Original Articles

Transforming growth factor β-induced peritoneal fibrosis is mouse strain dependent*

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ABSTRACT

Background. Encapsulating peritoneal sclerosis (EPS) is a rare but devastating complication of peritoneal dialysis. The etiology is unclear, but genetic predisposition may be a contributing factor. We used adenovirus-mediated gene transfer of transforming growth factor (TGF) β1 to the peritoneum in four genetically distinct laboratory mouse strains to assess differences in fibrogenic response.

Methods. Mice from four genetic backgrounds (C57BL/6J, DBA/2J, C3H/HeJ and SJL/J) received an intraperitoneal injection of an adenovirus expressing TGFβ1 (AdTGFβ1) or control adenovirus (AdDL) and were assessed 4 and 10 days after infection. Submesothelial thickening, angiogenesis and gene expression were quantified from peritoneal tissue. Protein was extracted from omental tissue and assessed for collagen, E-cadherin and TGFβ signaling pathway proteins.

Results. There was a graded response among the mouse strains to the peritoneal overexpression of TGFβ1. TGFβ1 induced a significant fibrogenic response in the C57BL/6J mice, whereas the SJL/J mice were resistant. The DBA/2J and the C3H/HeJ mice had intermediate responses. A similar graded response was seen in collagen protein levels in the omental tissue and in fibrosis-associated gene expression. TGFβ type 1 receptor and SMAD signaling pathways appeared to be intact in all the mouse strains.

Conclusions. There were significant differences in mouse strain susceptibility to peritoneal fibrosis, suggesting that genetic factors may play a role in the development of peritoneal fibrosis and possibly EPS. As early TGFβ1 signaling mechanisms appear to be intact, we hypothesize that fibrosis resistance in the SJL/J mice lies further down the wound-healing cascade or in an alternate, non-SMAD pathway.

INTRODUCTION

Encapsulating peritoneal sclerosis (EPS) is a rare but devastating complication of peritoneal dialysis (PD). In various epidemiological studies, the incidence of EPS is between 1 and 3.3% [1]. Duration of PD and younger age are consistent risk factors for EPS [2]. Cessation of PD and kidney transplantation may also be initiating events [3]. Some [4], but not all, studies [2] have identified peritonitis as a risk factor. EPS is more common in patients with high peritoneal membrane transport [5] and PD patients who develop EPS often show a decrease in ultrafiltration before EPS is apparent [6]. This ultrafiltration
dysfunction reflects a decrease in peritoneal osmotic conductance that has been related to fibrosis of the peritoneal membrane [7].

Many patients are exposed to peritoneal dialysate, but few develop EPS. This has led to the concept of a ‘two-hit’ mechanism. The first hit is thought to be uremia and PD therapy itself. The second hit may be peritonitis, inflammation, transplantation or a combination of events. It is also possible that the second hit is genetic; some patients may be predisposed to EPS due to a genetic constitution permissive for fibrotic pathophysiological responses.

The pro-fibrotic cytokine transforming growth factor (TGF)-β is an essential factor in progressive changes in the peritoneal membrane over time. Several studies have demonstrated an association between effluent TGFβ1 concentration and peritoneal membrane injury in patients on PD therapy [8, 9]. In an animal model, we have demonstrated that adenovirus-mediated gene transfer of TGFβ1 to the peritoneum results in functional and structural changes similar to those seen in patients on long-term PD [10]. Peritoneal fibrosis and angiogenesis have been demonstrated to be associated with epithelial to mesenchymal transition (EMT) of mesothelial cells [11] and we have shown that TGFβ1 induces a pattern of EMT similar to that which is seen in the peritoneal tissues of PD patients [12]. Furthermore, using a helper-dependent adenovirus to deliver longer duration TGFβ1 expression, we observed peritoneal changes suggestive of EPS in a rodent model [13].

The fibrogenic process is complex and involves collagen deposition, angiogenesis and EMT. The SMAD signaling pathway has a key role in fibrogenesis [14], but non-SMAD pathways have also been identified that have a role in peritoneal fibrosis [15].

There is evidence to suggest that genetic polymorphisms play some role in peritoneal membrane solute transport. Maruyama et al. [16] identified a polymorphism in the receptor for advanced glycation end products that was more common in patients with high peritoneal solute transport. Gillerot et al. [17] carried out a similar study and identified a polymorphism in the interleukin-6 gene that was predictive high solute transport. However, associations between gene polymorphisms and peritoneal fibrosis have not been identified.

