Emerging therapeutic potential of group 2 innate lymphoid cells in acute kidney injury

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Abstract

Acute kidney injury remains a global challenge and, despite the availability of dialysis and transplantation, can be fatal. Those that survive an acute kidney injury are at increased risk of developing chronic kidney disease and end stage renal failure. Understanding the fundamental mechanisms underpinning the pathophysiology of acute kidney injury is critical for developing novel strategies for diagnosis and treatment. A growing body of evidence indicates that amplifying type 2 immunity may have therapeutic potential in kidney injury and disease. Of particular interest are the recently described subset of innate immune cells, termed group 2 innate lymphoid cells. Group 2 innate lymphoid cells are crucial tissue-resident immune cells that maintain homeostasis and regulate tissue repair at multiple organ sites, including the kidney. They are critical mediators of type 2 immune responses following infection and injury. The existing literature suggests that activation of group 2 innate lymphoid cells and production of a local type 2 immune milieu is protective against renal injury and associated pathology. In this review, we describe the emerging role for group 2 innate lymphoid cells in renal homeostasis and repair. We provide an in-depth discussion of the most recent literature that use pre-clinical models of acute kidney injury and assess the therapeutic effect of modulating group 2 innate lymphoid cell function. We debate the potential for targeting these cells as novel cellular therapies in acute kidney injury and discuss the implications for future studies and translation.

Keywords

Group 2 innate lymphoid cells, ILC2, type 2 immunity, innate immunity, alternatively activated macrophages, acute kidney injury, ischemia-reperfusion injury.

Acute kidney injury (AKI), an abrupt decline in kidney function, is a common disorder with profound effects on mortality and morbidity [1]. Approximately 13 million people in the US are affected by AKI each year and approximately 2 million cases are fatal [2]. Meta-analysis of epidemiological studies demonstrated that the pooled incidence of AKI in hospital patients is 22% and AKI is independently associated with an up to 16-fold increase in the risk of death [3–5]. Indeed, the overall mortality of patients with AKI requiring renal replacement therapy was 46% [4]. Acute and chronic kidney disease are strongly interrelated, with patients recovering from AKI at increased risk of chronic kidney disease (CKD), and individuals with CKD have a substantially increased risk of AKI [6].

AKI can arise from a diverse array of pathophysiologic processes including hemodynamic compromise, nephrotoxic injury, autoimmune glomerulonephritis and urine outflow obstruction [7]. A common cause of AKI, however, is kidney ischemia, which induces hypoxic cell death in the most metabolically active components of the kidney, the tubular epithelium [8] (reviewed in [9]). Tubular epithelial injury results in necrosis and shedding of epithelia from the basement membrane, a process recognised as histopathological lesions termed acute tubular necrosis (ATN) [9–11]. Occlusion of tubular space by necrotic epithelial tissue results in glomerular injury and obsolescence [12]. The subsequent reduction in the quantity of functional nephrons (comprising afferent and efferent arteriole, glomerulus, tubule and collecting duct) leads to reduced glomerular blood flow and filtration, retention of waste products and impaired homeostasis of major electrolytes in the systemic circulation [11–13].

The Kidney Disease Improving Global Outcomes (KDIGO) initiative proposed the definition of AKI as an elevation in serum creatinine (SCr) within the prior 7 days, or by a reduction in urine output sustained over 6 hours [14]. However, with the recognition that increasing severity of AKI is associated with elevated risks of CKD and mortality, the Risk, Injury, Failure, Loss, End stage

(RIFLE) criteria were proposed to identify and grade injury based on the magnitude of SCr rise and urine output reduction [15]. When renal function is impaired for ≥3 months the patient may be defined as having CKD [16]. Therefore, a therapeutic window for reducing CKD exists by preventing AKI in high risk patients and improving regeneration or repair of the kidney following AKI. Importantly, the available therapies for AKI are of a supportive nature and do not treat the injury itself [1,17,18]. Hence, there is a clear need for an improved understanding of the cellular mechanisms that underpin the pathogenesis of AKI and CKD.

