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Human peritoneal mesothelial cells synthesize interleukin-6: Induction by IL-1β and TNFα

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Human peritoneal mesothelial cells synthesize interleukin-6: Induction by IL-1β and TNFα. Recent studies have demonstrated increased levels of IL-6 in the peritoneal cavity during CAPD peritonitis. The current investigation was initiated (i) to examine the human peritoneal mesothelial cell (HPMC) as a possible source of this secreted IL-6 and (ii) to characterize the released product and examine its regulation by other cytokines. Unstimulated HPMC under growth arrested conditions released IL-6 in a time dependent manner. After 24-hour HPMC IL-6 release (mean ± SEM, $N = 13$) (expressed as pg/μg cell protein) was 1.67 ± 0.33. Stimulation of HPMC with IL-1β or TNFα resulted in a time (increasing up to 48 hr) and dose dependent IL-6 generation. After 24 hours the levels induced by IL-1β and TNFα (both at 1000 pg/ml) were (mean ± SEM, $N = 13$) 19.08 ± 2.98 and 6.62 ± 1.72, respectively. Stimulation with combinations of IL-1β and TNFα resulted in additive increases in IL-6 release. This release could be inhibited by co-incubation with anti-IL-1β and/or anti-TNFα antibodies. The level of released HPMC IL-6 measured by immunometric assay (ELISA) correlated directly with that detected in the 7TD1 IL-6 bioassay ($r = 0.63$; $P < 0.001$). Western blot analysis of concentrated HPMC supernatants using specific anti-IL-6 antibody demonstrated immunoreactive bands at 23 and 28 Kd following IL-1β or TNFα treatment. PCR amplification of reverse transcribed HPMC mRNA using specific IL-6 primers demonstrated a single 465 base pair transcript, the expression of which was enhanced in a time dependent manner following treatment of HPMC with either IL-1β or TNFα. These data demonstrate for the first time that HPMC synthesize IL-6 and that its release can be regulated as a result of increased expression of specific mRNA and de novo protein synthesis by other cytokines. HPMC derived IL-6 might contribute directly to the cytokine network regulating intraperitoneal inflammatory responses associated with bacterial peritonitis.

Increasing evidence suggests that the mesothelial cell lining, the peritoneal cavity, plays a more than passive role during inflammatory events therein. In this respect we and others have demonstrated that it is a major source of prostaglandins in the peritoneal cavity [1–3]. In addition activated peritoneal macrophages can directly elevate mesothelial cell arachidonic acid metabolism at least partly as a result of their secretion of interleukin-1β (IL-1β) and tumor necrosis factor α (TNFα) [4].

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Macrophages form the highest proportion of resident phagocytic cells in the sterile peritoneal cavity and examination of dialysis effluent from patients during the beginning of a peritonitis episode, clearly indicates that this cell type forms the first line of defense against invading microorganisms [5]. These cells are capable of providing the initial signals for subsequent recruitment of neutrophils via secretion of chemotactic molecules such as leukotrienes and interleukin-8 [6–8]. The PMØ isolated from CAPD patients appear to behave as elicited or primed cells rather than as a resident macrophage population [6, 7]. This increases the potential for the intraperitoneal generation of pro-inflammatory substances, such as lipid mediators and cytokines, which may be capable of activating other cell populations [9, 10].

The role of the mesothelial cell during the initiation of peritoneal inflammation is poorly defined, and its contribution to the cytokine network has yet to be elucidated.

IL-6 is a polypeptide mediator produced by a variety of cell types, including T-cells, fibroblasts, epithelial and endothelial cells. Increased local levels of IL-6 have been demonstrated in many inflammatory conditions [11, 12] and exert a variety of systemic effects [13]. Actions include the induction of B-cell differentiation and immunoglobulin production and stimulation of acute phase protein synthesis by the liver [14, 15].

It has recently been demonstrated that dialysis effluent, collected during episodes of peritonitis, contains very large amounts of IL-6 bioactivity which is assumed to be derived mainly from invading inflammatory cells [16].

The present investigation set out to examine HPMC as the possible source of intraperitoneal IL-6 secretion, to characterize any released activity and examine its regulation by other cytokines (IL-1β and TNFα). We demonstrate that cultured human mesothelial cells express specific mRNA and release significant quantities of immunoreactive and bioactive IL-6. The mesothelium may be a potential source of this molecule during peritoneal inflammation.

Methods

All chemicals, unless otherwise stated, were obtained from the Sigma Chemical Company (Poole, Dorset, UK). Recombinant human IL-1β was a gift from Dr. D. Boraschi, Sclavo

Research Centre (Siena, Italy). Its specific activity was 2.5×10^7 U/mg as assessed in the EL-4 16/CTLL bioassay. Human TNFα was from BASF AG (Ludwigshafen, Germany). Its specific activity was 8×10^7 U/mg protein (as assessed in a 48 hr L929 bioassay in the absence of actinomycin D). All cytokine preparations were batched and stored at −70°C and freshly thawed for each experiment. Endotoxin contamination of recombinant material was <0.7 pg/μg protein as assessed by limulus amoebocyte lysate assay (Kabi Vitrum, Stockholm, Sweden). Goat anti-human IL-1β and goat anti-human IL-6 antibodies were a gift from The National Institute of Biological Standards and Control (NIBSC), Potters Bar (Herts, UK) and monoclonal anti-TNFα antibody (mono Ab 195) was provided by BASF AG.

