Basic Fibroblast Growth Factor 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 an important source for various cytokines and growth factors. This study was conducted to analyze the capacity of human peritoneal mesothelial cells (HPMCs) to synthesize and release basic fibroblast growth factor (bFGF) and to characterize its regulation by inflammatory cytokines. HPMC constitutively synthesized and released considerable amounts of bFGF as detected by a specific immunoassay. Almost 80% of bFGF (1547 ± 173 pg/ 10^5 cells) was localized intracellularly. Approximately 20% of the bFGF (357 ± 27 pg/ 10^5 cells) was associated with extracellular matrix components on the HPMC surface. Small amounts of bFGF ($<1\%$) were detectable in tissue culture supernatants (8.4 ± 1.4 pg/ 10^5 cells). Treatment of HPMCs with interleukin-1 β (IL-1 β ; 1 ng/ml) resulted in a significant increase in bFGF production. The intracellular bFGF content showed a rapid but only transient increase, which was significant above background levels after 24 hours (41% increase; $P < 0.05$). This increase in intracellular bFGF concentration was associated with an induction of the release of bFGF. Within 96 hours, the release of bFGF to the cell surface and into the supernatant increased by 58% (564 ± 52.4 pg/ 10^5 cells; $P < 0.01$) and by 214% (26.4 ± 3.2 pg/ 10^5 cells; $P < 0.001$), respectively. Neither tumor necrosis factor- α nor interferon- γ affected bFGF synthesis by HPMCs. Stimulation of HPMCs with IL-1 β increased steady-state levels of bFGF-specific mRNA. Immunohistochemical analyses of peritoneal tissue revealed constitutive expression of bFGF by HPMCs. This *in situ* expression proved to be most pronounced in areas of serosal inflammation in activated HPMCs. Our study demonstrates that HPMCs synthesize and release significant amounts of bFGF

and that the expression of this growth factor is significantly up-regulated by the proinflammatory cytokine IL-1 β . The data support the view that HPMCs are key regulators of abdominal disease processes such as peritonitis, peritoneal fibrosis, or peritoneal tumor metastasis. (Am J Pathol 1999, 155:1977–1984)

The peritoneal mesothelium is composed of a monolayer of specialized epithelial cells that extends over the entire surface of the abdominal cavity. It serves as a protective anatomical barrier and as a frictionless interface for the movement of organs and tissues, and it is involved in the formation and turnover of abdominal fluid.^{1,2}

Injury to the peritoneal cavity is a common phenomenon. It can be caused by trauma, surgery, infection, or various malignancies and is associated with serosal inflammation. Inflammatory disturbance of the integrity of the mesothelial cell lining triggers a series of reparative processes that may ultimately result in healing, peritoneal fibrosis, or intra-abdominal adhesions. Frequently, this is associated with serious or even fatal complications, like intestinal obstruction and ileus formation.^{1,2} The mechanisms of the peritoneal response to injury and the factors that determine the outcome of the reactive/reparative processes of the peritoneum are still poorly defined. Increasing evidence suggests that human peritoneal mesothelial cells (HPMCs) play a key role in the initiation and control of disease processes affecting the abdominal cavity.^{3–8} They are uniquely located to regulate the proliferation of submesothelial connective tissue cells and blood vessels, and they are potent producers of various regulatory cytokines like macrophage-colony stimulating factor, interleukin-1 β (IL-1 β), IL-6, and IL-8.^{9–15} We have previously shown that HPMCs also produce significant amounts of transforming growth factor- β (TGF- β), a mul-

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tifunctional growth factor that is considered to regulate peritoneal inflammation and repair processes.^{16,17} Several studies suggest that neoangiogenesis and tissue repair require an intimate interaction and cooperation of TGF- β with basic fibroblast growth factor (bFGF).^{18–22}

Basic FGF is the prototype of the fibroblast growth factor family, a group of structurally related polypeptides that presently consists of 18 members.^{23–27} Basic FGF shows high affinity for heparin and glycosaminoglycans and has been isolated from various normal and malignant tissues.^{23–32} This growth factor is mitogenic for various cell types including fibroblasts, smooth muscle cells, and endothelial cells, and it is one of the most potent inducers of the formation of mesenchyme and new blood vessels.^{23,24,29,30,33–35} However, up to now, the generation of bFGF by HPMCs has not been defined. Although HPMCs may be a potential source of bFGF, the regulation of its synthesis may rest with proinflammatory cytokines released by invading inflammatory phagocytes.

