The Pathophysiology of the Peritoneal Membrane

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ABSTRACT

The development of peritoneal dialysis (PD) as a successful therapy has and still depends on experimental models to test and understand critical pieces of pathophysiology. To date, the majority of studies performed in rat and rabbit models derive mechanistic insights primarily on the basis of interventional pharmacologic agents, blocking antibodies, or transient expression systems. Because body size no longer limits the performance of in vivo studies of PD, genetic mouse models are increasingly available to investigate the molecular and pathophysiologic mechanisms of the peritoneal membrane. We illustrate in this review how these investigations are catching up with other areas of biomedical research and provide direct evidence for understanding transport and ultrafiltration, responses to infection, and structural changes including fibrosis and angiogenesis. These studies are relevant to mechanisms responsible not only for the major complications of PD but also for endothelial biology, host defense, inflammation, and tissue repair processes.


Peritoneal dialysis (PD) is a life-sustaining therapy used by >100,000 patients with ESRD worldwide, accounting for approximately 10 to 15% of the dialysis population.1 The major obstacles to successful long-term PD are infection and deleterious functional alterations in the peritoneal membrane after exposure to dialysis solutions; this loss of dialysis capacity is responsible for increased morbidity and mortality. These alterations, involving approximately 50% of all PD patients, include progressive fibrosis, angiogenesis, and vascular degeneration associated with increased solute transport and loss of ultrafiltration (UF).2–4 In a small percentage of cases, a poorly defined but catastrophic fibrogenic response occurs primarily in the visceral peritoneum, leading to the onset of encapsulating peritoneal sclerosis (EPS) with an associated high mortality rate.5,6

In the past two decades, clinical improvements in therapy delivery and prescription have been introduced, including new dialysis solutions, improved connections, automated PD, and tailored antibiotic strategies. Although these advances reduce the incidence of peritonitis, infectious complications remain a problem, as does membrane failure. There is thus a growing need to understand the molecular basis of these membrane-degenerative events and a need to establish suitable experimental models to define better various aspects of the therapy. This includes a better understanding of transport mechanisms across the peritoneal membrane, improved definition of the response of the peritoneum to infection and inflammation, and deciphering the molecular mechanisms that drive peritoneal fibrosis and vascular damage that lead to membrane dysfunction.4 Many of these investigations using either in vivo animal models or in vitro cell-based systems are based on interventional studies with pharmacologic agents, with blocking antibodies, or in transient overexpression systems.7–10 Although these studies provide significant insight into peritoneal pathophysiology, it is widely acknowledged that they are limited in some cases by a lack of pathway specificity, adverse effects, and transient efficacy, so many questions remain to be addressed.11–13 Until recently, the body size of a species was considered a major limiting factor in performing in vivo studies relevant to PD; however, the development of new approaches for in vivo phenotyping14 coupled with molecular biology techniques provides the potential of using genetically modified mice for clinically relevant mechanistic studies addressing various key aspects of PD pathophysiology.15,16 The purpose of this brief review is to illustrate how these studies (primarily in rodent models) provide a more complete understanding of basic mechanisms and pave the way for the development of novel, specifically targeted diagnostic and therapeutic strategies aimed at reducing infection and improv-

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ing membrane survival and long-term outcomes in patients who are on PD.

**PERITONEAL TRANSPORT, AQUAPORINS, AND UF**

Once technical issues were overcome, mouse models were initially used to characterize the general structure of the visceral and parietal peritoneum that is effectively undistinguishable from that described in rats and humans.\(^{16,17}\) Furthermore, the specific distribution of the water channel aquaporin 1 (AQP1) and endothelial nitric oxide synthase (eNOS) in distinct vascular beds have been confirmed, and exposure of mice to standard glucose dialysis solutions yields equilibration curves for urea and glucose, sodium sieving, and a net UF that are remarkably similar to those obtained in rats and humans.\(^{16}\)

The capacity for UF across the peritoneal membrane is a major predictor of outcome and mortality in PD patients.\(^{18,19}\) According to the three-pore model, the major transport barrier of the membrane is the capillary endothelium, which contains ultrasmall pores (radius \(<3\AA\)) that facilitate the osmotic transport of water.\(^{20}\) Computer simulations have predicted these ultrasmall pores account for approximately 50% of the UF and explain the sodium sieving—the marked fall of the dialysate-to-plasma ratio of sodium during the first hour of PD with hypertonic dialysate.\(^{21}\) The identification of AQPs, a family of integral plasma membrane proteins conserved in bacteria, plants, and mammals, provided critical insights in the molecular mechanisms involving water permeation across biological membranes.\(^{22}\)