In diseases of other organs, genetic determinants of fibrosis have been reported. For example, polymorphisms in the TGFβ coding region [18] and the angiotensin II promoter region [19], along with an array of inflammatory mediators [20], have been associated with progressive liver fibrosis.

There is some evidence that mouse strains with different genetic backgrounds demonstrate differing fibrogenic responses. C57BL/6J mice are very susceptible to TGFβ1-induced lung fibrosis, while BALB/c mice are resistant [21]. One early observation involved the rate of healing of an ear punch wound in which a lupus-prone mouse, MRL/Ipr, was found to have accelerated wound healing that was related to the MRL genotype [22]. Differences in wound-healing responses have been observed in bleomycin- [23] and radiation-induced [24] pulmonary fibrosis. Strain-dependent fibrogenic responses have also been observed in models of kidney [25], liver [26] and heart [27] fibrosis.

The aim of the present study was to assess differences in peritoneal fibrosis in response to overexpression of TGFβ across a selection of distinct laboratory mouse strains. Differences in response across different mouse strains would suggest that genetics may play a role in human response to PD and in the development of EPS.

**MATERIALS AND METHODS**

**Animal model**

This study was performed in four strains of mice: C57BL/6J (B6), DBA/2J (D2), C3H/HeJ (C3H) and SJL/J (SJL). These strains were selected as they are commonly used experimental models, their respective genotypes and phenotypes are well documented and, most importantly, they represent four distinct arms of the mouse phylogenetic tree. Mice were purchased from the Jackson Laboratory (Bar Harbor, ME). All animal studies were performed according to the Canadian Council on Animal Care guidelines. Food and water were provided ad libitum.

**Animal studies**

Fifteen 5- to 6-week-old male mice from each of the four strains received an intraperitoneal injection 1.5 × 10⁸ pfu of a first-generation adenovirus expressing the active form of TGFβ1 (AdTGFβ1) diluted in 100 μL of phosphate-buffered saline [15] and were euthanized at Day 4 (n = 5) or Day 10 (n = 10). Thirteen mice from each strain received 1.5 × 10⁸ pfu of a control adenovirus (AdDL) that lacked transgene expression and were euthanized at Day 4 (n = 5) or Day 10 (n = 8) with isoflurane (MTC Pharmaceuticals, Cambridge, ON, Canada).

SJL mice (n = 20) also received a 2-fold dose of adenovirus (3 × 10⁹ pfu). Ten mice received 3 × 10⁸ pfu of the AdTGFβ1 preparation and, as a control, 10 mice received a combination of the two virus preparations −1.5 × 10⁸ pfu of AdDL + 1.5 × 10⁸ pfu of AdTGFβ1. These animals were euthanized at 4 and 10 days after infection. SJL mice also received adenoviruses expressing murine connective tissue growth factor (CTGF; AdCTGF) or human tissue inhibitor of metalloproteinase 1 (TIMP1; AdTIMP1). These adenoviruses were delivered at a dose of 1.5 × 10⁸ pfu in addition to AdTGFβ1 or AdDL (both at 1.5 × 10⁸ pfu). Five mice were treated in each group (n = 20) and euthanized 10 days after infection.

At the end of the experiment, 3 mL of 4.25% Dianene (Baxter Healthcare Corporation) was instilled in the peritoneum, and samples collected 15 min later. The fluid was centrifuged at 1500 rpm for 5 min and the supernatant frozen at −20°C. This effluent was evaluated for both active and total TGFβ1 concentration using enzyme-linked immunosorbent assay according to the manufacturer’s instructions (R&D Systems, Minneapolis, MN).

The entire anterior abdominal wall was resected and the upper portion of this tissue was formalin fixed. The lower portion was placed in 1 mL TRIzol (Invitrogen) for RNA extraction as previously described [28]. Omental tissue was frozen in liquid nitrogen for protein analysis.
**Histology and immunohistochemistry**

The formalin-fixed tissue was processed, paraffin embedded and cut into 5-µm sections. The sections were stained with Masson’s trichrome or for von Willebrand factor-related antigen (Dako, Carpinteria, CA) as previously described [28]. Eight to 10 images were taken per section and analyzed in a blinded fashion by image analysis software (Empix Imaging, Mississauga, ON, Canada). Vascular density was measured as

