine secretion

Growth-arrested cultures of KC were pretreated for 15 min with various concentrations (30–300 nM) of wortmannin before stimulation, with LPS added alone ($1\,\mu\mathrm{g\,ml^{-1}}$) or in combination with the optimally effective concentration of octreotide ($0.1\,\mathrm{ng\,ml^{-1}}$). Cell culture supernatants were collected after 24 h stimulation and MCP-1 was measured. Wortmannin inhibited LPS-induced secretion of MCP-1 from 862 ± 86 to $517 \pm 43\,\mathrm{pg\,ml^{-1}}$ in a concentration-dependent manner ($P<0.05$, Figure 5). Interestingly, the addition of

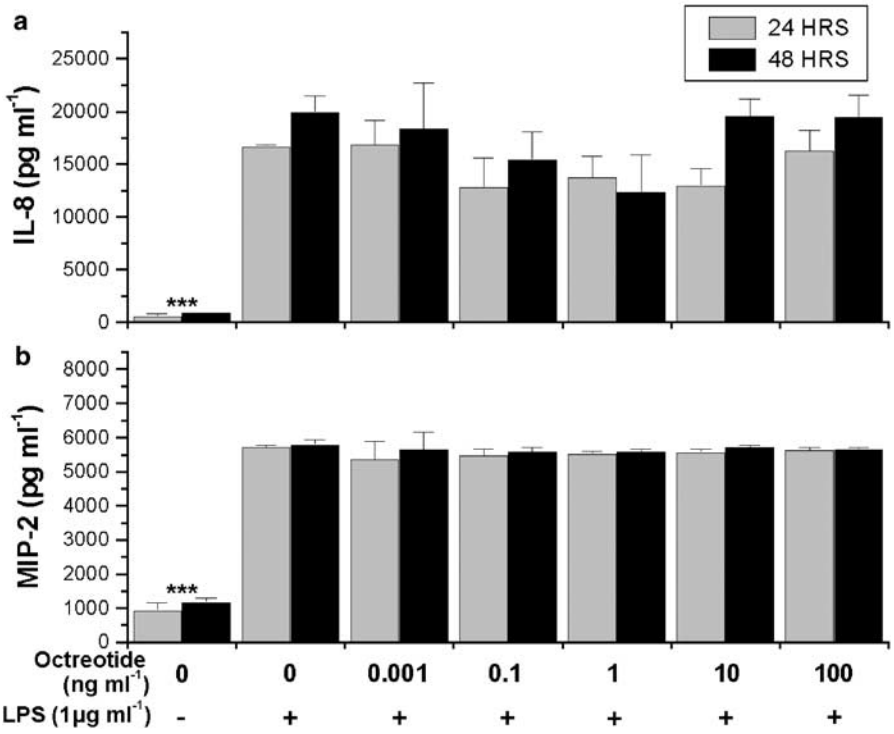

Figure 2 Octreotide has no effect on LPS-induced CXC chemokine secretion by KC. KC cultures were treated with vehicle, LPS, and LPS/octreotide, as described in 'Experimental protocol for chemokine evaluation'. Chemokines were measured in culture supernatants by ELISA. (a) IL-8 (GRO/CINC-1), (b) MIP-2. Values represent means ± s.e.m. of 4–7 experiments. * represents significance from the LPS-treated group, $*P<0.05$, $**P<0.01$, $***P<0.001$.

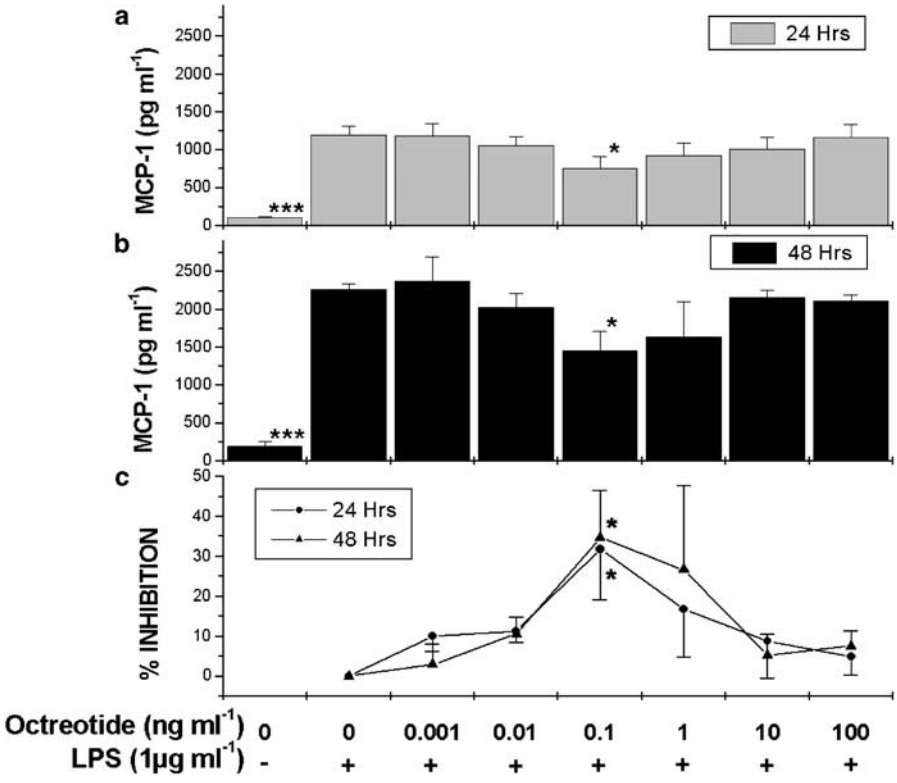

Figure 3 Octreotide inhibits LPS-induced MCP-1 secretion by KC. KC cultures were treated with vehicle, LPS, and LPS/octreotide, as described in 'Experimental protocol for chemokine evaluation'. MCP-1 was measured in culture supernatants by ELISA. (a) 24 h incubation experiments, (b) 48 h incubation experiments, (c) percentage of octreotide-induced inhibition of MCP-1 production by LPS-stimulated KC cultures. Values represent means ± s.e.m. of 4–7 experiments. * represents significance from the LPS-treated group, $*P<0.05$, $**P<0.01$, $***P<0.001$.

