ORIGINAL ARTICLES

IDENTIFICATION OF GENE TRANSCRIPTS IMPLICATED IN PERITONEAL MEMBRANE ALTERATIONS

Alena Parikova,¹ Aniek Vlijm,³ Irena Brabcova,² Marijke de Graaff,³ Dirk G. Struijk,³ Ondrej Viklicky,¹
and Raymond T. Krediet³

Department of Nephrology,¹ Transplant Laboratory,² Transplant Center, Institute for Clinical and Experimental Medicine, Prague, Czech Republic; and Division of Nephrology Department of Medicine,³ Academic Medical Center University of Amsterdam, Amsterdam, The Netherlands

◆ **Background:** Permanent stimulation of the peritoneum during peritoneal dialysis (PD) is likely to result in increased expression of genes encoding proteins involved in inflammation and tissue remodeling. Peritoneal fibrosis and neoangiogenesis may develop.

◆ **Objective:** To assess highly expressed genes potentially involved in peritoneal alterations during PD treatment using an animal model.

◆ **Methods:** A PD catheter was implanted in 36 male Wistar rats after 70% nephrectomy. The rats were divided into 3 groups, exposed to dialysis solution for 8 weeks, and sacrificed 2 weeks later. Group B was exposed to a buffer, group D was exposed to a 3.86% glucose-based dialysis solution, and in group D+H, a second hit of intraperitoneal blood on top of the dialysis solution was given to induce the development of peritoneal sclerosis. Before sacrifice, peritoneal function was assessed. Omental tissue was obtained for analysis of gene expression using RT-qPCR.

◆ **Results:** Fibrosis scores, vessel counts, and peritoneal function parameters were not different between the groups. Genes involved in the transforming growth factor beta signaling pathway, cell proliferation, angiogenesis, and inflammation were more expressed ($p < 0.05$) in the D+H group. Almost no differences were found between the control groups. We identified 4 genes that were related to peritoneal transport.

◆ **Conclusion:** Already a mid-term peritoneal exposure, when no microscopical and functional alterations are present, provokes activation of gene pathways of cell proliferation, fibrosis, neoangiogenesis, and inflammation.

KEY WORDS: Peritoneal dialysis; peritoneal membrane alterations; peritoneal transport; fibrosis; angiogenesis; inflammation; gene expression.

The continuous exposure to peritoneal dialysis (PD) solutions compromises the biological function of the peritoneal membrane. It is attributed to the combination of extremely high glucose concentrations and the presence of glucose degradation products (GDP) (1). Effects of low pH are overestimated because, in PD patients, values exceeding the toxic range are already present immediately after inflow of a lactate-buffered dialysis solution (2). Permanent exposure of peritoneal tissues during PD results in functional and morphologic alterations (3). At the cellular level, the alterations in leukocytes, mesothelial cells, endothelial cells, and fibroblasts functions lead to disturbances in the release of cytokines, chemokines, and growth factors, causing dysregulation of pro-inflammatory and profibrotic pathways, impaired host defense, and the induction of carbonyl and oxidative stress (3). Peritoneal fibrosis and neoangiogenesis develop. Morphological alterations of the peritoneal membrane subsequently affect functional characteristics of the peritoneum. Increases in peritoneal solute transport and a decrease in ultrafiltration capacity have been demonstrated in long-term PD patients (4).

The aim of the present study was to identify genes involved in peritoneal membrane alterations during PD treatment and to investigate the relationship between gene expression and functional characteristics of the peritoneal membrane using an experimental model, which was intended for development of encapsulating peritoneal sclerosis (EPS).

MATERIALS AND METHODS

The experimental model aiming to develop EPS was used for the study by Vlijm *et al.* (5). In short, administration of blood as the second hit to rats with renal failure treated with

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Correspondence to: Alena Parikova, Department of Nephrology, Transplant Center, Institute for Clinical and Experimental Medicine, Videnska, Czech Republic.

alena.parikova@ikem.cz

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PD for 8 weeks was hypothesized to develop relevant functional and morphological abnormalities corresponding with EPS in PD patients.

