Regulatory immune cells in kidney disease

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Am J Physiol Renal Physiol 295: F335–F342, 2008. First published April 16, 2008; doi:10.1152/ajprenal.00077.2008.—Lymphocytes and macrophages act as effector immune cells in the initiation and progression of renal injury. Recent data have shown that subpopulations of these immune cells (regulatory T lymphocytes and alternately-activated or regulatory macrophages) are potent modulators of tissue injury and repair in renal disease. Recent animal studies examining the therapeutic effect of these cells raise the exciting possibility that strategies targeting these cell types may be effective in treating and preventing kidney disease in humans. This review will describe their biological role in experimental kidney disease and therapeutic potential in clinical nephrology.

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Immune Responses in Kidney Disease

Classically, glomerulonephritis, the commonest cause of chronic kidney disease (CKD) and end-stage renal disease, is thought of as immune mediated, while other forms of renal injury are often described as nonimmune. In both circumstances, the immune system responds either to a cognate antigen (as in the case of glomerulonephritis) or to tissue injury involving recognition of damage and an ongoing inflammatory response. Thus the immune response found in CKD can be divided into two parts: 1) the cognate response, where T and B cells respond to a self protein leading to an immune response directed against the kidney or to some other antigen leading to chronic deposition of antigen antibody complexes in the kidney; and 2) the innate response, where inflammation related to injury drives an immune response.

Recent data show that immune cells may also limit the immune and inflammatory response and reduce tissue injury (see Table 1). Interfering with pathogenic mechanisms of disease by impairing recruitment of T lymphocytes into the diseased kidney or blockade of pathogenic T cell subsets can limit disease in a number of models of CKD. Other T cell subsets, which are protective or regulatory rather than pathogenic, are also recruited into diseased kidneys.

Regulatory cells were first described in the early 1970s as suppressor T cells that could suppress inflammatory T cell responses. Initial difficulties in demonstrating their existence in vivo were overcome with the advent of better markers of T cell subsets. The identification of a regulatory population of CD4+CD25+ T cells, which are protective or regulatory rather than pathogenic, is critical to its action (48) is critical to its action. CD4+CD25+ T cells, although these and CD25 have proved ineffective particularly in humans in defining functional regulatory T cells (Treg) subsets. In humans, the recent identification of CD127low expression has been the most definitive marker yet of Tregs (73). Other regulatory cell populations include γδ T cells, double negative T cells, and CD8 cell subsets (7). While regulatory cells comprise a small proportion of the total T lymphocyte population, they appear able to regulate key immune responses in autoimmune and inflammatory diseases, including CKD (3). They were first shown to have a regulatory effect in neonatally thymectomized mice (65). These mice are deficient in CD4+CD25+ T cells and develop a range of autoimmune diseases, including thyroiditis, colitis, and oophoritis (80).

The forkhead transcription factor Foxp3 is a key gene in Tregs. The recent discovery that mice lacking the gene Foxp3 (scurfin mice) are deficient in Tregs (38) and the in vivo regulatory phenotype found in T cells transduced with Foxp3 suggest that Foxp3 itself plays a vital role in the regulatory properties of these cells. Interaction of Foxp3 with other transcription factors such as NFAT (100) and Runt-related transcription factor 1 (60) or modification of Foxp3 by histone acetylation (48) is critical to its action (Foxp3 is discussed in more detail later). Tregs are known to suppress...
effectors and cytotoxic T lymphocyte responses by cell contact, cytokine production, and enhancement of their apoptosis (61, 69). Furthermore, Tregs also downmodulate the function and/or proliferation of other immune cells such as macrophages (53, 79), dendritic cells (43, 55), B cells (50), NK cells (21), and neutrophils (47).

The interaction between Tregs and nonimmune cells (e.g., parenchymal cells) is poorly understood. Parenchymal cells are active participants in immune injury. For example, in kidney disease tubular epithelial cells (TEC) have the capacity to act as antigen-presenting cells, interacting directly with effector inflammatory cells. In the normal kidney, TEC are physically separated from the interstitial and intravascular space by the tubular basement membrane, but this barrier is disrupted in disease, potentially allowing immune cells direct contact. TEC express major histocompatibility complex class I and II molecules and can process and present self and foreign antigen in the context of MHC molecules (23). TEC may act as nonprofessional antigen-presenting cells and cause lymphocyte activation (23) or downregulation (74). Similarly, other “nonimmune” parenchymal cells within the kidney (mesangial cells, endothelial cells) may also participate in immune injury. Research into whether and how Tregs might modulate immune responses via parenchymal cells should provide insights into mechanisms of their protective effect in renal injury.