Thus, this study was conducted to analyze the potential synthesis of bFGF by HPMCs and to examine its regulation in response to the inflammatory cytokines IL-1 β , tumor necrosis factor- α (TNF)- α , or interferon- γ (IFN- γ).

Materials and Methods

Isolation and Culture of HPMCs

HPMCs were obtained from omental tissue of consenting patients undergoing elective abdominal surgery. Mesothelial cells were isolated by a protocol described previously.³⁶ Briefly, small biopsy specimens of omental tissue (approximately 1 cm³) were rinsed in phosphate-buffered saline (PBS) at 4°C and immediately transported to the cell culture laboratory. The tissue was 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. Subsequently, the fat tissue was removed, and the collagenase solution containing the detached mesothelial cells was filtered through a 200- μ m cell strainer. Cells were then collected by centrifugation (400 $\times$ g, 10 minutes) and suspended in RPMI-1640 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 μ g/ml; Sigma Chemical Co., St. Louis, MO). These cells were then cultured in 25-cm² tissue culture flasks (Greiner, Kremsmuenster, Austria) at 37°C in a humidified atmosphere of 5% CO₂ in air. The purity and phenotype of the HPMC cultures were confirmed by flow cytometry using monoclonal antibodies against cytokeratin subtypes 8 and 18 (monoclonal antibody (mAb) CAM 5.2; Becton Dickinson, Mountain View, CA). A contamination by endothelial cells or phagocytes was excluded using mAbs against CD68 and CD34 (Becton Dickinson).¹⁴ Passage 2 or passage 3 cultures were used for experiments.

Induction of bFGF Production by HPMCs

For experiments, HPMCs were detached with trypsin/versene (0.05%/0.02%), washed once in medium, and seeded into 6-well tissue culture dishes in a volume of 3 ml of culture medium. After confluency, the monolayers were washed twice with fresh medium and then incubated for 24 to 96 hours in the presence or absence of the appropriate cytokines. Recombinant IL-1 β and recombinant TNF- α were purchased from Sigma. Recombinant IFN- γ was kindly provided by Dr. G. Adolf (E. Boehringer Institute, Vienna, Austria). Incubation of the cells with cytokines for up to 96 hours did not have any significant effect on cell viability as shown by trypan blue exclusion. All experiments were run in triplicate. At the end of incubation periods, supernatants and cells were removed, prepared, and stored for the analyses of bFGF protein levels.

Determination of bFGF by Enzyme-Linked Immunosorbent Assay (ELISA)

The level of bFGF production under different experimental conditions was determined by using the solid-phase ELISA Quantikine Human Basic Immunoassay (R&D Systems, Minneapolis, MN) as described previously.³² The assay was performed according to manufacturer's instructions. All ELISA measurements were routinely performed on an SLT 400 ATC ELISA reader (Lab Instruments, Salzburg, Austria). Basic FGF levels were determined extracellularly in supernatants and in the trypsin-releasable fraction (cell surface-bound, pericellular bFGF), as well as intracellularly in cellular extracts as described previously.³² Supernatants were removed from culture dishes, centrifuged to eliminate any residual cells (1500 $\times$ g, 10 minutes), and stored at -70°C until assayed. For the preparation of cell surface-bound pericellular bFGF, the HPMC monolayers were washed in PBS and enzymatically detached using 200 μ l of trypsin/versene (0.05%/0.02%) in PBS. Proteolysis was stopped after 5 minutes by adding equal amounts of culture medium containing 10% fetal calf serum, and aliquots were collected to determine cell number and cell viability, using a Casy cell counter (Casy, Schaefer, Reutlingen, Germany) and the trypan blue exclusion test. Thereafter, remaining cells were harvested by centrifugation (1500 $\times$ g, 5 minutes), and supernatants were frozen separately at -70°C for analyses. These supernatants contained the so-called trypsin-releasable fraction, ie, cell surface-bound pericellular bFGF-heparansulfate complexes, which are known to be resistant to proteolysis.^{32,37,38} To assure identical solutions for all samples, cellular extracts were prepared by resuspending remaining cell pellets in culture medium containing freshly added phenylmethylsulfonyl fluoride (1 mmol/L) and by subsequent homogenization with an ultrasound disintegrator (Branson Sonifier 250, Danbury, CT). Finally, cell debris was removed from lysates by centrifugation (1500 $\times$ g, 10 minutes), and cell extracts were collected and stored at -70°C