The first member of the AQP family to be identified, AQP1, is abundantly expressed in endothelial cells lining peritoneal capillaries, consistent with the predicted topology of ultrasmall pores (Figure 1).\(^{23,24}\) This hypothesis has been substantiated by Yang et al.,\(^{25}\) who showed that osmotically driven water transport across the peritoneum (estimated by a tracer dilution) was decreased in mice lacking AQP1. Ni et al.,\(^{26}\) used an infusion model to demonstrate that, in comparison with control littermates, AQP1 knockout mice lack sodium sieving and have a major decrease in UF, despite unchanged osmotic gradient (Figure 1). The use of AQP1-deficient mice thus validates the three-pore model and provides direct evidence for the role of water channels in PD. Increasing the expression (or function) of AQP1 in the peritoneum, with, for example, corticosteroid treatment, might thus be a potential approach to treating UF failure in PD patients.\(^{27}\)

![Figure 1](https://example.com/figure1.png)

**Figure 1.** Distribution and role of AQP1 in the peritoneal membrane. (A) Cross-section of the human parietal peritoneum stained for AQP1. AQP1 is detected in the endothelium lining peritoneal capillaries, venules, and small veins. m, mesothelium. Bar = 40 \(\mu\)m. (B) Immunogold electron microscopy on mouse visceral peritoneum unicryl sections shows a very strong signal for AQP1 in the plasma membrane and plasma membrane infoldings of capillary endothelial cells. Bar = 500 nm. (C and D) Effect of AQP1 deletion on the transport of water across the peritoneal membrane. Mice with a targeted deletion of Aqp1 are investigated using a peritoneal equilibration test essentially similar to that performed in patients. The dialysate-to-plasma ratio (D/P) of sodium (C) and the initial UF rates calculated from the first derivate of the best fitting curves for each mouse (D) are determined in Aqp1\(^{+/+}\) mice (purple symbols), Aqp1\(^{+/−}\) mice (blue symbols), and Aqp1\(^{−/−}\) mice (red symbols) during a 2-hour exchange with hypertonic dialysate. In comparison with Aqp1\(^{+/+}\) mice, mice lacking AQP1 show a complete loss of sodium sieving and significantly lower initial UF rates. Intermediate values of sodium sieving and initial UF rates are observed in Aqp1\(^{+/−}\) mice. Adapted from Ni et al.,\(^{26}\) with permission.
ACUTE PERITONITIS: ROLE OF NOS ISOFORMS

Acute peritonitis is characterized by an increased endothelial exchange area, with increased transport of small solutes and glucose, loss of proteins into the dialysate, and dissipation of the osmotic gradient, leading to UF failure.\textsuperscript{28,29} Vasooactive substances released during the inflammation reaction, particularly NO, play a role in these changes.\textsuperscript{30} Indeed, inhibition of NOS with \textsuperscript{NG}-nitro-L-arginine methyl ester improve UF and reverse permeability changes in rat and mouse models of acute peritonitis.\textsuperscript{8,31}

The three NOS isoforms—neuronal NOS (nNOS, NOS1), inducible NOS (iNOS, NOS2), and eNOS (NOS3)—are differentially expressed in the peritoneal membrane.\textsuperscript{32}

The neuronal and endothelial isozymes are constitutive isoforms, their activity being controlled by intracellular Ca\textsuperscript{2+} levels, whereas iNOS is quiescent until its transcription is activated by LPS and/or cytokines.\textsuperscript{33} The use of peritonitis models in mice lacking specific NOS isoforms demonstrates the importance of NO for structural and transport-related alterations induced by acute peritoneal infection or inflammation. The deletion of eNOS, which has no effect on peritoneal structure or transport at baseline, significantly attenuates the vascular proliferation and the inflammatory infiltrate (in a catheter-induced model of Gram-positive bacterial peritonitis), resulting in improved UF and reduced protein loss in the dialysate (Figure 2).\textsuperscript{15} Further investigations using a model of LPS-induced peritonitis in mice deficient for each NOS isoform confirm the specific role of eNOS in mediating increased solute transport and loss of UF associated with peritonitis, whereas neuronal NOS and iNOS have no effect.\textsuperscript{34} In contrast, iNOS null mice show more severe inflammatory changes and a trend toward increased mortality after LPS treatment. These data identify specific roles for NOS isoforms in the peritoneal membrane and suggest selective eNOS inhibition may improve peritoneal transport parameters and prevent vascular changes during acute peritonitis.