**CD4+ regulatory cells in kidney injury.** Studies of immune responses in renal injury have used models of both “immune-mediated” glomerulonephritis (e.g., Heymann’s nephritis; HN) and “nonimmune” toxin-mediated injury (e.g., adriamycin nephropathy; AN). In these models, studies of depletion of specific T cell subsets have given the first evidence of a role of regulatory cells in dampening immune-mediated renal injury.

In HN (a rat model of membranous glomerulonephritis), proteinuria was completely abolished by CD4 T cell or chronic CD8 T cell depletion and reduced by acute CD8 T cell depletion (62, 63). What is unclear is whether CD4 help of CD8 T cells occurs because of a breakdown of regulatory cell suppression of T cell activation. In AN (a rodent model of focal sclerosing glomerulonephritis, a leading cause of nephrotic syndrome in humans), we have shown that, in contrast to HN, depletion of CD4 T cells was damaging (87) and depletion of CD8 cells (91) was protective, suggestive of a protective role for a subset of CD4 T cells. As discussed below, depletion of γδ T cells also increased disease severity (99).

In another rat model of toxic renal injury induced by mercuric chloride, regulatory CD4 T cell clones producing transforming growth factor (TGF)-β have been found to be protective against injury, and these potentially are a regulatory subset (4). Additionally, the sensitivity of Lewis rats to HN induction (in comparison to Brown-Norway rats) may reflect a lower level of regulatory cell function (20).

AN is characterized by severe proteinuria and the development of renal failure. It is associated with macrophage and lymphocyte infiltration, reflecting an immune inflammatory response to the initial injury (44, 90). SCID mice (an immunodeficient strain of mice lacking functioning T and B cells) develop similar disease in AN to that of BALB/c mice, indicating that T cells are not necessary for the disease to develop (44). However, we found that reconstitution of SCID mice with CD4 T cells ameliorated disease (94). In contrast, reconstitution with the CD4 negative population containing CD8 T cells worsened disease. SCID mice were reconstituted with 3 × 10^6 CD4+ T cells/mouse 5 days after administration of adriamycin (ADR) at 5 mg/kg. At 6 wk, there was significantly less glomerulosclerosis, tubular injury, and proteinuria than in control SCID mice (P < 0.01). However, reconstitution of SCID mice with the CD4-ve fraction of splenocytes led to worse glomerulosclerosis, tubular injury, and proteinuria than in control SCID mice (P < 0.01). This suggests that within the CD4 subset of T cells are cells that can reduce inflammation and impact innate immunity. We found on histological examination of AN kidneys that CD4+ T cells coexpressing CD25+ were present within the renal interstitium.

To explore whether CD4+CD25+ T cells might have anti-inflammatory properties of their own or function through regulation of effector T cell subsets, we reconstituted SCID mice with sorted CD4+CD25+ T cells. Reconstitution with CD4+CD25+ T cells expressing high levels of Foxp3 reduced glomerulosclerosis and tubular injury compared with SCID mice with AN but no reconstitution. In addition, there was a significant fall in the number of macrophages in both the glomeruli and interstitium of SCID mice with AN that were reconstituted with Tregs compared with the AN alone group. Tregs inhibited lipopolysaccharide-induced macrophage activation in vitro, which was reversed by TGF-β. In vivo blockade of TGF-β using neutralizing antibodies significantly impaired the protective effect of Tregs. These findings suggest a TGF-β-dependent Treg-inhibitory interaction that can explain cognate-independent protection by Treg in renal injury (53).

In addition, CD4+CD25+ T cells were shown to have a protective role in an animal model of anti-glomerular basement membrane (anti-GBM) disease. Rosenkrantz’ group administered 1 × 10^6 CD4+CD25+ Tregs and then induced anti-GBM disease in mice with rabbit anti-mouse GBM antibody.
(98). Mice given Tregs had markedly reduced histological and functional renal injury compared with mice given CD4+CD25+ T cells. Tracking studies using green fluorescent protein (GFP)-labeled Tregs showed that Tregs localized in the renal-draining lymph nodes and spleen and not in the kidneys of nephritic animals. Treg-treated mice did not have reduced immune complex formation within the glomeruli, suggesting that Tregs did not affect the initiating phase of renal injury, but directly reduced end-organ damage by limiting kidney-specific immune cell activation within regional lymph nodes.