Table 1. Primer Sequences for bFGF and $\beta 2$ Microglobulin

Fragment	Primer sequence	Fragment length	cDNA bases
bFGF*	Sense 5'-GGCTTCTTCTGCGCATCCAC-3'	136bp	451-471
	Antisense 5'-GGTAACGGTTAGCACACTCCTTC-3'		586-563
$\beta 2$ -microglobulin*	Sense 5'-*ATGCCTGCCGTGTGAACCATGT-3'	284bp	306-372
	Antisense 5'-AGAGCTACCTGTGGAGCAACCT-3'		592-530

Both sense primers were labeled with the ABI fluorescent dye FAM at the 5' end.

*cDNA GeneBank accession numbers: bFGF, M279681; $\beta 2$ -microglobulin, AB021288.

until use. The lower detection limit of the ELISA was 5 pg/ml for human bFGF.

Determination of bFGF mRNA by Reverse Transcriptase-Polymerase Chain Reaction (RT-PCR)

A bFGF cDNA fragment and, as an internal control, a cDNA fragment of the housekeeping gene $\beta 2$ -microglobulin were coamplified in a duplex PCR, and the bFGF/ $\beta 2$ microglobulin ratio was determined using the Applied Biosystems Gene Scan Equipment (Applied Biosystems, Inc., (ABI), Foster City, CA). Total RNA was extracted using the SV-Total RNA isolation kit according to the suggestions of the supplier (Promega, Madison, WI). This guanidinium thiocyanate-solid phase adsorption-based RNA isolation procedure includes a DNase digestion step for isolation of genomic DNA-free RNA. Complementary DNA synthesis with 0.5 μ g of total RNA and random hexanucleotide primers was performed by a previously published protocol.^{39,40}

Duplex PCR was performed using 2 μ l of this cDNA solution in a final volume of 50 μ l of PCR reaction buffer (10 mmol/L Tris-HCl, pH 8.8, 1.5 mmol/L $MgCl_2$, 50 mmol/L KCl, 0.1% Triton X-100, 0.1 mmol/L of each deoxynucleotide triphosphate, 1.25 U Dynazyme Polymerase (Finnzyme, Oy, Finland) and 12.5 pmol of each primer). Amplification was started with the bFGF primers alone for 11 amplification cycles. Thereafter $\beta 2$ -microglobulin primers were added, and the amplification was continued for another 20 cycles. The thermocycler program used was as follows: A precycle of 2 minutes at 94°C followed by 31 cycles of 25 seconds at 94°C, 20 seconds at 96°C, 1 minute at 57°C, and 30 seconds at 73°C, and a postcycle of 2 minutes at 73°C. The oligonucleotide primers for bFGF and $\beta 2$ microglobulin cDNA fragments are given in Table 1. The primers hybridize to cDNA sequences encoded from different exons. The identity of amplified fragments was confirmed by DNA sequencing. Controls included PCR amplifications with and without cDNA from LNCaP cells that do not express bFGF.³² After amplification, samples were diluted in formamide. Fragments were then separated on a polyacrylamide sequencing gel, using the ABI 370A DNA sequencer, and fluorescence intensities of both fragments were measured by using the ABI GeneScan software. Results are expressed as a bFGF/ $\beta 2$ microglobulin ratio.

