Mesenchymal stromal cell–based therapies for acute kidney injury: progress in the last decade

Mesenchymal Stromal Cell-Based Therapies for Acute Kidney Injury: Progress in the Last Decade

[1]Barbara Fazekas, PhD and [1,2]Matthew D. Griffin, MB BCh, DMed

[1] Regenerative Medicine Institute (REMEDI) at CÚRAM Centre for Research in Medical Devices, School of Medicine, National University of Ireland Galway, Galway, Ireland

[2] Department of Nephrology, Saolta University Health Care Group, Galway University Hospitals, Galway, Ireland.

Address for correspondence: Prof. Matthew Griffin, National University of Ireland Galway, REMEDI, Biomedical Sciences, Corrib Village, Dangan, Galway, Ireland H91 TK33.

Email: matthew.griffin@nuigalway.ie

Phone: +353-91-495436

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Abstract

A little over 10 years ago, the therapeutic potential of mesenchymal stromal cells (MSC) for the treatment of acute kidney injury (AKI) was becoming widely recognized. Since then, there has been further intensive study of this topic with a clear translational intent. Over the past decade, many more animal model studies have strengthened the evidence that systemically or locally-delivered MSC ameliorate renal injury in sterile and sepsis-associated AKI (SA-AKI). Some of these pre-clinical studies have also provided a range of compelling new insights into the in vivo fate and mechanisms of action of MSC in the setting of AKI and other inflammatory conditions. Coupled with increased knowledge of the functional roles of resident and infiltrating immune cell mediators in determining the severity and outcome of AKI, the progress made during the last decade would appear to have significantly strengthened the translational pathway for MSC-based therapies. In contrast, however, the extent of the clinical experience with MSC administration in human subjects with AKI or SA-AKI has been limited to a small number of early-phase clinical trials which appear to demonstrate safety but have not thus far delivered a strong signal of efficacy. In this review, we summarize the most significant new developments in the field of MSC-based therapies as they relate to AKI and reflect on the key gaps in knowledge and technology that remain to be addressed in order for the true clinical potential of MSC and, perhaps, other emerging cellular therapies to be realized.

Keywords: Acute Kidney Injury, Stem Cell, Inflammation, Sepsis, Cell Therapy, Macrophages, Lymphocytes, Exosome, Cytokines, Chemokine

Introduction

Acute Kidney Injury (AKI) is a complex multifactorial disease that involves inflammation, ischemia and hypoxia leading to impaired blood flow to the kidneys which – through apoptosis and tissue necrosis – rapidly cause damage and functional failure of the kidneys[1, 2]. Acute kidney injury complicates up to 31% of hospital admissions[1, 2] and is associated with increased mortality – particularly when associated with sepsis and other ICU-requiring illness[3]. In those who survive, AKI commonly results in progression to chronic kidney disease (CKD) and the risk of CKD correlates with the severity of AKI[4, 5]. Among the main causes of AKI are nephrotoxicity, ischemia–reperfusion injury and, particularly in the ICU setting, sepsis[6, 7]. Although renal replacement therapy has been used for decades to support the life of patients with severe AKI, no specific therapies have yet been proven to consistently enhance renal functional recovery.

In recent years, the field of regenerative medicine has been recognized as offering the potential for true disease-modulating therapies to prevent or ameliorate AKI. In particular, administration of mesenchymal stem/stromal cells (MSC) has emerged as a promising novel therapeutic approach[8, 9]. Mesenchymal stromal cells are multipotent, fibroblast-like cells that can be isolated from bone marrow, adipose tissue, umbilical cord and other tissues. They are capable of supporting tissue homeostasis through their capacity to differentiate into several mature cell types as well as their inducible trophic properties[10]. In 2008, Humphreys and Bonventre published a critical review of the potential impact of MSC treatment on AKI based on the basic, animal model and human research that had been published to that point[11]. Among the points highlighted in this influential article were the following:

(a) Isolated MSC were recognized as an attractive cell therapy for AKI due to their demonstrated efficacy in multiple animal models of AKI and their capacity for expansion in culture and cryogenic preservation for later use “off the shelf”.

(b) Following intravenous administration, MSC were shown to be capable of homing to sites of injury, including the kidney.

(c) Although bone-marrow-derived MSC (BM-MSC) had been demonstrated to engraft within the kidneys and differentiate into renal parenchymal cells, such trans-differentiation occurs at very low frequency and with limited beneficial effect.

(d) The therapeutic effectiveness of MSC was more likely to be mediated by paracrine mechanisms, although the specific mechanisms and long-term consequences remained unclear.

This snapshot of the field a little over a decade ago suggested that clinical translation of MSC-based investigational medicinal products held substantial promise for improving AKI outcomes. It also posited that progress toward successful translational would be dependent upon the resolution of the key unanswered questions regarding their mechanisms of action and optimal therapeutic applications[12]. Since then the number of publications and patents related to MSC and AKI has continued to steadily increase (**Figure 1**).

In this review, we revisit the topic of MSC therapy for AKI with a focus on progress that has been made in the past decade toward better understanding and exploiting knowledge gaps that were recognized by Humphreys and Bonventre. Specifically, we examine the extent to which knowledge has advanced in regard to understanding of in vivo localization, survival and migration of MSC following administration; mechanisms of action of MSC in the setting of sterile and sepsis-associated AKI and documented clinical experiences with therapeutic MSC in patients with AKI.

New insights into the localisation, survival and migratory signals of exogenously administered MSC

Localization and homing to the site of injury: As noted in 2008, MSC migration to the injured kidneys and trans-differentiation into renal epithelium was observed only rarely and appeared unlikely to explain the rapid protective effects observed within the first 24 to 48 hours of AKI[13, 14]. Nonetheless, more recent applications of novel *in vivo* imaging techniques with enhanced sensitivity and accuracy such as magnetic resonance imaging (MRI), positron emission tomography (PET), single photon emission computerized tomography (SPECT) and whole-animal 3D microscopic resolution have been applied to better investigate full-body bio-distribution, pharmacokinetics and engraftment of MSC following various routes of administration, including intravenous (IV), intra-arteriolar (IA), intraperitoneal (IP), subcutaneous (SC) and intramuscular injection (IM)[15-21]. In one such study, IV-administered adipose-derived MSC (both live and heat-inactivated) were found not to migrate to an

inflammatory site with most remaining localized to the lungs[21]. Similar findings were made using post-mortem quantification of a MSC-delivered radioactive isotope to investigate the distribution of IV-administered MSC to individual tissue including the kidneys[16]. Taking the results of these and other studies together, it is now more clearly recognized that the large majority of MSC becomes trapped in the lungs following IV delivery with lower frequencies redistributed to the liver and spleen and only a small fractions likely to reach the kidneys or other organs[21-23]. Factors contributing to lung entrapment include the large size of MSC relative to lung capillary diameter, the extensive capillary network of the lungs and the strong adhesion properties of culture-expanded MSC[24-26]. Timing of delivery and number of cells administered may also influence the extent of MSC lung entrapment and re-distribution[15].