Thirty-six male Wistar rats were randomly assigned to 3 groups ($n=12/\text{group}$): 1) exposure to Dianeal (3.86% glucose; Baxter Healthcare, Castlebar, Ireland) for a period of 8 weeks followed by intraperitoneal administration of 20 mL of blood obtained by heart puncture of male Wistar donor rats as a second hit (experimental group D+H); 2) exposure to Dianeal only (control group D); and 3) exposure to a biocompatible, glucose-free buffer (Physioneal without glucose; Baxter, Nivelles, Belgium) (control group B). The rats of the experimental group D+H were sacrificed 2 weeks after administration of the second hit (Figure 1). All rats underwent a catheter implantation and a 1-step 70% nephrectomy to induce renal failure as described previously (6). After the nephrectomy, the animals received a nephroprotective diet to avoid too rapid development of renal failure. All 3 groups were infused twice daily for 8 weeks with 30 mL preheated and heparinized (5 IU/mL) dialysis solution on weekdays, 10 mL in the morning and 20 mL in the afternoon, and once daily with an infusion of 20 mL during the weekends. Blood samples were drawn under isoflurane anesthesia every 2 weeks by tail vein puncture to monitor renal function. A standard peritoneal permeability analysis adapted for the rat (SPARa) was performed before sacrifice (7). All SPARa's were performed during a 4-hour dwell with a 3.86% Physioneal solution (Baxter Healthcare, Dublin, Ireland). This SPARa is comparable to the SPA in humans and provides information on peritoneal transport characteristics (8). Prior to the SPARa, urine was collected in a metabolic cage for 24 hours to calculate residual renal function. Afterwards, the number of intraperitoneal adhesions was assessed at autopsy, and omental tissue was obtained. The protocol was approved by the committee on animal experiments of the Academic Medical Center University of Amsterdam, and national guidelines on animal research were followed.

MEASUREMENTS

Plasma, effluent, and urine concentrations of urea and creatinine were measured by means of enzymatic methods on automated analyzers (Hitachi, Boehringer Mannheim,

Figure 1 — The timetable of the various groups. D+H (Dianeal + hit) group, control B (Physioneal without glucose) group, and control group D (Dianeal). SPARa = standard peritoneal permeability analysis adapted for the rat.

Germany). Glucose was measured on an autoanalyzer (SMA II; Technicon, Terrytown, NJ, USA) by the glucose oxidase-peroxidase method.

CALCULATIONS

Renal function was assessed by creatinine and urea clearances using 24-hour urine samples obtained in the metabolic cage, and plasma samples obtained prior to the SPARa. Peritoneal solute and fluid transport parameters were calculated as described previously (9). Mass transfer area coefficients of urea (MTAC) were calculated according to the Waniewski model (10). Glucose absorption was calculated as the difference between the amount of glucose instilled and the amount recovered, relative to the amount instilled. Net ultrafiltration is the difference between in-situ intraperitoneal volume at 4 hrs and the initial intraperitoneal volume. Net ultrafiltration rate is net ultrafiltration volume divided by the dwell time.

HISTOPATHOLOGY

Omental tissue obtained from each rat after the SPARa was fixed in freshly prepared 4% paraformaldehyde. Paraffin-embedded tissues were stained with hematoxylin/eosin and picro-sirius red (PSR; Gurr, BDH, UK), providing a brick-red staining of fibrillary collagen, to assess the amount of fibrosis by semiquantitative scoring. Briefly, perivascular, intersegmental, and submesothelial areas were judged (grade 0 = normal presence of fibrous tissue, 1 = mild fibrosis, 2 = moderate fibrosis, and 3 = severe fibrosis) and combined with an overall fibrosis score that could have a maximum of 9. Platelet endothelial cell adhesion molecule-1 antibody (PECAM-1, goat-anti-rat CD31, Santa Cruz, California, USA) was used to count the number of vessels per microscopic field in omental tissue of the rats. The vessel density was scored by using computer-aided morphometrics.

