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Transforming Growth Factor-β Synthesis by Human Peritoneal Mesothelial Cells

Induction by Interleukin-1

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Peritoneal mesothelial cells are uniquely located to regulate cellular events in the peritoneal cavity and are a potentially important source for various cytokines. The present study was designed to elucidate the capacity of human peritoneal mesothelial cells (HPMCs) to synthesize and secrete the transforming growth factor (TGF)-β isoforms 1, 2, and 3 and to characterize their regulation by inflammatory cytokines. HPMCs constitutively released appreciable amounts of TGF-β1 and low amounts of TGF-β2 as detected by specific immunoassays. TGF-β1 levels secreted within 48 hours (45 ± 8.9 pg/10^5 cells) were 60-fold higher than TGF-β2 levels (0.9 ± 0.1 pg/10^5 cells), respectively. Treatment of HPMCs with interleukin (IL)-1β (10 ng/ml) resulted in a significant increase of both TGF-β1 (mean, 5-fold; P < 0.001) and TGF-β2 (mean, 6-fold; P < 0.01) generation. After 48 hours of IL-1β treatment the levels were 185 ± 17.1 pg/10^5 cells for TGF-β1 and 5.3 ± 1.5 pg/10^5 cells for TGF-β2, respectively. Neither tumor necrosis factor (TNF)-α nor interferon (IFN)-γ (both 10 ng/ml) affected TGF-β1 or TGF-β2 synthesis by HPMCs. TGF-β3 could not be detected in any of the supernatant media. Stimulation of HPMCs with IL-1β increased steady-state levels of TGF-β1- and TGF-β2-specific mRNA. Western blot analysis of supernatants revealed the presence of an immunoreactive band at 25 kd. Indirect competition assays confirmed receptor-binding activity of HPMC-derived TGF-β. Appreciable amounts of TGF-β were present in a bioactive form. Our results demonstrate that HPMCs synthesize the TGF-β isoforms 1 and 2 and that the levels of mRNA and protein release can be up-regulated by the proinflammatory cytokine IL-1β. (Am J Pathol 1996, 148:1679–1688)

The peritoneal cavity is lined by a single layer of mesothelial cells that rest on a sheet-like connective tissue membrane. Within the connective tissue layer is a vascular capillary network that lies in close proximity to the overlying mesothelium. Peritoneal mesothelial cells are thus uniquely located to respond to and regulate cellular events in the peritoneal cavity. The abdominal cavity can be involved by various inflammatory and neoplastic disease processes that are almost invariably associated with the formation of ascitic effusions. Both inflammatory and malignant ascitic fluids contain a variety of cytokines such as interleukin (IL)-1, tumor necrosis factor (TNF)-α, or IL-6 that may significantly affect the course of these diseases.[1–4] Recently, it has also been shown that significant amounts of bioactive transforming growth factor (TGF)-β can be detected in ascitic fluids.[5–8] The TGF-β superfamily consists of five similar molecules. The isoforms TGF-β1, -β2, and -β3 are produced by many mammalian cells and have also been identified in human serum and various tissues.[9,10] Much of the TGF-β in serum and tissues may, however, not be bioactive as it is secreted in an inactive form and must be cleaved from its precursor

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molecule to become activated. TGF-β is a multifunctional cytokine exerting diverse effects including the suppression of immune responses, stimulation of extracellular matrix formation, and modulation of cellular proliferation and differentiation.[9–15] In addition, it may affect important tumor cell functions like cell motility and invasiveness and thereby modulate tumor progression.[16,17]

However, the generation of ascitic TGF-β is not well defined. Increasing evidence suggests that the mesothelium, the largest resident intraperitoneal cell population, must be considered as one of the main sources of ascitic cytokines.[18–23] Although human peritoneal mesothelial cells (HPMCs) may be a potential source of TGF-β, the control of its synthesis is likely to rest with proinflammatory cytokines secreted from resident and/or invading phagocytes or metastasizing tumor cells.[18,24–26] The present study thus set out to characterize the synthesis of the TGF-β isoforms 1, 2, and 3 by HPMCs and to examine their regulation at both the mRNA and protein level in response to the inflammatory cytokines IL-1β, TNF-α, and IFN-γ.