Recently, there has been a resurgence of interest in the administration of MSC by the IP route[15, 20, 27, 28]. Interestingly, in animal models of haematological conditions, the effectiveness of MSC therapy was comparable or superior following IP compared to IV administration[15, 17], while, in cisplatin-induced AKI, renal function was similarly improved by IV, IP and sub-renal capsular MSC[16]. In the latter study, localization studies of GFP-expressing MSC, confirmed the absence of MSC migration into the renal parenchymal for all three routes of administration. In sepsis-induced cardiac dysfunction, similar small numbers of MSC were identified in the heart, liver, lung and kidneys following IP administration[20]. In the setting of experimental glomerulonephritis, injection of MSC into the renal artery led to glomerular and, to a lesser extent, intra-renal vessel localisation in association with improved glomerular healing[29]. Similarly, Sierra-Parraga recently demonstrated, in a pig model of renal I/R, that intra-renal arterial infusion of adipose-MSC resulted in significant retention up to 8 hours post-infusion primarily in an intra-glomerular distribution with smaller numbers in peri-tubular capillaries[30]. While bio-distribution results from animal models are likely to be relevant to human subjects, there remains a persistent gap in knowledge regarding the optimal administration protocols for MSC therapies in AKI.

The fate and survival of the administered MSC: Although the therapeutic effectiveness of MSC in AKI is now broadly accepted to be primarily mediated by paracrine mechanisms[16, 21], it would be reasonable to assume that prolonged presence of MSC at sites of injury or at other locations should be necessary for therapeutic efficacy. As described above, however, studies of the IV administration of human and rodent MSC in the setting of diverse disease models,

including AKI, have shown them to be largely eliminated within 24 hours of initial entrapment in the lungs[16, 17, 19, 21, 22, 31]. Recent reports by Galleu *et al.* and de Witte *et al.* suggest that this elimination occurs as a result of apoptotic cell death and subsequent phagocytosis of MSC by lung-resident myeloid cells (monocytes and macrophages)[17, 32]. Although this new insight into the short-lived nature of systemically administered MSC could be viewed as a major limitation to their use for AKI and other clinical applications, these studies have also raised the intriguing possibility that re-programming of myeloid cells by apoptotic MSC within the lungs is a key mechanism underlying their protective effects in acute and chronic inflammatory conditions[17, 21, 32]. As described in more detail later, a further mechanism whereby short-lived MSC could mediate prolonged disease-modulated effects is through the release of extracellular vesicles (EV) – lipid-bilayer-enclosed subcellular particles that contain a "cargo" of bioactive molecules and participate in cell-cell communication.[33-38] In particular, the small size of EV (30-120 nm) compared to intact MSC (30 μm) may allow them to avoid entrapment in the lungs and be rapidly distributed systemically and internalized by immune and epithelial cells within the kidneys[33, 36, 38].

Molecular signals regulating the migration potential of MSC: Directional migration of MSC is dependent on chemotactic signals from injured tissues[39]. The response of stromal cells to migratory stimuli and the mechanisms by which they sense and convert intracellular signals have been more clearly elucidated in the past decade, helping to better understand the migratory characteristics of therapeutically administered MSC. For example, directional migration of MSC is associated with the expression of CD44 cell-surface glycoprotein and stromal cell-derived factor 1 (SDF-1)–CXCR4 chemokine receptors[13, 40]. Interestingly, Liu et al. demonstrated that CXCR4 overexpression in BM-MSC increases their migration in mice with renal I/R following IV administration[40]. Increased numbers of CXCR4-overexpressing compared to control MSC were observed in the kidneys at 7 but not 14 days following administration. Indices of AKI severity were improved in recipients of CXCR4-overexpressing MSC suggesting than even a modest increase in intra-renal migration/retention may be beneficial. Transmigration of human MSC into the kidney or other vascularized organs may also require the interaction of vascular cell adhesion molecule-1 (VCAM-1) with very late antigen-4 (VLA-4) as well as the secretion of matrix metalloproteinase (MMP)-2 at the site of invasion[26, 41, 42]. The adherence process of MSC also involves the presence of platelets and

neutrophils – independently from each other – in physical contact with the endothelial cells where MSC are moving forward using a so-called non-apoptotic "membrane blebbing activity"[43, 44].

Further in vivo research is clearly required to determine whether the potential beneficial effects of MSC in AKI mediated through short-lived interactions with myeloid cells at distant sites and those mediated by MSC retained within the kidneys following targeted delivery or enhanced migration are mutually exclusive or can be combined for optimal therapeutic efficacy.

New insights into the mechanisms of action of MSC in AKI

General principles of AKI immune modulation by MSC: Despite the inability of IV administered MSC to distribute in large numbers to the kidneys, they have clearly ameliorated renal damage and dysfunction in multiple recent experimental models of AKI via predominantly paracrine immune modulatory/anti-inflammatory effects[16, 21, 23]. For example, Luo *et al.* reported that MSC administration resulted in higher survival rate, improved tubular function, reduced bacterial load in the blood and attenuated neutrophil infiltration of the kidneys in sepsis-associated AKI[23]. In this study, such effects were associated with re-balancing of pro- and anti-inflammatory mediators linked to the inhibition of intra-renal IL-17 secretion – illustrating how mechanism of action can be more precisely interrogated through detailed immunological analyses of pre-clinical in vivo models. Other recent mechanistic studies have called into question the requirement for MSC to remain viable in order to generate therapeutically beneficial paracrine effects. In a rat model of sepsis, Chang *et al.* reported that apoptotic MSC protected major organs from damage and improved survival[45]. Likewise, Luk *et al.* recently demonstrated that MSC rendered non-viable by heat inactivation modulated monocyte function, induced regulatory cytokine release and ameliorated AKI severity as effectively as live MSC[21]. The same group subsequently demonstrated that engulfment of apoptotic MSC in the lungs resulted in the generation of monocytes with an immune regulatory phenotype that subsequently re-distributed to the liver and other tissues[32]. Further extending this model of MSC mechanism of action in inflammatory disease, Galleu *et al.* reported that *in vivo* induction of MSC apoptosis in the lungs by cytotoxic lymphocytes was required for their beneficial effects in a mouse model of graft versus host disease (GvHD) [17].