RNA ISOLATION AND REAL-TIME QUANTITATIVE RT-qPCR

Omental tissue obtained after the SPARa was dipped into tissue storage reagent RNeasy (Sigma, St. Louis, MO, USA), stored, and transferred at -80°C for further gene expression analysis. High quality of total ribonucleic acid (RNA) was prepared from the small portion (1 mm^2) of tissue samples using RNeasy Fibrous Tissue Mini Kit (QIAcube Qiagen, Hilden, Germany). Quantification of total RNA and control of purity were performed by means of spectrophotometry method (NanoDrop 2000; Thermo Fisher Scientific, Waltham, MA, USA). Ribonucleic acid was then reverse-transcribed using Superscript Reverse transcriptase II (Invitrogen; Thermo Fisher Scientific, Waltham, MA, USA) and the synthesized cDNA was subjected to quantitative reverse transcription (RT-qPCR) analysis. Quantitative RT-PCR was performed using a custom-made Taqman low density array (TLDA) (ABI Prism 7900 H.T. Sequence Detection system; Applied Biosystems, Thermo Fisher Scientific, Waltham, MA, USA) with fast protocol to analyze 45 selected target genes

TABLE 1
List of the 45 Selected Genes Tested and
Their Presumed Functions

	Gene name
Angiogenesis	
EREG	Epiregulin
FGF1	Fibroblast growth factor 1
FGF2	Fibroblast growth factor 2
FIGF	Vascular endothelial growth factor D
PGF	Placental growth factor
TGFA	Transforming growth factor alpha
VEGFA	Vascular endothelial growth factor A
VEGFB	Vascular endothelial growth factor B
VEGFC	Vascular endothelial growth factor C
Cell proliferation	
BMP3	Bone morphogenetic protein 3
BMP4	Bone morphogenetic protein 4
BMP6	Bone morphogenetic protein 6
CSF1	Colony stimulating factor 1 (macrophage)
CSF2	Colony stimulating factor 2 (granulocyte-macrophage)
FGF9	Fibroblast growth factor 9
FGF10	Fibroblast growth factor 10
HGF	Hepatocyte growth factor
IGF1	Insulin-like growth factor 1
IGF2	Insulin-like growth factor 2
TFF1	Trefoil factor 1
Immune response	
CSF3	Colony stimulating factor 3 (granulocyte)
CXCL1	Chemokine (C-X-C motif) ligand 1
IL1a	Interleukin 1 alpha
IL1b	Interleukin 1 beta
IL2	Interleukin 2
IL4	Interleukin 4
IL7	Interleukin 7
IL12a	Interleukin 12 subunit alpha
IL18	Interleukin 18
LIF	Leukemia inhibitory factor
Other growth factors	
FGF7	Fibroblast growth factor 7
GDF10	Growth differentiation factor 10
IL11	Interleukin 11
IL3	Interleukin 3
INHHA	Inhibin alpha
Fibrosis	
BMP1	Bone morphogenetic protein 1
BMP7	Bone morphogenetic protein 7
EGF	Epidermal growth factor
FGF4	Fibroblast growth factor 4
FGF8	Fibroblast growth factor 8
INHBA	Inhibin beta A
PDGFA	Platelet derived growth factor alpha polypeptide
TGFB1	Transforming growth factor, beta 1
TGFB3	Transforming growth factor, beta 3

chosen on the basis of relevance to the study. Preconfigured array cards were used that targeted relevant genes for specific biological processes known to be involved in peritoneal changes i.e. angiogenesis, fibrosis, and inflammation, as summarized in Table 1. Quantitative RT-PCR based on TLDA technology was carried out as described elsewhere (11). Real-time RT-qPCR data were quantified using the SDS 2.4 software package (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA), and relative gene expression values were determined using the comparative $2^{-\Delta\Delta Ct}$ method of the Relative quantification (RQ) Manager Software v 1.2.1 (Applied Biosystems; Thermo Fisher Scientific, Waltham, MA, USA) with normalization to endogenous control (GAPDH). One of the samples with good expression profile on all of target genes ($Ct < 35$ in all detectors) was used as a calibrator. Marked gene expression was considered to be present when the difference in the expression of a specific gene was more than 2-fold among groups.