A brief overview of murine experimental models used to study AKI

Rodents, especially mice, are routinely used to model kidney injury and assess the cellular mechanisms due to ease of genetic manipulation and husbandry [19,20]. Male mice are primarily used as they display increased susceptibility to renal ischemia [21]. Renal ischemia-reperfusion injury (IRI) models are useful since ischemic injuries are common in renal transplantation [22]. Ischemia can be induced either in one (unilateral IRI) or both (bilateral IRI) kidneys under normothermic or hypothermic conditions [23–25]. Chemical-induced models of injury are also used, including adriamycin (doxorubicin) and cisplatin-induced nephropathy [26,27]. Both are well-documented to cause injury, inflammation and the development of fibrosis and glomerulosclerosis [26,27]. However, these compounds are administered systemically and therefore may have off-target effects on other organs independent of the renal injury. Though there are limitations in each experimental model, they have been indispensable for understanding immune responses and mechanisms of repair following AKI [19]. Indeed, models such as these have been used to begin to explore the functional role of a recently described subset of immune cells called innate lymphoid cells (ILCs).

Innate lymphoid cells

ILCs are categorized into three subsets based on their cytokine profiles and expression of specific transcription factors [28]. Group 1 ILCs (ILC1s) include the natural killer (NK) cell lineage and the non-NK ILC1s that resemble T helper (T_H)1 cells as they require the transcription factor T box 21 (*Tbx21*; T-BET) for their development and predominantly produce interferon (IFN)-γ [29]. Group 2 ILCs (ILC2s) resemble T_H2 cells as they require GATA binding protein 3 (GATA3) and produce type 2 cytokines, such as interleukin (IL)-4, IL-5, IL-9 and IL-13 [30,31]. Group 3 ILCs (ILC3s) comprise subsets that resemble T_H17 and T_H22 cells and require RAR-related orphan receptor gamma (*Rorc*; RORγT) and produce IL-17 and IL-22 [32,33].

Natural and inflammatory ILC2s

ILC2s are rare immune cells but are potent innate producers of type 2 cytokines in response to epithelial-derived alarmins, particularly IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) [30,31] (Figure 1). ILC2s are also required for the development of effective T_H2-mediated adaptive immune responses [34]. ILC2s have been suggested to exist in distinct states depending on the cytokines which drive their activation [35]. Under homeostatic conditions, natural ILC2s (nILC2s) are resident in most tissues, including the kidney, and respond primarily to IL-33 [36]. Indeed, nILC2s are defined as being IL-33 receptor (*Il1rl1*; ST2) positive [31]. Inflammatory ILC2s (iILC2s) however, are difficult to detect under homeostatic conditions but are rapidly induced in response to IL-25 [37]. The iILC2s represent a subpopulation of ILC2s that are ST2 negative [37]. There is evidence of plasticity between ILC states and it has been proposed that iILC2s can act as progenitors for nILC2s or even ILC3s [37,38]. These findings indicate that the tissue resident pool of nILC2s may be replenished by iILC2s and that resident cells may change in response to certain insults.

ILC2s coordinate innate and adaptive immune responses

ILC2s are resident in murine and human kidneys and exhibit similar type 2 effector cytokine profiles [39,40]. IL-4 is produced by ILC2s in response to alarmins from epithelia, helminth infection or activation of pattern recognition receptors [41,42]. Interestingly, ILC2s express the IL-4 receptor and are sensitive to IL-4 through positive feedback, which can further enhance ILC2 activation and cytokine production [43]. IL-5 is constitutively produced by ILC2s in adult mammals, which is required to maintain homeostasis of eosinophils and in turn maintain homeostasis of macrophages [36,39]. ILC2s also produce significant amounts of IL-13 which is required for recruitment of eosinophils [34,39]. Additionally, IL-13 induces production of mucins that are required for tissue homeostasis [44–47]. IL-4 and IL-13 are also critical for the polarization of macrophages towards the alternatively activated state (AAM; also known as M2) which can dampen excessive inflammatory immune responses and aid with epithelial recovery following insults within the kidney and other organs [48] (reviewed in [49]).