Materials and Methods

Isolation and Culture of HPMCs

HPMCs were obtained from omental tissue of consenting patients undergoing elective abdominal surgery. Cells were isolated by modification of a method described previously.[27] Briefly, small biopsies (approximately 1 cm^3) of omental tissue were rinsed in phosphate-buffered saline (PBS), transferred to a 0.05% solution of collagenase I (Worthington, Boehringer, Mannheim, Germany), and allowed to float for 1.5 to 2 hours at 37°C. Thereafter, the fat tissue was removed and the collagenase solution containing detached mesothelial cells was filtered through a 200-mesh steel sieve. Cells were then collected by centrifugation (10 minutes at 400 × *g*), suspended in Eagle's minimal essential medium (Gibco, Paisley, UK) supplemented with 10% fetal calf serum, penicillin/streptomycin (100 IU/ml, 100 mg/ml; Seromed, Berlin, Germany), and polymyxin B (5 μg/ml; Sigma Chemical Co., St. Louis, MO) and grown to confluence in 25-cm^2 culture flasks (Falcon, Oxnard, CA) at 37°C in a humidified atmosphere of 10% CO_2 in air. The mesothelial cell phenotype and purity of the cultures was confirmed by flow cytometry using monoclonal antibodies (MAbs) against cytokeratins 8 and 18 (MAb CAM5.2, Becton Dickinson, Mountain View, CA) and vimentin (MAb V9, Dakopatts, Glostrup, Denmark) and the demonstration of a uniform coexpression of both intermediate filaments. A contamination by peritoneal phagocytes or endothelial cells was excluded using MAbs against CD34 and CD68 (Becton Dickinson) as described previously.[23] Cells used for experiments were either passage 2 or passage 3.

Induction of TGF-β Production by HPMCs

For experiments, HPMCs were detached with trypsin/versene (0.05%/0.02%), washed once in medium, seeded, and grown to confluence in 24-well tissue culture dishes in a 1-ml volume of culture medium. Confluent monolayers were then washed three times with serum-free culture medium and incubated for 3 to 48 hours in the absence or presence of the appropriate cytokines in serum-free medium. Recombinant IL-1β was provided by Dr. P. Lomedico, Hoffman LaRoche, Nutley, NJ. Human recombinant TNF-α and recombinant IFN-γ were kindly provided by Dr. G. Adolf, E. Boehringer Institute, Vienna, Austria. At the end of the incubation period supernatants were removed, concentrated 10-fold using Centriprep-10 concentrators (Amicon, Beverly, MA), and stored at −70°C until assayed. The remaining cells were then enzymatically detached, and the cell number of the samples was determined using a Coulter Counter (Luton, UK). Incubation of the cells with cytokines for up to 48 hours did not have any significant effect on cell viability as shown by trypan blue exclusion. All culture conditions were run in duplicate. Supernatants for Western blot analysis and indirect competition assays were derived from HPMCs grown in 75-cm^2 culture flasks (Falcon).

Determination of TGF-β Production by Enzyme-Linked Immunosorbent Assay (ELISA)

To determine the level of TGF-β production under the different experimental conditions cell culture supernatants were assayed using specific sandwich enzyme immunoassays as described previously.[28] Briefly, concentrated supernatants were precipitated with ammonium sulfate and extracted with HCl-ethanol. Protein pellets were resuspended in binding buffer (150 mmol/L NaCl, 100 mmol/L Tris-HCl (pH 7.4), 0.05% Tween 20, 1 mg/ml bovine serum albumin V) and added to 96-well microtiter plates that were coated with a mouse monoclonal anti-TGF-β antibody (Genzyme, Cambridge, MA). After incubation at room temperature for 1 hour and careful washes with PBS, the second antibody was added

(immunoglobulin (Ig)G fraction from chicken against TGF-β1, IgG fraction from rabbit against TGF-β2 (both from R&D Systems, Minneapolis, MN), or IgG fraction from rabbit against TGF-β3 (Santa Cruz Biotechnology, Santa Cruz, CA)). After another incubation for 1 hour at room temperature and repeated washes with PBS, the third antibody was added (phosphatase-labeled anti-rabbit IgG or anti-chicken IgG, both from Kirkegaard and Perry, Gaithersburg, MD), followed after 1 hour by several washes with PBS and final addition of phosphatase substrate p-nitrophenyl phosphate. Extinction at 405 nm was measured photometrically (SLT reader, Anthos Labtec Instruments, Salzburg, Austria). Ultrapure human TGF-β1, porcine TGF-β2, and recombinant human TGF-β3 were used as standards (R&D Systems). Cross-reactivity was less than 1%. All tests were performed in duplicate. All data for TGF-β production detected by ELISA are expressed as picograms per 10^5 cells.

Western Blot Analysis

TGF-β1 proteins in supernatants from HPMCs were further characterized by Western blot analysis using a detection system with a rabbit antibody against human TGF-β1 (Promega, Madison, WI). Supernatants were concentrated sixfold by ultrafiltration over an Amicon 5 concentrator (Amicon), and 50-μl samples were transiently acidified by addition of 1 μl of 1 N HCl, followed by incubation for 1 hour at 4°C and neutralization by the addition of 1 μl of 1 N NaOH. The samples were subjected to electrophoresis on a 1.0-mm 12% sodium dodecyl sulfate gel. Semi-dry blotting on nitrocellulose membrane (BA 85, Schleicher & Schuell, Dassel, Germany) was performed using a Fast-Blot system (Biometra, Goettingen, Germany) for 15 minutes at a constant current of 140 mA. All other procedures were done according to the recommendations of the manufacturer including the final detection with an anti-rabbit IgG (Fc) conjugated with alkaline phosphatase. TGF-β1 purified from human platelets (R&D Systems) was used as a standard.