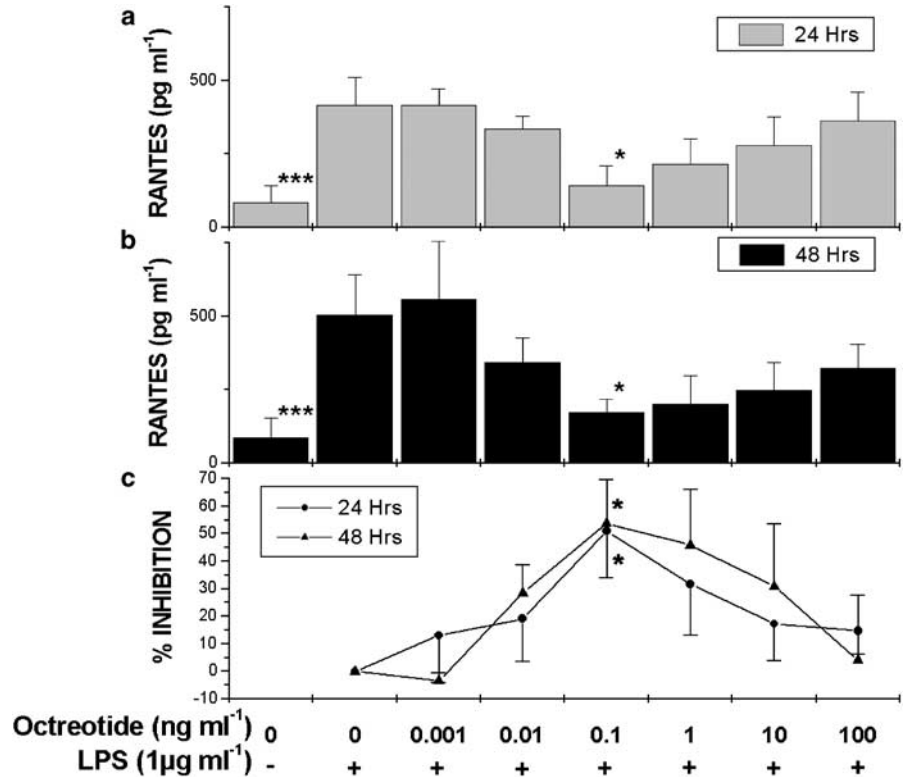

Figure 4 Octreotide inhibits LPS-induced RANTES secretion by KC. KC cultures were treated with vehicle, LPS, and LPS/octreotide, as described in 'Experimental protocol for chemokine evaluation'. RANTES was measured in culture supernatants by ELISA. (a) 24 h incubation experiments, (b) 48 h incubation experiments, (c) percentage of octreotide-induced inhibition of RANTES production by LPS-stimulated KC cultures. Values represent means ± s.e.m. of 4–7 experiments. * represents significance from the LPS-treated group, $*P<0.05$, $**P<0.01$, $***P<0.001$.

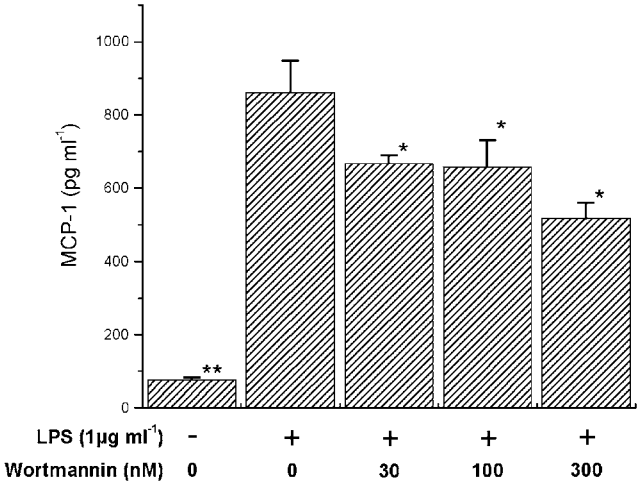

Figure 5 PI3-kinase inhibition suppresses LPS-induced MCP-1 secretion by KC. KC cultures were pretreated with wortmannin and stimulated with vehicle or LPS, as described in 'Experimental protocol for chemokine evaluation'. Chemokines were measured in culture supernatants by ELISA. Values represent means ± s.e.m. of three experiments. * represents significance from the LPS-treated group, $*P<0.05$.

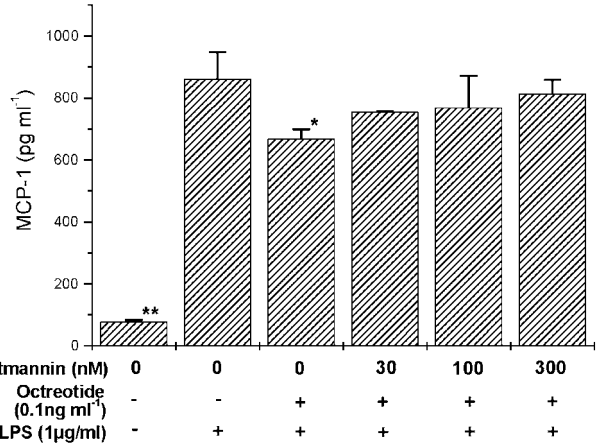

Figure 6 PI3-kinase inhibition prevents octreotide suppression of LPS-induced MCP-1 secretion by KC. KC cultures were pretreated with wortmannin and stimulated with vehicle, LPS or LPS/octreotide, as described in 'Experimental protocol for chemokine evaluation'. Chemokines were measured in culture supernatants by ELISA. Values represent means ± s.e.m. of three experiments. * represents significance from the LPS-treated group, $*P<0.05$.

wortmannin 15 min before octreotide/LPS treatment was found to prevent the inhibitory effects of octreotide on MCP-1 secretion in a concentration-dependent manner (Figure 6).

CC chemokine mRNA expression

Growth-arrested cultures of KC were stimulated or not with 1 $\mu g\,ml^{-1}$ LPS, 0.1 $ng\,ml^{-1}$ octreotide (the concentration of octreotide with the strongest inhibitory effect on chemokine

secretion), or the combination of 1 μg ml^{-1} LPS with 0.1 ng ml^{-1} octreotide. Following incubation for 24 h, total RNA was extracted from the monolayer cultures and the mRNA expression of MCP-1 and RANTES was assessed by multiplex semiquantitative RT–PCR using ribosomal 18S RNA as the internal control. Incubation with LPS increased mRNA expression of both MCP-1 and RANTES, as shown by the increased MCP-1 18 s^{-1} and RANTES 18 s^{-1} ratios observed following LPS stimulation (Figures 7 and 8, respectively). Octreotide alone exhibited an inhibitory effect on mRNA expression of MCP-1 and RANTES when compared to control expression of the two chemokines (Figures 7 and 8). In concordance with our results in the protein level, we observed a partial inhibitory effect of octreotide on LPS-induced mRNA expression of both MCP-1 and RANTES when LPS and octreotide were added together in the medium (Figures 7 and 8).