In human disease, there is evidence of antigen-specific Tregs in Goodpasture’s syndrome. Here, the antigen is type IV collagen. While the disease is active, collagen-specific T cells are inflammatory, but once the disease subsides, collagen-specific T cells become regulatory (67). The original peptide mapping showed that an IL-10-producing population of CD4 T cells evolved in this disease, further proof that CD4+CD25+ T cells in this disease can inhibit antigen-specific proliferation (6, 67).

Histone/protein deacetylase inhibitors. Another way of modulating Treg function in kidney disease is to enhance their function and/or numbers in vivo. Histone/protein deacetylase (HDAC) inhibitors are promising in this regard. HDAC inhibition was shown recently to enhance Treg numbers and control inflammatory bowel disease and prevent organ rejection (81). Mishra and colleagues (54) demonstrated a protective effect of HDAC inhibition in a murine lupus nephritis model; modulation of Tregs may have been a mechanism of protection.

γδ T cells. γδ T cells are a subset of T cells that have a different and more restricted T cell receptor (TCR) than the more common αβ T cells. They are found predominantly in tissues such as skin and intestine, where they appear to have a regulatory or anti-inflammatory function. They have both inflammatory and regulatory/anti-inflammatory features and appear to function as a bridge between innate and cognate immune systems (26). Specific subsets of γδ cells have been found to colonize the kidney and urogenital tract and expand in disease.

We have demonstrated that γδ T cell depletion leads to worsening of renal disease in AN. This is linked to a specific TCR expressing δ1γδ chains and a highly restricted CDR3 region. These cells were found in greater numbers within the kidney in a variety of models of renal disease. They expressed a number of NK cell receptors, including NKG2D, that were capable of binding to nonclassic “stress” ligands and released the regulatory cytokine TGF-β (99).

Foxp3-transduced Tregs in renal injury. Recently, the forkhead/winged helix transcription factor Foxp3 has been shown to act as a master control gene for the development and function of Tregs (18, 31, 38). Studies have indicated that Foxp3 is specifically expressed by CD4+CD25+ cells and programs their development and function (18, 38, 59, 72). In humans, mutation of the Foxp3 gene results in immunodysregulation, polyendocrinopathy, enteropathy, and X-linked syndrome (“IPEX”), an X-linked immunodeficiency syndrome associated with autoimmunity in multiple endocrine organs (8, 95). A frameshift mutation of Scurfin, the mouse Foxp3, results in early lethality due to hyperactivation of T cells in “Scurfy mice” (mice that die from autoimmune disease within 3 wk of age) (5). The recent description of the GFP-Foxp3 mouse suggests that Foxp3 expression identifies the Treg population and is expressed in αβCD4 T cells (19). Hence more severe pathology is found in Foxp3-deficient mice (Scurfy mice) compared with those depleted of CD4+CD25+ T cells that develop autoimmune disease at a much slower rate. This suggests that Foxp3-expressing T cells may be more effective Tregs than CD4+CD25+ T cells alone (2, 19). Initial studies showed that expression of Foxp3 in CD4 T cells leads to regulatory features, and transfer in vivo is associated with reversal of autoimmune phenotypes (31, 38). Thus gene transfer of Foxp3 has the potential to produce therapeutic Tregs.

We have previously examined the effect of Foxp3-transduced Tregs on AN, a mouse model of chronic proteinuric renal disease. Tregs were created by retroviral gene transfer of Foxp3 to naive T cells (93). Our results showed that transduction of Foxp3 induces a regulatory phenotype in these T cells, and Foxp3-transduced T cells inhibit the proliferation of CD4+CD25- cells in vitro. Furthermore, transduced Tregs protected against renal functional and structural injury in vivo in this murine model. Therefore, cellular therapy using Tregs to reconstitute or strengthen regulatory function is an attractive option for many immune-mediated diseases. Effective gene transfer of rodent Tregs from naive T cells in vitro and in vivo through retroviral Foxp3 transduction brings the prospect of a novel approach for treating CKD. Gene therapy directed at the kidney has been difficult because of the problems of delivery of vector to the kidney; these results suggest that gene therapy by transduction of immune cells may overcome this barrier to treatment.

Analysis of Foxp3 transgenic or knockout mice has further established an essential role for Foxp3 in regulatory T cell development. In Foxp3 transgenic mice, the number of CD4+CD25+ Tregs is increased; both CD4+CD25+ and CD8+ T cells that express Foxp3 exhibit immunosuppressive activity (5, 38). Like Scurfy mice, Foxp3-deficient mice show hyperreactivity of T cells. This finding is due to a deficiency in the development of CD4+CD25+ Tregs (18). Thus Foxp3 appears to be a reliable marker for Tregs as it has been shown to be the master regulator specific for the development and function of these cells.