In Situ Detection of bFGF

Basic FGF was detected *in situ* by immunohistochemistry in normal and inflamed human omental tissue of consenting patients. Acutely inflamed peritoneal tissue was derived from two patients with salpingitis and two patients with perforating diverticulitis. The tissue was fixed overnight in 4% formalin, dehydrated, and embedded in paraffin. Immunohistochemical staining was performed by a streptavidin-biotin peroxidase protocol as described previously.³² Briefly, a polyclonal rabbit antibody directed against amino acids 40-63 of the amino-terminal region of bFGF (Santa Cruz Biotechnologies, Santa Cruz, CA) was applied to tissue sections (dilution of 1:100 in PBS) inside a humidified chamber at room temperature. After incubation for 60 minutes and two washes in PBS to remove excess antibody, sections were treated with a biotinylated goat anti-rabbit immunoglobulin (Dakopatts, Glostrup, Denmark) at a dilution of 1:500 for 60 minutes. This procedure was followed by incubation with peroxidase-labeled streptavidin (1:800 in PBS) for 30 minutes. The enzymatic reaction was then developed in a freshly prepared solution of 3'-diaminobenzidine (0.5 mg/ml) and 0.1% H_2O_2 for 5 minutes. Finally, sections were counterstained with hemalaun, dehydrated, cleared in xylene, and mounted. The specificity of the immunohistochemical detection was controlled by the addition of a 100-fold excess of human recombinant bFGF (Life Technologies, Inc., Paisley, UK) to the primary antibody solution before incubation. Serial sections were furthermore stained with H&E and immunohistochemically with the mAb CAM 5.2 to locate and define the mesothelium.

Statistical Analyses

Data were analyzed using the Mann-Whitney *U* test. All statistical analyses were performed on an Apple Macintosh computer using Statview version 4.2 (Abacus Concepts, Berkeley, CA).

Results

Synthesis of bFGF by HPMCs

Basic FGF was measured by a specific ELISA in mesothelial cell cultures from five different donors. In all cases, HPMCs were shown to constitutively produce con-

Figure 1. Cellular content and release of bFGF by HPMCs. Cells were treated for 96 hours with the proinflammatory cytokines IL-1 β , TNF- α , or IFN- γ (1 ng/ml each). bFGF was measured in duplicate by ELISA in the supernatants (Supernatant), the trypsin-releasable fraction (Pericellular), and cellular extracts (Intracellular). Results are the mean of bFGF expressed as pg/10⁵ cells, from HPMCs cultured from five separate donors. *** $P < 0.001$, ** $P < 0.01$.

siderable amounts of bFGF (Figure 1). Almost 80% of the protein was localized intracellularly. The mean level detected in the cytoplasm within a culture period of 96 hours was 1547 ± 173 pg/10⁵ cells (all mean values are reported $\pm$ SE). Approximately 20% of the bFGF was associated with extracellular matrix components on the cellular surface of the HPMCs and could be released by trypsin treatment. The mean level detected within this trypsin-releasable pericellular compartment was 357 ± 27 pg/10⁵ cells. Only small amounts of the bFGF synthesized by HPMCs (<1%) were found in the supernatant. The mean level within 96 hours of culture was 8.4 ± 1.4 pg/10⁵ cells.

Induction of bFGF by IL-1 β

Treatment of HPMCs with the proinflammatory cytokine IL-1 β resulted in a significant elevation of both the production and release of bFGF (Figure 1). After 96 hours, the mean level induced by IL-1 β (1 ng/ml) was 26.4 ± 3.2 pg/10⁵ cells in supernatants and increased by 214% ($P < 0.001$). The amount of pericellular bFGF increased by 58% ($P < 0.01$), resulting in a mean level of 564 ± 52.4 pg/10⁵ cells. The cytoplasmic content of bFGF was also increased with a mean level of 1886 ± 269 pg/10⁵ cells. However, this was not significant when analyzing the cells at 96 hours of culture. Neither TNF- α nor IFN- γ (1 ng/ml each) had any significant effect on bFGF synthesis by HPMCs.

The induction of bFGF by IL-1 β was time-dependent (Table 2). The intracellular bFGF content increased rapidly and was already significantly above background generation by 24 hours (2136 ± 137 pg/10⁵ cells; $P < 0.05$). However, within the following 72 hours, the amount of intracellular bFGF showed a continuous decline and did not significantly differ from the controls. Conversely, bFGF levels in supernatants increased continuously with time in culture, and they were maximally elevated 96 hours after initiation of the inductive treatment with IL-1 β . The level of surface-bound bFGF also rapidly increased over the baseline level, but without significant relation to the culture periods.