Furthermore, we explored the possible effect of the phosphatidylinositol 3 (PI3)-kinase inhibitor LY294002 on the mRNA expression of MCP-1 and RANTES by the isolated KC. Growth-arrested cultures of KC were pretreated for 15 min with various concentrations (1, 10, 30 μM) of LY294002 before stimulation with 1 μg ml^{-1} LPS. Following incubation for 24 h, total RNA was extracted from the monolayer cultures, and the mRNA expression of MCP-1 and RANTES was assessed by multiplex semiquantitative RT–PCR, under the same conditions as previously described. In concordance with our results in the protein level using LY294002 instead of wortmannin, we observed a concentration-dependent inhibition of the LPS-induced MCP-1 and RANTES mRNA expression, as shown in Figures 9 and 10, respectively.

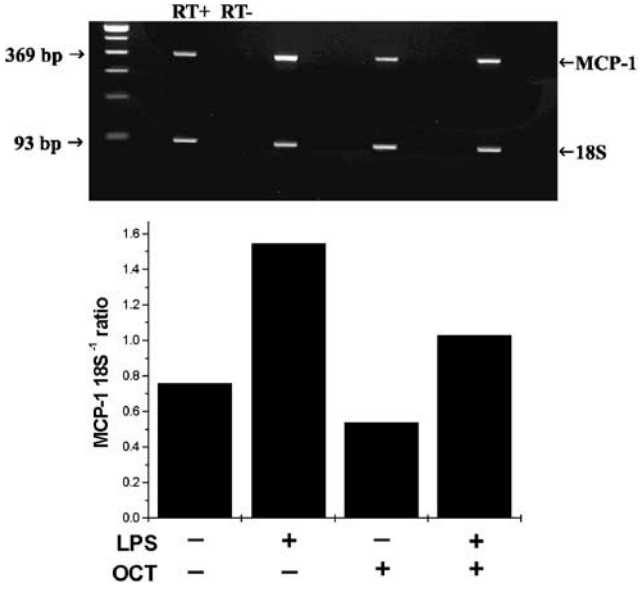

Figure 7 Octreotide inhibits MCP-1 mRNA expression by 'resting' and LPS-stimulated KC. KC cultures were stimulated with vehicle, 1 μg ml^{-1} LPS (LPS), 0.1 ng ml^{-1} octreotide (OCT) or the combination of 1 μg ml^{-1} LPS with 0.1 ng ml^{-1} OCT for 24 h. Total mRNA was extracted from monolayer cultures following stimulation for 24 h, and MCP-1 mRNA expression was assessed with a semiquantitative RT–PCR, using specific primers for MCP-1 and 18s rRNA, as described under 'RT–PCR'. The upper panel is the electrophoresis of the PCR products on agarose gels stained with ethidium bromide, and the lower panel is the densitometric analysis showing the relative expression of MCP-1 mRNA expressed as the MCP-1 to 18s ratios. Data are from a single experiment representative of at least three others.

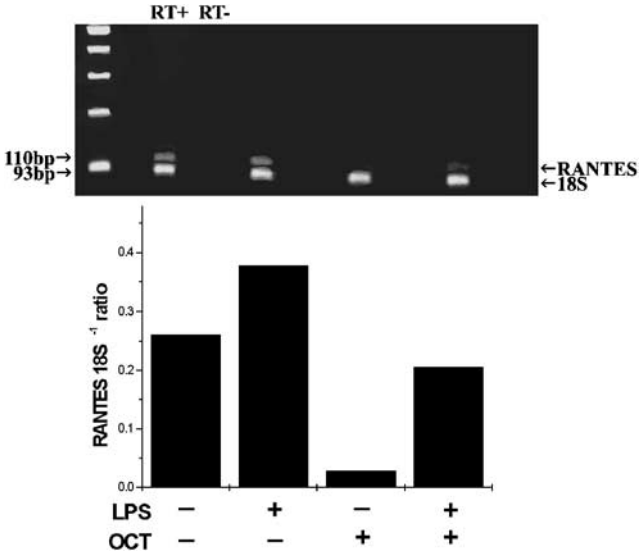

Figure 8 Octreotide inhibits RANTES mRNA expression by 'resting' and LPS-stimulated KC. KC cultures were stimulated with vehicle, 1 μg ml^{-1} LPS (LPS), 0.1 ng ml^{-1} octreotide (OCT) or the combination of 1 μg ml^{-1} LPS with 0.1 ng ml^{-1} octreotide for 24 h. Total mRNA was extracted from monolayer cultures following stimulation for 24 h, and RANTES mRNA expression was assessed with a semiquantitative RT–PCR, using specific primers for RANTES and 18s rRNA, as described under 'RT–PCR'. The upper panel is the electrophoresis of the PCR products on agarose gels stained with ethidium bromide, and the lower panel is the densitometric analysis showing the relative expression of MCP-1 mRNA expressed as the MCP-1 to 18s ratios. Data are from a single experiment representative of at least three others.

Discussion

In the present study, we have demonstrated chemokine production by isolated rat KC. KC, stimulated with vehicle, secreted a basal amount of the CXC chemokines, IL-8 (GRO/CINC-1) and MIP-2, and the CC chemokines, MCP-1 and RANTES, while activation with LPS induced a significant increase of the chemokine production. CC chemokine mRNA expression studies also demonstrated a basal expression of MCP-1 and RANTES by 'resting' KC, which was upregulated following stimulation with LPS. Treatment with octreotide, applied at 'physiological' nanomolar concentrations (Chowers *et al*., 2000), was found to inhibit basal and LPS-induced mRNA expression, as well as the LPS-induced CC chemokine secretion, while this somatostatin analogue was without effect on CXC chemokine production. Previous studies have shown that the *in vitro* application of the natural neuropeptide at concentrations in the nanomolar range may deactivate chemotactic responses of human monocytes and leukocytes (Pawlikowski *et al*., 1987; Wiedermann *et al*., 1993). However, it is the first time, to our knowledge, that differential inhibition of the mRNA expression and secretion of chemotactic molecules by somatostatin analogues have been shown on resident liver macrophages.