REGULATION OF PERITONEAL INFLAMMATION AND LEUKOCYTE TRAFFICKING

Acute peritonitis is well described in PD patients and studied in murine models. It begins with infiltration of leukocytes into the peritoneal membrane, starting with a rapid accumulation (within 12 to 24 hours) of neutrophils, which are progressively cleared and replaced by a population of mononuclear cells, monocytes and/or macrophages, and lymphocytes. This temporal switch in the pattern of leukocyte recruitment plays a critical role in the clearance of infection.\textsuperscript{35} IL-6 is a key mediator involved in this regulation, because IL-6 also suppresses the accumulation of neutrophils in other inflammatory tissues.\textsuperscript{36} This IL-6–mediated response depends on the presence of its soluble IL-6 receptor (sIL-6R), which forms a ligand–receptor complex allowing IL-6 signaling in cell types lacking the cognate IL-6R, through the ubiquitously expressed transducing molecule, gp130. Through this mechanism, the sIL-6R/IL-6 complex (so called transsignaling) modulates the expression of specific chemokines and adhesion molecules and regulates the process of apoptosis, thereby influencing leukocyte recruitment (Figure 3).\textsuperscript{37–40} This control primarily results from chemotactic cytokine production by the mesothelial cells lining the peritoneal cavity.\textsuperscript{41} These cells contribute to proinflammatory cytokine-driven activation and synthesize large amount of IL-6 during inflammation;\textsuperscript{42} however, they do not express the cognate IL-6R and are thus regulating chemokine synthesis only when exposed to the agonistic sIL-6R/IL-6 complex (Figure 3).\textsuperscript{38}

Figure 2. The role of eNOS in transport and structural changes induced by acute peritonitis is investigated using a 5-day catheter-induced peritonitis model in wild-type mice (eNOS WT-p) or littermates lacking eNOS (eNOS KO-p). The lack of eNOS is reflected by a significant reduction in the vascular proliferation (A, staining with the endothelial marker CD31; C, morphometry analyses) and a marked reduction in the transport of small solutes (B, dialysate-to-plasma ratio (D/P) for urea during a 2-hour dwell, n = 6 mice in each group; C, area under curve [AUC]) and in the loss of protein in the dialysate (C). Data compiled from Ni et al.\textsuperscript{15}
For gaining insights into the mediators controlling the pattern of leukocyte recruitment during peritoneal inflammation, a mouse model of acute peritoneal inflammation was established by using a controlled dose of cell-free supernatant of *Staphylococcus epidermidis*, a major cause of PD-associated peritonitis. The model recapitulates the pattern of inflammatory events encountered during human PD peritonitis, with early activation of proinflammatory cytokines (TNF-α, IL-1, and IFN-γ) and subsequent changes in chemokine expression and the rapid recruitment of neutrophils and their subsequent replacement by monocytes. By combining clinical and in vitro investigations with studies in IL-6 null mice, it is believed that the initial attraction of neutrophils by proinflammatory cytokine-driven expression of CXC chemokine, MIP-1/KC, is followed by the release of sIL-6R shed from neutrophils, facilitating the formation of sIL-6R/IL-6 complexes. In turn, these complexes suppress the release of other CXC chemokines, ensuring clearance of neutrophils, and simultaneously promoting the secretion of the CC chemokines, such as monocyte chemotactic protein 1 (MCP-1) and RANTES, triggering the recruitment of mononuclear leukocytes. Further studies using IFN-γ null mice establish that IFN-γ is also involved in controlling the initial recruitment of neutrophils, by affecting the local activities of IL-6 and IL-1β, but also in the promotion of their apoptosis and clearance. Using knock-in mice expressing mutant forms of the IL-6 signal transducer molecule gp130, McLoughlin et al. showed that IL-6/sIL-6R signaling also selectively promotes T cell recruitment into the peritoneal membrane through a gp130-dependent, STAT1/3-dependent activation pathway. Taken together, these studies provide useful insight into the actions of IL-6 and its soluble receptor during acute inflammation and suggest that while the transition from innate immunity to acquired immunity facilitates the resolution of inflammation and the clearance of bacterial infection in the peritoneum, dysregulation of this pathway as occurs in chronic inflammation or after repeated infections also contributes to inflammation-induced peritoneal damage. These studies provide clear evidence for therapeutic intervention to reduce inflammation and to promote the clearance of bacterial infections (N.T. and S.A. Jones, unpublished data).