The production and release of bFGF in response to IL-1 β was also dose-dependent (Table 3). A slight in-

Table 2. Time-Dependent Generation of bFGF by HPMCs

	Time in culture (hours)		
	24	48	96
Supernatant			
Control	8 $\pm$ 2	6 $\pm$ 2	6 $\pm$ 1
IL-1 β (1 ng/ml)	17 $\pm$ 2	22 $\pm$ 2*	27 $\pm$ 3 [†]
Pericellular			
Control	434 $\pm$ 84	403 $\pm$ 38	369 $\pm$ 29
IL-1 β (1 ng/ml)	503 $\pm$ 43	480 $\pm$ 34	499 $\pm$ 49
Intracellular			
Control	1506 $\pm$ 163	1356 $\pm$ 129	1456 $\pm$ 120
IL-1 β (1 ng/ml)	2136 $\pm$ 137 [†]	1813 $\pm$ 222	1558 $\pm$ 236

Results are the means ($\pm$ SE) of bFGF expressed as picograms per 10⁵ cells, from HPMCs cultured from three separate donors.

* $P < 0.01$, [†] $P < 0.05$ when compared with the respective controls.

crease in bFGF levels in the various compartments was already apparent with an IL-1 β dose of 0.01 ng/ml. At a dose of 1 ng/ml, the inductive effect of IL-1 β was maximal.

RT-PCR Analysis of HPMC mRNA

To study bFGF gene expression, mRNA was isolated, reverse transcribed from control and cytokine-treated HPMCs, and subjected to PCR amplification. Basic FGF-specific transcripts of 136 bp were detected in all cultures analyzed (Figure 2). To compare the expression of bFGF mRNA under control conditions with cultures treated with IL-1 β , we determined bFGF mRNA in relation to the mRNA of the housekeeping gene β 2-microglobulin. Treatment of HPMCs with IL-1 β resulted in a rapid increase of bFGF mRNA. As shown in Figure 3, this was already detectable after 3 hours of IL-1 β treatment (1 ng/ml). After 12 hours, the mean bFGF signal was increased 3.5-fold when compared with untreated cultures of HPMCs. Thereafter, levels remained elevated (2.2-fold) up to 24 hours.

In Situ Detection of bFGF in Human Peritoneal Tissue

Basic FGF was detected *in situ* in a series of peritoneal biopsies obtained from morphologically normal or inflamed omental tissue. Normal HPMCs lining the peritoneal surface exhibited a flat morphology and showed a constitutive but weak expression of bFGF (Figure 4). The protein was primarily detected intracellularly. In biopsies that were altered by inflammation, the mesothelium exhibited a marked increase of the bFGF protein expression. This was most pronounced when the mesothelial cell lining was actively infiltrated by inflammatory cells. In these areas, the mesothelial cells showed cellular swelling, loss of polarization, and intense cytoplasmic expression of bFGF. In submesothelial tissue, regardless of whether we were analyzing normal or inflamed peritoneum, we noticed only weak bFGF expression in occasional capillary endothelial cells. When an excess of human recombinant bFGF was added to the antibody solution before incubation, immunostaining was com-

Table 3. Dose Effect of IL-1 β on the 96-Hour Generation of bFGF by HPMCs

Compartment	Control (ng/ml)	IL-1 β (ng/ml)			
		0.01	0.1	1	10
Supernatant	8 $\pm$ 1	10 $\pm$ 1	23 $\pm$ 4*	26 $\pm$ 2*	24 $\pm$ 3*
Pericellular	326 $\pm$ 37	400 $\pm$ 33	475 $\pm$ 51	563 $\pm$ 87 [†]	564 $\pm$ 88 [†]
Intracellular	1536 $\pm$ 286	1572 $\pm$ 215	1947 $\pm$ 284	2013 $\pm$ 448	1639 $\pm$ 401

Results are the means ($\pm$ SE) of bFGF expressed as picograms per 10⁵ cells, from HPMCs cultured from three separate donors.

* $P < 0.01$, [†] $P < 0.05$ when compared with the respective controls.

pletely inhibited, indicating that the immunoreaction was specific.

Discussion

Increasing evidence suggests that the peritoneal mesothelial cell lining must be considered as a central ele-

ment of the cytokine network controlling disease processes of the abdominal cavity.⁴ HPMCs are uniquely located to interact with noxious agents, inflammatory cells, and the cellular components of the submesothelial connective tissue, and HPMCs constitute a bioactive cellular membrane that is an important source of a variety of cytokines.^{6,7,9-15} The present data further support this concept.