STATISTICAL ANALYSIS

Data are presented as medians and 75% confidence intervals (CI), unless stated otherwise. Since only 6 from 45 selected genes passed normality using the Kolmogorov-Smirnov test, possible differences between the 3 groups were assessed with Kruskal-Wallis analysis, and followed by Dunn's post-hoc test for genes with a p value ≤ 0.01 . Spearman's rank correlation coefficient was used to analyze possible correlations between genes and peritoneal transport parameters.

RESULTS

Two rats in each group were dropped from the study due to catheter- or nephrectomy-related problems and 1 rat was

TABLE 2
Peritoneal Solute and Fluid Transport Parameters
Measured During the SPARa

	D+H (n=8)	D (n=7)	B (n=6)
MTAC _{urea} ($\mu\text{L}/\text{min}$)	339 (311–351)	262 (194–320)	264 (221–353)
GA (%)	61 (59–67)	62 (55–68)	55 (46–64)
TCUFR ($\mu\text{L}/\text{min}$)	84 (79–90)	71 (66–80)	85 (77–101)
NUFR ($\mu\text{L}/\text{min}$)	64 (56–75)	60 (47–70)	72 (59–98)
ELAR ($\mu\text{L}/\text{min}$)	18 (15–24)	6 (2–21)	8 (5–22)

SPARa = standard peritoneal permeability analysis adapted for the rat; D = Dianeal group; D+H = Dianeal + hit group; B = Physioneal without glucose (buffer); hit : 20 mL of blood obtained by heart puncture of male Wistar donor rats administered intraperitoneally; MTAC = mass transfer area coefficient; GA = glucose absorption; TCUFR = transcapillary ultrafiltration rate; NUFR = net ultrafiltration rate; ELAR = effective lymphatic absorption rate. An explanation of TCUFR and ELAR is given in reference 7. All solutions were provided by Baxter. Data are expressed as medians and interquartile ranges.

excluded because of the presence of more severe renal impairment than the others. Renal urea, median 0.71 mL/min (95% CI 0.64 – 0.76 mL/min) and creatinine clearance, median 1.80 mL/min (95% CI 1.70 – 1.94 mL/min, were similar in all 3 groups allowing a fair comparison. The remaining 29 rats underwent a SPARa. Eight rats did not complete the SPARa, apparently due to severe hypovolemia. Therefore, peritoneal transport was assessed in 21 rats. No differences were present in solute and fluid transport parameters between the groups as shown in Table 2.

Autopsy and peritoneal tissue collection could be performed in 29 rats. Fibrosis scores and vessel counts were not different

between the groups; however, intraperitoneal adhesions were more frequently present in the D+H experimental group ($p < 0.001$) as shown in Table 3. Table 4 shows the mRNA expression of the investigated genes. Nine genes were expressed significantly differently ($p \leq 0.01$). For all of these, the differences were between the 2nd hit group and both other groups. Marked expression in the D+H group was present for 6 of these: vascular endothelial growth factor D, bone morphogenetic protein 6, colony-stimulating factor 2 (granulocyte-macrophage) (CSF-2), inhibin alpha (INHA), epidermal growth factor, inhibin beta A. The expression of hepatocyte growth factor, interleukin 12

TABLE 3
Macroscopic and Histological Assessment of Omentum

	D+H (n=10)	D (n=10)	B (n=9)
Macroscopic adhesions	3.5 (2.8–5.3)*	1.0 (0.8–2.0)	1.0 (0.8–1.0)
Overall fibrotic score	4.5 (3.0–6.3)	5.0 (3.5–5.0)	4.0 (3.0–6.0)
Blood vessels (no/field)	34.0 (26.6–43.4)	36.5 (24.9–45.7)	33.5 (24.9–43.8)

D+H = Dianeal + hit group; D = Dianeal group; B = Physioneal without glucose; hit = 20 mL of blood obtained by heart puncture of male Wistar donor rats administered intraperitoneally.