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**FIGURE 1:** Active and total TGFβ1 in peritoneal effluent 4 and 10 days after infection with AdTGFβ1 or control adenovirus (AdDL). (A and B) Four days after adenovirus infection, all groups of animals treated with AdTGFβ1 had significantly increased concentration of active (A) and total (B) TGFβ in the peritoneal effluent. However, there was significantly less TGFβ1 in the peritoneal effluents of the SJL and the C3H mice treated with AdTGFβ1 compared with the B6 mice. (C and D) Ten days after adenovirus infection, the SJL mice had significantly elevated peritoneal effluent of active TGFβ (C), and all groups continued to have elevated levels of total TGFβ1 (D).

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**FIGURE 2:** (A) The submesothelial thickness was significantly increased in all groups treated with AdTGFβ1 except for the SJL mice, which showed minimal response. (B) The angiogenic response (vascular density in the first 200 µm of submesothelial tissue) was increased significantly by AdTGFβ1 in all mouse strains except the SJL mice.

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*Peritoneal fibrosis and mouse strain*
FIGURE 3: Representative histology of the peritoneal membrane taken from the anterior abdominal wall of the B6, D2, C3H and SJL mice treated with AdDL and AdTGFβ1. In the top right section, the various structures are labeled including p, peritoneal cavity; m, mesothelial cell layer; sm, submesothelium and mu, muscles of the abdominal wall. Control adenovirus (AdDL, top panels) has no effect on peritoneal structure. TGFβ1 induces submesothelial proliferation and fibrosis. This effect was observed in a graded manner with the B6 mice demonstrating the clearest changes, and the SJL mice having very little structural change.

FIGURE 4: In Day-10 samples, mRNA was extracted from the anterior abdominal wall and gene expression was assessed by quantitative RT–PCR. (A) Type 1 collagen and (B) Timp1 mRNA was increased by AdTGFβ1 in all mouse strains except the SJL mice. In the animals treated with control adenovirus (AdDL), Timp1 gene expression was increased in the SJL mice compared with the other three strains (B). (C) Snail1 and (D) Snail2 gene expression was increased significantly in the B6 mice. In the C3H mice, Snail1 was increased, but not Snail2. Neither Snail1 nor Snail2 was increased in the SJL mice.
the number of vessels in the first 200 μm below the mesothelial cell layer.

**Gene expression**

Quantitative real-time polymerase chain reaction (PCR) for the alpha 2 chain of Type 1 collagen (Col1A2), murine and human Timp1, SNAIL (Snai1), SLUG (Snai2), fibronectin (Fn1), vascular endothelial growth factor (Vegf), E-cadherin (Cdh1) and Ctgf was performed using an ABI Prism 7500 Sequence Detector (Applied Biosystems, Foster City, CA) as previously described [28]. mRNA quantification was referenced to 18s and glyceraldehyde 3-phosphate dehydrogenase (Gapdh) with similar results. The data referenced to Gapdh are shown.

**Protein analyses**

Protein was extracted from frozen omental tissue and 20 μg/lane was analyzed by western blot for Type 1 collagen (Sigma Canada Ltd., Oakville, ON, Canada), E-Cadherin (BD Biosciences, Mississauga, ON, Canada), phosphorylated SMAD2 (pSMAD2) (EMD Millipore, Billerica, MA, USA), phosphorylated SMAD3 (pSMAD3) and total SMAD2/3 (Cell Signaling Technology, Danvers, MA), and Type 1 TGFβ receptor (R&D Systems). Band density was quantified and normalized to β-actin.

**Statistical analyses**

All data are shown as mean ± SD. The data were analyzed by one-way analysis of variance (ANOVA) with the Tukey post hoc correction (between groups) or by the t-test (AdTGFβ1 versus control). A two-way ANOVA was used to test for the overall effect of TGFβ and mouse strain (SPSS, IBM, Armonk, NY).

**RESULTS**

The concentrations of active and total TGFβ1 were measured in the peritoneal effluent (Figure 1). Four days after administration of adenovirus, all AdTGFβ1-treated animals had significantly increased levels of both active and total TGFβ versus AdDL-treated controls (Figure 1A and B). There were some differences between groups, with the B6 mice having the highest levels, and C3H and SJL mice having lower concentrations. By Day 10, all AdTGFβ1-treated animals continued to
have increased peritoneal effluent total TGFβ1 concentration compared with AdDL-treated controls (Figure 1D), while only SJL mice continued to have significantly elevated levels of active TGFβ (Figure 1C).