We demonstrate that human peritoneal mesothelial cells express specific mRNA and synthesize immunoreactive bFGF and that the production of total bFGF can be significantly increased by stimulation of HPMCs with the proinflammatory cytokine IL-1 β . The induction of intracellular bFGF by IL-1 β showed a rapid but transient increase, which was statistically significant after 24 hours. In addition, our data show that the production and release of bFGF can be increased in a dose-dependent manner by IL-1 β . RT-PCR analyses confirmed the capacity of HPMCs to synthesize bFGF and revealed an increased amount of bFGF-specific mRNA transcripts after treatment with IL-1 β . Immunolocalization showed that bFGF protein expression by HPMCs was markedly increased in acute inflammation of the peritoneum.

Previous *in vitro* studies have focused on the compartmentalization of bFGF in mammalian cells, and they have demonstrated that bFGF is distributed intracellularly and extracellularly in a ratio of approximately 4:1.^{32,37,38} The present data are in agreement with these observations. *In vitro*, almost 80% of bFGF was localized in the cytoplasm of HPMCs. This was confirmed by immunohistochemical analyses of HPMCs in tissue sections, which revealed predominantly cytoplasmic staining. Only minimal amounts of this growth factor were released into tissue culture supernatants. This is probably because bFGF lacks a specific signal peptide region for secretion.⁴¹ Cellular injury and/or death has been proposed as a possible mechanism for the release of bFGF.⁴² However, in cell culture experiments in which cell death could be excluded, it has been shown that bFGF can be actively released via exocytosis.⁴³ Vlodavsky and coworkers suggested alternative mechanisms, such as transport in association with cell surface or matrix components.^{31,44} It has been shown recently that HPMCs synthesize and secrete significant amounts of proteoglycans, which may provide a basis for the release of bFGF by these cells.⁵ The concept that bFGF is transported to the cell surface in association with cellular matrix proteins is supported by the observation that about 20% of total bFGF was found in the pericellular, trypsin-releasable compartment of HPMCs. Despite their role in the release of bFGF, mesothelial proteoglycans may furthermore act to se-

Figure 2. Detection of bFGF-specific transcripts by RT-PCR. Total RNA was isolated from HPMCs from three separate donors, reverse transcribed to cDNA, and specific cDNA fragments of the bFGF and the β 2-microglobulin cDNAs were amplified in a duplex PCR, using fluorescence-labeled primers as described in Material and Methods. The products were separated on a sequencing gel by means of the ABI 370A sequencer and quantified by using ABI GeneScan software. The figure presents a black and white picture of the virtual gel picture obtained with ABI GeneScan software, showing the bFGF and the β 2-microglobulin fragments amplified from three samples.¹⁻³ All PCR reactions were performed in duplicate.

Figure 3. Quantification of bFGF mRNA expression in HPMC treated with IL-1 β . HPMCs were treated with IL-1 β for 3, 12, and 24 hours, respectively. bFGF-specific mRNA levels were determined quantitatively as described in Material and Methods in relation to the housekeeping gene β 2-microglobulin. Results are expressed as bFGF/ β 2-microglobulin ratios and represent the mean values of mesothelial cultures derived from three separate donors.

Figure 4. Immunohistochemical detection of bFGF in peritoneal tissue. **A-C:** Normal peritoneal tissue covered by a flat layer of mesothelial cells. **A:** The cells are identified by their expression of the cytokeratin subtypes 8 and 18. **B:** Section stained with H&E. **C:** Serial section next to section in **B** stained with a polyclonal antibody against bFGF. HPMC showed a weak expression of bFGF. Weak positivity was also noticed in capillary endothelial cells of the submesothelial tissue. **D-F:** Acute peritoneal inflammation. **D:** HPMCs are infiltrated by polymorphonuclear cells and covered by an exudate of fibrin. **E:** The cells appear activated with enlarged cytoplasm and are highlighted by staining for cytokeratin subtypes 8 and 18. **F:** Serial section next to section in **E** revealed increased expression of bFGF in activated HPMCs. Granulocytes were negative. Only weak positivity was noticed in the submesothelial tissue. Original magnification, $\times 400$.