The number of macroscopic adhesions was counted by autopsy. The amount of fibrosis in picro-sirius red-stained omental tissue is shown as assessed in different areas: perivascular, intersegmental, and submesothelial. Maximum score per area was 3 resulting in a maximum over-all score of 9. Also, the number of vessels of five microscopic fields in platelet endothelial cell adhesion molecule-1 antibody-stained omental tissue is shown. Data are expressed as medians and interquartile ranges.

* $P < 0.001$ compared to both control groups.

TABLE 4
A Comparison of Quantitative mRNA Expression in Omental Tissue Between Experimental Groups

Gene ^a	D+H (n=10)	D (n=10)	B (n=9)	Kruskal-Wallis p value
Angiogenesis				
EREK	1.3 (0.8–2.8)	0.5 (0.1–1.6)	0.3 (0.2–1.2)	0.11
FGF1	0.8 (0.5–1.1)	0.5 (0.3–0.6)	0.6 (0.4–0.8)	0.06
FGF2	0.7 (0.4–0.8)	0.4 (0.3–0.6)	0.4 (0.2–0.5)	0.08
FIGF	2.6 (1.7–4.0) ^b	0.9 (0.7–1.3)	1.3 (0.9–1.9)	0.01
PGF	2.7 (1.6–3.7) ^c	1.7 (0.8–2.3)	1.1 (0.7–1.9)	0.04
TGFA	0.7 (0.3–1.5)	1.4 (0.8–2.8)	1.3 (0.9–2.0)	0.15
VEGFA	1.1 (0.7–1.6)	1.3 (0.6–1.6)	0.9 (0.5–1.5)	0.71
VEGFB	1.1 (0.7–1.6)	1.3 (1.0–1.8)	0.9 (0.6–1.4)	0.30
VEGFC	1.4 (1.1–1.9)	1.2 (0.6–1.3)	1.2 (0.7–1.4)	0.06
Cell proliferation				
BMP3	0.6 (0.4–2.4)	0.6 (0.2–3.8)	0.6 (0.2–2.8)	0.78
BMP4	1.9 (1.8–3.6)	1.7 (1.3–2.1)	1.1 (0.9–1.4)	0.09
BMP6	2.1 (1.3–3.5) ^b	0.7 (0.1–1.2)	0.4 (0.1–1.0)	0.01
CSF1	1.5 (1.2–1.8)	1.2 (0.5–1.5)	1.0 (0.6–1.5)	0.11
CSF2	2.7 (0.6–5.0) ^b	0.6 (0.2–1.4)	0.6 (0.2–1.1)	0.01
FGF9	1.1 (0.6–1.7)	0.9 (0.7–1.1)	0.8 (0.6–1.5)	0.81
FGF10	1.5 (1.0–3.2)	0.9 (0.1–1.7)	0.5 (0.3–1.0)	0.09
HGF	1.3 (1.0–1.8) ^b	0.4 (0.3–0.6)	0.3 (0.2–0.6)	0.01
IGF1	1.2 (0.8–1.5)	1.5 (0.8–1.6)	1.0 (0.9–1.8)	0.75
IGF2	3.9 (2.7–4.6)	2.9 (1.9–4.5)	1.4 (1.0–5.3)	0.29
TFF1	0.3 (0.1–0.5)	0.4 (0.1–0.7)	0.1 (0.0–0.7)	0.60