Determination of Secreted TGF-β Activity by Indirect Competitive Radioreceptor Assay

TGF-β receptor reactivity was determined by competition with ^{125}I-labeled TGF-β (Biomedical Technology, Stoughton, MA) for specific binding on A549 human lung carcinoma cells as described previously.[12] This competition assay detects all TGF-β species known. Briefly, A549 cells were seeded into 24-well tissue culture dishes at a concentration of 1×10^5 cells per well in Iscove's modified Eagle's medium supplemented with 10% fetal calf serum. After incubation overnight in a 5% CO_2 atmosphere at 37°C the cells were washed three times with binding buffer (Iscove's modified Eagle's medium, 1 mg/ml bovine serum albumin, and 40 mmol/L HEPES buffer, pH 7.4) at 25°C. Samples of conditioned media were either directly used in the assay or activated by transient acidification with HCl to a final concentration of 125 mmol/L over 1 hour, followed by neutralization with NaOH. Thereafter, binding buffer (200 μl) containing 100 pmol/L ^{125}I-labeled TGF-β and serial dilutions of HPMC-conditioned medium or various concentrations of TGF-β were added, and the cells were incubated for 2 hours at 25°C in sealed plastic bags. Cells were then washed with ice-cold Hank's balanced salt solution containing 1 mg/ml bovine serum albumin and solubilized for 30 minutes at 37°C with a prewarmed 750-μl Triton solution (20 mmol/L HEPES, 1% Triton X-100, and 10% glycerol). Radioactivity of aliquots was determined in a LKB gamma counter. The concentration of TGF-β in conditioned medium was calculated by interpolation on the standard curve with TGF-β. Specific binding of ^{125}I-labeled TGF-β was determined in the presence and absence of a 100-fold excess of TGF-β. Data are expressed as picograms per 10^5 cells.

TGF-β mRNA Analyses by Reverse Transcriptase Polymerase Chain Reaction (RT-PCR)

Total cellular RNA from HPMCs grown as described above was isolated by the phenol hot procedure.[29] A total of 100 ng of RNA was reverse transcribed into cDNA in a final volume of 20 μl in buffer (10 mmol/L Tris-HCl (pH 8.3), 50 mmol/L KCl, 5 mmol/L $MgCl_2$, 0.1% w/v gelatin) containing 2 μl of deoxynucleotide triphosphates (2.5 mmol/L; Pharmacia, Uppsala, Sweden), 1 μl of 3′ primer (20 μmol/L), and 2.5 U of AMV reverse transcriptase (Stratagene, Heidelberg, Germany). After incubation for 30 minutes at 52°C and 1 minute at 99°C, the mixture was cooled on ice and subjected to the PCR reaction by adding 1 μl of each 5′ and 3′ primer, 2 μl of deoxynucleotide triphosphates, 68 μl of H_2O, 8 μl of 10× buffer (as described above, except that the final concentration of MgCl was 1.5 mmol/L), and 0.5 μl of Taq polymerase (Stratagene). Amplification was performed according to the following schedule. For TGF-β1, the 1st cycle was 5 minutes at 95°C, 1 minute at 57°C, and 1 minute at 72°C; the 2nd through 29th cycles

were 1 minute at 95°C, 1 minute at 57°C, and 1 minute at 72°C; and the final cycle was 1 minute at 95°C, 1 minute at 57°C, and 10 minutes at 72°C. For TGF-β2, the 1st cycle was 4 minutes at 95°C, 50 seconds at 53°C, and 1 minute at 72°C; the 2nd through 29th cycles were 1 minute at 95°C, 50 seconds at 53°C, and 1 minute at 72°C; the final cycle was 1 minute at 95°C, 50 seconds at 53°C, and 10 minutes at 72°C. For β_2-microglobulin, the 1st cycle was 10 minutes at 70°C, 3 minutes at 4°C, 1 minute at 95°C, 1 minute at 65°C, and 1 minute at 72°C; the 2nd through 24th cycles were 1 minute at 95°C, 1 minute at 65°C, and 1 minute at 72°C; and the final cycle was 1 minute at 95°C, 1 minute at 65°C, and 11 minutes at 72°C. The oligonucleotide primers were as follows: TGF-β1, 5′ primer CAGAAATACAGCAACAATTCCTGG, corresponding to bases 1358 to 1381 of the TGF-β1 cDNA, and 3′ primer TTGCAGTGTGTTATCCCTGCTGTC, corresponding to bases 1520 to 1543 of the TGF-β1 cDNA, product of 186 bp[30]; TGF-β2, 5′ primer GTTTTTCTGTTGGGCATTGA, corresponding to bases 304 to 323 of the TGF-β2 cDNA, and 3′ primer TCTTCTGGGGGACTGGTGAG, corresponding to bases 615 to 634 of the TGF-β2 cDNA, product of 331 bp[31]; β_2-microglobulin, 5′ primer CAGCAAGGACTGGTCTTTCTATCTCTTGTA, corresponding to bases 201 to 230 of the β_2-microglobulin cDNA, and 3′ primer GGAGCAACCTGCTCAGATACATCAAAACATGG, corresponding to bases 510 to 539 of the β_2-microglobulin cDNA, product of 338 bp.[32]