**TRANSGENIC MICE USED FOR CELLULAR STUDIES**

A major interest of transgenic mice is the possibility of harvesting cells to develop primary cultures to investigate the role of specific molecules in a given cell population. This approach has been used to investigate the role of Toll-like receptor 4 (TLR4) in murine peritoneal mesothelial cells (MPMC) exposed to inflammation. Kato et al. developed primary cell cultures of MPMC derived from either C3H/HeN mice (wild-type; LPS sensitive) or C3H/HeJ mice (that lack the response to LPS). Using this system, they observed the induction of MCP-1 and macrophage inflammatory protein 2 (MIP-2) by MPMC stimulated with lipid A depends on the expression of TLR4. Furthermore, leukocyte recruitment into the peritoneal cavity and the production of MCP-1 and MIP-2 in response to LPS are significantly increased in C3H/HeN mice as compared with C3H/HeJ mice. Thus, TLR4 is directly involved in the production of chemokines by mesothelial cells, suggesting that TLR4-mediated pathways reduce the detrimental consequences of peritoneal inflammation. Recent studies also showed that treatment with the soluble form of TLR2 modulates peritoneal inflammation and leukocyte recruitment and does not have a negative impact on bacterial clearance in a peritoneal infection model. These data suggest that therapeutic intervention against inflammation can be achieved without compromising peritoneal host defense.
FIBROSIS PATHWAYS, ANGIOGENESIS, AND EPITHELIAL-TO-MESENCHYMAL TRANSITION

Studies have demonstrated that peritoneal mesothelial cells undergo epithelial-to-mesenchymal transition (EMT) after exposure to injury or associated growth factors (Figure 4) to form fibroblasts. Furthermore, EMT of peritoneal mesothelial cells is associated with angiogenic stimuli and altered solute transport. Angiogenesis and fibrosis seem to be intimately linked through common initiating growth factors and inflammatory cytokines and the EMT process. Understanding the mechanisms of fibrosis and the interaction with angiogenesis is therefore important to developing therapeutic strategies to preserve the peritoneum as a dialysis membrane.

Epithelial-to-Mesenchymal Transition

EMT is an essential process in embryogenesis, beneficial in normal wound healing, but is pathogenic in malignancy and fibrosis. There is increasing evidence to suggest that treatment to prevent EMT may also ameliorate peritoneal fibrosis and angiogenesis and therefore preserve the peritoneal membrane. EMT is a cellular program consisting of a loss of cell–cell and cell–matrix interaction and cell polarity, cytoskeletal rearrangement, and basement membrane degradation with subsequent migration or invasion. Recently, biomarkers for EMT have been categorized and include the loss of the epithelial adhesion protein E-cadherin and upregulation of mesenchymal markers such as fibroblast-specific protein 1.

E-cadherin expression is regulated at multiple levels, including gene expression and both extracellular and intracellular protein cleavage. E-cadherin gene expression is suppressed by a family of regulatory proteins, including zinc finger DNA–binding proteins Snail, Slug, Twist, ZEB1, and ZEB2. These proteins are, in turn, regulated by growth factors such as PDGF, TGF-β, and Wnt proteins.

The complex pathways involved in EMT have been investigated in transgenic mouse models, but these have yet to be studied in the peritoneum to any extent. Smads are key signal transduction proteins involved in TGF-β signaling, and the role of Smad3 in EMT is somewhat controversial. In the kidney, Smad3 is essential for EMT, but EMT is observed in Smad3 knockout mice with local ocular lens overexpression of TGF-β. EMT has been studied in mesothelial cell cultures, which reveal a mammalian target of rapamycin (mTOR) may have a role in peritoneal EMT, and the mTOR inhibitor, rapamycin, has been shown to maintain E-cadherin expression in the face of TGF-β–induced EMT. Likewise, bone morphogenic protein 7 acts as a TGF-β antagonist and in mesothelial cell culture reverses EMT.

Invasion is essential in the full EMT process. Matrix metalloproteinase 2 and MMP-9 have been studied extensively, because they are gelatinases with specificity for basement membrane–associated collagen type IV. Aside from this collagenase activity, MMPs have a variety of other effects, including cleavage of growth factor precursors and growth factor–binding proteins and altering activity of other receptors and proteases.

Metalloproteinase inhibitors have been evaluated in a mouse model as a potential strategy to prevent EMT in the peritoneum. However, the role of metalloproteinase inhibitors in preventing peritoneal fibrosis remains to be determined.