TABLE 4 (cont'd)

Gene	D+H (n=10)	D (n=10)	B (n=9)	Kruskal-Wallis p value
Immune response				
CSF3	0.5 (0.4–0.6)	0.6 (0.1–2.1)	0.5 (0.2–1.1)	0.90
CXCL1	1.2 (0.6–2.3) ^b	0.7 (0.1–1.3)	0.7 (0.2–1.1)	0.01
IL1a	0.3 (0.2–0.7)	0.8 (0.2–2.4)	0.7 (0.3–2.3)	0.33
IL1b	2.1 (1.4–4.5)	1.1 (0.9–1.4)	1.6 (1.0–2.4)	0.10
IL2	1.0 (0.6–1.7)	0.5 (0.2–0.7)	0.5 (0.2–1.1)	0.06
IL4	0.8 (0.6–2.3) ^c	0.3 (0.0–1.6)	0.1 (0.0–0.2)	0.01
IL7	1.2 (1.0–1.6)	0.7 (0.4–1.9)	0.5 (0.3–1.3)	0.09
IL12a	1.9 (1.3–2.9) ^b	0.9 (0.6–1.9)	1.1 (0.6–1.4)	0.01
IL18	1.9 (1.1–2.7) ^b	0.4 (0.2–1.0)	0.7 (0.4–1.3)	0.01
LIF	2.2 (1.1–4.0) ^c	0.7 (0.4–0.9)	0.6 (0.2–1.0)	0.02
Other growth factors				
FGF7	0.8 (0.6–1.6)	0.7 (0.5–0.9)	0.7 (0.4–1.1)	0.50
GDF10	1.3 (0.6–1.8)	0.9 (0.6–1.3)	0.7 (0.6–1.0)	0.29
IL11	3.3 (1.9–5.6) ^c	1.8 (1.2–3.4)	1.3 (0.6–1.7)	0.02
IL3	0.6 (0.1–0.9) ^c	0.4 (0.0–0.2)	0.1 (0.0–0.3)	0.01
INHA	3.6 (1.7–5.4) ^b	1.3 (0.7–2.1)	0.9 (0.5–1.5)	0.01
Fibrosis				
BMP1	2.4 (1.8–3.9) ^d	0.8 (0.4–2.1)	1.1 (0.8–1.9)	0.03
BMP7	1.3 (0.6–2.0)	1.3 (0.6–1.5)	0.9 (0.7–1.4)	0.99
EGF	3.1 (1.8–4.3) ^b	1.0 (0.8–2.4)	1.2 (0.9–1.8)	0.01
FGF4	1.0 (0.9–1.1)	1.0 (0.9–1.1)	1.0 (0.9–1.1)	0.49
FGF8	1.3 (0.1–1.9)	0.7 (0.6–3.0)	0.5 (0.1–1.0)	0.24
INHBA	3.7 (2.8–5.0) ^b	0.8 (0.2–1.1)	0.6 (0.3–1.2)	0.01
PDGFA	1.4 (1.1–1.6) ^d	0.8 (0.6–0.9)	1.0 (0.7–1.2)	0.01
TGFB1	2.2 (1.6–3.0)	1.5 (1.1–2.0)	1.5 (1.3–2.4)	0.06
TGFB3	1.9 (0.9–2.9)	1.3 (0.8–2.2)	1.1 (0.9–1.3)	0.27

D+H = Dianeal + hit group; D = Dianeal group; B = Physioneal without glucose; hit = 20 mL of blood obtained by heart puncture of male Wistar donor rats administered intraperitoneally.

Relative quantities of specific gene expression relative to that of the housekeeping gene and the calibrator sample are expressed as medians and interquartile ranges.

^a See Table 1 for gene names.

^b Significant ($p \leq 0.05$) for the comparison between D+H vs B and D in Dunn's post-hoc test.

^c Significant ($p \leq 0.05$) for the comparison between D+H vs B in Dunn's post-hoc test.