Quantification of TGF-β1 and TGF-β2 mRNA by Competitive RT-PCR

For quantification of the TGF-β1 and TGF-β2 mRNA signals obtained from HPMCs under treatment with IL-1β, a competitive PCR was developed (manuscript in preparation). Briefly, *in vitro* synthesized cRNA was used as a standard. This cRNA was transcribed by T7-polymerase (Boehringer Mannheim, Mannheim, Germany) from a PCR-II plasmid containing a partial TGF-β1 cDNA (1142 to 1561) deleted in the region 1258 to 1351 (final length of 326 bp) or a partial TGF-β2 cDNA (304 to 635) deleted in the region 435 to 462 (final length of 304 bp). Varying amounts (0.25 to 1000 pg) of the respective mutated cRNA were added as a competitive template. RT-PCR was performed as described above. After amplification, separation by standard agarose gel (3%) electrophoresis, and staining with ethidium bromide, a photograph was taken and scanned densitometrically using an AGFA Arcus scanner. The amount of the TGF-β1 or TGF-β2 mRNA from HPMCs was calculated by comparison with the cRNA standard of known concentration. For the quantitative PCR, the following primers were used: TGF-β1, 5′ primer GCCGACTACTACGCCAAGGA, corresponding to bases 1142 to 1161, and 3′ primer AACCCGTTGATGTCCACTTG, corresponding to bases 1541 to 1560, product of 419 bp for wild-type and 326 bp for deletion mutant[30]; TGF-β2, 5′ primer GTTTTTCTGTTGGGCATTGA, corresponding to bases 304 to 323, and 3′ primer TCTTCTGGGGGACTGGTGAG, corresponding to bases 615 to 634, product of 331 bp for wild-type and 304 bp for deletion mutant.[31]

Statistical Analyses

Data were analyzed by nonparametric procedures. Cytokine-treated cultures were compared with cultures under control conditions using the Mann-Whitney *U*-test.

Results

Synthesis of Isoforms TGF-β1 and TGF-β2 by HPMCs

The various TGF-β isoforms were measured by specific ELISA in supernatants of mesothelial cell cultures from six different donors. In all cases, unstimulated confluent HPMCs were shown to constitutively release appreciable amounts of the isoform TGF-β1 (Figure 1). The mean (±SEM) level achieved within 48 hours was 45.2 ± 8.9 pg/10^5 cells. By contrast, the isoform TGF-β2 was secreted in much lower amounts and production was detectable in only three of six separate HPMC cultures. The mean (±SEM) level secreted within 48 hours was 0.9 ± 0.1 pg/10^5 cells. Mean TGF-β1 levels were 60-fold higher than the amount of secreted TGF-β2. TGF-β3 could not be detected in any of the supernatants analyzed.

Cytokine Induction of TGF-β1 and TGF-β2

Treatment of HPMCs with the proinflammatory cytokine IL-1β resulted in a significant elevation of the secretion of both TGF-β1 and TGF-β2. After 48 hours, the mean (±SEM) TGF-β1 levels induced by IL-1β (10 ng/ml) were 185 ± 17.1 pg/10^5 cells and increased fivefold ($P < 0.001$) over baseline values. Secretion of TGF-β2 was increased sixfold ($P < 0.01$) resulting in a mean (±SEM) production of 5.3 ± 1.5 pg/10^5 cells (Figure 1). Neither TNF-α nor IFN-γ (10 ng/ml each) had any effect on the generation of either TGF-β1 or TGF-β2. TGF-β3 was not

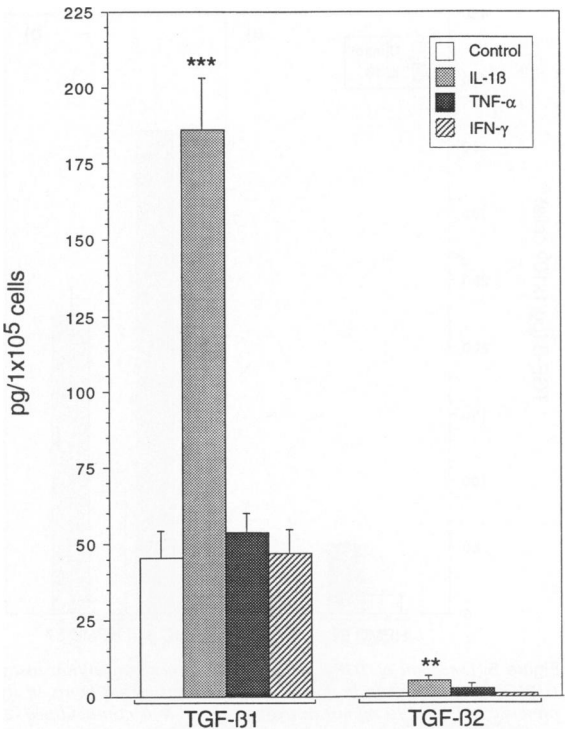

Figure 1. *Secretion of isoforms TGF-β1 and TGF-β2 by HPMCs. Cells were stimulated for 48 hours with the proinflammatory cytokines IL-1β, TNF-α, or IFN-γ (10 ng/ml each). TGF-β1 and TGF-β2 were measured in duplicate by ELISA. Results are the means (±SEM) of TGF-β release expressed as pg/10⁵ cells, from HPMCs cultured from six separate donors.* ***P < 0.001; **P < 0.01.