Figure 4. Peritoneal mesothelial cells undergo EMT. The peritoneum of mice was stained for the epithelial marker cytokeratin in green and the mesenchymal marker α-smooth muscle actin (α-SMA) in red. (A through C) In animals exposed to TGF-β, cells with epithelial characteristics (teal arrows) are intermingled with cells expressing both epithelial and mesenchymal markers (white arrows) and fully differentiated myofibroblasts (red arrows). (D through F) Unexposed animals reveal a single mesothelial cell layer positive for cytokeratin with no α-SMA expression. Nuclei are counterstained with DAPI (blue). Magnification, ×200.
model of peritoneal fibrosis using repeated injections of chlorhexidine gluconate. The MMP inhibitor suppresses peritoneal fibrosis and angiogenesis, but the effect on EMT was not evaluated.

Peritoneal Membrane Fibrosis and Angiogenesis

The most consistent change observed in the peritoneal tissues of a patient who is on PD is an increase in the submesothelial thickness associated with peritoneal fibrosis and angiogenesis (Figure 5). In some rare cases, peritoneal fibrosis manifests as an aggressive encapsulation of the bowels (EPS) with significant associated morbidity and mortality. Fibrosis and angiogenesis seem to occur together in peritoneal tissues, and interventions that reduce angiogenesis also reduce fibrosis.

The cause of peritoneal fibrosis is not clear, but both human biopsy studies and animal studies suggested that uremia alone induces fibrotic changes in the peritoneum. An ongoing focus of research concerns the biocompatible nature of PD fluids and their possible fibrogenic effects. Aside from a low pH and lactate buffer, standard dialysis fluids have a high concentration of glucose and contain glucose degradation products (GDPs) as a result of heat sterilization. High concentration of glucose alone induces fibrogenic growth factors in peritoneal mesothelial cells in culture. Both in vivo and in vitro studies find that GDPs induce fibrosis and angiogenesis in the peritoneum. The uremic milieu, along with nonphysiologic PD solutions, leads to the appearance of advanced glycation end-products (AGEs) in the peritoneal tissues. These AGEs bind to a cognate receptor (RAGE), and this direct interaction induces fibrosis. The interaction between fibrosis and angiogenesis may occur at the level of inducing cytokines; TGF-β and inflammatory cytokines induce vascular endothelial growth factor and angiogenesis. Likewise, using RAGE null mice, Schwenger et al. demonstrated that GDPs induce both fibrosis and angiogenesis.

At the cellular level, the fibroblast is a key mediator of peritoneal fibrosis. Selective depletion of fibroblasts using a transgenic mouse with the thymidine kinase gene driven by a fibroblast-specific promoter demonstrated that selective depletion of fibroblasts decreases fibrosis and angiogenesis.

EPS has been identified as a potential important complication of PD; however, the incidence remains controversial. This clinical concern has led to a need to develop realistic animal models of EPS. The standard model used to date includes a daily injection of chlorhexidine gluconate. We recently developed a novel model using a helper-dependent adenovirus expressing TGF-β1 (HDAdTGFβ1). Unlike the first-generation adenovirus, the helper-dependent adenovirus demonstrates prolonged expression. HDAdTGFβ1 administered as a single injection to the peritoneum of mice leads to a progressive fibrosis and bowel encapsulation and suppresses weight gain similar to the clinical course in EPS.

CONCLUSIONS AND PERSPECTIVES

The examples outlined herein reveal how the use of transgenic mouse and cellular models has already made a significant impact on defining basic mechanisms that operate in the peritoneal membrane. The development of transgenic mice for pathways and molecules relevant to specific diseases together with the possibility of investigating minute biologic samples for numerous parameters simultaneously explains why the use of such models is set to transform research into practice. To date, studies in null mice and cells derived from these animals provide direct evidence for the role of specific molecules in peritoneal fibrosis.
mechanistic insights into the transport properties of the peritoneal membrane, the role of cytokines and chemokines in regulating peritoneal inflammation, bacterial clearance and leukocyte recruitment, and pathways involved in structural and fibrogenic alterations that contribute to treatment failure (Figure 5).

As more of these models become available to target other relevant pathways and with the application of multiplex assay and DNA/RNA array technologies in these models, it will become possible to assess the interactive relationships of various physiologic and pathophysiologic pathways in the peritoneum in relation to systemic parameters. Mouse models also offer a vital preclinical resource in which the testing of various therapeutic strategies, arising from the mechanistic approaches mentioned herein, can be evaluated. Limitations of such models should be kept in mind, including the various growth and metabolic rates, the effect of the genetic background, and the possibility of adaptive mechanisms. Despite these limitations, they nevertheless offer a tremendous resource that is poised to transform peritoneal research and lead to targeted interventions to prolong PD therapy.

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DISCLOSURES

None.

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