The strains demonstrated a graded response of submesothelial thickening with the B6 and D2 mice having the greatest increase in thickness, while the SJL mice were fairly resistant to TGFβ1-induced fibrosis (Figures 2A and 3). The B6, D2 and C3H mice all had increased vascularization after AdTGFβ1 treatment compared with AdDL-treated mice (Figures 2B and 3). There was no significant difference between the mouse strains in their response. The SJL mice did not demonstrate any increase in submesothelial vascularization in response to TGFβ1.

**Gene expression**

The gene expression data revealed a strong upregulation of fibrosis-related genes in the B6, D2 and C3H mice at Day 10 (Figure 4). There was no significant regulation of these genes in the SJL mice treated with AdTGFβ1 compared with control adenovirus-treated animals. Type 1 collagen gene expression was significantly increased in the B6, D2 and C3H mice, but not in the SJL mice (Figure 4A). There was a baseline difference in Timp1 gene expression in the AdDL-treated animals, with the SJL mice having significantly higher baseline expression (Figure 4B). The genes encoding the zinc finger regulatory proteins essential to EMT, namely Snai1 and Snai2, were both upregulated by TGFβ1 in the B6, D2 and C3H mice, but not affected in the SJL mice (Figure 4C and D).

Likewise, at Day 10, fibronectin was significantly upregulated by AdTGFβ1 in the B6, D2 and C3H mice, but not affected in the SJL mice (Figure 5A). There was no significant regulation of Vegf (Figure 5B), or Cdh1 (Figure 5C) gene expression by AdTGFβ1 in any of the mouse strains. By two-way ANOVA, Ctgf expression was significantly increased by TGFβ1 and different among the strains (Figure 5D).

**Protein expression**

Protein expression of type 1 collagen and E-cadherin was assessed by western blot of omental tissue (representative blot shown in Figure 6). Ten days after infection, the B6 mice treated with AdTGFβ demonstrated a significant increase in omental collagen protein that was not seen in the other groups (Figure 6B). E-cadherin was significantly downregulated in the B6 mice by AdTGFβ1 treatment (Figure 6C), whereas in the SJL mice, E-Cadherin protein was significantly increased by AdTGFβ1.
In order to assess SMAD signaling and TGFβ receptor expression as possible direct causes of strain-specific differential response to TGFβ1, we quantified phosphorylated SMAD3 (pSMAD3), phosphorylated SMAD2 (pSMAD2) and TGFβ receptor 1 (TGFβR1) expression in omental tissue (Figure 7). At Day 4, we saw that AdTGFβ1 infection significantly increased pSMAD3 expression compared with control and the effect was greatest in the SJL mice (Figure 7B). There was also a significant difference at baseline, with the SJL mice having higher expression of pSMAD3 in the AdDL-treated animals. pSMAD2 expression was also increased by exposure to TGFβ1 (Figure 7C). TGFβR1 expression did not change between strains or with exposure to TGFβ1 (Figure 7D).

To determine whether SJL resistance to fibrosis could be overcome by increasing the dose of AdTGFβ1, the SJL mice were injected with AdTGFβ1 at a dose of $3 \times 10^8$ pfu controlled with AdTGFβ1 + AdDL (both at $1.5 \times 10^8$ pfu). These animals were euthanized at Day 4 and 10. The active and total TGFβ1 concentrations in the peritoneal effluents are shown in Figure 8A and B. The SJL mice demonstrated high levels of active TGFβ1 in the effluent at Day 10 after treatment with the increased dose of AdTGFβ1. As seen in Figure 8, doubling the dose of AdTGFβ1 did not recreate the fibrogenic phenotype in the SJL mice as observed in the B6 mice. Neither submesothelial thickness (Figure 8C) nor vascular density (Figure 8D) was affected by doubling the adenovirus dose in the SJL mice.

Four days after infection, omental tissue was taken from these mice and assayed from pSMAD2 and pSMAD3 (Figure 9). Both SMAD2 and SMAD3 phosphorylation was significantly greater than that seen in the B6 mice. In the SJL
FIGURE 8: The SJL mice were administered $3 \times 10^8$ pfu of AdTGFβ1 in an attempt to overcome resistance to fibrosis. The increased dose of AdTGFβ led to sustained high levels of active (A) and total (B) TGFβ expression 10 days after infection. The increased dose of AdTGFβ1 did not lead to an increase in submesothelial thickening (C) or vascularization (D). The B6 and SJL mice treated with a single dose of adenovirus are shown for comparison. † $P < 0.01$ compared with all other groups.

