Peritoneal dialysis is a widely used mode of renal replacement therapy in which preservation of the structural and functional integrity of the peritoneal membrane is critical for continued success. Progressive scarring, or fibrosis, in the peritoneal membrane is now well described in peritoneal dialysis patients, but its extent is variable. While some patients survive for long periods on peritoneal dialysis, others suffer from ‘fibrosis-related’ membrane failure, or rarely, more severe complications such as encapsulating peritoneal sclerosis (EPS). The reasons for these variations in responses are unlikely to be explained by variations in the therapy, which has remained largely unchanged over the past 20 years, and are suggestive of a genetic component to the susceptibility of individuals to different outcomes. In this issue of NDT, Margetts et al. [1] describe how they used genetically different mouse strains to examine the variability in responses to a defined, constant profibrotic stimulus in a model of peritoneal fibrosis. The data highlight a possible genetic linkage to susceptibility to fibrosis that adds significant insights into our understanding of the mechanisms driving peritoneal damage.

The key to continued success of peritoneal dialysis as a therapy remains in preservation of the performance of the peritoneal membrane as a dialysing organ. In health, the visceral and parietal peritoneum and its phospholipid-rich secretions and anti-friction surfaces facilitate bowel motility within the abdominal cavity. The outer surface of the whole comprises a single layer of mesothelial cells, specialized to provide a low friction and non-adhesive surface. The mesothelium that lines different outcomes. In this issue of NDT, Margetts et al. [1]

the peritoneal membrane sits on the sub-mesothelial compact zone, comprising a collagen-rich extracellular matrix in which larger blood vessels and capillaries are sited. Peritoneal dialysis is associated with a spectrum of alterations in the morphology of the peritoneal membrane which includes alterations in the mesothelial cell morphology, thickening of the sub-mesothelial compact zone and progressive vascular damage (vasculopathy) [2]. Cross-sectional studies have linked these changes to long-term glucose exposure and episodic infection [2]. These alterations to the peritoneal membrane associate with alterations in peritoneal solute transport, loss of ultrafiltration and eventual technique failure. More recent data suggest that the changes in the mesothelium play a key role in driving the changes in peritoneal structure and function. Data primarily from animal models and corroborated with the limited clinical studies suggest that the acquisition of a fibroblast-like phenotype by mesothelial cells, so-called trans-differentiation or epithelial-mesenchymal transition, is key in driving changes in extracellular matrix turnover and fibrogenesis (reviewed in [3]).

As the genesis of peritoneal fibrosis is insidious without overt clinical symptoms, except in cases of EPS, there are at present no tests that can predict its onset, nor are there any therapeutic approaches other than a switch of renal replacement therapy modality from peritoneal dialysis to haemodialysis when membrane function is seriously compromised. In the case of EPS, surgery can be used to relieve intestinal obstruction, but while outcomes have improved from this intervention the risk of mortality from the condition remains significant. The clinical consequences of peritoneal fibrosis are thus clear, but the processes that initiate and drive it in peritoneal dialysis patients remain less well defined.

In keeping with fibrogenesis in many other organs, peritoneal fibrosis recapitulates what may be broadly characterized as a wound healing response. One of the key drivers of wound healing is transforming growth factor beta-1 (TGF-β1), and the over-expression of this cytokine is central to scarring in many pathological contexts. TGF-β1 was memorably described as 'The dark side of tissue repair' by Border et al. [4], who were amongst the first to show the link between TGF-β1 and fibrosis in their pioneering studies of kidney disease [5]. TGF-β1 has also been shown, in animal models at least, to be an important determinant of fibrosis in the peritoneal cavity. TGF-β1 is expressed at high concentrations in the effluent of peritoneal dialysis patients in response to dialysis and during infection [6]. Mesothelial cells when exposed to TGF-β1 in vitro exhibit pro-fibrotic changes, notably including increased plasminogen activator inhibitor-1 expression, proposed to lead to disordered matrix degradation [7].

Previous work by Margets et al. has demonstrated that over-expression of TGF-β1, or pro-inflammatory cytokines, in the rat peritoneum using an adenoviral vector-driven system led to fibrogenic changes (fibrosis and vascular proliferation) reminiscent of those seen in long-term peritoneal dialysis patients [8, 9]. In subsequent studies in mice, longer-term over-expression, using a helper-dependent adenoviral vector system, led to progressive fibrosis with adhesion formation and encapsulation of bowel that the authors equated to changes seen during EPS in humans [10]. These studies provided a clear link between the expression of TGF-β1 and the genesis and progression of peritoneal fibrosis.