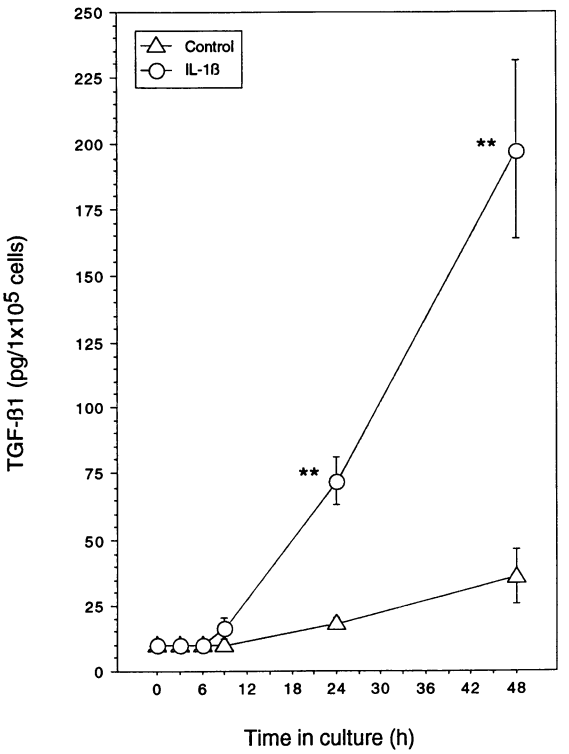

Figure 2. *Time-dependent generation of TGF-β1 by HPMCs. HPMCs were treated for various time periods with IL-1β (10 ng/ml) or cultured without IL-1β. Data are the means (±SEM) of TGF-β1 release expressed as pg/10⁵ cells, from HPMCs cultured from three separate donors.* **P < 0.01.

detectable in any of the cytokine-treated HPMC cultures.

The induction of TGF-β secretion by IL-1β was time dependent. The release of TGF-β1 was significantly ($P < 0.01$) above background generation by 24 hours and continued to rise up to 48 hours (Figure 2). TGF-β2 was below the detection limit in the time course before 48 hours of incubation. The release of TGF-β1 in response to IL-1β was also dose dependent. A significant induction was already achieved with an IL-1β dose of 0.01 ng/ml ($P < 0.05$). At doses of 1 ng/ml IL-1β and above, the generation of TGF-β1 was maximal and showed a plateau (Figure 3).

Western Blot Analysis

The identity and molecular weight of secreted TGF-β1 was further characterized by Western blot analysis of concentrated (times six) HPMC supernatants. By the use of a specific anti-TGF-β1 antibody we detected an immunoreactive band at 25 kd after stimulation of HPMCs with IL-1β (Figure 4).

Determination of Secreted TGF-β Activity by Indirect Competitive Radioreceptor Assay

An indirect competition assay was used to analyze receptor reactivity of HPMC-derived TGF-β. These experiments were performed using HPMCs from two different donors. As shown in Figure 5, significant amounts of TGF-β activity could be detected in acidified supernatants of untreated control cultures. Treatment of HPMCs with IL-1β (10 ng/ml) resulted in a marked increase of TGF-β activity in the respective supernatants. The IL-1β-induced elevation of measured activity was 4-fold and 2.4-fold in the different HPMC cultures, respectively. Analyses of non-acidified samples revealed that TGF-β is also released in a biologically active form. Levels detected in these samples were 7 and 15% of those detected in acidified samples, respectively. This proportion remained essentially unaltered in cultures treated with IL-1β.

RT-PCR Analysis of HPMC mRNA

HPMC mRNA isolated from control and cytokine-stimulated cells was reverse transcribed and sub-