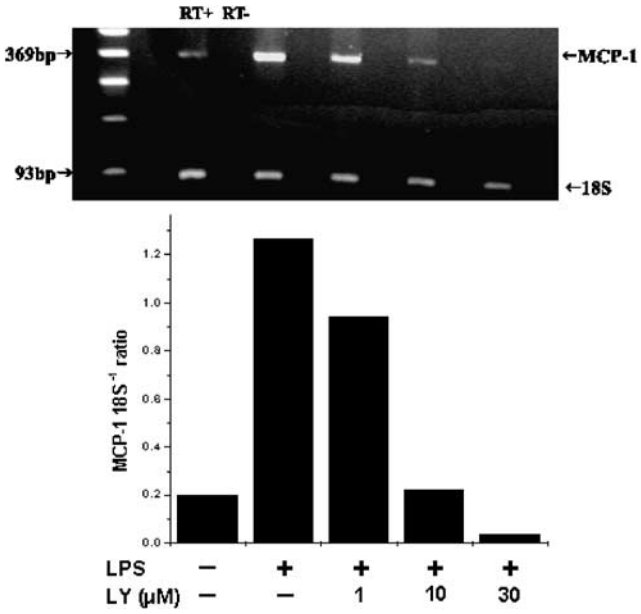

Figure 9 PI3-kinase inhibition suppresses MCP-1 mRNA expression by KC. KC cultures were pretreated for 15 min with different concentrations of LY294002 (LY), and stimulated with 1 μg ml^{-1} LPS (LPS) for 24 h. Total mRNA was extracted from monolayer cultures following stimulation for 24 h and MCP-1 mRNA expression was assessed with a semiquantitative RT–PCR, using specific primers for MCP-1 and 18s rRNA, as described under 'RT–PCR'. The upper panel is the electrophoresis of the PCR products on agarose gels stained with ethidium bromide, and the lower panel is the densitometric analysis showing the relative expression of MCP-1 mRNA expressed as the MCP-1 to 18s ratios. Data are from a single experiment representative of at least three others.

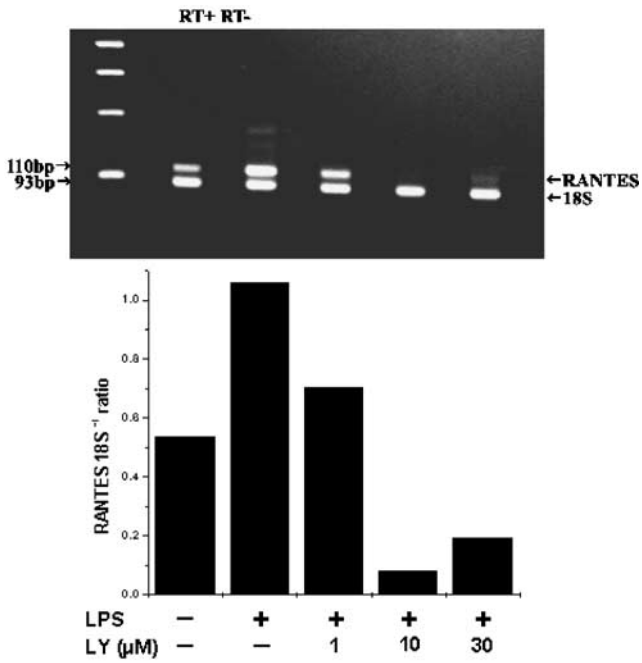

Figure 10 PI3-kinase inhibition suppresses RANTES mRNA expression by KC. KC cultures were pretreated for 15 min with different concentrations of LY294002 (LY), and stimulated with 1 μg ml^{-1} LPS (LPS) for 24 h. Total mRNA was extracted from monolayer cultures following stimulation for 24 h, and RANTES mRNA expression was assessed with a semiquantitative RT–PCR, using specific primers for MCP-1 and 18s rRNA, as described under 'RT–PCR'. The upper panel is the electrophoresis of the PCR products on agarose gels stained with ethidium bromide, and the lower panel is the densitometric analysis showing the relative expression of RANTES mRNA expressed as the MCP-1 to 18s ratios. Data are from a single experiment representative of at least three others.

Inflammatory cell infiltration is a common feature of liver diseases (Ajuebor & Swain, 2002), but the mechanisms that regulate cell recruitment to the liver are poorly understood. Chemokines and their receptors play a crucial role in immune and inflammatory responses, by directing a certain population of leukocytes to the site of inflammation. Chemokine expression in the liver is induced in almost all types of pathological conditions, and a correlation exists between chemokines released and the predominant leukocyte population infiltrating the liver (Marra, 2002). Overexpression of the CXC chemokines IL-8 and CINC in the hepatic tissue has been associated with the neutrophilic infiltration and the degree of tissue inflammation observed in acute alcoholic hepatitis, viral hepatitis, cirrhosis, and experimental models of liver allograft rejection (Sheron *et al*., 1993; Yamaguchi *et al*., 1997; Shimoda *et al*., 1998; Polyak *et al*., 2001). MIP-2 and KC have been implicated in the development of ischaemia–reperfusion liver injury, and neutralization of these molecules reduced neutrophilic infiltration and hepatocellular damage (Lentsch *et al*., 1998).

Among the CC chemokines, MCP-1 has been found to induce recruitment of activated monocytes and macrophages within the liver in experimental models of acute and chronic inflammation (Kuziel *et al*., 1997; Dambach *et al*., 2002), and to contribute to liver neutrophilic infiltration *via* the induction of ICAM-1 (Yamaguchi *et al*., 1998). Another CC chemokine, RANTES, has been found to be expressed at high levels in the liver of patients with hepatitis C. RANTES has been implicated in the recruitment of activated T cells to the areas of piecemeal necrosis, and its expression has been correlated with hepatic inflammation and the response to interferon therapy (Kusano *et al*., 2000; Promrat *et al*., 2003). Thus, agents able to modify hepatic chemokine production may prove to be useful therapeutic options in the future.

Increased production of the MIP-1, MCP-1, and RANTES has been observed in isolated KC after *in vivo* LPS challenge. KC depletion has been shown to reduce the production of MIP-1, MCP-1, RANTES, MIP-2, and KC, resulting in attenuation of liver injury following LPS or ischaemia–reperfusion treatment (Bukara & Bautista, 2000; Mosher *et al*., 2001). We have shown that isolated KC produce detectable amounts of the CXC chemokines CINC/IL-8, MIP-2, and the CC chemokines MCP-1 and RANTES, and the production was significantly increased in the presence of LPS. We have also shown that the observed increase of CC chemokine secretion is accompanied by induction of their mRNA expression, suggesting that upregulation of chemokine production by LPS could be attributed at least in part to transcriptional upregulation of the MCP-1 and RANTES genes.

Treatment with octreotide had no effect on 'basal' secretion of IL-8, MIP-2, and MCP-1 on the concentrations tested. A nonsignificant negative effect was observed on basal RANTES production following stimulation with 1 and 100 ng ml^{-1} of octreotide. However, octreotide was found to

inhibit LPS-induced CC chemokine secretion. The inhibitory effect of octreotide on chemokine production shows a typical 'bell-shaped' dose–response relationship in which the induced effects are lost at higher ligand concentrations. The maximum effect of octreotide was observed at a concentration of $0.1\,ng\,ml^{-1}$. When this concentration was used to further evaluate the effects of octreotide CC chemokine mRNA expression, a suppressive effect was observed on 'basal' and LPS-induced mRNA expression for both MCP-1 and RANTES. These results indicate that octreotide might exert its inhibitory effects on CC chemokine secretion by down-regulating the transcription of MCP-1 and RANTES genes.