^d Significant ($p \leq 0.05$) for the comparison between D+H vs D in Dunn's post-hoc test.

subunit alpha, and interleukin 18 ranged between 1.3 and 1.9. More detailed examination showed that none of these 9 genes was markedly upregulated after exposure to the buffer or to Dianeal. The intraomental mRNA expression of 3 of the markedly expressed genes was related to peritoneal transport (CSF2, leukemia inhibitory factor, INHA). Additionally, chemokine (C-X-C motif) ligand 1 (CXCL1) showed a correlation with peritoneal small solute and fluid kinetics, as shown in Figure 2. Statistical significance of these relationships was not reached for only CXCL1 versus glucose absorption ($p = 0.07$) and for INHA versus net ultrafiltration ($p = 0.18$).

DISCUSSION

The present study in rats with chronic kidney failure and daily exposure to a dialysis solution, followed by a 2nd hit, has some similarities with PD patients, but with a relatively

short duration of peritoneal exposure and the inclusion of a control group, only exposed to a "biocompatible" buffer. This may explain the absence of functional and morphological differences between the B and the D group. The number of peritoneal blood vessels was higher than in another study with a mid-term exposure duration (12), but this is likely an effect of kidney failure, as we showed previously (6). In line with these observations, no upregulation of the omental mRNA expression of any transcript was present after exposure to Dianeal.

Although no control group with kidney failure without exposure was investigated in the present study, the absence of a difference between the D and B groups and the absence of a marked upregulation of any of the genes investigated make it likely that uremia *per se* was the main reason for the slight upregulation of the growth factors vascular endothelial growth factor C (VEGFC), placental growth factor and interleukin-11.

Figure 2 — The relationships between INHA, LIF, CXCL1, CSF2, and parameters of solute and fluid transport. INHA = inhibin alpha; LIF = leukemia inhibitory factor; CXCL1 = growth-regulated alpha protein; CSF2 = colony stimulating factor 2; GA = glucose absorption; MTAC = mass transfer area coefficient of urea; NUFR = net ultrafiltration rate. Each dot represents a rat.

This is supported by data from the literature, showing that a uremic state itself induced by subtotal nephrectomy leads to increased expression of collagen-3 and of some growth factors, like VEGF and fibroblast growth factor-2 (13).

In contrast, the 2nd hit was associated with an increased number of adhesions and a large number of upregulated genes from all 5 categories, again without other detectable functional and morphologic peritoneal alterations. The importance of a

2nd hit for the development of EPS in patients has emerged from morphological studies (14) and clinical observations in PD patients (15). The relatively short duration of peritoneal exposure in the present study may explain why the morphologic changes were absent, despite the marked effects on omental gene expression of those involved in fibrosis, angiogenesis, growth factors, and immune responses. These will be discussed below.

Vascular endothelial growth factor is likely involved in peritoneal angiogenesis, judged from its upregulation by glucose (16), its relationships with peritoneal transport (17), and its involvement in epithelial to mesenchymal transition of mesothelial cells (18). Although no marked upregulation was present for the VEGF a, b, and c genes, this was evident for the d gene (FIGF), which is involved in lymphangiogenesis (19), and for the other angiogenic family member placental growth factor. Additionally CXCL1, which activates VEGF signalling in gastric cancer cells (20), appeared to be related to peritoneal transport in the present study.

Transforming growth factor β (TGF β) is one of the most potent regulators of the production and deposition of extracellular matrix and also stimulates angiogenesis (21). The 2 phenomena are related in patients who exhibit peritoneal alterations after long-term exposure to dialysis solutions (22,23). In accordance, the TGF β 1 gene was markedly expressed. The so-called TGF β superfamily consists of over 30 members, including bone morphogenetic proteins (BMP) and growth differentiation factors (GDF) (24). Bone morphogenetic protein-1 plays a role in the regulation of extracellular matrix formation (25). In agreement with the long-term morphologic alterations, we found a markedly increased omental expression of BMP1, but not of growth differentiation factors.