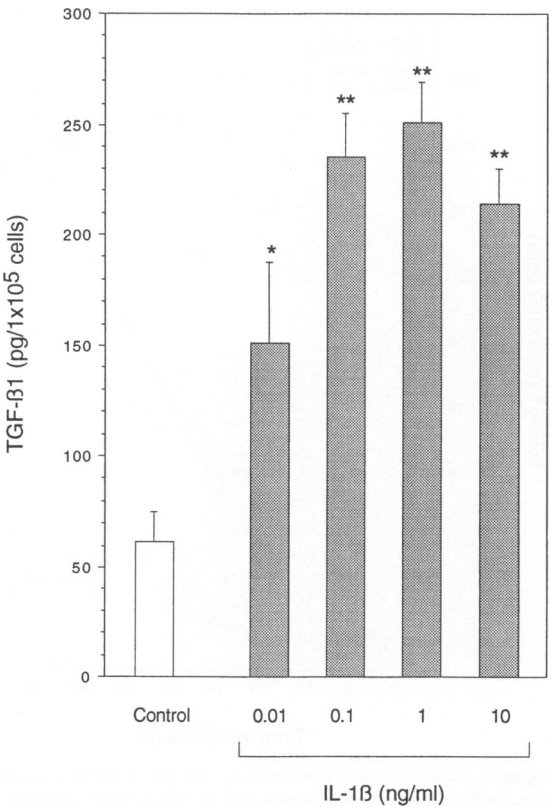

Figure 3. *Dose effect of IL-1β on the 48-hour generation of TGF-β1 by HPMCs. The data presented are the means (±SEM) of TGF-β1 release expressed as pg/10^5 cells, from HPMCs cultured from three separate donors.* **P < 0.01; *P < 0.05.

jected to PCR amplification to study the expression of TGF-β1 and TGF-β2 genes. In all cultures analyzed, these experiments demonstrated the constitutive expression of TGF-β1-specific transcripts of 186 bp and TGF-β2-specific transcripts of 331 bp (Figure 6). Treatment of HPMCs with IL-1β resulted in an increase of both TGF-β1 and TGF-β2 mRNA levels. Competitive PCR analyses were used to quantify the increase of the TGF-β1 and TGF-β2 mRNA levels after IL-1β stimulation in a time-dependent manner. As shown in Table 1, after 12 hours of treatment with IL-1β, the mean TGF-β1 mRNA signal was increased

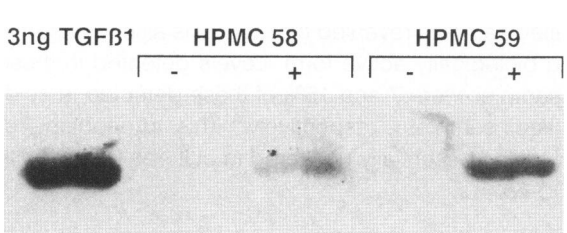

Figure 4. *Detection of TGF-β1 by Western blot analysis. Supernatants (sixfold concentrated) from HPMCs from two separate donors cultured for 48 hours in the presence of 10 ng/ml IL-1β (+) or without IL-1β (−) were analyzed. Human TGF-β1 purified from platelets was used for comparison.*

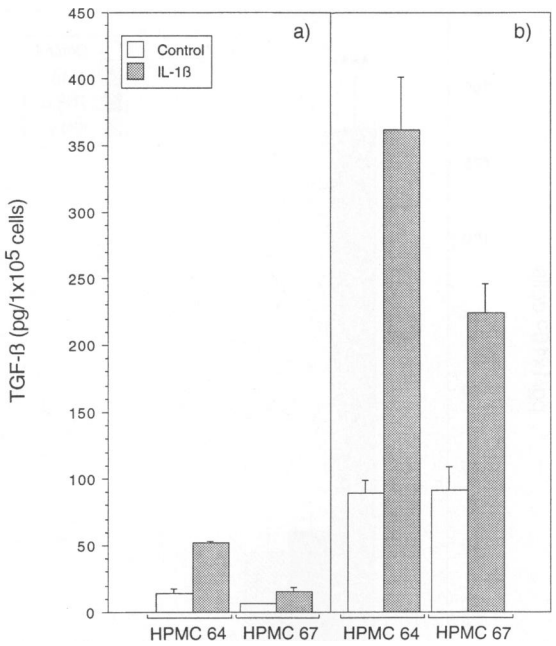

Figure 5. *Detection of TGF-β activity by indirect competition assay. HPMCs from two separate donors were cultured for 48 hours in the presence of IL-1β (10 ng/ml) or without IL-1β, and non-acidified* (a) *and acidified* (b) *supernatants were analyzed to detect TGF-β-receptor binding. The competition assay detects all TGF-β species known. TGF-β1 purified from human platelets was used as a standard. Data are the means (±SEM) of TGF-β1 activity, expressed as pg/10^5 cells, of five measurements.*

12-fold when compared with untreated HPMC cultures. Thereafter, levels remained elevated (approximately 10-fold) up to 48 hours. TGF-β2 mRNA levels were already increased after 3 hours of IL-1β treatment (mean 5.2-fold), although its induction was less pronounced than the induction of TGF-β1 mRNA (Table 1). The results of representative competitive RT-PCR analyses are shown in Figure 7.