Interestingly, as early as 2005, Thum *et al.* had proposed a "dying stem cell hypothesis" to explain how transplanted progenitors cells modulated the inflammatory response associated with acute myocardial infarction having undergone apoptosis[46]. One common thread to such insights into MSC *in vivo* mechanism of action is the early "cross-talk" that occurs between administered MSC (either live or dead) and resident myeloid cells encountered at the first localization site [32, 47]. Also of interest from a mechanistic perspective, the group of Camussi has demonstrated that recovery from AKI after MSC administration may be mediated by the MSC-released microvesicles (MVs)[48, 49]. **Figure 2** illustrates recently-elucidated concepts for the potent paracrine effects of short-lived, therapeutic MSC in acute inflammatory conditions including AKI as a result interactions with myeloid cells at the first localization site following administration. With these mechanistic concepts of immune modulation in mind, we focus below on summarizing the role of immune cells in AKI[50, 51] and the extent to which MSC administration may counteract the immunological basis of AKI in sterile or sepsis-associated settings.

Non-sepsis-associated AKI: Circulating and resident immune cells are critical mediators of tissue injury and repair in experimental models of non-sepsis-associated ("sterile") AKI due to IRI, nephrotoxicity and other causes. Neutrophils and monocytes circulate in increased numbers in the blood. Infiltrating monocytes differentiate into pro-inflammatory (M1) macrophages or dendritic cells (DC) depending on their response to intra-renal stimuli[52]. Subsequently, macrophages may undergo transition from M1 to an anti-inflammatory (M2) phenotype supporting tubular repair[53]. Resident renal mononuclear phagocytes (with phenotypic and functional features that overlap between those of macrophages and DC) also mount complex responses to tissue injury[54-56]. Concomitant with these myeloid cell responses, lymphocyte populations, including T-cells, B-cells and natural killer T (NK-T)-cells also become mobilized and/or activated within the kidneys during sterile AKI. In particular, increased numbers of intra-renal $CD4^+$ (helper) or $CD8^+$ (cytotoxic) T cells have been consistently documented[57-60]. The role of $CD4^+$ helper T-cells may be particularly important for post-AKI repair and functional recovery[57, 58, 61]. More recently, CD4/CD8 double negative (DN) T-cell have also been shown to be an important kidney-resident early responder to AKI[62]. In the mouse renal IRI model, DN T-cells significantly ameliorated AKI severity through an IL-10-dependent T helper type 2 (Th2)-like immune mechanism. Similarly, $CD4^+CD25^+FoxP3^+$

regulatory T-cells (Treg) migrate to the kidneys and to play a protective role early after the onset of ischemic or nephrotoxic AKI[63, 64]. Cytokines and chemokines are key soluble mediators of inflammation and immune cell infiltration and activation during sterile AKI. Following acute injury, increased concentrations of pro- and anti-inflammatory cytokines, such as interleukin (IL)-6, IL-10, IL-17, interferon (IFN)-γ and tumour necrosis factor (TNF)-α become detectable in the circulation and kidneys, driving myeloid cell and Th1-, Th2-, Th17- and Treg-type lymphocytic responses[50, 59, 64]. Other inducible mediators, such as Prostaglandin E2 (PGE2) and its EP receptors, also influence AKI severity and outcome through modulatory effects on immune response[65, 66].

Recent reports have provided new mechanistic insights into MSC-mediated modulation of the immunopathology of sterile AKI (**Figure 3A**). For example, infusion of MSC in animals with renal IRI resulted in increased intra-renal Treg and their deletion inhibited MSC therapeutic efficacy[31]. Interestingly, IL-17A - typically viewed as a pro-inflammatory cytokines - upregulated cyclooxygenase (COX)-2 expression and PGE2 production by MSC. As recently shown by Bai *et al.*, pre-treatment of MSC with IL-17A prior to administration to animals with renal IRI resulted in promotion of Treg in the circulation, spleen and kidneys and in superior protection of renal function compared to untreated MSC. Inhibition of COX-2 reversed these beneficial effects[67]. In another recent study, IP administration of MSC-conditioned medium resulted in reduced renal infiltration by monocytes and B-cells in a mouse model of unilateral ureteral obstruction[68]. Although the mechanistic basis for these effects was not identified, the finding is in keeping with systemically-driven paracrine effects of MSC on immunological mediators of AKI. Modulation of the functional programming of mononuclear phagocyte subpopulations has also emerged as a consistent mechanistic theme in sterile AKI models. Geng *et al.* demonstrated that MSC ameliorate rhabdomyolysis-induced AKI via switching macrophages from M1 to M2 phenotype[69]. Consistent with this macrophage re-programming mechanism, multiple renal IRI studies have demonstrated that MSC reduce expression of pro-inflammatory mediators [IL-6, IL-1β, TNF-α, IFN-γ, ICAM-1, inducible nitric oxide synthase (iNOS)] and increase expression of anti-inflammatory mediators (IL-10, bFGF, TGF-α) in kidneys and/or circulation[31, 67, 70]. Finally, in the cisplatin-induced model of nephrotoxic sterile AKI, MSC expression of the enzyme heme oxygenase-1 (HO-1), which is known to counter-

regulate acute tissue injury[71, 72], was shown to be necessary for their secretion of anti-inflammatory and cyto-protectant mediators[73].

1. *Sepsis-associated AKI*

Acute kidney injury is a common end-organ manifestation of sepsis. Similar to sterile AKI, sepsis-associated AKI (SA-AKI) is associated with systemic and localized activation of innate and adaptive immune responders[74]. However, recent studies of human subjects and experimental animal models suggest that the respective roles of individual immune effector cells and soluble mediators may differ or take on greater significance for SA-AKI compared to sterile AK[64]. For example, Maravista *et al*. observed increased numbers of IL-17-producing (Th17-type) $CD4^+$ T-cells in peripheral blood mononuclear cells (PBMC) of SA-AKI patients compared to those with uncomplicated sepsis or CKD. Furthermore, IL-17 release by PBMC was higher in non-surviving SA-AKI patients compared to survivors[75]. The induction of anti-inflammatory mechanisms may also play disparate roles in the pathogenesis of SA-AKI and sterile AKI. In a comparative study of a mouse model of SA-AKI [caecal ligation and puncture (CLP)] and renal IRI, Lee *et al.* observed that SA-AKI was associated with greater increases of plasma IL-10, splenic Treg, immune cell apoptosis and apoptosis/caspase-3 activity in the kidneys[64]. In contrast to renal IRI, depletion of Treg and caspase 3 inhibition were associated with improved renal structure and function in SA-AKI[64]. Dysregulated neutrophil migration and cytotoxic functions may also contribute to failure of tissue repair mechanisms in sepsis[76, 77]. Thus, interventions designed to suppress innate immune responses and enhance anti-inflammatory mechanisms may have distinctly different effects in sterile and SA-AKI. A common thread, however, may relate to the role of monocyte/macrophage infiltration and macrophage polarization in kidney injury and repair. As recently shown by Li *et al.* in the rat CLP model, depletion of alternatively-activated (M2) macrophages that accumulated in the kidneys 72 hours after onset of sepsis resulted in increased AKI severity and reduced renal function[78].