Isolation and culture of human peritoneal mesothelial cells

Human peritoneal mesothelial cells (HPMC), obtained from the omental tissue of consenting patients undergoing elective abdominal surgery, were isolated and characterized essentially as described previously. The cells cultured were pure mesothelial cells as assessed by their uniform cobblestone appearance at confluence, by the presence of surface microvilli, by the lack of staining for factor VIII related antigen and the uniform positive staining for cytokeratins 8 and 18 [3]. The presence of contaminating macrophages was excluded following examination of Fc receptor expression (using a red cell rosetting assay [3]) and Ia (class II MHC) antigen immunostaining, both of which were negative in all mesothelial cell cultures examined. Cells were maintained in Ham's F12 medium (CM) (ICN Biomedicals Ltd, High Wycombe, Bucks, UK) supplemented with penicillin (100 U/ml), streptomycin (100 mg/ml), L-glutamine (2 mM) (Gibco BRL Life Technologies Ltd, Uxbridge, UK), transferrin (5 μg/ml), insulin (5 μg/ml), hydrocortisone (0.4 μg/ml) (all from Sigma) and 10% vol/vol fetal calf serum (FCS) (ICN Biomedicals Ltd). Cells were passaged using trypsin:EDTA:glucose (0.125% wt/vol:0.01% wt/vol:0.1% wt/vol). All data presented are from experiments performed with cells from the second passage which had previously been growth arrested. There was no difference in reactivity of mesothelial cell cultures irrespective of passage number, cells were used from the second passage and not later to maximize cell numbers but to avoid using cultures containing senescent cells [3].

Establishment of growth arrested HPMC

HPMC were grown to confluence in rat tail collagen treated [17] 250 ml flasks or in multi-well plates and then transferred to CM containing 0.1% vol/vol FCS (rest medium) for 48 hours prior to stimulation. Previous experiments had demonstrated that at this time point the cells were maintained in a viable (as assessed by the lack of LDH release) but nonproliferative state (data not shown). Under these conditions the cells could be maintained for up to 96 hours without any significant loss of viability.

Western blotting

Synthesis and molecular weight characterization of secreted IL-6 was examined by Western blot analysis using specific sheep anti-human IL-6 (NIBSC). Ten to 40 μl of 10× concentrated (Centricon 3 micro-concentrators, Amicon Ltd, Stonehouse, UK) culture supernatants from growth arrested control and cytokine stimulated HPMC and recombinant IL-6 (10 μg) were subjected to electrophoresis in 1.5 mm thick 5 to 20% gradient SDS polyacrylamide gels (mini-Protean II, BioRad Laboratories Ltd, Hemel Hempstead, UK). After electrophoresis the gels were equilibrated in blot buffer (7.81 mM Tris, 60 mM glycine 20% vol/vol methanol, pH 8.3) and transblotted for four hours at 0°C at 200 mA constant current on to pre-wetted nitrocellulose (Immobilon P; Millipore UK Ltd, Watford, UK). The blotted membrane was placed in blocking buffer (PBS containing 1% wt/vol bovine serum albumin (BSA; ICN Biomedicals Ltd) and 0.1% vol/vol Tween 20 overnight at 4°C. The blots were washed (×6) with PBS and incubated overnight at 4°C with the primary antibody (1:1500) dissolved in PBS, Tween 20 (0.1% vol/vol) BSA (1% wt/vol), and then incubated (60 min at room temperature) with alkaline phosphatase conjugated rabbit anti-goat IgG (1:5000), dissolved in Tris-buffered saline containing 0.1% vol/vol BSA.

The blot was washed again (×4 with PBS-Tween 20) and developed following incubation with 50 ml of substrate buffer (0.1 M Tris-HCl pH 9.5, 0.1 M NaCl and 5 mM $MgCl_2$) containing nitroblue tetrazolium (0.33 mg/ml), 5-bromo-4-chloro-3 indolyl phosphate (BCIP) (170 μg/ml). The blots were dried overnight and photographed. The molecular weights of developed bands were compared to those of prestained SDS-PAGE standards (low range; Bio-Rad Laboratories Ltd, Hemel Hemstead, UK).

RNA isolation, reverse transcription and PCR amplification

HPMC were grown to confluence in 24 well plates and growth arrested as previously described. Total cellular RNA was extracted from both control and cytokine treated HPMC following lysis with 4 M guanidine isothiocyanate and centrifugation through 5.7 M cesium chloride in 0.1 M EDTA [18]. As a result of the limited amount of material available IL-6 mRNA expression was examined using PCR of reverse transcribed HPMC mRNA.

Two micrograms of total RNA sediment was dissolved in 5 μl water containing 75 ng oligo dT (Pharmacia Biosystems Ltd, Milton Keynes, UK) by heating to 65°C. At room temperature 4 μl of 3× reverse transcription buffer (United States Biochemicals, Cleveland, Ohio, USA) containing 0.125 U RNAase blocker (Stratagene Ltd, Cambridge, UK) was added and mixed thoroughly. Reverse transcription was performed after addition of 3.5 μl of substrate enzyme mix (18 nmol dNTP's (Pharmacia Biosystems Ltd) and 5 U reverse transcriptase (Stratagene Ltd) at 37°C for one hour. The reaction mixture was diluted to 100 μl with water and stored at −20°C.

PCR amplification was performed in a total volume of 25 μl (5 μl of reverse transcription product and 20 μl of master mix) 0.6 U Taq polymerase (Perkin-Elmer Ltd, Beaconsfield, UK) 20 μM of each primer, 20 nM dNTP's (Gene Amp-kit; Perkin-Elmer Ltd). The reaction mix was overlayed with 20 μl mineral oil. Various amplification cycles (20 to 35) were carried out starting with denaturation at 94°C for one minute, followed by primer annealing at 60°C for one minute, and extension at 72°C for one minute. Extension time was incremented by 5 sec/cycle. The terminal product was kept at 4°C.