questerate and protect bFGF within the extracellular matrix.³¹ However, *in vivo*, HPMC-derived bFGF may become available by direct release, possibly also due to cellular injury, or by an indirect release of the peptide through degradation of extracellular matrix components by inflammatory or tumor cells.³¹

The synthesis of bFGF has previously been described in various normal and malignant cell types.²³⁻³² Interestingly, our data demonstrate that HPMCs are a particularly rich source of bFGF. When comparing the production per cell, the amount of intracytoplasmic bFGF in HPMCs proved to be 12- to 50-fold higher than the amount detected in other cell types like arterial smooth muscle cells or prostate carcinoma cells.^{32,37}

The induction of the synthesis and release of bFGF by IL-1 β during inflammatory processes may play an important role, because IL-1 β can be released *in vivo* by invading or resident phagocytes. The most compelling evidence for its importance in peritoneal injury is the increase in bFGF expression in HPMCs in inflamed peritoneum. Overexpression of bFGF in inflammatory conditions has also been demonstrated in other organs like the pancreas and prostate and, in particular, in wound healing of the skin.^{32,45-47} In dermal wound healing, bFGF expression has been described almost exclusively in keratinocytes but not in the dermal granulation tissue, suggesting that the recovering surface epithelium is the most important source of this cytokines *in vivo*.⁴⁵ It is interesting that, in the peritoneum, an inner surface of the body, we detected bFGF also primarily within the covering epithelial lining, ie, the mesothelium.

The production of bFGF by human peritoneal mesothelial cells and its induction by IL-1 β raise important considerations about the biological significance. bFGF is a protein with mitogenic, angiogenic, and chemotactic

properties, and it is likely that it is involved at multiple levels in the control of peritoneal inflammation and repair processes. Recently, it has been shown that the proliferation of mesothelial cells is stimulated by bFGF *in vitro* and even more pronounced *in vivo*, which may constitute an important mechanism for the recovery of the integrity of the mesothelial cell lining after injury.⁴⁸

Both inflammatory and neoplastic disease processes affecting the abdominal cavity are associated with pronounced neoangiogenesis and stroma formation.^{1,7,49} One of the most important targets for HPMC-derived bFGF may thus be endothelial cells of the submesothelial capillary vessels. bFGF is an important inducer of neoangiogenesis, and it may contribute to the concerted expression of cytokines and proteolytic enzymes required for capillary formation.³⁵ Recent studies have shown that bFGF induces the expression of vascular endothelial cell growth factor in forming capillaries that may be an important mechanism to boost angiogenesis in the peritoneal lining tissues.⁵⁰ Within the newly formed vascularized connective tissue, the effects of bFGF may finally be counteracted by TGF- β , which may reverse the invasive stage of angiogenesis and contribute to vascular remodeling and the formation of quiescent capillaries.¹⁸⁻²⁰ TGF- β released from activated HPMCs may possibly be involved in this process.¹⁷

HPMC-derived bFGF may also be crucially involved in the development of fibrosis and adhesions within the peritoneum.^{12,51} It is a potent mitogen and chemoattractant for fibroblasts and may directly affect the submesothelial mesenchyme.^{23,24,30,34} Increasing evidence suggests, however, that fibrosis requires a complex cooperation of bFGF with TGF- β .^{21,22} Using an experimental model of subcutaneous tissue fibrosis, Shinozaki and coworkers have demonstrated that persistent fibrosis

was achieved only by the simultaneous application of bFGF and TGF- β .²² Recently, it has been shown that, in human kidney tubular epithelial cells, bFGF is able to induce the secretion of preformed TGF- β . TGF- β , in turn, induced bFGF gene expression and protein synthesis in these cells, and it was postulated that this positive feedback loop may be involved in the progressive nature of tissue fibrosis and scarring.²¹ The observation that, in HPMCs, the synthesis and release of both bFGF and TGF- β are up-regulated by the proinflammatory cytokine IL-1 β is intriguing and tempts us to suggest that similar positive feedback mechanisms may exist in HPMCs.¹⁷

In conclusion, the data presented here further support the concept of HPMCs as a central element of the cytokine network operating in diseases of the abdominal cavity. Detailed analyses of the interaction of bFGF and TGF- β and the interdependence of their synthesis and release by HPMCs are currently under investigation.

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