Several recent studies using mouse and rat CLP or LPS administration have documented and mechanistically explored the protective effects of MSC in sepsis[27, 47, 79-83] and SA-AKI[23, 28, 47, 81-84]. These have generally provided evidence that systemic administration of MSC ameliorates the severity of SA-AKI by modulating the balance between pro-inflammatory and anti-inflammatory states (**Figure 3B**). The studies mostly utilized same-species BM-MSC[23, 47, 81, 83],

though some utilized adipose-derived MSC[80], dermal-derived MSC[79] or human MSC[27, 28]. Routes of administration included IV[23, 47, 79, 81-84] and IP[27, 28, 80] given 0-6 hours post-sepsis induction. Reported beneficial effects included lower serum creatinine[23, 47, 83, 84] and blood urea nitrogen[23, 83], higher GFR[28, 84], reduced tubular injury[23, 28, 47, 81] and reduced renal cell apoptosis[83]. Effects of MSC were primarily evaluated at 24 hours post-sepsis induction [23, 28, 81, 84] with only two studies evaluating effects at 96 hours[47] or multiple time-points[82]. Within the kidneys, MSC administration was shown to be associated with lower levels of IL-6, IL-1α, IFN-γ and NFκB activity and higher VEGF[23, 28]. Luo *et al.* also demonstrated markedly reduced intra-renal transcripts for pro-inflammatory chemokines (CCL2, CCL3, CXCL1, CXCL2, CXCL5)[23]. Consistent with this, reduced renal infiltration of neutrophils[23] and monocyte/macrophages[28] has been reported. Systemically, MSC resulted in reduced circulating concentrations of IL-2, IL-6, IL-1β, TNF-α, IFN-β, IFN-γ and MCP-1[27, 40, 83, 85]. An important early role for IL-10 has been consistently identified in the effects of MSC on sepsis and SA-AKI. Systemic IL-10 concentration is increased in MSC recipients[27, 82] as early as 6 hours post-CLP and subsequently falls by 12-24 hours[83, 85]. Several studies provide evidence that this early induction of IL-10 is likely to be mediated by MSC cross-talk with monocytes/macrophages via an iNOS/COX/PGE2 axis[47, 80, 86]. Driven by NF-κB signalling, iNOS-induced COX2 mediates high-level production of PGE2 which binds to macrophage EP2 and EP4 receptors and stimulates their secretion of IL-10 secretion[47]. During sepsis, IL-10 production enhances phagocytic functions of neutrophils and macrophages, reduces their renal infiltration[23, 47, 80, 81, 85] and promotes the conversion of intra-renal M1-like macrophages ($F4/80^+/iNOS^+/CD206^-$) to an M2-like phenotype ($F4/80^+/iNOS^-/CD206^+$)[84]. Reduced macrophage-derived TNFα and IL-12 secondarily results in reduced systemic and intra-renal activation of Th1-type T-cells[47, 79, 80] and likely contributes to decreased NK cell numbers in early sepsis[82].

Several other aspects of MSC immune modulatory effects in sepsis and SA-AKI require further investigation. In contrast to findings in sterile AKI models, the beneficial effects of MSC in sepsis and SA-AKI appear to be independent of HO-1 as BM-MSC administered into HO-1-deficient mice improved survival[81]. There is also limited understanding of the influence of MSC administration on effector and regulatory T-cell populations in SA-AKI[47, 79, 80]. Finally, it should be acknowledged that there are currently limited data available on the mid-to-long-

term outcomes for MSC administration in models of sepsis[47, 82] and on the effects of later or multiple dosing of MSC in experimental SA-AKI[45, 81].

The potential therapeutic effects of MSC-derived extracellular vesicles in AKI

With the widespread acceptance of a predominantly paracrine, disease modulatory mechanism of action of MSC in inflammatory diseases, there has been increasing interest in the potential for products secreted or released by MSC to be developed as therapeutic alternatives to whole cell products. The most compelling progress in this area has come from the investigation of MSC-derived EVs[33]. As a result of their biogenesis within intracellular multi-vesicular bodies or by extrusion directly from the surface membrane, EV may interact with target cells via surface-expressed ligands, transfer surface receptors and deliver intracellular signalling proteins, messenger RNAs, micro RNAs, bioactive lipids and glycans[33, 36, 37]. In relation to AKI, systemically-administered EV isolated from MSC have been reported in a number of recent studies to show beneficial effects in models of AKI[33-35, 38, 86-90]. For example, human MSC-EV significantly reduced morphological injury and apoptosis while enhancing tubular cell proliferation in various mouse or rat sterile AKI model[34, 35, 86-89]. Although the underlying mechanisms of EV-associated renal regeneration are far from fully elucidated, it is likely that specific vesicles subtypes have greater therapeutic than others. For examples, Bruno *et al.*, demonstrated that only the "exosome-enriched" fraction of MSC-EVs prepared by a differential centrifugation protocol conveyed beneficial effects in a glycerol-induced model of rhabdomyolysis-associated AKI. Profiling studies revealed that the cargo of this EV fraction was characterized by specific mRNAs and microRNAs involved in regulating cell cycle, proliferation and anti-apoptotic pathways that could explain their pro-repair effects[87]. In other studies, EVs effects on AKI models or renal tubular epithelial cell injury have been shown to be due to miRNA-dependent inhibition of gene products involved in inflammation, matrix receptor interaction, and cell adhesion; through promotion of anti-oxidative and pro-angiogenic pathways and through induction of autophagy[34, 86, 88, 89]. The protective role of EV-delivered microRNA components, such as miR-146a and miR-223 has also been confirmed in a mouse polymicrobial sepsis model[90, 91]. Although the therapeutic benefits of MSC-EV in human subjects remain to be confirmed, the impressive, mechanistically-plausible results observed in animal models, combined with emerging

translational programs for EV-based therapies[92], make this a fascinating area for future development.