ILC2s express the inducible T-cell co-stimulator (ICOS) in addition to its ligand (ICOSL; ICOSLG in human) and ICOS-ICOSL interactions between ILC2-ILC2, or between ILC2s and T_H lymphocytes, enhances cytokine production [50–52] (Figure 1). However, ICOS-ICOSL interactions between ILC2s and induced regulatory T cells (iT_{reg}) has the opposite effect [53] (Figure 1). ILC2s have also been demonstrated to induce the expansion of T_H2 and T_{reg} *via* the expression of the costimulatory molecule TNF superfamily member 4 (*Tnfsf4*; OX40L) [54]. Through the production of IL-13, ILC2s can increase the activation of T_H2 cells indirectly through dendritic cells that produce the T-cell attracting chemokine CCL17 [34,55]. Additionally, IL-13 can recruit macrophages and other phagocytes to the area and cause dendritic cells to migrate to lymph nodes and prime naïve T-cells to differentiate into T_H2 cells [56,57]. The interaction between ILC2s and T cell subsets is complex and tightly regulated to allow heterogeneous function in different contexts and locations.

Diversity of ILC2s

In addition to cytokines, ILC2s also produce the growth factor amphiregulin (AREG), which promotes tissue repair [58,59]. In the lung, AREG is essential for resolution of tissue homeostasis following viral respiratory infection [60]. Excessive AREG signaling can drive fibrosis in tissues such as the lung and kidney, and AREG inhibition may be beneficial in this context [61,62]. Recent literature suggests that AREG is also produced by kidney ILC2s and is renoprotective [63]. ILC2s have also been shown to express the antigen presenting molecule major histocompatibility complex class II (MHCII) [56,57]. This allows them to act as antigen presenting cells (APCs) to stimulate the adaptive immune system [56,57]. Recent advances have shown that ILC2s can be neuronally regulated, specifically by the neuropeptide neuromedin U (NMU), which signals through the surface NMU receptor 1 (NMUR1) to drive their activation and expansion [64–66] (Figure 1).

There is also a growing body of evidence suggesting ILC2s exhibit sexual dimorphism. Depending on the tissue, ILC2s express androgen and estrogen receptors and this signaling can alter their activity [67–71] (Figure 1). Androgens suppress lung ILC2s and appear to inhibit differentiation of ILC2 progenitors from the bone marrow, whereas estrogen has the opposite effect on uterine ILC2s [67–71]. Interestingly, multiple reports have found that male mice have fewer tissue resident ILC2s within multiple organs, though it is not yet known whether this is the case in humans [67,69,71]. Male mice are also more susceptible to kidney injury [21,72]. It is currently unknown whether this discrepancy in the number of ILC2s under homeostatic conditions in males is causal or merely coincidental. Immune-related factors and signalling pathways such as IFN-γ, IL-27 and signal transducer and activator of transcription (STAT)-1 can also suppress the activity of ILC2s [73–75] (Figure 1). This may be a mechanism of how pathogens can suppress host repair responses. These immune-related factors may also prove to be avenues for modulating ILC2s, either to increase or decrease their activity depending on the disease context (Figure 1); similar to that described for type 2-mediated allergic diseases [76].

ILC2s as a potential cellular therapy in renal injury

ILC2s have recently been shown to have protective effects in mouse models of IRI [77]. Administration of recombinant mouse IL-25 prior to IRI induction resulted in the expansion and activation of ILC2s, which reduced tubular damage, blood urea nitrogen (BUN) and SCr following IRI [77]. To further confirm the role of ILC2s in AKI, ILC2s were isolated from naïve mice and stimulated *ex vivo* with IL-25 [77]. These activated iILC2s were then adoptively transferred into recipient mice one day prior to the induction of IRI [77]. Mice that received iILC2s had significantly reduced tubular damage, BUN and SCr [77]. Additionally, *in vivo* IL-25-stimulated ILC2s were able to induce an AAM phenotype when co-cultured with kidney resident or bone-marrow derived macrophages *ex vivo* [77]. This suggests that ILC2s may induce protective effects *via* the induction of AAMs. However, it is difficult to conclude whether ILC2s are constitutively renoprotective without introduction of exogenous IL-25 [77]. Furthermore, iILC2s are predominantly responsive to IL-25 and are not considered to be the resident population of ILC2s within peripheral tissues [37].