Table 1. Amplification primers

Gene	Oligonucleotide sequence	Product size	Reference
Cyclophillin	3′-CATCTGCACTGCCAAGACTG CTGCAATCCAGCTAGGCATG-5′	326	[19]
IL-6	3′-TACATCCCTCGACGGATCTC GCTACATTTGCCGAAGAGCC-5′	465	[20]
α-Actin	3′-TCTCCACAACCCTCTGCACC GGAGCAATGATCTTGATCTT-5′	234	[29]

Analysis of PCR products

One-tenth of the PCR reaction product was separated by flat bed electrophoresis in 1.5% wt/vol agarose gels (BioRad Laboratories Ltd) stained with ethidium bromide and photographed. The negatives were scanned using a densitometer (Model 620 video densitometer, Bio-Rad Laboratories Ltd) and the density of the bands compared to those of the housekeeping gene.

Oligonucleotide synthesis

Oligonucleotides were automatically synthesized (Applied Biosystems, USA). Matrix bound oligonucleotides were split and de-protected by incubation in NH_4OH (33% vol/vol) at 55°C overnight, lyophilized and used directly. The sequences of the amplification primers are in Table 1.

Induction of IL-6 production by HPMC

HPMC monolayers were grown to confluency in collagen coated 24-well plates (Falcon; Becton-Dickinson UK Ltd, Oxford, UK), and growth arrested for 48 hours in CM containing 0.1% vol/vol FCS as previously described, washed ×4 with rest medium, and then incubated at 37°C in the presence or absence of the appropriate stimulus. At specific time intervals, HPMC supernatants were removed, centrifuged at 12,000 × g and then stored at −70°C until assayed. At the end of the incubation period the cells were washed with PBS (pH 7.3) (Dulbecco; Oxoid Ltd, Basingstoke, UK) and the cellular protein solubilized with 0.1 N NaOH. Total cellular protein was estimated in these supernatants using the modified Bradford method [21]. Repeated cell counts revealed that 1 μg of cellular protein was equivalent to 3.76 ± 0.56 × 10^5 cells (N = 5). All data for IL-6 production are expressed as pg/μg of cellular protein.

Cytokine synthesis measurements

Synthesised IL-6 was measured in the culture supernatants of control and simulated (growth arrested) HPMC by specific sandwich-ELISA. Briefly, 96-well microtiter plates were coated with affinity purified rabbit anti-mouse IgG (Dakopats, Hamburg, FRG) in PBS for one hour at room temperature, the plates were washed ×3 with PBS Tween 20 0.05% vol/vol (PBS-Tween) and incubated with mouse monoclonal anti-human IL-6 antibody (primary antibody) (Serva, Heidelburg, Germany), recombinant IL-6 standard (British Biotechnology Ltd, Oxford, UK) or test supernatants. The plates were washed ×3 with PBS-Tween and incubated with biotinylated goat anti-human IL-6 (R&D Systems, Minneapolis, Minnesota, USA) for 30 minutes at 30°C. The detection system consisted of Streptavidin-peroxidase (Calbiochem, Frankfurt, Germany) and 3,3′5,5′-tetramethyl benzidine (Fluka, Buchs, Switzerland). Once the color had developed the optical density was assessed at 450 and 630 nm in a microtiter plate reader (MR 5000; Dynatech Laboratories Ltd, Billingshurst, Sussex, UK). The IL-6 concentration in the samples was calculated by comparison with standard concentrations ranging from 20 to 2000 pg/ml. The sensitivity of the assay was 20 pg/ml. There was no apparent cross reactivity in this assay with IL-1α, IL-1β, TNFα, TNFβ, IL-2 or IL-4 (British Biotechnology Ltd.) when these were added to blanks in concentrations up to 50 ng/ml. HPMC supernatants containing IL-6, as measured by ELISA, were subsequently subjected to bioactivity measurement using the 7TD1 bioassay as previously described [22].

Statistical analysis

All statistical analyses were performed by using the Wilcoxon signed rank test for paired non-parametric data, a P value of less than 0.05 being considered as significant. All data are presented as mean (±SEM).

Results

Synthesis of IL-6 by HPMC

Unstimulated non-proliferating HPMC released IL-6 in a time dependent manner which was significantly above background by 12 hours and reached a plateau between 24 and 48 hours. After 24 hours the mean (±SEM) release of IL-6 was 1.40 ± 0.97 pg/μg cellular protein (N = 5; Fig. 1 A and B).

Cytokine induction of HPMC IL-6

Stimulation of non-proliferating HPMC with IL-1β or TNFα enhanced the time dependent generation of IL-6. This release became significant above background levels after a lag phase of three hours with both IL-1β (1000 pg/ml) ($z = 2.203$; $P < 0.01$) and TNFα (1000 pg/ml) ($z = 2.203$; $P < 0.01$) and continued to rise over the whole time course studied.

The release of IL-6 in response to IL-1β and TNFα was also dose dependent. After 24 hours significant release above background levels was achieved with doses of IL-1β above 5 pg/ml ($z = 1.96$, $P < 0.05$) and with TNFα doses above 10 pg/ml ($z = 2.66$; $P < 0.01$; Fig. 2). Maximal generation of HPMC IL-6 was stimulated with an IL-1β dose of 1000 pg/ml when the release was 19.08 ± 0.33 pg/μg, N = 13 cellular protein, which was 11-fold above background levels ($z = 2.66$; $P < 0.01$). The release in response to TNFα continued to rise over the whole dose range tested; at a dose of TNFα of 5000 pg/ml the released IL-6 levels had reached 7.84 ± 1.78 pg/μg, N = 13, fivefold above background levels ($z = 2.023$; $P < 0.01$).