The kinetics of the LPS-induced chemokine suppression are characteristic of an octreotide-mediated effect, as described in literature for octreotide uptake by somatostatin-receptor (sst)-positive cell lines and octreotide-inhibitory effects on cell proliferation and secretion (Setyono-Han *et al*., 1987; Kusterer *et al*., 1994; de Jong *et al*., 1999). The bell-shaped form of a dose–response curve is not unusual for pharmacological processes. This may indicate that more than one mechanism of inhibition may exist and this effect may be even cell-specific. The phenomenon resembles G-coupled receptor-mediated responses, which are characterized by a rapid ligand-induced uncoupling of G-proteins from the receptor with increasing ligand concentration, leading to prolonged receptor desensitization (Schindler *et al*., 1998; Oomen *et al*., 2002).

Another possible explanation may be the different affinity of octreotide for different somatostatin receptors. Octreotide binds with high affinity to somatostatin receptors sst2 and with lower affinity to sst3 and sst5, while it does not bind to sst1 and sst4 (Kubota *et al*., 1994). So, one possible explanation may be that octreotide, when present in high concentration, binds to functionally different receptors with less affinity for the ligand such as the sst3 and sst4, which modify the end result. Moreover, previous investigators have found that somatostatin is efficiently coupled in a negative manner to adenylate cyclase through sst5 receptors, but, at higher agonist concentrations, the receptor can also mediate activation of adenylate cyclase by a mechanism apparently involving Galphas protein activation (Carruthers *et al*., 1999). Thus, a biphasic effect can also be expected from coupling of the sst receptors to different second messengers, depending on the agonist concentration. Lastly, the kinetics of the inhibition by octreotide appears similar to biological effects elicited following activation of PI3-kinase pathways. Previous investigators have found a 'bell-shaped' concentration–response relationship in D-3 phosphatidylinositol lipid production, PI3-kinase activity, and finally monocyte chemotaxis following agonist-induced PI3-kinase activation (Turner *et al*., 1998). Different agonist concentrations may activate different components of the PI3-kinase pathway. This might be a protective mechanism against excessive exposure of these cells to high agonist levels.

In the present study, octreotide was found to have a differential effect on chemokine secretion. Although octreotide treatment inhibited mRNA expression and secretion of CC chemokines, it had no effect on CXC chemokine production. Differential regulation of CC and CXC chemokines has been previously shown following stimulation of intestinal epithelial cell lines with INFγ and TNFα, in which case upregulation of CC chemokines was found, while there was no effect on CXC chemokine expression (Kim *et al*., 2002). Moreover, differential inhibition patterns of CC and CXC chemokine production have been reported following treatment with protein kinase C inhibitors of different specificity (Jordan *et al*., 1996). The differential inhibitory effect of octreotide observed in the present study represents an interesting observation relevant to previously published data on chemokine regulation that requires further study. However, the exact mechanism of differential regulation and polarized secretion of chemokines is a broad and complex subject that cannot be addressed extensively in the present work.

The intracellular signalling pathways that take place during KC activation by LPS are currently being investigated. LPS-induced signal transmission requires binding to specific cellular receptors, including toll-like receptors and results on activation and stimulation of a wide spectrum of host-defensive systems (Fenton & Golenbock, 1998). This requires the involvement of multiple signalling molecules in transduction pathways; for example, protein-tyrosine kinase (PTK), LPS receptor-associated serine/threonine kinase, Ras, Raf-1, IκB kinase, MEK, mitogen-activated protein kinases (MAPKs) (Weinstein *et al*., 1991; Ulevitch & Tobias, 1995), etc.

Recent studies have shown that both Toll-like receptors (TLR)-2 and TLR4 signalling activate the PI3-kinase pathways which have been shown to act both positively and negatively on NF-κB-dependent gene expression in monocytes and macrophages (Guha & Mackman, 2002). In our *in vitro* experimental model, treatment of isolated KC with $0.1\,ng\,ml^{-1}$ of octreotide reduced LPS-induced production of MCP-1. Pretreatment with the PI3-kinase inhibitor wortmannin also resulted to a concentration-dependent reduction of MCP-1 production. Furthermore, we show that LY294002, another PI3-kinase inhibitor, downregulated mRNA expression of MCP-1 and RANTES in a concentration-dependent manner. This might suggest that PI3-kinase pathways act positively on the LPS-induced KC responses, possibly through upregulation of transcription of MCP-1 and RANTES genes.

Interestingly, when cells were treated with both substances at optimally effective concentrations, MCP-1 secretion returned to levels prior to inhibition. The PI3-kinases have already been implicated in the intracellular signalling of somatostatin receptor agonists (Sakanaka *et al*., 1994; Medina *et al*., 2000), but not in the inhibitory properties of somatostatin on chemokine secretion so far. We have previously shown that IL-13 or IL-4 significantly reduce RANTES and MCP-1 secretion by HT-29 cells *via* activation of a wortmannin-sensitive PI3-kinase pathway (Kolios *et al*., 1999). Although such an assumption seems tempting, we feel that further studies are needed to address this question.

Direct immunoregulatory effects of somatostatin have previously been reported mainly on cytokine and nitric oxide production. *In vitro* studies have shown that somatostatin inhibits NF-κB activation and TNFα and IL-8 production in human pancreatic periacinar myofibroblasts and bacteria-induced IL-1 and IL-8 production by intestinal epithelial cells (Chowers *et al*., 2000; Andoh *et al*., 2002). Somatostatin and octreotide have also been found to exert a direct immunomodulatory effect by suppressing spontaneous TNFα and NO production by isolated rat KC and TNFα, IL-1 beta, IL-6, IL-12, and IL-8 secretion by LPS-activated human monocytes (Peluso *et al*., 1996; Chao *et al*., 1999; Komorowski *et al*., 2001).

