T Lymphocytes and Acute Kidney Injury: Update

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Abstract

The immune system is among the key pathogenic factors in acute kidney injury (AKI). Various immune cells, including dendritic cells, natural killer T cells, T and B lymphocytes, neutrophils and macrophages are involved. Conventional CD4⁺ lymphocytes are well established to participate in early injury, and CD4⁺CD25⁺FoxP3 regulatory T cells are protective and can accelerate repair. A newly identified kidney T cell receptor + CD4⁺CD8[−] (double-negative) T cell has complex functions, including potentially anti-inflammatory roles in AKI. In this mini review, we summarize the data on the role of lymphocytes in AKI and set the stage for further mechanistic studies as well as interventions to improve outcomes.

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(compared to abundant lymphocyte infiltration in interstitial nephritis or allograft rejection) on the pathophysiology of AKI, experimental models have been used to carefully examine detailed leukocyte trafficking data, plus cause-and-effect roles of different lymphocyte populations [1]. This review will focus on T lymphocytes in AKI. Due to limitations in the manuscript size, the authors will focus on select studies, and the reader is referred to more comprehensive reviews on inflammation during AKI for a more inclusive representation of the published data.

Early Studies Demonstrating a Role for T Cells as Mediators of AKI

Leukocytes, particularly neutrophils, have been implicated in ischemia-reperfusion injury (IRI) of various organs. Initially meant to attenuate neutrophil trafficking to the postischemic kidney, rats were pretreated with monoclonal antibodies against leukocyte adhesion receptors CD11a and CD11b. Though rats were protected from AKI, anti-neutrophil serum in the same study was not

Introduction

Acute kidney injury (AKI) is characterized by both cellular and humoral inflammation. To better understand the role of small numbers of infiltrating lymphocytes

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protective. To explain this inconsistency, the authors postulated that leukocytes other than neutrophils, like macrophages, lymphocytes, and dendritic cells, expressing CD11/CD18 integrins were mediating AKI [2]. A follow-up study in mice deficient in both CD4 and CD8 T cells demonstrated an attenuated injury response to IRI, and T cells increased their adherence to hypoxia-reoxygenated renal tubular epithelial cells in vitro [3].

Harnessing the power of mouse immunology to better understand AKI, athymic mice (nu/nu) and CD4^{-/-} mice were relatively protected from IRI, and adoptive transfer of T cells restored injury – providing direct proof for a role of T cells in AKI [4]. The costimulatory molecule CD28 and T cells producing interferon (IFN)- γ were also pathogenic. These data are consistent with human kidney biopsy samples during transplant acute tubular necrosis, where T cells are seen clogging up the medullary vasa recta [5]. An alternative approach targeting T cells in AKI was performed by blockade of the T cell CD28-B7 costimulatory pathway, providing further evidence that T cells were early mediators [5]. Anti-B7-1 antibody, but not anti-B7-2, markedly reduced leukocyte accumulation in the kidney and improved renal function in rats after warm IRI, suggesting a predominant role of the B7-1 pathway in the protection against renal IRI through inhibition of T cell adherence and activation. B7-1 expression was identified on endothelial cells of the ascending vasa recta in postischemic human kidney (within 1 h), suggesting a potential role of T cell costimulation in renal IRI [6].

Mechanistic Studies on T Cells in AKI

With the finding that T cells, particularly CD4⁺ cells, were mediating AKI, it was hypothesized that CD4 T cells that functionally differentiated into a Th1 (IFN- γ producing) phenotype were pathogenic and those with the Th2 [interleukin (IL)-4 producing] phenotype were protective. To test this hypothesis, studies were performed that demonstrated that STAT4^{-/-} mice, which are deficient in IFN- γ production, were protected from renal IRI, while STAT6^{-/-} mice, which are IL-4 deficient, had a more severe course [7]. This study demonstrated a complex role for T cells in AKI, much like in more traditional immune diseases, where T cells could have either a pathogenic or protective phenotype depending on the subtype.

An emerging area of attention is the high incidence of transition of AKI to chronic kidney disease (CKD) in AKI survivors, occurring particularly in the elderly, those with

underlying kidney disease, or those with a prolonged bout of AKI. A detailed examination of severe ischemic AKI leading to CKD in mice demonstrated that persistent renal and extrarenal immune responses occurred after a single episode of severe IRI. Late immunologic changes during CKD development at 6 weeks in these mice included increased CD4⁺ infiltration, and upregulation of IL1- β and RANTES (regulated upon activation, normal T-cell expressed and secreted). Spleens from ischemic mice, but not sham-operated mice, had significant increases in T cell IFN- γ production, even though they had similar numbers of CD4 and CD8 cells. Taken together, this shows that these immune processes resulting from kidney injury could have long-term consequences on renal disease progression [8]. Thus, the hypothesis developed that IRI could expose normally sequestered antigens that would in turn lead to proliferation of antigen-recognizing pathogenic lymphocytes. This led to experiments demonstrating that adoptive transfer of lymphocytes from IRI mice (donors) with significant fibrosis and cellular inflammation traffic to normal kidneys in recipient mice and directly mediate the development of albuminuria [9].