Combined cytokine stimulation

Stimulation of non-proliferating HPMC with combinations of IL-1β and TNFα resulted in the generation of IL-6 above the levels generated by each cytokine alone. The level of release, however, was not significantly different from the additive values of the two cytokines when each was used alone (data not shown). Peak stimulation of IL-6 in these experiments was obtained at the highest combination of cytokine concentrations tested (IL-1β, 5000 pg/ml + TNFα 50 pg/ml) when the 24 hour release was 21.52 ± 8.16 pg/μg cell protein (mean ± SEM, N =

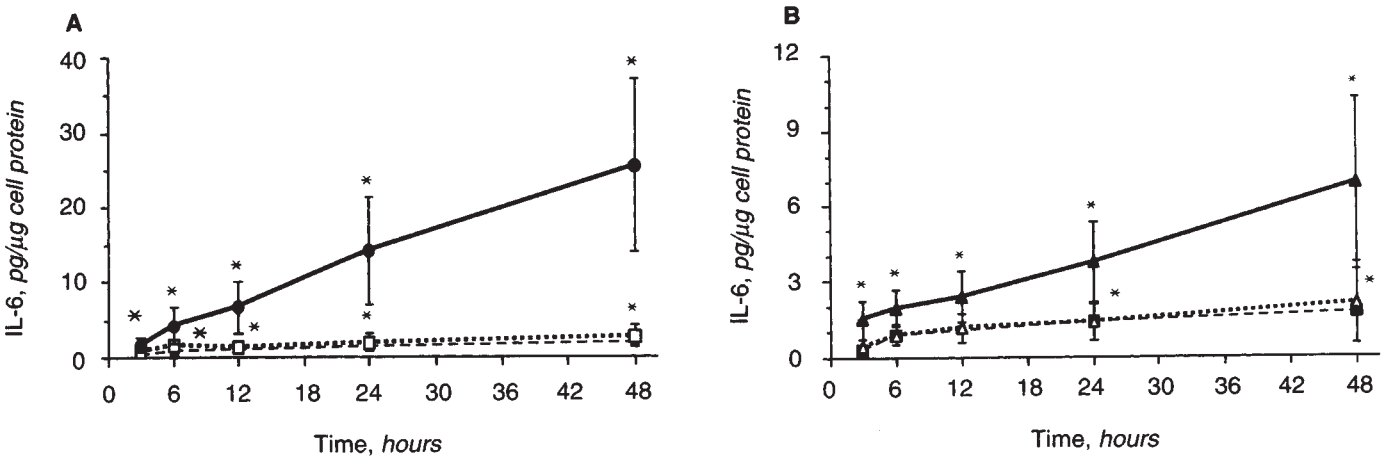

Fig. 1. A. *Time dependent generation of IL-6 from unstimulated HPMC (--■--), or from HPMC stimulated with IL-1β [10 (--□--) and 1000 (–●–) pg/ml].* The data presented are the (mean ± SEM) IL-6 release expressed as pg/μg cell protein, from five separate experiments with HPMC prepared from separate omental specimens. **B.** Time dependent generation of IL-6 from unstimulated HPMC (--■--), or HPMC stimulated with TNFα [10 (--△--) and 1000 (–▲–) pg/ml]. The data presented are the (mean ± SEM) IL-6 release expressed as pg/μg cell protein, from five separate experiments with HPMC prepared from separate omental specimens. * represents a statistically significant difference ($P < 0.05$) compared to unstimulated IL-6 release at the same time point.

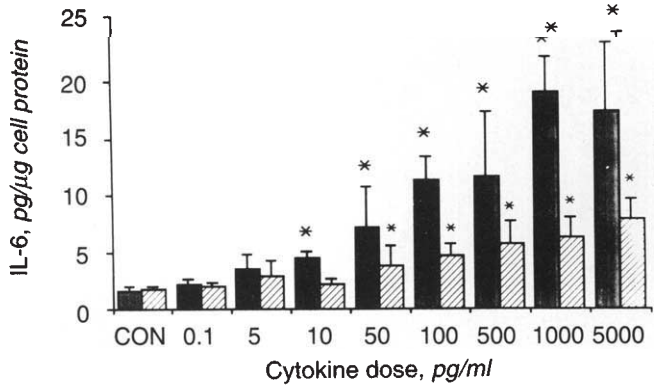

Fig. 2. *Dose effect of IL-1β (■; 0.1 to 5000 pg/ml) and TNFα (▨; 0.1 to 5000 pg/ml) on the 24 hour generation of IL-6 from HPMC.* The data presented are the (mean ± SEM) IL-6 release expressed as pg/μg cell protein, from 13 separate experiments with HPMC prepared from separate omental specimens. * represents a statistically significant difference ($P < 0.05$) compared to the control unstimulated IL-6 release value.

5). In these experiments the levels of IL-6 generated in response to individual stimulation with the highest concentrations of IL-1β (5000 pg/ml) or TNFα (5000 pg/ml) tested were 17.32 ± 5.99 and 7.84 ± 1.78 pg/μg cellular protein ($N = 5$), respectively.