Previous *in vivo* studies have shown that somatostatin significantly attenuates galactosamine-induced liver injury in rats (Limberg & Kommerell, 1983) and reduces tissue injury in experimental chronic colitis in rats (Lamrani *et al*., 1999). Interestingly, octreotide treatment has been reported to decrease connective tissue formation, improve vascular changes associated with hepatic schistosomiasis (Mansy *et al*., 1998) and exert antifibrotic effects in the CCl4 model of liver fibrosis (Fort *et al*., 1998). There are recent studies, implicating MCP-1 in the pathogenesis of liver fibrosis *via* stimulation of hepatic stellate cell (HSC) migration and activation of HSC intracellular signalling (Marra *et al*., 1999). Therefore, suppression of the MCP-1 and RANTES production might conceivably result in reduction of monocyte-macrophage recruitment and suppression of HSC activation and migration in inflamed liver parenchyma. Octreotide significantly suppressed MCP-1 and RANTES production by LPS-activated KC in our *in vitro* experimental model. Our data justify the need for further *in vivo* studies in order to explore the physiological significance of our observations, and the possible immunoregulatory and therapeutic effects of octreotide in liver disorders.

References

AJUEBOR, M.N. & SWAIN, M.G. (2002). Role of chemokines and chemokine receptors in the gastrointestinal tract. *Immunology,* **105,** 137–143.

ANDOH, A., HATA, K., SHIMADA, M., FUJINO, S., TASAKI, K., BAMBA, S., ARAKI, Y., FUJIYAMA, Y. & BAMBA, T. (2002). Inhibitory effects of somatostatin on tumor necrosis factor-alpha-induced interleukin-6 secretion in human pancreatic periacinar myofibroblasts. *Int. J. Mol. Med.,* **10,** 89–93.

BAYON, L.G., IZQUIERDO, M.A., SIROVICH, I., VAN ROOIJEN, N., BEELEN, R.H. & MEIJER, S. (1996). Role of Kupffer cells in arresting circulating tumor cells and controlling metastatic growth in the liver. *Hepatology,* **23,** 1224–1231.

BONE-LARSON, C.L., SIMPSON, K.J., COLLETTI, L.M., LUKACS, N.W., CHEN, S.C., LIRA, S., KUNKEL, S.L. & HOGABOAM, C.M. (2000). The role of chemokines in the immunopathology of the liver. *Immunol. Rev.,* **177,** 8–20.

BUKARA, M. & BAUTISTA, A.P. (2000). Acute alcohol intoxication and gadolinium chloride attenuate endotoxin-induced release of CC chemokines in the rat. *Alcohol,* **20,** 193–203.

CARRUTHERS, A.M., WARNER, A.J., MICHEL, A.D., FENIUK, W. & HUMPHREY, P.P. (1999). Activation of adenylate cyclase by human recombinant sst5 receptors expressed in CHO-K1 cells and involvement of Galphas proteins. *Br. J. Pharmacol.,* **126,** 1221–1229.

CHAO, T.C., CHAO, H.H., LIN, J.D. & CHEN, M.F. (1999). Somatostatin and octreotide modulate the function of Kupffer cells in liver cirrhosis. *Regul. Pept.,* **79,** 117–124.

CHOWERS, Y., CAHALON, L., LAHAV, M., SCHOR, H., TAL, R., BAR-MEIR, S. & LEVITE, M. (2000). Somatostatin through its specific receptor inhibits spontaneous and TNF-alpha- and bacteria-induced IL-8 and IL-1 beta secretion from intestinal epithelial cells. *J. Immunol.,* **165,** 2955–2961.

DAMBACH, D.M., WATSON, L.M., GRAY, K.R., DURHAM, S.K. & LASKIN, D.L. (2002). Role of CCR2 in macrophage migration into the liver during acetaminophen-induced hepatotoxicity in the mouse. *Hepatology,* **35,** 1093–1103.

DE JONG, M., BREEMAN, W.A., BERNARD, B.F., VAN GAMEREN, A., DE BRUIN, E., BAKKER, W.H., VAN DER PLUIJM, M.E., VISSER, T.J., MACKE, H.R. & KRENNING, E.P. (1999). Tumour uptake of the radiolabelled somatostatin analogue [DOTA0, TYR3]octreotide is dependent on the peptide amount. *Eur. J. Nucl. Med.,* **26,** 693–698.

EL SALHY, M., STENLING, R. & GRIMELIUS, L. (1993). Peptidergic innervation and endocrine cells in the human liver. *Scand. J. Gastroenterol.,* **28,** 809–815.

FAUSTO, N., LAIRD, A.D. & WEBBER, E.M. (1995). Liver regeneration. 2. Role of growth factors and cytokines in hepatic regeneration. *FASEB J.,* **9,** 1527–1536.

FENTON, M.J. & GOLENBOCK, D.T. (1998). LPS-binding proteins and receptors. *J. Leukoc. Biol.,* **64,** 25–32.

FORT, J., OBERTI, F., PILETTE, C., VEAL, N., GALLOIS, Y., DOUAY, O., ROUSSELET, M.C., ROSENBAUM, J. & CALES, P. (1998). Antifibrotic and hemodynamic effects of the early and chronic administration of octreotide in two models of liver fibrosis in rats. *Hepatology,* **28,** 1525–1531.

GUHA, M. & MACKMAN, N. (2002). The phosphatidylinositol 3-kinase-Akt pathway limits lipopolysaccharide activation of signaling pathways and expression of inflammatory mediators in human monocytic cells. *J. Biol. Chem.,* **277,** 32124–32132.

JORDAN, N.J., KOLIOS, G., ABBOT, S.E., SINAI, M.A., THOMPSON, D.A., PETRAKI, K. & WESTWICK, J. (1999). Expression of functional CXCR4 chemokine receptors on human colonic epithelial cells. *J. Clin. Invest.,* **104,** 1061–1069.

JORDAN, N.J., WATSON, M.L., YOSHIMURA, T. & WESTWICK, J. (1996). Differential effects of protein kinase C inhibitors on chemokine production in human synovial fibroblasts. *Br. J. Pharmacol.,* **117,** 1245–1253.

KARALIS, K., MASTORAKOS, G., CHROUSOS, G.P. & TOLIS, G. (1994). Somatostatin analogues suppress the inflammatory reaction *in vivo*. *J. Clin. Invest.,* **93,** 2000–2006.

KIM, J.M., KIM, J.S., JUN, H.C., OH, Y.K., SONG, I.S. & KIM, C.Y. (2002). Differential expression and polarized secretion of CXC and CC chemokines by human intestinal epithelial cancer cell lines in response to *Clostridium difficile* toxin A. *Microbiol. Immunol.,* **46,** 333–342.

KOLIOS, G., WRIGHT, K.L., JORDAN, N.J., LEITHEAD, J.B., ROBERTSON, D.A. & WESTWICK, J. (1999). C–X–C and C–C chemokine expression and secretion by the human colonic epithelial cell line, HT-29: differential effect of T lymphocyte-derived cytokines. *Eur. J. Immunol.,* **29,** 530–536.