FIGURE 9: The SJL mice were treated with twice the dose of AdTGFβ ($3 \times 10^8$ pfu). (A) Four days after infection, the mice were euthanized and pSmad3 and pSmad2 were measured in protein extracted from omental tissue. There was a significant increase in pSmad3 (B) and pSmad2 (C) expression in the SJL mice treated with higher dose of AdTGFβ1 compared with the B6 mice treated with AdTGFβ ($1.5 \times 10^8$ pfu).
mice treated with $3 \times 10^8$ pfu of AdTGFβ1, there was a further increase in SMAD2 and SMAD3 phosphorylation, but this was not significant compared with the SJL mice treated with $1.5 \times 10^8$ pfu.

Gene expression from the SJL mice treated with a higher dose of adenovirus vector was evaluated (Figure 10). Genes associated with fibrosis ($Fn1$, $Snai1$, $Snai2$) were not enhanced in the SJL mice receiving twice the adenovirus dose as the C57BL/6 mice. The SJL mice treated with a higher dose of adenovirus vector were compared with the SJL mice treated with a single dose of adenovirus, as shown in Figure 10.

Figure 10: Gene expression at Day 10 for the B6 mice treated with $1.5 \times 10^8$ pfu of AdTGFβ1 or the SJL mice treated with $1.5$ or $3.0 \times 10^8$ pfu of AdTGFβ1. (A) The increased dose of AdTGFβ1 did not lead to an increase in fibronectin (B), Snai1 (B) or Snai2 (C) gene expression in the SJL mice. (D) Vegf gene expression was not regulated significantly by any treatment. The B6 and the SJL mice treated with a single dose of adenovirus are shown for comparison. *P < 0.01 compared with all other groups except where noted (in B).

Figure 11: The SJL mice were treated simultaneously with AdTGFβ1 and an adenovirus expressing CTGF or TIMP1 each delivered at $1.5 \times 10^8$ pfu. Infection with both AdTIMP1 or AdCTGF and AdTGFβ1 did not reverse the resistance to fibrosis seen in the SJL mouse. Submesothelial thickness (A) and vascular density (B) was not affected.
mice (Figure 10A–C). Vegf gene expression was not regulated at Day 10 (Figure 10D).

Previous work by our group has suggested that CTGF [29] or TIMP1 [21] may be responsible for the difference in fibrogenic response among mouse strains. In order to assess this further in the SJL mice, we co-expressed TGFβ1 and CTGF or TIMP1 in the peritoneum. Neither CTGF nor TIMP1 reversed the fibrosis resistance seen in the SJL mice. Specifically, there was no increase in submesothelial thickness (Figure 11A) or angiogenesis (Figure 11B) in animals treated with both adenoviruses simultaneously.

Furthermore, we assessed these animals for changes in gene expression. In animals treated with CTGF, we could detect an increase in Ctgf mRNA (Figure 12A). Neither Fn1 (Figure 12B) nor Vegf (Figure 12C) gene expression was increased by dual adenovirus administration. Likewise, expression of Snai1, Snai2 and Timp1 mRNA (Figure 13A–C) was increased in the B6 mice treated with AdTGFβ1, but the addition of AdCTGF or AdTIMP1 to AdTGFβ1 did not alter gene expression in the SJL mice. Human TIMP1 protein shares 74% homology with murine TIMP1 and previous work demonstrated that human AdTIMP1 induces predictable responses in mice [30]. The human TIMP1 expressed by our AdTIMP1 was not detected by the murine primers used, so we confirmed TIMP1 expression using human primers (Figure 13D).

**DISCUSSION**

These results show significant support for a strain difference in response to TGFβ1 administration. B6 mice respond strongly to this stimulus with an extensive wound healing response—fibrosis, angiogenesis and evidence of EMT. There was a graded response of the D2 and the C3H mice, whereas there was virtually no response to TGFβ1 in the SJL mice.