IL-6 bioactivity

The supernatants from non-proliferating unstimulated and cytokine stimulated HPMC were assessed for IL-6 bioactivity in the 7TD1 proliferation assay. These estimations correlated directly with the data obtained with the same samples measured in the IL-6 ELISA ($R^2 = 0.631$, $P < 0.01$, $N = 50$).

Antibody inhibition studies

The specificity of the IL-1β and TNFα stimulation of HPMC IL-6 was confirmed in antibody inhibition experiments with specific anti-cytokine antibodies (Fig. 3 A and B). Co-incubation of HPMC with IL-1β or TNFα in the presence of a fixed dose (that calculated to inhibit 100 pg/ml of the respective cytokine) of polyclonal anti-IL-β antibody or monoclonal anti-TNFα antibody resulted in inhibition of IL-6 generation at all cytokine doses tested. At an IL-1β concentration of 100 pg/ml, IL-6 synthesis was reduced by a mean (±SEM, $N = 3$) of 83.08 ± 10% (Fig. 4A). At a TNFα concentration of 100 pg/ml IL-6 release was reduced by 84.7 ± 9.3% (mean ± SEM, $N = 3$; Fig. 3B).

Western blot analysis

Western blot analysis of concentrated (×10) HPMC supernatants, using specific polyclonal anti-IL-6 antibody, revealed the expression of immunoreactive bands at approximately 23 and 28 Kd following stimulation of HPMC with either IL-1β or TNFα (Fig. 4).

PCR amplification of HPMC mRNA

PCR amplification of reverse transcribed HPMC mRNA, using specific IL-6 primers resulted in the generation of a single 465 base pair IL-6 transcript. Prior stimulation of HPMC with IL-1β (Fig. 5A) or TNFα (Fig. 5B) resulted in a time dependent induction of specific IL-6 transcript as assessed densitometrically (Fig. 5C), when compared to 24-hour unstimulated control HPMC mRNA levels (lane 1). In separate experiments HPMC were exposed to either control medium or IL-1β (1000 pg/ml) or TNFα (1000 pg/ml) for six hours at 37°C. At this time point IL-6 specific mRNA was demonstrable in both unstimulated and cytokine treated HPMC. In cytokine treated HPMC the levels of IL-6 specific mRNA were, however, markedly increased as assessed densitometrically. Under the same conditions the level of the housekeeping gene α-actin mRNA remained unaltered (Fig. 6A and B).

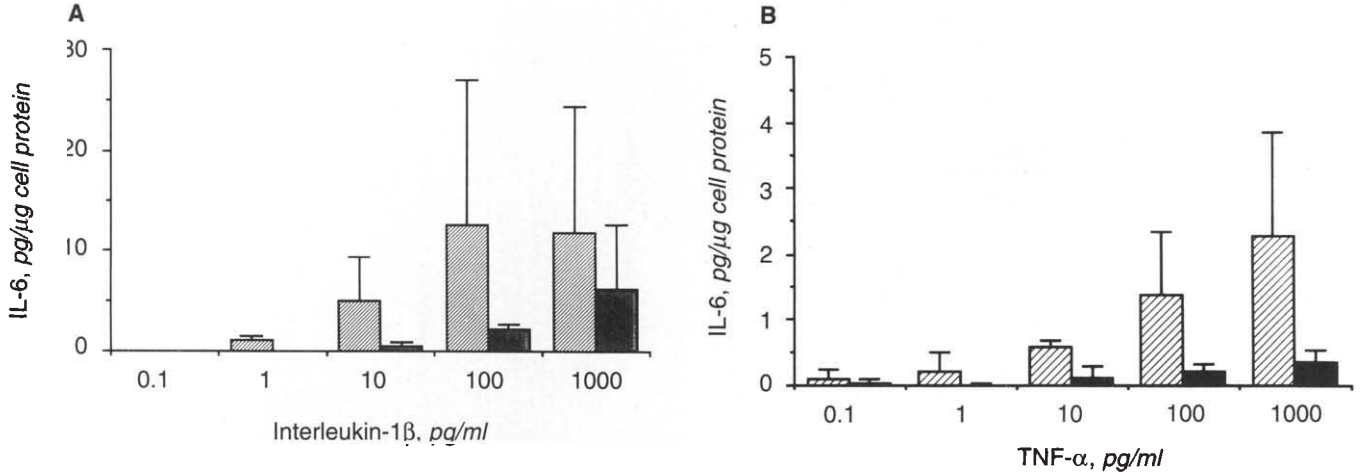

Fig. 3. *Antibody inhibition of cytokine induced HPMC IL-6 release.* **A.** Dose effect of IL-1β (▨; 0.1 to 1000 pg/ml) on the release of IL-6 from HPMC in the absence and in the presence of a neutralizing dose (sufficient to inhibit 100 pg/ml IL-1β) of polyclonal sheep anti-human IL-1β (■). Data represent the (mean ± SEM) five separate experiments with HPMC prepared from separate omental specimens. **B.** Dose effect of TNFα (▨; 0.1 to 1000 pg/ml) on the release of IL-6 from HPMC in the absence and in the presence of a neutralizing dose (sufficient to inhibit 100 pg/ml IL-1β) of monoclonal anti-human TNFα (■). Data represent (mean ± SEM) five separate experiments with HPMC prepared from separate omental specimens.