Genetic factors have previously been linked to baseline variability in peritoneal transport characteristics [11], but the extent to which an individual’s genetic make-up may predispose to a scarring (or fibrotic) response in peritoneal dialysis remains uncertain. Margetts et al. have addressed this experimentally, by comparing the responses with the in vivo over-expression of TGF-β1 in the peritoneum of four strains of mice. They found that C57BL/6j mice were susceptible to fibrosis, SJL/j mice were resistant and two other strains had an intermediate phenotype. These pro-fibrotic changes occurred in these mice despite apparently intact TGF-β1 signalling in all animals, and mirror the strain-specific variation in fibrotic responses in other models. These data provide clear evidence for heterogeneity of peritoneal responses to TGF-β1, and are in keeping with an underlying genetic component to the risk of scarring in the peritoneum. Interestingly, however, whilst previous studies have highlighted a propensity to TGF-β1-driven pulmonary fibrosis in C57BL/6 mice [12], this strain appears to be resistant to renal fibrosis in the remnant kidney model [13]. These data suggest that such an underlying genetic propensity to fibrosis may be limited to specific stimuli or organs. This apparent complexity of genetic risk of fibrosis is perhaps not surprising when one considers the likely underlying mediators and mechanisms.

The mechanisms by which strain-specific variation in response to TGF-β1 occur remains an open question. Mice were purchased from the same source and were cared for in the same facility, making systematic environmental differences unlikely. It is, thus, likely that genetically determined differences in TGF-β1 responses amongst the strains underlie the differences in the extent of scarring uncovered. TGF-β1 signalling was shown to be intact in all strains, through the measurement of phosphorylation of Smad proteins. These are the key intermediary molecules that transduce TGF-β1 signals from receptor to nucleus. The data, however, suggest some tantalizing variation in basal and stimulated levels of Smad phosphorylation between the strains, and further experiments examining both Smad- and non-Smad-signalling downstream of TGF-β1 in these animals might definitively identify the mechanisms underlying differences in the extent of scarring seen in these animals.

The TGF-β1 pathway itself is complex and subject to significant tuning of response by other pathways and mediators. Regulation at the levels of TGF-β1 activation, intracellular signalling and interaction of transcription factors with DNA (reviewed in [14, 15] and [16]) lead to changes in the way that cells respond to TGF-β1 signals. Other cytokines are also capable of altering TGF-β1 responses. BMP-7 may be a key antagonist of TGF-β1-dependent fibrotic changes in the peritoneum, and the balance between BMP- and TGF-β-sIGNALs may be the core regulator of mesothelial cell phenotype [17]. In other cellular contexts, for example the kidney, pro-inflammatory cytokines such as IL-1 and IL-6 may also play key roles in dictating TGF-β1 responses [18]. There are, therefore, numerous loci potentially responsible for altering overall fibrosis pathway activity and scarring responses to TGF-β1 signals that may account for the heterogeneity of mouse strain response to TGF-β1 identified in these
It is also important to recognize that understanding the complexity of peritoneal fibrosis in peritoneal dialysis patients extends beyond the over-expression of TGF-β1. The peritoneum in peritoneal dialysis patients is challenged with a complex perturbation of the internal milieu, including uraemia, sub-clinical inflammation and continuous exposure to the constituents of the dialysis solutions, notably glucose and its degradation products, and acidic pH. Alteration of the panoply of cytokines and growth factors is well documented in the peritoneal cavity of peritoneal dialysis patients, which together with regular washout of endogenous secreted compounds is believed to contribute to changes in the resident cell phenotype, extracellular matrix turnover and membrane fibrosis that occur over months or years. Episodes of peritoneal infection further destabilize the homeostatic environment, with recruitment and activation of the various sub-populations of cells and their degradation products, and acidic pH. Alteration of the internal milieu, including complex perturbation of the glucose to the constituents of the dialysis solutions, notably glucose and its degradation products, and acidic pH. Alteration of the panoply of cytokines and growth factors is well documented in the peritoneal cavity of peritoneal dialysis patients, which together with regular washout of endogenous secreted compounds is believed to contribute to changes in the resident cell phenotype, extracellular matrix turnover and membrane fibrosis that occur over months or years. Episodes of peritoneal infection further destabilize the homeostatic environment, with recruitment and activation of the various sub-populations of cells and their degradation products, and acidic pH. Alteration of the internal milieu, including complex perturbation of the peritoneum linked to genetic variation in response pathways and mediators not directly measurable by the peritoneum.