Following this initial study, the role of ILC2s were investigated in a model of doxorubicin-induced nephropathy [78]. A similar approach was used to activate ILC2s in this model, however, the cytokine IL-33 was administered for 5 days, starting the day after doxorubicin administration [78]. IL-33 induced partial protection against the typical features of doxorubicin-induced nephropathy such as tubular inflammation and fibrotic-like remodeling [78]. It was demonstrated that these effects occur independently of adaptive immunity using mice deficient in T and B cells (*Rag2*$^{-/-}$), and mice deficient in T, B and ILCs (*Rag2*$^{-/-}$*Il2rcg*$^{-/-}$) [78]. However, it is also possible that non-ILC, ST2 expressing cells such as basophils, eosinophils and mast cells may have important roles in protection against renal insult [79–83]. As the ILC-deficient animals lacked T and B cells in addition to all ILC subsets, not just ILC2s, it is difficult to conclude that ILC2s are required [78]. In

this study, IL-33-activated ILC2s were also able to induce an AAM phenotype, which is consistent with the mechanism of protection following IL-25 administration [77,78]. Importantly, ILC2s have been found in human kidney biopsies, which identifies the potential for these cells to be targeted in humans [78]. However, alterations in ILC2 number, function and phenotype in human kidney diseases requires further investigation.

The potential complementary effects of IL-2 and IL-33 were investigated using models of chemical-induced injury, by doxorubicin and cisplatin, in addition to IRI [84]. In each model, treatment with IL-2 and IL-33, prior to the induction of injury, protected against injury through the induction of both T_{reg} and ILC2s [84]. In addition, a novel hybrid cytokine created from IL-2 and IL-33 (IL233) conferred improved protection against injury compared to either cytokine alone [84]. In some experiments, IL233 was sufficient to prevent animals from becoming moribund following IRI, while all vehicle treated mice were culled within 3-4 days following injury as a humane intervention [84]. These results are exciting proof-of-principal evidence for prophylactic treatment and intermediate intervention, although it is unlikely this particular therapy will be a viable option in humans. The main challenges are that IL-33 has multiple targets and cannot currently be administered directly to the kidney without the use of invasive techniques. For patients with type 2-mediated allergic diseases, such as asthma, systemic activation of ILC2s and other IL-33 responsive cells would likely be detrimental and potentially life-threatening.

Most recently, the therapeutic potential of ILC2s in IRI has been further elucidated [63]. IL-33 treatment or transfer of IL-33-stimulated ILC2s was sufficient to significantly reduce SCr, tubular injury and increase survival to 100% out to 28 days post injury [63]. In other studies, treatment with murine IL-33, or recombinant human IL-33 in humanized mice, prior to injury, induced similar protective effects, although only the day following injury was assessed [63]. Loss of function studies were also performed by depletion of ILC2s in T/B cell-deficient (*Rag1*$^{-/-}$) mice by treatment

with anti-CD90 [63]. These studies demonstrated that the protective effects of IL-33 were significantly diminished in the absence of ILC2s [63]. However, these mice were also depleted of other CD90 expressing cells including T_{reg} cells, which may also be critical for this renoprotection [63,83,85]. Furthermore, a complex bidirectional relationship exists between ILC2s and T_{reg} [63,83,85]. This concept is supported by studies examining the IL233 hybrid cytokine [84]. Interestingly, depletion of ILC2s and other CD90 expressing cells did not worsen the severity of injury [63].