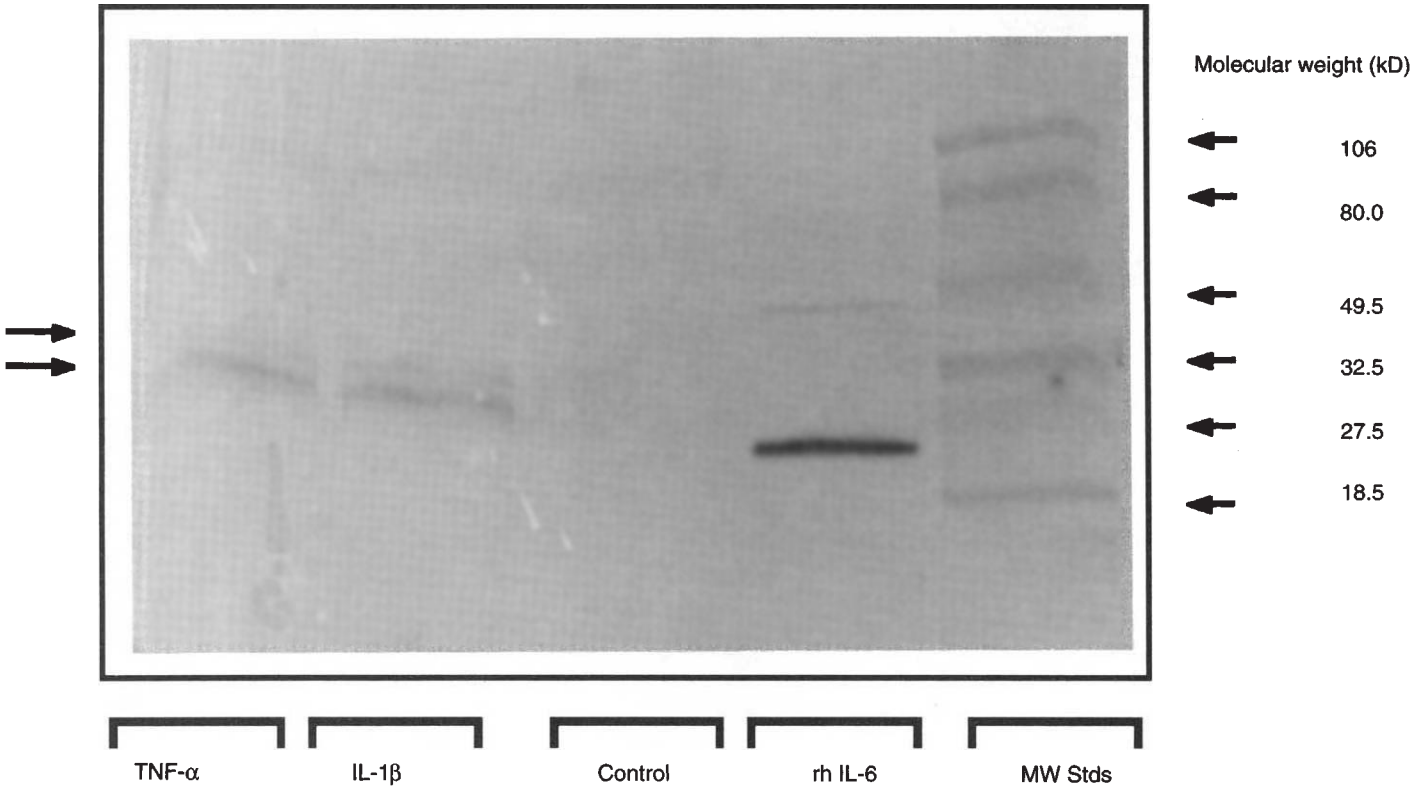

Fig. 4. *Western blot analysis of concentrated HPMC 18 hour supernatants and recombinant IL-6 using polyclonal sheep anti-human IL-6 antibody.* HPMC were either unstimulated or stimulated with IL-1β (1000 pg/ml) or TNFα (1000 pg/ml). Data are presented from a single representative experiment of three performed with HPMC prepared from separate omental specimens.

Discussion

The present study demonstrates for the first time that non-proliferating HPMC express specific IL-6 mRNA and synthesize immunoreactive and bioactive IL-6. The capacity of HPMC to synthesize IL-6 was confirmed by PCR analysis of HPMC mRNA transcripts using specific IL-6 oligonucleotide primers, which demonstrated a 465 base pair single band of complementary DNA with a maximal induction between three and 12 hours following IL-1β or TNFα stimulation. Western