Loser’s group (51) also studied the effect of Foxp3-transduced Tregs in autoimmune CD40L transgenic mice. Treated mice had lower titers of antinuclear antibody, less renal immunoglobulin deposition, and improved renal function compared with controls. While a comprehensive assessment of renal histology and function was not performed, this study does suggest a protective role of Tregs against lupus nephritis.

Mast Cells

Both Rosenkranz and Blank in differing models of glomerulonephritis have shown a clear protective role for mast cells, whereas other groups had shown a pathogenic role (28, 37, 84). Interestingly, the mast cell has recently been shown to be crucial for Treg maintenance in tissues through the cytokine IL-9, and this indirect role of mast cells in maintaining Tregs may explain their protective effect in the two published reports (52).
Regulatory Macrophages

Macrophages are key mediators in the pathogenesis of most types of primary and secondary human kidney diseases (16, 71). The density of macrophage accumulation correlates with the degree of renal dysfunction and is predictive of disease progression (1). Similarly, many studies in both immune and nonimmune-initiated models of kidney disease have shown a clear association between macrophage accumulation and the development of renal injury (11, 13). A variety of strategies have been used to examine the role of macrophages in experimental renal injury, including adoptive transfer of macrophages, the systemic depletion of macrophages, blockade of molecules involved in monocyte recruitment into the inflamed kidney, and gene modification of macrophages (14, 30, 39, 45, 89).

Macrophages are a diverse and dynamic population of cells, which have the capacity to perform a wide range of critical functions. Originally, activated macrophages were considered as cells that secreted inflammatory mediators and killed intracellular pathogens (58). These macrophages were also defined as classically activated macrophages (M1) following stimulation by LPS or IFN-γ in vitro. Now, increasing evidence indicates that macrophages are able also to modulate immune responses through anti-inflammatory cytokines or regulation of T cell function (22). For example, decidal macrophages have been shown to possess an immunoinhibitory function at the maternal-fetal interface. These cells express indoleamine 2,3-dioxygenase, which is able to block maternal T cell activation against the fetus (27). In vitro, macrophages incubated with IL-4, IL-13, or glucocorticoids can develop a regulatory phenotype (also known as alternatively activated or M2 macrophages), which produce anti-inflammatory cytokines and inhibit T cell proliferation.

The adoptive transfer of both nonactivated and IFN-γ-activated macrophages can worsen inflammation in animal models of acute renal injury (29, 33, 34). In contrast, inhibition of NF-κB in macrophages leads to an anti-inflammatory phenotype, whichameliorated renal injury in an animal model of glomerulonephritis (96). In addition, macrophages transfected with anti-inflammatory cytokines have been shown to reduce renal injury in animal models of glomerulonephritis (41, 85, 97). Whereas these studies support the importance of macrophages in mediating renal injury, they all suffer from the limitation that the strategies employed affect more than just macrophages (36, 82).

We studied the effect of adoptive transfer of macrophages in SCID mice with AN. Macrophages freshly isolated from spleens and peritoneal cavities of mice were cultured in the presence of cytokines, skewed toward a proinflammatory (LPS, designated M1) or anti-inflammatory (IL-4 and IL-13, designated M2 or Mreg) phenotype. Macrophages were transfused into mice 5 days after ADR injection, when renal injury is already established in this model. Mregs significantly protected mice against renal injury, in contrast to M1-injected mice, which had greater injury than seen in ADR-alone controls. The already established in this model. Mregs significantly protected

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the patients on hemodialysis (78) as well as patients with class III and IV lupus nephritis (17). However, patients with lupus nephritis showed a significant tubulointerstitial infiltrate of DCs (17). Similarly, in an animal model of nephrotoxic glomerulonephritis, DCs accumulated in the tubulointerstitial compartment (42). These data suggest that in chronic renal disease, DCs are recruited from the circulation into sites of renal injury. To define the role of DCs in renal injury, Scholz and colleagues (70) examined the effect of renal DCs in a murine model of human necroting glomerulonephritis. By depleting DCs using diphtheria toxin in diphtheria toxin receptor-labeled CD11c/GFP transgenic mice, they demonstrated a remarkable worsening of functional and histological renal injury. The likely (but not proven) mechanism for the protective effect of renal DCs was that ICOS-L expressed on DCs induced IL-10 production by infiltrating CD4+ Th1 cells (70); previous studies have shown that IL-10 can reduce the severity of renal injury (9, 40, 56). These data suggest that in renal injury, DCs are recruited from the circulation and accumulate within the kidney, acting to dampen further tissue injury. Their distribution within the kidney places them as ideal sentinels, monitoring for antigens that may provoke damaging immune responses. Further studies in other models of renal injury are needed to elucidate the precise role of DCs in the kidney.