Clinical experiences with MSC in acute kidney injury

Administration of autologous and allogeneic MSC to patients with kidney disease has shown good safety and tolerability in several clinical trials[93, 94]. However, the number of trials in which evidence for efficacy of MSC therapy in AKI can be examined is very limited. As summarized in **Table 1**, there have been two completed trials in this area, two withdrawn or terminated, one trial currently recruiting and one not yet open for recruitment. The first Phase 1 clinical trial (NCT00733876) to be conducted involved 15 patients at high risk of developing severe AKI following on-pump cardiac surgery who received an allogeneic, BM-MSC product infused via the suprarenal aorta. Outcomes for only the first five study subjects were published, with none developing severe AKI or requiring dialysis immediately or later after surgery. One patient died suddenly 26 days after surgery but this was not considered to be related to the MSC administration[95]. Subsequently, a multi-center, randomized, placebo-controlled, double-blind Phase 2 clinical trial (NCT01602328) was completed. In this study 156 post-cardiac surgery patients with clinical evidence of early post-operative AKI were randomized and 135 were treated with cells (n=67) or placebo (n=68)[94]. As reported by Swaminathan *et al.*, human allogeneic BM-MSC (AC607) administered via the intra-arterial route did not reduce the time to recovery of kidney function, provision of dialysis, or mortality compared with placebo-treated controls[94]. All patients were followed up for up to 90 days with no significant differences between groups identified for longer-term kidney function (serum creatinine concentration (sCr) and glomerular filtration rate), in-hospital mortality or intensive care/total hospital length of stay. Numerically, the number of patients who died or required dialysis following treatment with AC607 was higher. As the results were discordant with the beneficial effects associated with MSC administered by a similar route in relevant pre-clinical AKI models[18, 19], the authors questioned whether there were key variables such as administration timing, MSC formulation or method of delivery that had not been replicated in the clinical trial. A specific point of debate is whether the use of a risk prediction algorithm or alternative biomarkers capable of identifying AKI in advance of a rise in sCr could allow for earlier, more efficacious delivery of MSC[96].

Additional clinical experiences with MSC therapies for AKI are likely to become available in the next few years. For example, an ongoing Phase 1b/2a trial that aims to enrol up to 32 patients with AKI requiring continuous renal replacement (NCT03015623) is evaluating the safety and tolerability of an extracorporeal biologic/device combination product containing allogeneic human MSC. With a randomized, double-blind, sham-controlled design and a proposed follow-up period of 6 months, the trial has the potential to generate preliminary evidence of efficacy compared to continuous renal replacement therapy alone[97]. Trials of MSC therapies in sepsis may also yield important insights into their potential efficacy for SA-AKI. As for other clinical conditions, there is mounting evidence that MSC can be administered safely to patients with sepsis. For example, in a Phase 1 safety and tolerability study (CISS, NCT02421484) with a dose escalation design, groups of 3 septic patients each were sequentially treated with allogeneic MSC at doses of $0.3x10^6$ cells/kg, $1x10^6$ cells/kg and $3x10^6$ cells/kg along with conventional treatment and were compared with 21 conventionally-treated control patients. No pre-specified MSC infusion–associated or serious unexpected adverse event occurred during follow-up period[98]. While no clear signal of improved outcome for MSC-treated patients was observed, modest alterations in plasma cytokine profiles were observed in the highest MSC dose cohort compared to controls[99]. Although 56% and 38% of MSC-treated and control patients respectively had “renal failure”, AKI outcomes were not reported. A follow-up Phase II clinical trial enrolling 114 patients is planned (NCT03369275). Results of an open-label, randomized Phase 1b/2a trial of IV allogeneic MSC (n=14) compared to conventional therapy alone (n=13) in patients with neutropenia and septic shock have been published in abstract form only[100]. In this study, which also did not report renal functional outcomes, MSC therapy was associated with increased survival at 28 days but not at 3 months. A number of Phase 1/2 trials have also been completed or initiated in patients with acute respiratory distress syndrome (ARDS) which is often accompanied by AKI[101, 102]. Although these trials also bring the potential for further investigation of MSC therapy on AKI severity and outcome in meaningful numbers of patient (for example the START trial in which 60 patients with ARDS were treated with allogeneic MSC or placebo in blinded fashion[103]), none have thus far reported analysis results of renal functional indices or AKI biomarkers.

Overall, these limited recent trial results display that our knowledge of the mechanisms of action, potency indicators and responder phenotypes underlying AKI amelioration by MSC remain insufficient for effective clinical translation. In this regard, recent mechanistic studies

offer evidence that immunological screening prior to clinical trial enrolment might enable the identification of patients more likely to respond to MSC-based therapies. As described earlier, Galleu *et al.* have shown experimentally that MSC apoptosis induced by cytolytic lymphocytes followed by uptake ("efferocytosis") by recipient phagocytes is indispensable for beneficial immunomodulation to occur in GvHD[17]. This potential mechanism of action along with the authors' demonstration that anti-MSC cytolytic activity[17] and serum elevation in PGE2 following IV administration of MSC correlate with response to therapy in patients with acute GvHD[104] suggests at least one novel approach to assaying for and monitoring MSC responsiveness in acute conditions such as AKI.

Conclusions and Future Directions

As we have reviewed here, the past 10 years have seen continued strong research interest in the beneficial effects of MSC therapies in AKI. Numerous studies in pre-clinical models of AKI have increased the evidence base that administration of MSC (or their released products) is associated with improved renal structure and function. Furthermore, progress in understanding the systemic and renal-resident immunological responses to both sterile and SA-AKI have greatly enhanced our ability to identify immune modulatory mechanisms underlying these benefits. Advances in *in vivo* cell tracking and, especially, in the *in vivo* analysis of MSC fate and mechanisms of action through modulating key mediators of inflammation have begun to address some of the important knowledge gaps identified by Humphreys and Bonventre in 2008. Despite this progress, it cannot be ignored that the clinical experiences outlined in the preceding section represent a modest delivery on the promise that was recognized at that time. Indeed, the field of MSC therapeutics appears to have reached a cross-reads at which leading opinions differ in regard to the validity and clinical value of the term MSC as well as the best strategies for harnessing their demonstrated mechanisms of action[105-107].