Discovery of Resident CD4⁺CD25⁺ Regulatory T Cells and T Cell Receptor + CD4–CD8– in the Kidney

In order to better study the mechanisms by which low numbers of infiltrating T cells in the mouse kidney could modify AKI and subsequent progression to CKD, a tissue digestion and sieving technique followed by flow cytometry was developed [10]. Using this technique, despite extensively flushing of the kidney, a population of CD4⁺CD25⁺ regulatory T (T_{reg}) cells was found in the normal kidney, which increased gradually after AKI. Another population, previously unknown to reside in the kidney, the T cell receptor (TCR)+ $\alpha\beta$ CD4–CD8– [double-negative (DN)] T cells, were discovered and numbers changed early after IRI [11]. Given that CD4⁺CD25⁺ T_{reg} cells were known to be anti-inflammatory, it was hypothesized that they could accelerate repair from AKI. Mice treated with CD4⁺CD25⁺ T_{reg} depleting PC61 monoclonal antibody 24 h after ischemia showed increased tubular damage in the cortex and outer medulla, and reduced cortical tubular proliferation, demonstrating that T_{reg} cells were required for normal repair [12]. In complementary experiments, T_{reg} cells were infused into wild-type mice starting 24 h after initiation of AKI, and there was a significant improvement in tubular repair. T_{reg} cells

Fig. 1. Immune cells in AKI – overview and introduction of the DN T cell. Immune cells reside in the kidney steady state and increase after IRI. T cells contribute to increased vascular permeability after IRI and important roles are played in AKI by dendritic cells (DCs), macrophages, NK and NKT cells, B cells and neutrophils. During the reperfusion phase, these cells adhere to activated endothelium

and other cells responding to cytokines, chemokines, oxygen free radicals, complement, coagulant factors, and other mediators. This triggers the inflammatory cascade and increases cytokine levels (IFN- γ and TNF- α , for example) that mediate inflammation after ischemia. TLRs = Toll-like receptors. Panels represent the outer medulla (modified with permission [1]).

reduced proinflammatory cytokine production by other immune cells, a possible mechanism by which they accelerated repair after AKI. CD4+CD25+ T_{reg} cells were also found to be pathogenic in the early injury phase of ischemic AKI in a series of elegant studies both depleting and modifying these cells. Partial depletion of T_{reg} cells with an anti-CD25 monoclonal antibody has been performed and showed worsened ischemic kidney damage. FoxP3+ T_{reg} -deficient mice accumulated a greater number of leukocytes, while T_{reg} repletion significantly attenuated renal

injury and leukocyte accumulation. Adoptive transfer of wild-type T_{reg} cells into RAG-1 knockout mice also prevented kidney injury; however, transfer of IL-10-deficient T_{reg} cells did not [13].

Despite exciting studies on CD4+CD25+ T_{reg} cells, it was intriguing that the most abundant T cell type in normal mouse kidney was the DN T cell. DN T cells represent a rare subset that normally comprises 1–5% of TCR $\alpha\beta$ cells in the peripheral blood and lymphoid organs. Nonetheless, DN T cells comprise a significant component of T

cells residing in the non-lymphoid tissues. They are found in high numbers in mucosal tissue (small intestine and a major subset in the large intestine) [14], female reproductive tract (dominant lymphocyte population; 70–90% in naïve C57BL/6 mice [15]), epithelial organs (kidney, liver, and skin), and bone marrow in the steady state. In the kidney, DN T lymphocytes comprise a high percentage ($23 \pm 4\%$; $18\text{--}32\%$; $p < 0.001$) of T cells with a high proportion of activated CD69+, an effector/memory CD44+CD62L-phenotype, and a distinct TCR repertoire [11].

Potential Role of DN T Cells in the Maintenance of Normal Kidney Homeostasis and Possible Players as Immune Modulators of Repair/Resolution from AKI

DN T cells are best known for their accumulation in secondary lymphoid organs of humans and mice bearing loss-of-function mutations (*lpr* and *gld* mutations) in the Fas death pathway. These mutations also cause a lupus-like syndrome and DN T cells produce IL-17. In lupus nephritis patients, DN T cells are found in greater numbers in the peripheral blood and produce IL-17 [16], which is pathogenic in systemic lupus erythematosus. Children bearing dominant interfering mutations in the Fas pathway develop a syndrome similar to that in *lpr* and *gld* mice and referred to as autoimmune lymphoproliferative disease. Whether DN T cells play an immunoregulatory role in this disease is currently unknown. The underlying mechanism is still unclear, especially the role of DN T cells in the protection against organ-specific autoimmunity.

Significant expansion and accumulation of DN T cells in the periphery occur in allotransplantation, autoimmune diseases, cancers, and in response to certain infectious diseases. It also occurs in consequence to certain spontaneous mutations in humans and mice and in many

TCR transgenic mice, *gld* mice, which accumulate DN T cells in the periphery due to homozygous point mutations in the Fas ligand, are more resistant to ischemic kidney injury than age-matched controls [17]. These results provide insight into a promising new target for therapeutic intervention in kidney diseases.

Both inflammatory and anti-inflammatory functions have been assigned to expanded DN T cells in cases of autoimmune diseases and genetic alterations. Peripherally expanded DN T cells may serve as surrogates for tissue-resident DN T cells to facilitate investigation.

DN T cells with regulatory properties are increasingly being reported, notably after allogeneic hematopoietic stem cell transplantation with a potential role for DN T cells in human allohematopoietic stem cell transplantation with graft-versus-host disease.

Conclusion

Despite initial skepticism, the bulk of data in the field demonstrates an important role for immune cells in the early pathogenesis of AKI. There is increasing evidence that immune cells are also participating in normal and misguided repair after AKI, with implications for CKD development. There are many important subtypes of leukocytes in AKI, including dendritic cells, macrophages, natural killer (NK) cells, NKT cells, B cells, T cells and neutrophils (fig. 1) [1]. Besides the pathogenic CD4+ cell, an important mediator is the anti-inflammatory CD4+CD25+ T_{reg} that has the potential to be harnessed to decrease early injury and also enhance repair. There is also excitement about a newly discovered kidney T cell which has emerged as a potential player, the DN T cell. The role and functions of this and other T cells are in the process of being unveiled. An improved method to study these DN T cells will aid in this process [18].

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