In conclusion, Margetts et al. have provided excellent evidence for a genetic basis to heterogeneity of response to TGF-β1 in the peritoneal cavity. These are important data, in the context of understanding susceptibility to membrane damage. Investigation of the TGF-β1 signalling network and its downstream targets will be a fruitful area for further study in this regard and will doubtless be applied to other pathways as this research expands. Such dissection of the impact of disease phenotypes in well-defined mouse strains, including the use of knock-out and knock-in strains, will be key in unlocking possible differences in signalling and transcriptional responses to TGF-β1 and other mediators, which might give clues as to what drives the genesis of fibrosis and to what allows it to be established such that it impacts on peritoneal dialysis treatment. Studies with biocompatible solutions and those aimed at understanding the impact of infection on peritoneal dialysis patients will also benefit from these approaches, with the aim of producing meaningful advances in preventative, diagnostic and therapeutic interventions that prolong peritoneal dialysis survival.

(See related article by Margetts et al. Transforming growth factor β-induced peritoneal fibrosis is mouse strain dependent. Nephrol Dial Transplant 2013; 28: 2015–2027.)

REFERENCES


14. Leask A. Signaling in fibrosis: targeting the TGF beta, endothelin-1 and CCN2 axis in scleroderma. Front Biosci 2009; 1: 115–122


Nrf2 implicated as a novel therapeutic target for renal regeneration after acute kidney injury

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In the kidney, renal tubular epithelial cells are metabolically very active and packed with mitochondria to fuel transporters such as the Na/K ATPase. As a result, tubular cells are exposed to high levels of metabolic byproducts in the form of reactive oxygen species (ROS). At the same time, due to their high-oxygen consumption, they are particularly vulnerable to ischaemic injury.

Acute kidney injury (AKI) can result from mechanical trauma, ischaemia/reperfusion, sepsis, toxins or nephrotoxic medication. AKI can lead to acute tubular necrosis (ATN) through accumulation of ROS and/or nephrotoxins in tubular epithelial cells which overwhelm cellular defenses of these cells [1, 2]. As a response to this, the body will launch damage control measures, including inflammation and stimulation of tissue repair and regeneration [3]. How tubular regeneration is accomplished is a subject of ongoing controversy but likely it involves dedifferentiation and proliferation of resident tubular epithelial cells, proliferation of intra-tubular stem/progenitor cells characterized by expression of progenitor markers (ALDH+, CD24+ CD133+), or by extra-tubular stem/progenitor cells, e.g. from bone marrow [4, 5]. If injury is not too extensive, the tubular epithelium can often be regenerated and kidney function restored completely [6]. However, severe injury or incomplete recovery of kidney function can lead to progressive deterioration into chronic kidney disease and eventually renal failure with the need for renal replacement therapy. Development of therapies for the prevention of ATN or the acceleration of tubular regeneration could have significant clinical impact.

AKI survival requires damaged and regenerating cells to deal with multiple metabolic challenges imposed by hypoxia as well as high levels of oxidative stress. A preconditioning strategy using hypoxia inducible factor (HIF)-stabilizing prolyl hydroxylase domain inhibitors [7] was shown to prevent ischaemia-reperfusion injury. The HIF-1α pathway is considered to mainly address hypoxia responses, but recently, a link has been suggested between HIF-1α and the transcription factor NF-E2-related factor 2 (Nrf2), which is mainly involved in the response to oxidative stress [8–10].

In this issue of NDT, a next step towards supportive therapies for AKI is made by Hans-Joachim Anders’ group [11]. They describe an ex vivo model of tubular injury and regeneration for the screening of compounds that promote tubular cell survival and tubular repair. In this model, they observed that the dietary phytochemical sulforaphane (SFN) promotes survival during isolation, outgrowth and migration of tubular epithelial cells. This protective effect involved upregulation of several Nrf2-dependent antioxidant enzymes and of transformed mouse 3T3 cell double minute 2 (Mdm2), which promotes cell survival and growth by inactivating p53. Previously, the same group demonstrated that Mdm2 promotes tubular regeneration after post-ischaemic tubular necrosis in mice [12].

Nrf2 is the master regulator of genes encoding antioxidant and phase II drug metabolizing enzymes [13]. Moreover, Nrf2 has anti-inflammatory properties [14]. Consistent with its function, Nrf2 is highly expressed in tissues that are regularly

Received for publication: 6.7.2012; Accepted in revised form: 25.7.2012

doi: 10.1093/ndt/gft202