Previous work has demonstrated unique phenotypic characteristics of SJL mice. They have a muscle phenotype that is expressed at advanced age related to decreased expression of dysferin [31]. They are used to study experimental allergic encephalopathy as they are immunologically susceptible to this injury [32]. Pertinent to our observations, SJL mice are 'slow healers' in an ear punch wound-healing model [22]. They also lack susceptibility to atherosclerosis when exposed to a high-fat diet [33]. These last two features support our observations that SJL mice have a fundamental defect in the wound-healing response. Further work has mapped this resistance to wound healing to a region of chromosome 9 [34]. In our experiments, we identified interesting differences in baseline Timp1 gene expression and pSMAD3 expression in the SJL mice.

We examined whether early events from adenovirus infection to receptor activation could be playing a role in the
difference in fibrogenic response. At Day 4, the transgene expression in the SJL mice was significantly less than in the other strains. At Day 10, however, active TGFβ1 expression persisted in the SJL mice, whereas it returned to baseline levels in the other mouse strains. We were concerned that this altered TGFβ1 kinetics—lower but prolonged expression—may explain the differences in fibrogenic response. However, SMAD3 phosphorylation was strongest in SJL mice at Day 4—suggesting that signaling was intact and enhanced in the SJL mice. Furthermore, doubling the dose of adenovirus still did not induce any significant fibrogenic response in the SJL mice (Figure 8).

We also assessed levels of TGFβR1, which were not different between mouse strains. We, therefore, conclude that the fibrogenic defect in SJL mice is not in the AdTGFβ1 infection or transgene expression, and not in the early signaling pathways. There were clearly differences in downstream expression of fibrosis-related genes—suggesting a defect further down the cascade of molecular signals in the wound-healing response.

Previous work by our group assessing differences in fibrogenic responses in B6 and BALB/c mice suggested that CTGF [29] or TIMP1 [21] may be involved. We, therefore, overexpressed both of these factors along with TGFβ1 to assess whether this could reverse the fibrogenic resistance of the SJL mice. The SJL mice remained resilient to these potent fibrogenic stimuli.

Interestingly, there was evidence of a defect in the EMT response. Essentially, there was a lack of increase in Snai1 and Snai2 gene expression in the SJL mice treated with AdTGFβ1. This was associated with a lack of downregulation of E-cadherin protein in the SJL mice. In fact, E-cadherin protein response was increased by TGFβ1 exposure in the SJL mice, suggesting a possible protective mechanism.

The EMT process is complex with many potential modifiers. E-cadherin is a key intercellular adhesion molecule and its loss is indicative of epithelial release. E-cadherin gene expression is suppressed by a family of regulatory proteins including zinc finger DNA-binding proteins SNAIL1 and SNAIL2 [35]. These proteins are, in turn, regulated by growth factors such as platelet-derived growth factor (PDGF), TGFβ and wnt. E-cadherin associates with the cytoskeletal network through the structural protein β-catenin. In turn, β-catenin is regulated by glycogen synthase kinase 3β. This leads to the involvement of a variety of signaling pathways distinct from TGFβ, including wnt, PDGF, Notch and mammalian target of rapamycin [15]. Mobilization of transdifferentiated epithelial...
Peritoneal vascularization has been shown to be an essential component of peritoneal membrane injury and associated with peritoneal solute transport [37]. We have found peritoneal angiogenesis associated with TGFβ1 exposure [10], but others have identified glucose [38], and mesothelial cells undergoing EMT [39] as sources of VEGF and angiogenesis. We did not find Vegf mRNA to be strongly regulated 10 days after infection, in keeping with our previous observations [10]. We did, however, find that the SJL mice were resistant to TGFβ1-induced angiogenesis. This may be related to an overall lack of response to TGFβ1, or may represent a secondary effect, perhaps through a lack of EMT response seen in the SJL mice.

Overall, our findings support the hypothesis that genetic variance may explain some of the predisposition to peritoneal fibrosis and to the subsequent development of EPS. The difference in pro-fibrotic response among the mouse strains, particularly the striking lack of response in the SJL mice, is very intriguing. Data on the genes involved in this differential response remain unclear and therefore, further investigation is needed. The availability of a dense single nucleotide polymorphism map for the mouse genome and knowledge of strain-dependent genetic variation provides an ideal model in which to explore and identify the genetic contribution to peritoneal fibrosis.

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### CONFLICT OF INTEREST STATEMENT

C.H. is an employee of Baxter Healthcare. P.J.M. is a Canadian Institutes of Health Research clinician scientist.


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