# IL-17A is a novel player in dialysis-induced peritoneal damage

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**The classical view of the immune system has changed by the discovery of novel T-helper (Th) subsets, including Th17 (IL-17A-producing cells). IL-17A participates in immune-mediated glomerulonephritis and more recently in inflammatory pathologies, including experimental renal injury. Peritoneal dialysis patients present chronic inflammation and Th1/Th2 imbalance, but the role of the Th17 response in peritoneal membrane damage has not been investigated. In peritoneal biopsies from dialyzed patients, IL-17A immunostaining was found mainly in inflammatory areas and was absent in the healthy peritoneum. IL-17A-expressing cells included lymphocytes (CD4+ and γδ), neutrophils, and mast cells. Elevated IL-17A effluent concentrations were found in long-term peritoneal dialysis patients. Studies in mice showed that repeated exposure to recombinant IL-17A caused peritoneal inflammation and fibrosis. Moreover, chronic exposure to dialysis fluids resulted in a peritoneal Th17 response, including elevated IL-17A gene and protein production, submesothelial cell infiltration of IL-17A-expressing cells, and upregulation of Th17 differentiation factors and cytokines. IL-17A neutralization diminished experimental peritoneal inflammation and fibrosis caused by chronic exposure to dialysis fluids in mice. Thus, IL-17A is a key player of peritoneum damage and it may be a good candidate for therapeutic intervention in peritoneal dialysis patients.**

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Chronic kidney disease is reaching epidemic proportions and unfortunately the current treatments at best only delay disease progression, most patients evolving to end-stage kidney disease, needing dialysis until kidney transplantation can be achieved.[1] Peritoneal dialysis (PD) is a safe and cost-effective form of dialysis, a technique characterized by a shorter half-life than hemodialysis because of glucotoxic damage by PD fluids (PDFs) to the peritoneal membrane, leading to sclerosis. Improving the survival of the peritoneal membrane may provide access to dialysis to a high number of patients in developing nations. Chronic exposure to nonphysiological PDFs and episodes of infection cause inflammation and damage to the peritoneal membrane, which undergoes a loss of the mesothelial cell monolayer, submesothelial fibrosis, angiogenesis, and hyalinizing vasculopathy.[2,3] Nevertheless, peritoneal inflammation caused by PDF is a complex phenomenon, and its systemic repercussion is still poorly understood. For this reason, therapies that preserve the integrity of the peritoneal membrane and enlarge the time that patients could receive PD treatment are necessary.

In the 1980s, after an intense research on T-helper (Th) function in mice, the theory that immune regulation involves homeostasis between Th1 and Th2 activity emerged.[4] The Th1/Th2 hypothesis relies largely on the cytokine patterns of these two cell subtypes. Interferon-γ is the signature cytokine of Th1 cells and interleukin-4 (IL-4) is the archetypal cytokine of Th2 cells.[4,5] Uremia and dialysis have been associated with an impaired immune response, an altered Th1/Th2 balance, and the release of proinflammatory cytokines.[6,7] Chronic inflammation is a well-recognized complication in PD patients. Among the plethora of pro-inflammatory and antiinflammatory cytokines found elevated in PD patients' serum, the antiinflammatory cytokine IL-10, the proinflammatory cytokines IL-6, and tumor necrosis factor-α may have important roles in the development of Th imbalances, contributing to cardiovascular disease and wasting syndrome in those patients.[8] Some authors have speculated that the effector memory T cells

from the peritoneal cavity of PD patients form a part of the first line of defense against invading pathogens, thus activating the Th1-polarized response.[9] $CD4^+$ T cells have an important role in the initiation of immune responses by providing help to other cells and by taking on a variety of effector functions during immune reactions. Upon antigenic stimulation, naive $CD4^+$ T cells activate, expand, and differentiate into different Th effector subsets.[4,5] The intensive research conducted in recent years has challenged the classical view of the immune system. Novel $CD4^+$ Th subsets, including Th17 cells and $CD4^+/CD25^{high}/Foxp3^+$ regulatory T cells, have been described, expanding the Th1/Th2 paradigm.[10] Accumulating data suggest that Th17 cells are highly proinflammatory, and IL-17A, as an effector cytokine, is involved in the pathogenesis of host defense, autoimmune responses (including rheumatoid arthritis, inflammatory bowel diseases, and multiple sclerosis), and more recently in chronic inflammatory diseases, such as atherosclerosis and renal diseases.[10–12] However, there are no studies about the potential role of the Th17 response in PD complications. Our aim was to investigate whether IL-17A could be involved in the damage of the peritoneal membrane caused by chronic exposure to PDF, by studying human peritoneal biopsies and effluents from PD patients, and experimental models in mice, including the one that resembles human PD therapy.