Peritoneal inflammation mediated by cytokine release is often present in PD (26–30). Intraperitoneal granulocyte-macrophage colony-stimulating factor (CSF2) causes a flux of macrophages into the peritoneum of stable PD patients (31). In our analysis, we found a marked upregulation of omental mRNA expression of IL1b and of CSF2.

Leukemia inhibitory factor (LIF) is a polyfunctional glycoprotein cytokine, belonging to the IL6 family (32). It has hematopoietic, neuronal, and endocrine functions (33) and is involved in tubular regeneration after experimental acute renal failure (34). However, LIF is not an important factor in the composition of the extracellular matrix *in vivo* (35), nor in possible effects on cultured mesothelial cells after induction of acute bacterial peritonitis (36). The upregulation omental LIF mRNA in the present study can therefore not be explained by its known functions discussed above. However, it is known that LIF mRNA expression is increased by exposure to high glucose concentrations (37). It is speculative if this is the only cause for the increased expression, because it was only found after the 2nd hit and not in the D group. The same may apply to inhibin alpha expression, as it is stimulated by and thereby dependent on insulin.

Epidermal growth factor is another factor known to be present in the peritoneal cavity. It induces morphological changes of mesothelial cells toward a fibroblastic phenotype (38). Heparin-binding EGF-like growth factor (HB-EGF) is a member of the EGF family influenced by exposure to high glucose and hyperosmolarity (39). Exposure to high glucose concentrations may therefore be the cause of the marked omental mRNA expression of EGF found in the present analysis.

Our finding that omental mRNA expression of CSF2, LIF, INHA, and CXCL1 were related to peritoneal small solute

transport, and therefore negatively with ultrafiltration, was unexpected and may seem odd, but may be explained by the factors involved in their upregulation. Inflammation causes an increase in peritoneal transport. The observed upregulation of CSF2, in combination with its relationship with peritoneal transport, suggests an important role for this cytokine. Exposure to high glucose concentrations can influence peritoneal transport (16); our results point to important effects of LIF and INHA upregulation in this process. Vascular endothelial growth factor is related to peritoneal transport (17); chemokine (C-X-C motif) ligand stimulates VEGF. The relationship between mRNA CXCL and peritoneal transport makes it likely that the latter is more important in transport regulation than VEGF itself.

Our study has some weak points, like the absence of groups without chronic renal failure, a group with chronic renal failure without exposure, and the short duration of exposure in the other groups. However, most animal studies are restricted to a follow-up of only 4 weeks. Another potential weakness is the use of multiple (45 genes) comparisons in the 3 groups of animals. Also, it must be taken into account that data obtained in a rat model may not be fully representative of the situation in patients. Furthermore, omental tissue was used because it usually shows most abnormalities and EPS is a disease of the visceral peritoneum. Strong points are that we are not aware of any other study investigating effects of peritoneal exposure on mRNA expression of a large number of genes and on any analysis trying to relate gene expression to functional characteristics of peritoneal transport. Nonetheless, it has to be noted that the associations between some gene expression and transport parameters, although interesting, should be considered cautiously, especially because they were unexpected and the relevance of this finding needs confirmation by other studies.

In conclusion, the present study showed that intraperitoneal expression of some genes involved in angio-, fibrogenesis, and immune response precedes morphologic and functional alterations of peritoneal membrane after a 2nd hit with blood. Analysis of the various factors showed that those upregulated by glucose exposure were markedly expressed after this hit, suggesting that inflammation enhances the effects of glucose on peritoneal tissues. The most important factors are probably those that are not only regulated, but also related to peritoneal transport. Further investigations are obligatory to establish the clinical relevance of CSF2, LIF, INHA, and CXCL1 in PD. Studies in PD patients are required for better insight in their function during treatment.

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DISCLOSURES

The authors have no financial conflicts of interest to declare.

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