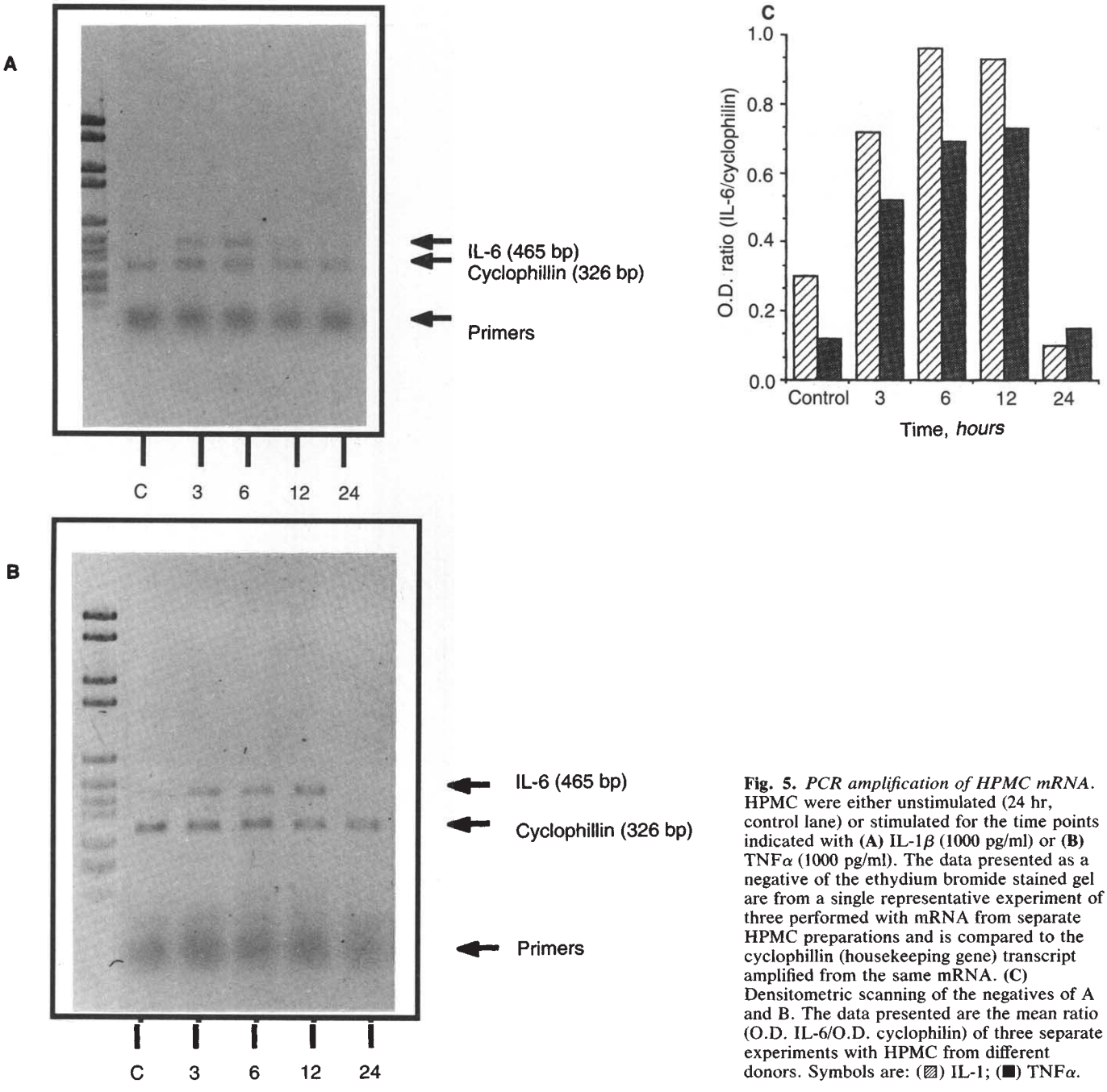

Fig. 5. *PCR amplification of HPMC mRNA.* HPMC were either unstimulated (24 hr, control lane) or stimulated for the time points indicated with **(A)** IL-1β (1000 pg/ml) or **(B)** TNFα (1000 pg/ml). The data presented as a negative of the ethydium bromide stained gel are from a single representative experiment of three performed with mRNA from separate HPMC preparations and is compared to the cyclophillin (housekeeping gene) transcript amplified from the same mRNA. **(C)** Densitometric scanning of the negatives of A and B. The data presented are the mean ratio (O.D. IL-6/O.D. cyclophilin) of three separate experiments with HPMC from different donors. Symbols are: (▨) IL-1; (■) TNFα.

blot analysis of concentrated HPMC supernatants using specific anti-human IL-6 antibody identified immunoreactive bands at approximately 23 and 28 Kd in cytokine treated HPMC, these are of similar molecular mass to those demonstrable in control and stimulated monocyte supernatants and to those previously described for IL-6 in other cell types [23]. The cultured cells were pure mesothelial cells as assessed morphologically (light and electron microscopy), by the uniform positive immunofluorescent staining for cytokeratins 8 and 18 and lack of staining for factor VIII related antigen [3]. It has been suggested that cells derived from digestion of omental tissue might be microvascular endothelial cells; recent evidence, however, suggests that these cells are in fact are of mesothelial and not endothelial origin [24–26]. The possibility of contamination of mesothelial cell culture by adherent macrophages was excluded by the fact that these cells do not usually survive passage and by the lack of Ia antigen, Fc receptor expression in all cell cultures examined.

The secretion of IL-6 protein from HPMC was time dependent and occurred in a constitutive manner. Previous studies have established that both IL-1β and TNFα are potent and specific inducers of HPMC prostaglandin production [4]. In