## RESULTS

### Expression of IL-17A in the peritoneum of PD patients

There was almost no detectable IL-17A immunostaining in healthy human peritoneum or in predialysis biopsies of PD patients, whereas IL-17A-positive staining was found in all biopsies from PD patients studied (Figure 1a–c). The quantification of IL-17A immunostaining showed a positive Spearman's correlation with the duration of PD treatment ($\rho = 0.673$; $P < 0.05$; $n = 17$ patients, Figure 1d), inflammation, and fibrosis, and a negative correlation with mesothelial integrity ($\rho = 0.553$, $\rho = 0.609$, $\rho = -0.520$, respectively; $P < 0.05$). In serial peritoneum sections of a PD patient, IL-17A-positive staining was associated with inflammatory cell infiltration (including monocytes/$CD68^+$, $CD3^+$, and $CD4^+$ T cells) in the submesothelial zone (Figure 2a). IL-17A-expressing cells include T lymphocytes (Th17 cells), γδ lymphocytes, mast cells, and neutrophils (Figure 2b and c).

IL-17A effluent concentrations were significantly increased in PD patients treated for more than 3 years (Figure 3), showing a positive Spearman's correlation with the duration of PD treatment ($\rho = 0.335$; $P < 0.05$, $n = 41$), as observed with IL-17A immunostaining in the peritoneum, therefore allowing noninvasive IL-17A monitoring.

### IL-17A upregulates proinflammatory and profibrotic factors in the peritoneum *in vivo*

A single intraperitoneal IL-17A dose was injected in mice, and the peritoneal membrane was studied after 10 days. IL-17A induced the presence of inflammatory cells in the submesothelial zone, including monocytes, $CD3^+$, $CD4^+$, and neutrophils (Figure 4a and b). IL-17A injection also increased peritoneal gene expression of proinflammatory factors (including adhesion molecules and chemokines) and fibrotic-related factors, such as transforming growth factor-β (TGF-β), connective tissue growth factor, the extracellular matrix protein fibronectin (FN), and the fibroblast marker α-smooth muscle actin (α-SMA) (Figure 4c–e). To evaluate whether IL-17A could induce peritoneal fibrosis, mice received repeated IL-17A injections (weekly) and the peritoneum membrane was studied after 35 days. IL-17A caused peritoneal membrane thickness associated with elevated FN deposition and positive immunostaining of activated fibroblast markers, such as FSP-1 and α-SMA (Figure 5a and b). Upregulation of some fibrotic-related proteins was confirmed by western blotting (Figure 5c). These data suggest that IL-17A could directly contribute to peritoneal inflammation and fibrosis.

### Chronic exposure of the peritoneal membrane to PDF induces a local production of IL-17A in an experimental mouse model

To further study whether Th17 cells are involved in the peritoneal membrane damage caused by chronic exposure to PDF, we have used an experimental mouse model, which resembles the human situation.[13,14] Daily standard PDF instillation in mice increased peritoneal IL-17A tissue levels compared with saline-instilled control mice, observed at day 7 and remaining elevated after 30 days. The lack of IL-17A induction in saline-instilled mice suggests a PDF-mediated effect. In contrast, tissue levels of interferon-γ and IL-4 (Th1 and Th2 hallmark cytokines, respectively) were not increased (Figure 6a). Upregulation of IL-17A mRNA levels was also found at 7 and 30 days of daily PDF instillation (Figure 6b). In peritoneal sections of PDF-treated mice, $IL\text{-}17A^+$-expressing cells were found, whereas there were no positive cells in control mice (Figure 6c). IL-17A-expressing cells include Th17 ($CD4^+$ T cells) and γδ lymphocytes (Figure 6d). Our data indicate that daily PDF instillation elicited the presence of IL-17A-producing cells (Th17 and γδ lymphocytes) in the peritoneal membrane, and suggest that these cells are the source of IL-17A.

Exposure of the peritoneal membrane to PDF induces the migration of inflammatory cells to the peritoneal cavity. These cells, and their produced cytokines, can be obtained by peritoneal washing, and are representative of the inflammatory process. In the peritoneal lavage, IL-17A levels were increased in mice that received PDF instillation compared with controls (Figure 7a). Moreover, IL-17A peritoneal lavage levels were correlated with peritoneal membrane thickness (Spearman's $\rho = 0.795$; $P < 0.0001$; Figure 7b).

### Peritoneal activation of Th17-related cytokines and transcription factors in response to PDF in mice

To explore whether PDF exposure regulates the upstream mechanisms involved in Th17 cell activation in the peritoneum, the main factors involved in Th17 differentiation, the