KOMOROWSKI, J., JANKIEWICZ-WIKA, J., STEPIEN, T., KUZDAK, K. & STEPIEN, H. (2001). Octreotide inhibits the secretion of interleukin-12 from mononuclear cells in human peripheral blood (PBMCs) *in vitro*. *Horm. Metab. Res.,* **33,** 689–690.

KOUROUMALIS, E., SKORDILIS, P., THERMOS, K., VASILAKI, A., MOSCHANDREA, J. & MANOUSOS, O.N. (1998). Treatment of hepatocellular carcinoma with octreotide: a randomised controlled study. *Gut,* **42,** 442–447.

KUBOTA, A., YAMADA, Y., KAGIMOTO, S., SHIMATSU, A., IMAMURA, M., TSUDA, K., IMURA, H., SEINO, S. & SEINO, Y. (1994). Identification of somatostatin receptor subtypes and an implication for the efficacy of somatostatin analogue SMS 201-995 in treatment of human endocrine tumors. *J. Clin. Invest.,* **93,** 1321–1325.

KUSANO, F., TANAKA, Y., MARUMO, F. & SATO, C. (2000). Expression of C–C chemokines is associated with portal and periportal inflammation in the liver of patients with chronic hepatitis C. *Lab. Invest.,* **80,** 415–422.

KUSTERER, K., BUCHHEIT, K.H., SCHADE, A., BRUNS, C., NEUBERGER, C., ENGEL, G. & USADEL, K.H. (1994). The somatostatin analogue octreotide protects against ethanol-induced microcirculatory stasis and elevated vascular permeability in rat gastric mucosa. *Eur. J. Pharmacol.,* **259,** 265–271.

KUZIEL, W.A., MORGAN, S.J., DAWSON, T.C., GRIFFIN, S., SMITHIES, O., LEY, K. & MAEDA, N. (1997). Severe reduction in leukocyte adhesion and monocyte extravasation in mice deficient in CC chemokine receptor 2. *Proc. Natl. Acad. Sci. U.S.A.,* **94,** 12053–12058.

LAMBERTS, S.W., VAN DER LELY, A.J., DE HERDER, W.W. & HOFLAND, L.J. (1996). Octreotide. *N. Engl. J. Med.,* **334,** 246–254.

LAMRANI, A., TULLIEZ, M., CHAUVELOT-MOACHON, L., CHAUSSADE, S., MAUPRIVEZ, C., HAGNERE, A.M. & VIDON, N. (1999). Effects of octreotide treatment on early TNF-alpha production and localization in experimental chronic colitis. *Aliment. Pharmacol. Ther.,* **13,** 583–594.

LENTSCH, A.B., YOSHIDOME, H., CHEADLE, W.G., MILLER, F.N. & EDWARDS, M.J. (1998). Chemokine involvement in hepatic ischemia/reperfusion injury in mice: roles for macrophage inflammatory protein-2 and KC. *Hepatology,* **27,** 1172–1177.

LIMBERG, B. & KOMMERELL, B. (1983). Beneficial effect of somatostatin on galactosamine induced liver injury. *Regul. Pept.,* **7,** 187–193.

LIU, P., MCGUIRE, G.M., FISHER, M.A., FARHOOD, A., SMITH, C.W. & JAESCHKE, H. (1995). Activation of Kupffer cells and neutrophils for reactive oxygen formation is responsible for endotoxin-enhanced liver injury after hepatic ischemia. *Shock,* **3,** 56–62.

MANSY, S.S., YEHIA, H.A., HASSAN, M.M., HASSAN, E.A., YOUSSEF, M.M., HADI, A.A. & MACKENZIE, C.D. (1998). Octreotide decreases connective tissue formation and improves vascular changes associated with hepatic schistosomiasis. *J. Egypt. Soc. Parasitol.,* **28,** 23–44.

MARRA, F. (2002). Chemokines in liver inflammation and fibrosis. *Front. Biosci.,* **7,** d1899–d1914.

MARRA, F., ROMANELLI, R.G., GIANNINI, C., FAILLI, P., PASTACALDI, S., ARRIGHI, M.C., PINZANI, M., LAFFI, G., MONTALTO, P. & GENTILINI, P. (1999). Monocyte chemotactic protein-1 as a chemoattractant for human hepatic stellate cells. *Hepatology,* **29,** 140–148.

MCINTOSH, C.H. (1985). Gastrointestinal somatostatin: distribution, secretion and physiological significance. *Life Sci.,* **37,** 2043–2058.

MEDINA, D.L., TORO, M.J. & SANTISTEBAN, P. (2000). Somatostatin interferes with thyrotropin-induced G1-S transition mediated by cAMP-dependent protein kinase and phosphatidylinositol 3-kinase. Involvement of RhoA and cyclin E x cyclin-dependent kinase 2 complexes. *J. Biol. Chem.,* **275,** 15549–15556.

MOSHER, B., DEAN, R., HARKEMA, J., REMICK, D., PALMA, J. & CROCKETT, E. (2001). Inhibition of Kupffer cells reduced CXC chemokine production and liver injury. *J. Surg. Res.,* **99,** 201–210.

NIEDERMUHLBICHLER, M. & WIEDERMANN, C.J. (1992). Suppression of superoxide release from human monocytes by somatostatin-related peptides. *Regul. Pept.,* **41,** 39–47.

NOLAN, J.P. (1981). Endotoxin, reticuloendothelial function, and liver injury. *Hepatology,* **1,** 458–465.

OOMEN, S.P., VAN HENNIK, P.B., ANTONISSEN, C., LICHTENAUER-KALIGIS, E.G., HOFLAND, L.J., LAMBERTS, S.W., LOWENBERG, B. & TOUW, I.P. (2002). Somatostatin is a selective chemoattractant for primitive (CD34(+)) hematopoietic progenitor cells. *Exp. Hematol.,* **30,** 116–125.

PAWLIKOWSKI, M., STEPIEN, H., KUNERT-RADEK, J., ZELAZOWSKI, P. & SCHALLY, A.V. (1987). Immunomodulatory action of somatostatin. *Ann. N.Y. Acad. Sci.,* **496,** 233–239.

PELUSO, G., PETILLO, O., MELONE, M.A., MAZZARELLA, G., RANIERI, M. & TAJANA, G.F. (1996). Modulation of cytokine production in activated human monocytes by somatostatin. *Neuropeptides,* **30,** 443–451.

POLYAK, S.J., KHABAR, K.S., PASCHAL, D.M., EZELLE, H.J., DUVERLIE, G., BARBER, G.N., LEVY, D.E., MUKAIDA, N. & GRETCH, D.R. (2001). Hepatitis C virus nonstructural 5A protein induces interleukin-8, leading to partial inhibition of the interferon-induced antiviral response. *J. Virol.,* **75,** 6095–6106.