DCs are able to regulate immune responses because of their unique relationship with Tregs. This partnership is crucial to the control of immune responses by these two cell types. DCs can act in a regulatory capacity by activating Tregs, thereby dampening immune responses. DCs can present specific antigens (e.g., pancreatic islet antigens) to polyclonal Tregs and convert them to antigen-specific Tregs. Antigen-specific Tregs are more potent and specific than polyclonal Tregs at dampening organ-specific immune responses (83). DCs can convert naive T cells to Tregs and expand existing Tregs via release of IL-10 and increased expression of indoleamine 2,3-dioxygenase (46, 57, 76). On the other hand, Tregs can downregulate the antigen-presenting capacity of DCs and render them anti-inflammatory (43, 55). Additionally, DCs become regulatory in response to many other factors, including IL-10 (15), TGF-β (86), granulocyte-macrophage-CSF and IL-4 (49, 64), vasoactive intestinal peptide (10, 68), and apoptotic cells (78). The synergism between DCs and Tregs mediates the regulatory properties exhibited by both cell populations and will be important to understand when therapies aimed at treating and preventing renal inflammation are designed.

To our knowledge, no studies have addressed the potential benefits of adoptive transfer of regulatory DCs in any form of renal injury. Modulation of DC number and function has great potential to be used as a therapeutic strategy for patients with kidney disease.

Potential Applications in Human Renal Diseases

To date, therapies using modulation or adoptive transfer of regulatory immune cells have not been tested in human subjects to either treat or prevent renal disease. Our current summary of knowledge is solely derived from animal studies, and so their applicability to human renal disease is uncertain. The manipulation of regulatory cells to treat and prevent CKD has great potential, however, major hurdles will need to be overcome before treatments such as adoptive transfer of regulatory cells can be used for treatment in humans.

First, achieving adequate purity and numbers of Tregs from humans for adoptive transfer has been difficult to achieve thus far. Identifying specific surface markers of regulatory cells (e.g., CD127low in FoxP3-positive Tregs) and optimizing culture methods for Tregs (e.g., use of IL-2 and anti-CD28 to expand Treg cell numbers in vitro) will help achieve these ends. The choice between polyclonal and antigen-specific regulatory cells is difficult: the former can be harnessed in high cell numbers but are nonspecific, whereas the latter are specific but difficult to culture.

Second, we need to optimize the dose, timing, and frequency of use of regulatory cells in human renal disease. Research has focused on the effect of regulatory cells given before or early after the onset of renal injury, whereas their effect in advanced disease is not known. Further studies will need to look at their effectiveness late in renal injury. Additionally, renal disease has many causes (both immune and nonimmune), and so regulatory cell immunotherapy may be useful only for selected patients; the challenge will be in defining this responsive group. In an animal model of type 1 diabetes, administration of Tregs to nonobese diabetic (NOD) mice soon after disease onset is less effective than before onset, but protection is still achievable using antigen-specific Tregs (83). Selecting suitable patients for regulatory cell immunotherapy will involve knowing which patients are at highest risk of renal disease, and identifying patients with renal disease at an early stage when response to therapy is more likely.

Third, suppressing the immune system with regulatory cells brings with it the associated risks of immunosuppression, i.e., malignancy and infection, both due to immunosuppression per se and also transfer of unwanted tumor or infectious agents with the regulatory cells. To address this issue, antigen-specific regulatory cells could provide more specific protection against the damaged organ without systemic immunosuppression. In addition, optimization of methods of detection of infectious agents may reduce the risk of transmitting infection. However, only a few types of renal disease such as Goodpasture’s syndrome and Alport’s syndrome are known to be caused by a single antigen, whereas more common renal diseases such as membranous nephritis have no defined antigen.

Therefore, while it is an exciting prospect that regulatory cells could be used to dampen or even prevent renal disease, greater understanding of this therapeutic strategy, the diseases being treated, and vigorously designed clinical trials are required before these therapies can be applied generally to humans with renal disease.

Conclusions

The last 5 years have seen an exponential growth in our understanding of regulatory immune cells and their role in autoimmune disease, malignancy, and transplantation tolerance. Recent data have demonstrated a protective role of regulatory T lymphocytes, macrophages, mast cells, and dendritic cells in dampening glomerular and tubulointerstitial inflammation in a number of models of renal injury. Future research will need to concentrate on how best to harness this ability to protect against renal injury while avoiding systemic immunosuppression. Such studies may pave the way for the
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