Discussion

A variety of cellular components are believed to participate in the peritoneal response to injury, including resident and invading inflammatory cells, submesothelial connective tissue cells, blood vessels, and the mesothelial cell lining. The mesothelial cell layer serves as an anatomic barrier between the peritoneal cavity and the underlying connective tissue and is thought to play a key role in the transit of cells and in the formation and turnover of peritoneal fluid. More recent observations indicate that HPMCs are also an important source of regulatory cytokines such as IL-1, IL-6, and IL-8.[18–23]

The present data demonstrate that human peritoneal mesothelial cells express specific mRNA and synthesize immunoreactive TGF-β1 and TGF-β2 and that

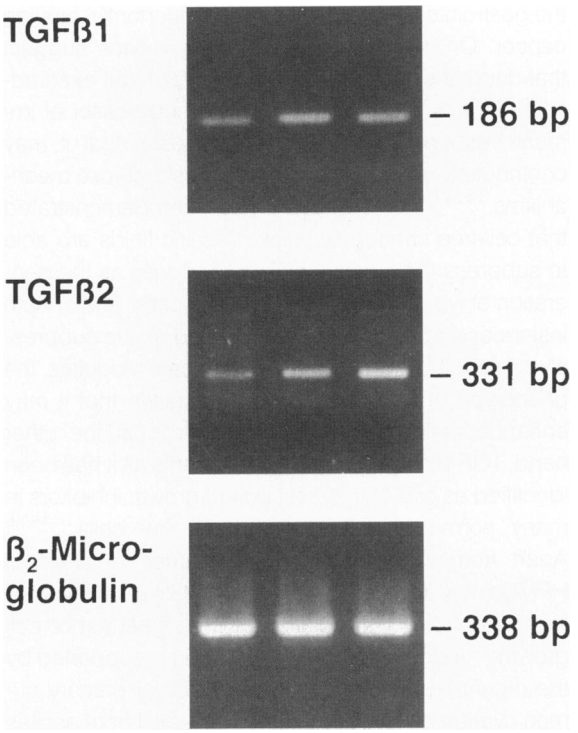

Figure 6. *Detection of TGF-β1-specific transcripts of 186 base pairs and TGF-β2-specific transcripts of 331 base pairs by RT-PCR. mRNA was isolated from HPMCs from three separate donors cultured for 24 hours. Detection of β_2-microglobulin mRNA was performed to ensure integrity of the mRNA.*

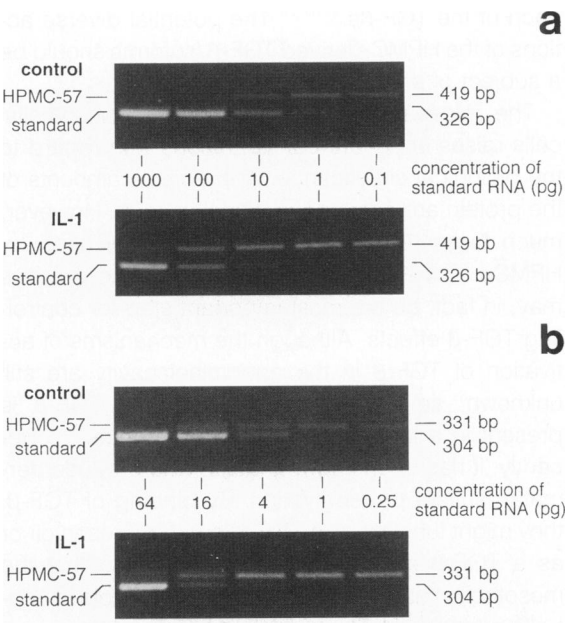

Figure 7. *Competitive RT-PCR of TGF-β1 mRNA* (a) *and TGF-β2 mRNA* (b) *in HPMCs cultured for 48 hours with IL-1β (10 ng/ml) or without IL-1β (control). Varying concentrations of standard RNA were added as competitive template.*

the secretion of both TGF-β isoforms can be markedly increased by treatment of HPMCs with IL-1β. TGF-β1 was secreted in appreciable amounts in a time-dependent and constitutive manner, whereas TGF-β2 levels were much lower, being detectable in only three of six separate HPMC cultures. The induction of TGF-β secretion by IL-1β appeared to be time and dose dependent. Western blot analyses of medium conditioned by HPMCs identified an immunoreactive band at 25 kd, which is of similar molecular mass to that previously described for TGF-β1 in other cell types.[9,10] An indirect competition assay demonstrated receptor-binding activity of HPMC-derived TGF-β. The capacity of HPMCs to synthesize TGF-β was confirmed by RT-PCR analysis of HPMC mRNA transcripts. Using specific oligonucleotide primers, we identified TGF-β1- and TGF-β2-specific transcripts that were significantly increased in amount after IL-1β treatment.

Table 1. *Densitometric Quantification of TGF-β1 and TGF-β2 mRNA Expression in HPMCs Treated with IL-1β*

Time in culture (hours)	Relative expression of mRNA	
	TGF-β1 mRNA	TGF-β2 mRNA
3	1.9 ± 0.7	5.2 ± 1.7
12	12.1 ± 3.9	4.2 ± 1.7
24	8.9 ± 2.5	6.6 ± 2.6
48	9.5 ± 2.9	4.6 ± 2.0

The mRNA expression of untreated cultures at each time point was set as 1, and the respective levels under IL-1β treatment (10 ng/ml) are given as relative values. Data are the means ± SD of two separate experiments using HPMCs from different donors.