From our perspective, in regard to progress toward MSC therapies for diverse diseases over the past decade, we highlight two overarching areas of advancement that support a continued translational effort and at least cautious optimism for future successes in AKI: (a) Basic insights into MSC mechanisms of action in animals models are beginning to be linked to biological observations in human "responders" participating in clinical trials – creating the

potential to develop much-needed assays for patient screening and post-administration monitoring as well as assays for confirming the “potency” of administered cells. (b) The manufacturing and regulatory pathways as well as the infrastructure and know-how for conducting clinical trials of MSC-based therapies in patients with complex diseases including sterile and SA-AKI have been established at academic centres and biotechnology/biopharma companies in multiple countries and have yielded a clear track record of safety and feasibility – an achievement that falls short of prior expectations but also represents an essential platform for more effectively translating the emerging mechanistic insights. Although it remains difficult to foresee in which clinical areas the next breakthroughs for disease-modulating cellular therapies such as MSC will emerge, we anticipate that much of continued research is this area will remain relevant to the goal of reducing morbidity and mortality from AKI.

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Figure legends

Figure 1. Graphical representations of the numbers of research papers and patents between 2008 and 2018 that were identified using the search term ["Acute kidney injury" AND ("mesenchymal stromal cell" OR “mesenchymal stem cell”)]. The search was conducted using the Scopus database (www.scopus.com). Duplicate patent entries were removed.

Figure 2. Illustrations of three potential mechanisms whereby interactions between short-lived MSC may mediated prolonged immune modulatory/anti-inflammatory effects via interaction with resident myeloid cells (monocytes/macrophages) at the site of initial localization following administration. **A**) Direct (contact-dependent) and/or indirect (non-contact dependent) cross-talk between viable (“live”) MSC and resident myeloid cells resulting in the production of multiple soluble mediators by both cell types. **B**) Phagocytosis of non-viable (“dead”) MSC by resident myeloid cells resulting in induced release of anti-inflammatory/pro-repair factors by the re-programmed myeloid cells. **C**) Release and systemic dissemination of microvesicles containing anti-inflammatory/pro-repair factors by MSC under the influence of myeloid-derived soluble mediators.

Abbreviations: MSC = Mesenchymal Stromal Cell. The figure was created with Biorender.com.

Figure 3. Illustrations of recently-documented mechanisms of immunomodulation by MSC in experimental AKI under sterile (**A**) and sepsis-associated (**B**) conditions. Mechanistic aspects of the systemic (Extra-renal, Upper Panels) and intra-renal (Lower Panels) effects are separately depicted. Red text indicates key soluble mediators produced directly by MSC or by MSC-interacting immune cells that have been documented to play key roles in MSC beneficial effects in vivo. **(A)** In sterile AKI, MSC infusion results in increased numbers of Treg (*via* COX-PGE2 axis), promotion of IL-10-dependent switching of macrophages from M1 pro-inflammatory to M2 anti-inflammatory phenotypes and reduced expression of pro-inflammatory mediators (e.g. TNFα, IFNγ, IL-6 etc.) both systemically and intra-renally. Roles of IL-17 and HO-1 in inducing these MSC effects have been described. Within the kidneys, MSC infusion is also associated with reduced monocyte and B-cell infiltration and increased expression of anti-inflammatory mediators (IL-10, bFGF, TGF-α). **(B)** In sepsis-associated AKI, MSC infusion leads to increased IL-10 concentration that is likely to be mediated by MSC cross-talk with monocytes/macrophages via an iNOS/NF-κB/COX/PGE2 pathway. Prolonged

IL-10 production enhances phagocytic functions of neutrophils and macrophages, while reducing their renal infiltration. Suppression of systemic NK cell responses has also been reported. MSC infusion has been shown to promote M1 to M2 transition (through binding of PGE2 to EP2 and EP4 receptors), to inhibit Th1-type $CD4^+$ T-cell responses and to reduce production of various soluble pro-inflammatory cytokines both systemically and intra-renally (grey boxes). Inflammatory injury within the kidneys is also limited by inhibition of IL-17 production with associated reduction in key pro-inflammatory chemokines (CXCL1, CXCL2, CXCL5, CCL2, CCL3).

Abbreviations: MSC = Mesenchymal Stromal Cell; M1 = M1-type macrophage; M2 = M2-type macrophage; T reg = Regulatory T cell; EP2 = Prostaglandin E2 receptor 2; EP4 = Prostaglandin E2 receptor 4; HO-1 = Heme oxygenase-1; COX = Cyclooxygenase; PGE2 = Prostaglandin E2; NK = Natural killer cell; TNF-α = Tumour necrosis factor alpha; IFN-β = Interferon beta; IFN-γ = Interferon gamma; IL-2 = Interleukin-2; IL-6 = Interleukin-6; IL-1α = Interleukin-1 alpha; IL-1β = Interleukin-1 beta; MCP-1 = Monocyte chemoattractant protein-1; iNOS = Inducible nitric oxide synthase; bFGF = basic fibroblast growth factor; TGF-α = Transforming growth factor alpha; BCL-2 = B-cell lymphoma 2; CCL-2 = C-C motif chemokine ligand 2; CCL-3 = C-C motif chemokine ligand 3; CXCL-1 = Chemokine (C-X-C motif) ligand 1; CXCL-2 = Chemokine (C-X-C motif) ligand 2; CXCL-5 = Chemokine (C-X-C motif) ligand 5. The figure was created with Biorender.com.

Table 1: Summary of clinical studies registered on ClinicalTrials.gov website and meeting the search terms [("AKI or acute kidney injury") or ("sepsis or septic shock") and ("MSC or mesenchymal stromal cell"), most recent search date October 23rd 2019].