PROMRAT, K., MCDERMOTT, D.H., GONZALEZ, C.M., KLEINER, D.E., KOZIOL, D.E., LESSIE, M., MERRELL, M., SOZA, A., HELLER, T., GHANY, M., PARK, Y., ALTER, H.J., HOOFNAGLE, J.H., MURPHY, P.M. & LIANG, T.J. (2003). Associations of chemokine system polymorphisms with clinical outcomes and treatment responses of chronic hepatitis C. *Gastroenterology,* **124,** 352–360.

REICHLIN, S. (1983). Somatostatin. *N. Engl. J. Med.,* **309,** 1495–1501.

REUBI, J.C., WASER, B., MARKUSSE, H.M., KRENNING, E.P., VANHAGEN, M. & LAISSUE, J.A. (1994). Vascular somatostatin receptors in synovium from patients with rheumatoid arthritis. *Eur. J. Pharmacol,* **271,** 371–378.

ROLLINS, B.J. (1997). Chemokines. *Blood,* **90,** 909–928.

SAKANAKA, C., FERBY, I., WAGA, I., BITO, H. & SHIMIZU, T. (1994). On the mechanism of cytosolic phospholipase A2 activation in CHO cells carrying somatostatin receptor: wortmannin-sensitive pathway to activate mitogen-activated protein kinase. *Biochem. Biophys. Res. Commun.,* **205,** 18–23.

SALLUSTO, F., MACKAY, C.R. & LANZAVECCHIA, A. (2000). The role of chemokine receptors in primary, effector, and memory immune responses. *Annu. Rev. Immunol.,* **18,** 593–620.

SCHINDLER, M., KIDD, E.J., CARRUTHERS, A.M., WYATT, M.A., JARVIE, E.M., SELLERS, L.A., FENIUK, W. & HUMPHREY, P.P. (1998). Molecular cloning and functional characterization of a rat somatostatin sst2(b) receptor splice variant. *Br. J. Pharmacol.,* **125,** 209–217.

SETYONO-HAN, B., HENKELMAN, M.S., FOEKENS, J.A. & KLIJN, G.M. (1987). Direct inhibitory effects of somatostatin (analogues) on the growth of human breast cancer cells. *Cancer Res.,* **47,** 1566–1570.

SHERON, N., BIRD, G., KOSKINAS, J., PORTMANN, B., CESKA, M., LINDLEY, I. & WILLIAMS, R. (1993). Circulating and tissue levels of the neutrophil chemotaxin interleukin-8 are elevated in severe acute alcoholic hepatitis, and tissue levels correlate with neutrophil infiltration. *Hepatology,* **18,** 41–46.

SHIMODA, K., BEGUM, N.A., SHIBUTA, K., MORI, M., BONKOVSKY, H.L., BANNER, B.F. & BARNARD, G.F. (1998). Interleukin-8 and hIRH (SDF1-alpha/PBSF) mRNA expression and histological activity index in patients with chronic hepatitis C. *Hepatology,* **28,** 108–115.

SU, G.L. (2002). Lipopolysaccharides in liver injury: molecular mechanisms of Kupffer cell activation. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **283,** G256–G265.

SU, G.L., GOYERT, S.M., FAN, M.H., AMINLARI, A., GONG, K.Q., KLEIN, R.D., MYC, A., ALARCON, W.H., STEINSTRAESSER, L., REMICK, D.G. & WANG, S.C. (2002). Activation of human and mouse Kupffer cells by lipopolysaccharide is mediated by CD14. *Am. J. Physiol. Gastrointest. Liver Physiol.,* **283,** G640–G645.

TEN BOKUM, A.M., HOFLAND, L.J. & VAN HAGEN, P.M. (2000). Somatostatin and somatostatin receptors in the immune system: a review. *Eur. Cytokine Netw.,* **11,** 161–176.

TURNER, S.J., DOMIN, J., WATERFIELD, M.D., WARD, S.G. & WESTWICK, J. (1998). The CC chemokine monocyte chemotactic peptide-1 activates both the class I p85/p110 phosphatidylinositol 3-kinase and the class II PI3K-C2alpha. *J. Biol. Chem.,* **273,** 25987–25995.

ULEVITCH, R.J. & TOBIAS, P.S. (1995). Receptor-dependent mechanisms of cell stimulation by bacterial endotoxin. *Annu. Rev. Immunol.,* **13,** 437–457.

VALATAS, V., XIDAKIS, C., ROUMPAKI, H., KOLIOS, G. & KOUROUMALIS, E.A. (2003). Isolation of rat Kupffer cells; a combined methodology for highly purified primary cultures. *Cell Biol. Int.,* **27,** 67–73.

VAN HAGEN, P.M., HOFLAND, L.J., TEN BOKUM, A.M., LICHTENAUER-KALIGIS, E.G., KWEKKEBOOM, D.J., FERONE, D. & LAMBERTS, S.W. (1999). Neuropeptides and their receptors in the immune system. *Ann. Med.,* **31** (Suppl 2), 15–22.

WEINSTEIN, S.L., GOLD, M.R. & DEFRANCO, A.L. (1991). Bacterial lipopolysaccharide stimulates protein tyrosine phosphorylation in macrophages. *Proc. Natl. Acad. Sci. U.S.A.,* **88,** 4148–4152.

WIEDERMANN, C.J., REINISCH, N. & BRAUNSTEINER, H. (1993). Stimulation of monocyte chemotaxis by human growth hormone and its deactivation by somatostatin. *Blood,* **82,** 954–960.

WINWOOD, P.J. & ARTHUR, M.J. (1993). Kupffer cells: their activation and role in animal models of liver injury and human liver disease. *Semin. Liver Dis.,* **13,** 50–59.

YAMAGUCHI, Y., ICHIGUCHI, O., MATSUMURA, F., AKIZUKI, E., MATSUDA, T., OKABE, K., YAMADA, S., LIANG, J., MORI, K. & OGAWA, M. (1997). Enhanced expression of cytokine-induced neutrophil chemoattractant in rat hepatic allografts during acute rejection. *Hepatology,* **26,** 1546–1552.

YAMAGUCHI, Y., MATSUMURA, F., TAKEYA, M., ICHIGUCHI, O., KURATSU, J.I., HORIUCHI, T., AKIZUKI, E., MATSUDA, T., OKABE, K., OHSHIRO, H., LIANG, J., MORI, K., YAMADA, S., TAKAHASHI, K. & OGAWA, M. (1998). Monocyte chemoattractant protein-1 enhances expression of intercellular adhesion molecule-1 following ischemia–reperfusion of the liver in rats. *Hepatology,* **27,** 727–734.

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