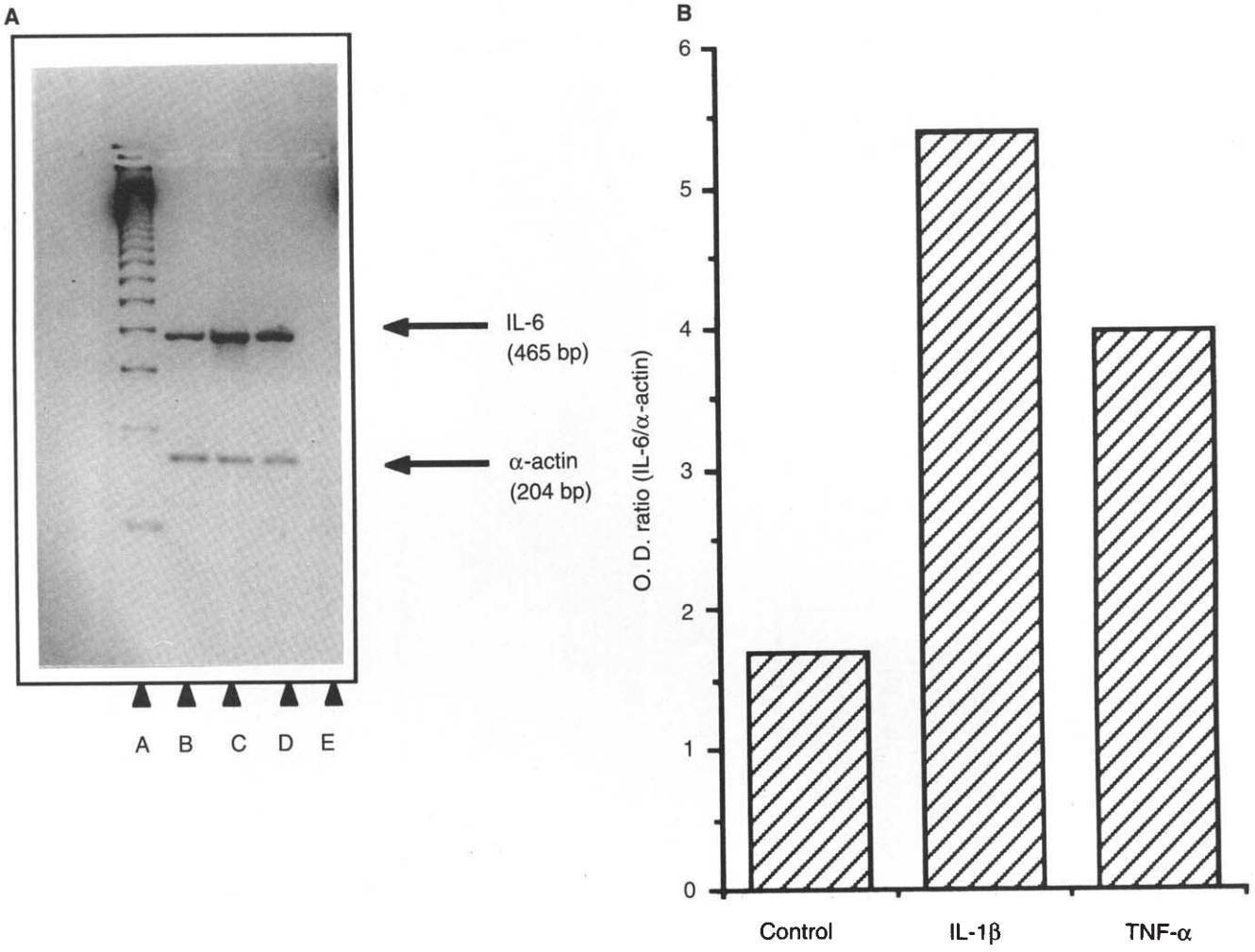

Fig. 6. A. *PCR amplification of reverse transcribed HPMC RNA.* The RNA was extracted, reverse transcribed and PCR amplified for IL-6 and α-actin as described in methods and materials. Data presented are from a negative of the ethydium bromide stained gel from a single representative of three performed. Cells were treated for 6 hours with control medium (lane B), interleukin-1β (1000 pg/ml) (lane C) or TNFα (1000 pg/ml) (lane D). The products were subsequently electrophoresed, stained with ethydium bromide, photographed and compared to DNA standards (lane A). In addition the specificity of the reaction was confirmed in tubes where no cDNA was present (lane E). **B.** Densitometric scanning of the negative of A. The data presented are the mean ratio (O.D. IL-6/O.D. α-actin) of three separate RT/PCR experiments with HPMC from separate donors.

keeping with this specific stimulatory activity, both IL-1β and TNFα induced a time and dose dependent secretion of IL-6 from HPMC. That this effect was cytokine specific was demonstrated in co-incubation experiments utilizing specific anti-IL-1β and anti-TNFα antibodies. Under these conditions IL-1β and TNFα triggered IL-6 synthesis could be inhibited following incubation with the relevant antibody. Incubation of HPMC with combined doses of IL-1β and TNFα resulted in additive increases in IL-6 generation. Western blot analysis of IL-1β and TNFα stimulated HPMC supernatants confirmed the presence of increased levels of immunoreactive IL-6.

Goldman et al [16] have recently demonstrated low IL-6 levels in uninfected peritoneal dialysis effluent which are dramatically elevated during episodes of *Staphylococcal* peritonitis. The authors suggest that the cellular source of this increased IL-6 secretion are the activated peritoneal macrophages which migrate into the peritoneal cavity during peritonitis. The potential of HPMC to synthesize IL-6 in response to the pro-inflammatory cytokines IL-1β and TNFα suggests, however, that the observed rise in intraperitoneal IL-6 occurring during CAPD peritonitis might, at least in part, be of mesothelial origin.

The precise function of high intra-peritoneal IL-6 levels is, as yet, unknown, although recent evidence suggests that its secretion may be an anti- rather than pro-inflammatory event. This proposal is supported by the finding that IL-6 has the potential to suppress the expression of mRNA for other cytokines (IL-1α/β and TNFα) [27, 28]. It is, therefore, tempting to speculate that increased intraperitoneal levels of IL-6 may serve as a negative feedback signal for the production of IL-1 and TNFα from activated peritoneal macrophages, thus participating in controlling the inflammatory response in the peritoneal cavity.

The present findings suggest that in the clinical setting of

CAPD the peritoneal membrane not only serves as the permeability barrier through which ultrafiltration occurs, but might also be directly involved in a cytokine network within the inflamed peritoneum, via its secretion of IL-6.

Acknowledgments

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