Our understanding of the innate immune system and its role in the resolution and repair of tissue injury is increasing. However, it is far from complete and further characterization of the immunological interactions, particularly in kidney disease, is required for translation into clinical practice. Recent studies provide a strong rationale to further explore the role of ILC2s in AKI. Whilst these provide substantial steps forward in our understanding of the immunology of AKI, it is also important to consider that *in vivo* or *ex vivo* stimulated cells may establish an artificial state that is not representative of their normal functions in human AKI patients. Collectively, these studies show that artificially increasing ILC2s provides protection from AKI and decreases pathology (Table 1). Since *Rag1*$^{-/-}$, *Rag2*$^{-/-}$ and *Rag2*$^{-/-}$*Il2rcg*$^{-/-}$ mice are deficient in T and B lymphocytes, the next important step to is show that the effects are maintained by selective depletion of ILC2s whilst preserving the adaptive populations; an approach described by Oliphant and colleagues [56]. This is particularly important as a recent report suggests ILCs may be redundant, since there was no susceptibility to any particular human disease in patients with ILC lymphopenia [86]. There are also conflicting reports which suggest that therapeutic use of IL-33 may be detrimental in AKI, discussed below.

Therapeutic potential of IL-33-induced ILC2 activation; a double-edged sword

Using a cisplatin model, IL-33 treatment was detrimental and led to increased SCr and histopathology [87]. Administration of a soluble form of the IL-33 receptor, ST2, significantly reduced these features [87]. Consistent results were achieved using IL-33 and soluble ST2 within an IRI model, and a similar conclusion was reached using unilateral ureter obstruction [88,89]. Mice deficient in either IL-33 or ST2 (*Il33*$^{-/-}$ or *Il1rl1*$^{-/-}$) were partially protected against the remodeling and fibrotic changes following injury, and *Il33*$^{-/-}$ mice were also protected against features of injury following IRI [89,90].

The dosage of IL-33, length of administration and/or the type of renal injury may drastically alter the response exhibited to be advantageous or deleterious. IL-33 treatment was beneficial at a dose of 0.3µg per day for 5 days, but was detrimental at a lower dose of 0.05µg per day for 14 days [63,88]. Whilst both studies utilized models of IRI, there were differences in the ischemia timeframe and in the surgery itself [63,88]. These contrasting conclusions could potentially be explained by the composition of the immune cells within the kidney and circulation at the time of IL-33 treatment. It is emerging that diverse microbiomes in experimental animals in different facilities has substantial impact on immune responses in multiple contexts. One way around this may be to perform similar studies in germ-free mice and mice that are "wild-type" with diverse microbiomes.

Concluding remarks

Amplification of ILC2s using IL-33 may be effective in attenuating the deleterious consequences of AKI in preclinical models. However, since IL-33 may be both beneficial and detrimental, more selective strategies are required to target ILC2s to delineate their role in kidney injury and disease. The crucial next step is to investigate whether these strategies are sufficient to prevent or delay progression to chronic disease and end stage renal failure. It is also plausible that ILC2-activating therapies could be detrimental if assessed at later time points due to the exacerbation of fibrosis as a

result of uncontrolled AAM activation. If indeed ILC2-activating therapies are proven to be effective in the prevention of chronic disease, there is still the issue of targeting therapeutics directly to ILC2s and only within the kidney. We envision that future therapeutic strategies could involve isolating circulating immune cells, such as ILC2s, from patients followed by *ex vivo* stimulation to promote activation and translocation to the kidney, and finally re-introduction to the patient. However, further studies are required to better define the diverse function of ILC2s in kidney injury and disease.

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Statement of author contributions

GJMC drafted the manuscript and figures. SL, AVD, SHJ and PMH provided critical review of the manuscript. MRS conceptualized, reviewed and edited the manuscript. All authors provided final approval of the submitted and published versions.