Non-sepsis-associated acute kidney injury

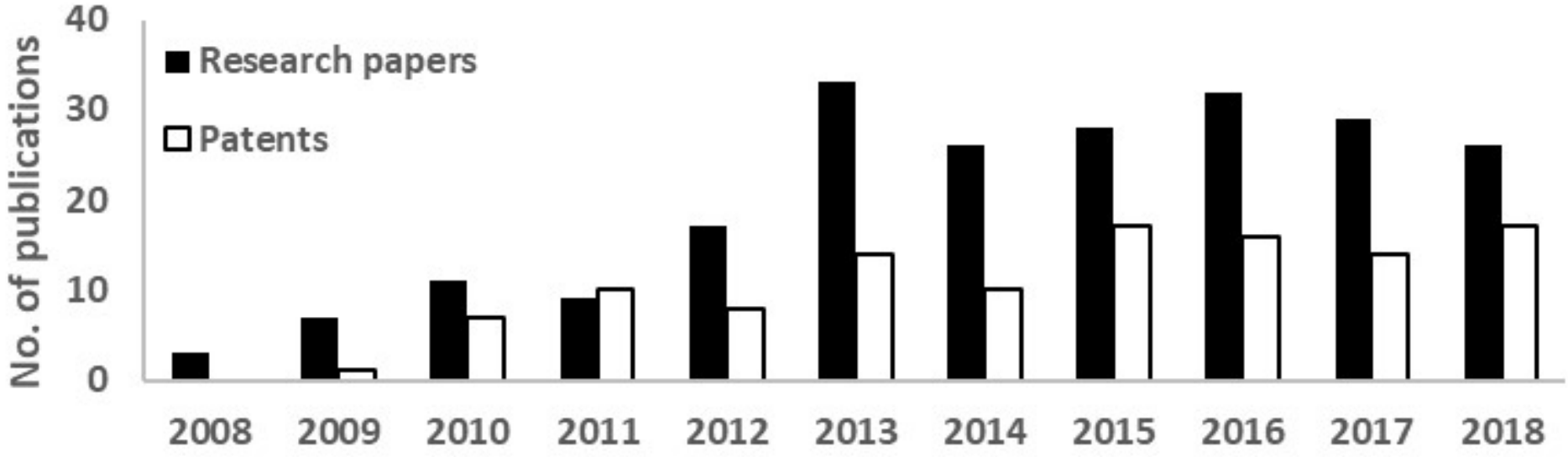
NCT ID no.	Title	Phase	Treatment	Outcome measures (1) Primary (2) Secondary	Recruitment	Enrolment number	Start date	Completion date
NCT00733876	Allogeneic Multipotent Stromal Cell Treatment for Acute Kidney Injury Following Cardiac Surgery	I	Allogeneic BM-MSC: single administration of low, intermediate and high dose in avg. $2x10^6$ cells/kg body weight into suprarenal aorta†	(1) Absence of MSC-specific adverse or serious adverse events	Completed	15	August 2008	October 2013
NCT01602328	A Study to Evaluate the Safety and Efficacy of AC607 for the Treatment of Kidney Injury in Cardiac Surgery Subjects (ACT-AKI)	II	Allogeneic BM-MSC (AC607): single dose of $2x10^6$cells/kg or vehicle into suprarenal aorta	(1) Time to kidney recovery (sCr) (2) All-cause mortality or dialysis	Terminated	156	June 2012	August 2014
NCT01275612	Mesenchymal Stem Cells in Cisplatin-Induced Acute Renal Failure in Patients with Solid Organ Cancers	I	Allogeneic BM-MSC: single dose of 1, 2 or $5x10^6$ cells/kg IV	(1) sCr at 15 days post-cisplatin infusion (2) urinary NGAL, NAG	Withdrawn*	9	November 2010	March 2018
NCT03015623		I-II		(1) Safety and tolerability	Recruiting	24	June	

	A Study of Cell Therapy for Subjects with Acute Kidney Injury Who Are Receiving Continuous Renal Replacement Therapy		Allogeneic MSC-containing extracorporeal device (SBI-101) or sham device				2017	December 2018

Sepsis/septic shock with acute kidney injury

NCT ID no.	Title	Phase	Treatment	Outcome measures (1) Primary (2) Secondary	Recruitment	Enrolment number	Start date	Completion date
NCT02421484	Cellular Immunotherapy for Septic Shock: A Phase I Trial (CISS)	I	Allogeneic BM-MSC: single dose of 0.3, 1 or $3x10^6$ cells/kg or placebo IV	(1) Safety and tolerability	Completed	30	June 2016	Oct 2018
NCT03369275	Cellular Immunotherapy for Septic Shock (CISS2)	II	Allogeneic BM-MSC: single dose of $300x10^6$ cells or placebo IV	(1) reduction in days on mechanical ventilation or renal replacement therapy or vasopressors, safety and tolerability (2) multiple, including organ failure scores	Not yet recruiting	114	March 2018	Oct 2020