The synthesis of TGF-β has previously been described in numerous cell types including macrophages, fibroblasts, endothelial cells, and pleural mesothelial cells.[9,10,14,30,33,34] Our data further support the concept that peritoneal mesothelial cells by the release of cytokines may be actively involved in the regulation of inflammatory or neoplastic diseases affecting the peritoneum. Considering the huge surface area of the abdominal cavity, it is tempting to suggest that the lining mesothelium may be a prominent source of TGF-β detected in ascitic fluids.[5–8] The induction of TGF-β secretion by IL-1β seems to be of considerable importance as *in vivo* IL-1β may be released by resident and invading phagocytes as well as, in the case of malignancy, by floating or invading tumor cells and thus potently foster TGF-β synthesis and secretion by HPMCs.[18,24–26] Previous studies have shown that the synthesis of various cytokines by HPMCs is induced by the proinflammatory cytokines IL-1β and TNF-α.[18–23] TGF-β secretion, however, was stimulated only by IL-1β, possibly suggesting a more conservative regulation of this cytokine. Both isoforms, TGF-β1 and TGF-β2, have been shown to display similar activity and potency in many different cells. However, some cells respond selectively, indicating additional specific roles for

each of the TGF-βs.[9,10,14] The potential diverse actions of the HPMC-derived TGF-β isoforms should be a subject of additional investigations.

The release of TGF-β by peritoneal mesothelial cells raises important considerations with regard to the biological significance. Appreciable amounts of the protein are secreted in bioactive form. However, much larger quantities of TGF-β are released by HPMCs in the latent form. The activation process may, in fact, be the most important step for controlling TGF-β effects. Although the mechanisms of activation of TGF-β in the abdominal cavity are still unknown, several reports confirm that TGF-β is present in a bioactive form in ascitic fluids.[5–8] Recently, it has been shown that HPMCs produce dermatan sulfate proteoglycans. By binding of TGF-β, they might function as a TGF-β long-term reservoir or as a TGF-β clearance system, indicating that the mesothelium itself may play an important role in controlling bioavailability of secreted TGF-β.[35]

TGF-β is an important regulator of the immune and inflammatory systems and exhibits pro- and anti-inflammatory activities.[14] It potently chemoattracts leukocytes and induces IL-1 mRNA expression.[36] After initiation of an inflammatory response, however, it exerts anti-inflammatory effects including inhibition of neutrophil and T lymphocyte adhesion to the endothelium, deactivation of macrophages, and antagonism of TNF-α and IL-1 function.[14,37–43] These anti-inflammatory properties of TGF-β may *in vivo* be further enhanced by IL-6, a cytokine that is produced in large quantities by IL-1- or TNF-α-stimulated HPMCs.[20,23,44] The pivotal homeostatic importance of TGF-β in the regulation of inflammation is highlighted by the recent finding that in transgenic mice both targeted disruption and overexpression of the TGF-β1 gene result in multifocal excessive inflammatory reactions.[45–48] In mice lacking the TGF-β1 gene, inflammation also affects serosal surfaces of internal organs supporting the view that HPMC-derived TGF-β is involved in the initiation and resolution of inflammatory responses in the peritoneum.[45]

More than any other growth factor, TGF-β has profound effects on the synthesis and degradation of the extracellular matrix. It stimulates the synthesis of all major matrix proteins and decreases matrix degradation.[9,10,49] Despite their location on the peritoneal surfaces, HPMCs might thus, via the release of TGF-β, play an important role in the promotion of peritoneal repair processes and also be involved in fibroproliferative reactions leading to peritoneal adhesions or peritoneal fibrosis.[50–52]

The peritoneal cavity represents a route for dissemination of various neoplasms including carcinomas of the gastrointestinal tract and, most importantly, ovarian cancer. On one hand, several observations suggest that under these circumstances TGF-β might exert adverse effects. As TGF-β is a potent suppressor of immune responses, it has been proposed that it may contribute to a weakening of local host defense mechanisms.[14,37,38,53,54] In fact, it has been demonstrated that cell-free cancer-associated ascitic fluids are able to suppress the function of T cells as well as the generation of lymphokine-activated killer cells, and in both instances ascitic TGF-β was identified as the suppressive factor.[5,7,8] In addition, TGF-β can modulate the phenotype of tumor cells and it is known that it may enhance tumor cell invasiveness.[16,17] On the other hand, TGF-β may exert beneficial effects as it has been identified as one of the most potent growth inhibitors in many normal and malignant epithelial cells.[9–15,55] Apart from their function as a barrier to invasion, HPMCs may thus, via the release of TGF-β, be involved in the control and regulation of peritoneal tumor cell growth.[16] Indeed, the latter hypothesis is supported by the recent finding that the proliferation of primary human ovarian cancer cell cultures obtained from ascites is strongly inhibited by TGF-β.[56]

The data presented support the concept that HPMCs are significantly incorporated in the cytokine network operating in peritoneal diseases. Additional studies are necessary to better define the precise functions of HPMC-derived TGF-β.

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