List of abbreviations

AAM, alternatively activated macrophage; AKI, acute kidney injury; APC, antigen presenting cell; AREG, amphiregulin; ATN, acute tubular necrosis; BUN, blood urea nitrogen; CKD, chronic kidney disease; ICOS, inducible T-cell co-stimulator; ICOSL, inducible T-cell co-stimulator ligand; ILC, innate lymphoid cell; ILC1, group 1 innate lymphoid cell; ILC2, group 2 innate lymphoid cell; ILC3, group 3 innate lymphoid cell; iILC2, inflammatory group 2 innate lymphoid cell; IRI, ischemia-reperfusion injury; iT_{reg}, induced regulatory T cell; nILC2, natural group 2 innate

lymphoid cell; NMU, neuromedin U; NMUR1, NMU receptor 1; SCr, serum creatinine; TSLP, thymic stromal lymphopoietin

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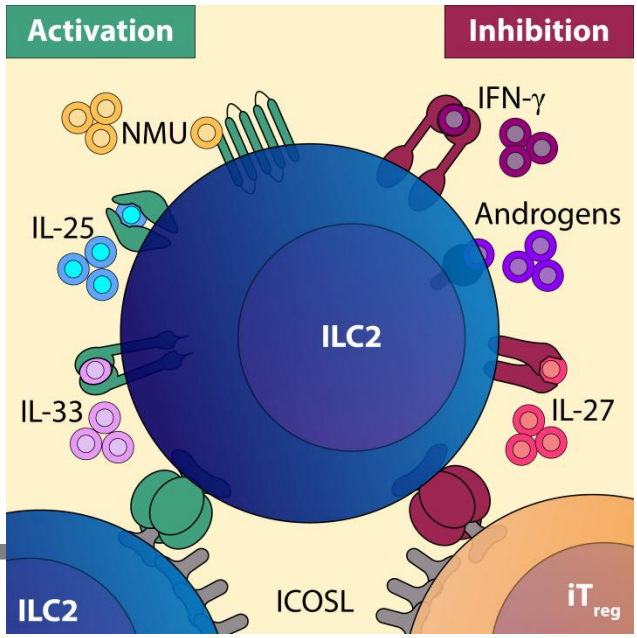
Figure Legend

Figure 1. Factors that regulate ILC2 function. Crucial factors and associated receptors that modulate group 2 innate lymphoid cells (ILC2s) to activate (green) or inhibit (red) their survival, proliferation and cytokine production. For acute kidney injury, increasing the activity of neuromedin U (NMU), IL-25, IL-33 and thymic stromal lymphopoietin (TSLP), and decreasing the activity of androgens, IL-27 and interferon-γ (IFN-γ) may be advantageous. Inducible T-cell costimulator (ICOS), however, can be excitatory for ILC2-ILC2 interactions, or inhibitory for induced regulatory T-lymphocyte (iT_{reg})-ILC2 interactions depending on co-stimulatory factors from the host cell.



Tables

Table 1: Methods of *in vivo* group 2 innate lymphoid cell expansion in experimental AKI

Method of ILC2 expansion	Timing	Model	Endpoint	Reference
0.3µg rmIL-25, for 5 days OR 2_x10^5 ILC2s (*ex vivo* rmIL-25 stim), once	Prophylactic	30min, bilateral IRI	Day 1	[77]
0.4µg rmIL-33, for 5 days	Therapeutic	12mg/kg doxorubicin, i.v	Day 14	[78]
66pmol rmIL-2 & rmIL-33 OR 66pmol IL233, each for 5 days	Both	24/26min, bilateral IRI; 20mg/kg cisplatin, i.p; 10mg/kg doxorubicin, i.v	IRI=Day 1/9/28; Cis/Dox=Day 4	[84]
0.3µg rmIL-33, for 5 days OR 1_x10^6 ILC2s (*ex vivo* rmIL-33 stim), once	Both	30/38min, bilateral IRI; 35min, bilateral IRI($Rag^{-/-}$)	Day 1/28	[63]

Summary of the main experimental approaches for ILC2 expansion in the kidney by administration of ILC2-activating recombinant mouse (rm) cytokines or adoptive transfer of purified group 2 innate lymphoid cells (ILC2s) either before (prophylactic) or after (therapeutic) the induction of injury. 8-12 week old, male, wild-type BALB/c or C57BL/6 mice were used in each study unless specified. In each study, expanding ILC2s was deemed beneficial.