[†] Abbreviations: BM-MSC = bone marrow-derived mesenchymal stromal cell, sCr = serum creatinine concentration; NGAL = neutrophil gelatinase-associated lipocalin, NAG = N-acetyl-p- D glucosaminidase.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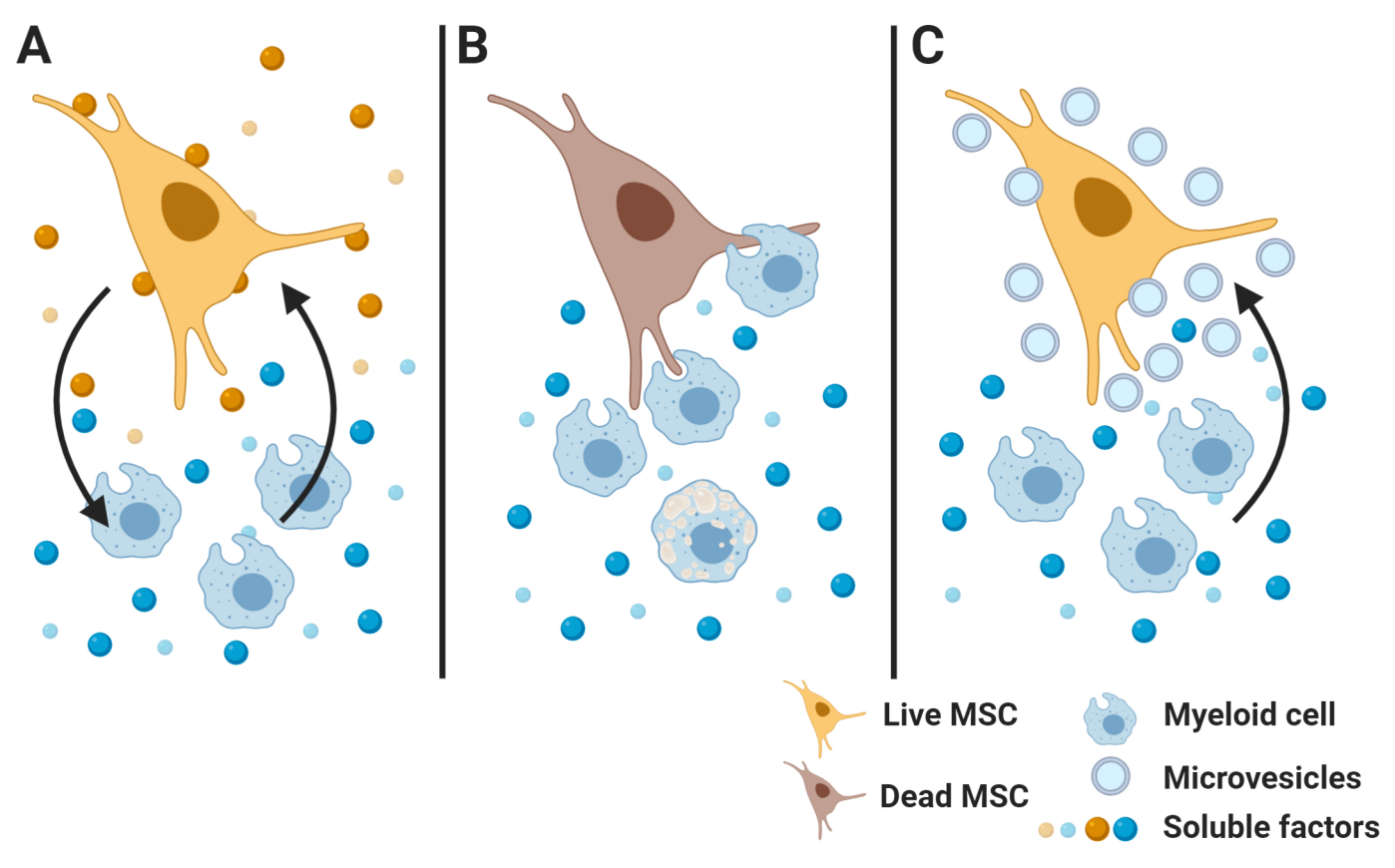
A
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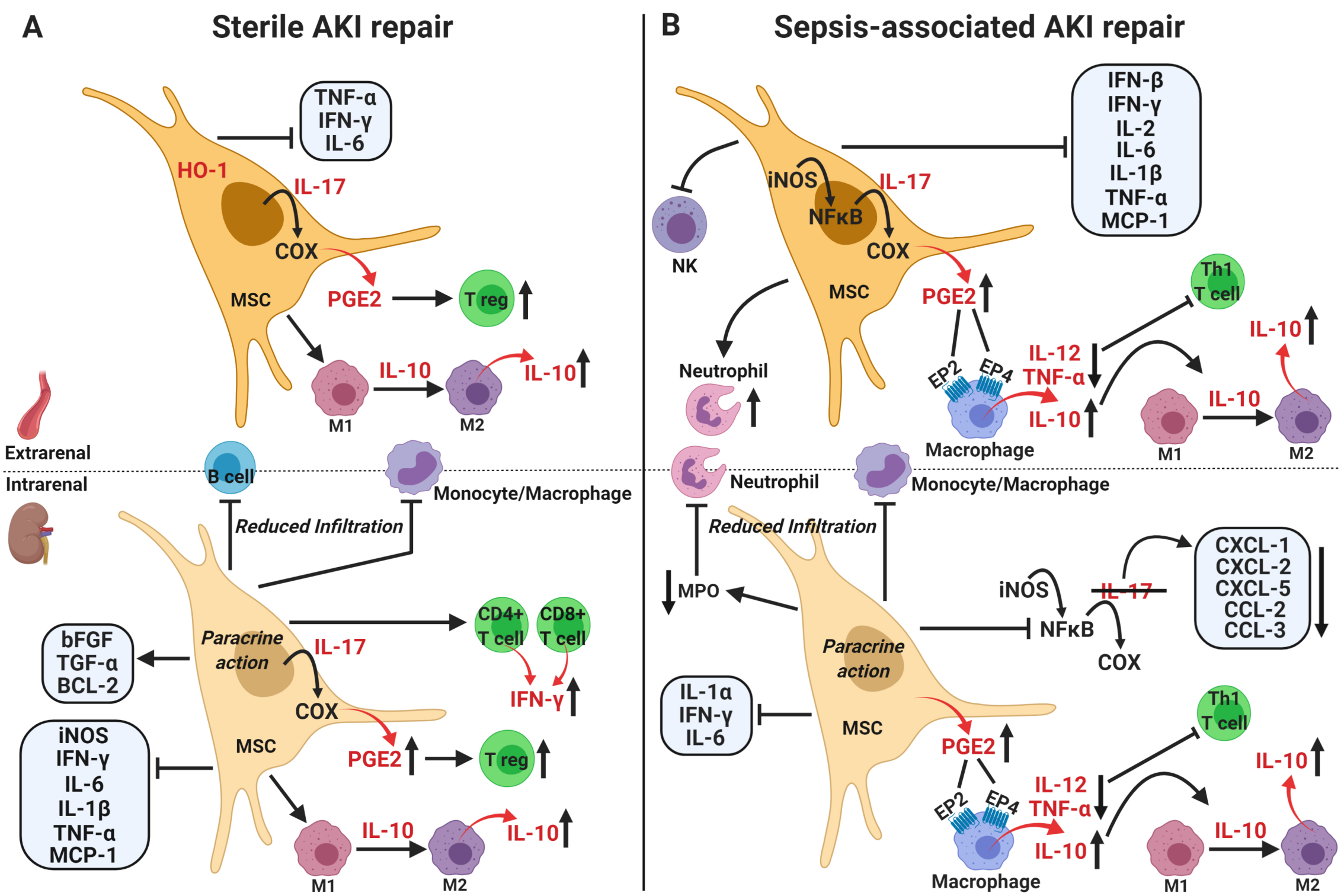
Live MSC
Dead MSC
Myeloid cell
Microvesicles
Soluble factors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TNF-α
IFN-γ
IL-6
HO-1
IL-17
COX
MSC
PGE2
T reg
IL-10
M1
M2
IL-10
Extrarenal
Intrarenal
B cell
Monocyte/Macrophage
Reduced Infiltration
bFGF
TGF-α
BCL-2
Paracrine action
IL-17
CD4+ T cell
CD8+ T cell
IFN-γ
COX
MSC
iNOS
IFN-γ
IL-6
IL-1β
TNF-α
MCP-1
PGE2
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B
Sepsis-associated AKI repair
IFN-β
IFN-γ
IL-2
IL-6
IL-1β
TNF-α
MCP-1
NK
iNOS
NFκB
IL-17
COX
MSC
Neutrophil
PGE2
EP2
EP4
IL-12
TNF-α
IL-10
Th1 T cell
IL-10
Macrophage
M1
IL-10
M2
Neutrophil
Monocyte/Macrophage
Reduced Infiltration
MPO
Paracrine action
iNOS
IL-17
NFκB
COX
CXCL-1
CXCL-2
CXCL-5
CCL-2
CCL-3
IL-1α
IFN-γ
IL-6
MSC
PGE2
EP2
EP4
IL-12
TNF-α
IL-10
Th1 T cell
IL-10
Macrophage
M1
IL-10
M2