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The role of macrophages during acute kidney injury: destruction and repair

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Abstract

Acute kidney injury (AKI) is defined by a rapid decline in renal function. Regardless of the initial cause of injury, the influx of immune cells is a common theme during AKI. While an inflammatory response is critical for the initial control of injury, a prolonged response can negatively affect tissue repair. In this review, we focus on the role of macrophages, from early inflammation to resolution during AKI. These cells serve as the innate defense system by phagocytosing cellular debris and pathogenic molecules; and bridging communication with the adaptive immune system by acting as antigen-presenting cells and secreting cytokines. While many immune cells function to initiate inflammation, macrophages play a complex role throughout AKI. This complexity is driven by their functional plasticity: the ability to polarize from a “pro-inflammatory” phenotype to a “pro-reparative” phenotype. Importantly, experimental and translational studies indicate that macrophage polarization holds promise as a novel therapeutic strategy to promote repair during AKI. A thorough understanding of the biological roles these phagocytes play during both injury and repair is necessary to understand the limitations while furthering the therapeutic application.

Keywords

AKI; zebrafish; macrophage; neutrophils; polarization

Introduction

Acute kidney injury (AKI) encompasses a spectrum of disease mechanisms that ultimately result in a rapid decline in renal function. Clinically, the etiologies are classified as pre-renal (e.g., hypovolemia and sepsis), intrinsic (e.g., drugs and toxins), and post-renal causes (e.g., obstruction or malignancy). Despite the array of initial insults, activation of the immune

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defense systems is a common thread, with immune cells playing a prominent role from initiating injury to promoting tissue repair [1].

The first phase of immune cell recruitment begins when injured renal tubular epithelial cells (RTECs) increase expression of damage-associated molecular pattern (DAMP) molecules, Toll-like receptors (TLRs), and other alarmins [1]. These signals recruit the first responders of the innate immune system – neutrophils, natural killer cells, activated macrophages, and resident macrophages to the site of injury within 24 hours [2]. Most innate immune cells extravasate from the vascular system to the site of injury to engulf cellular debris, as well as further propagating immune response to recruit adaptive immune cells. Unlike most innate immune cells, resident macrophages reside in the kidney even during steady state for immunosurveillance [3]. During injury, resident macrophages will act to engulf cellular debris as additional bone-marrow derived macrophages and monocytes are recruited [4]. The rapid recruitment of immune cells is critical for the regenerative response in the kidney and is also conserved across multiple organ systems [5].

The timeline and balance of invading leukocytes appears to be critical and it has been shown that persistence of the immune response can lead to further damage. In particular, different subtypes of macrophages (called polarization), which will be discussed in this review, have gathered much attention for their roles in progression to chronic diseases in various organs, including the kidney [6, 7]. Without proper regulation of macrophage driven inflammation, tissue repair cannot be successful. This review will outline the major recruitment pathways involved in macrophage polarization, the roles macrophages play in tissue repair, clinical trials focused on pathways important for macrophage polarization, an alternative experimental model using zebrafish, and future directions for further expanding our knowledge of the immune response during AKI.

Macrophage origin and function

Macrophages were once viewed as a single population of phagocytic cells derived from the bone marrow [8]. Later studies determined there were two distinct lineages from which macrophages originate: embryonic and bone marrow [3]. These two populations have varying roles not only during mammalian development but also in response to injury in adults, where various lineages of macrophages play complex roles in both response to the initial insult and resolution of the damaged tissue.

Embryonic macrophages express the PU.1 transcription factor and are derived from the hematopoietic stem cell (HSC) lineage within the yolk sac. Later in development HSCs migrate from the yolk sac to the bone marrow, which becomes the site of hematopoiesis in mammals. Embryonic macrophages function in innate immune protection and regulate fetal architecture by promoting vascularization, clearing apoptotic cells, supplying cellular matrix components (laminin, triggrin, type IV collagen, and proteoglycans), and providing cues for RBC maturation [9]. Another crucial function for embryonic macrophages is to migrate to sites of developing organs and mature into various types of resident macrophages, such as the Kupffer cells in the liver. In each organ, resident macrophages have specific roles to maintain the steady state, including clearing cells undergoing apoptosis and engulfing cells to remodel tissue architecture during development. Over the course of an organism’s life

resident macrophages self-replenish, carry out immunosurveillance, and other organ-specific functions [10]. For example, Kupffer cells play a role in liver detoxification, and in the brain microglia carry out synaptic pruning during development and adulthood [3]. Resident macrophages, along with the bone marrow-derived macrophages, assume different phenotypes during injury events to initiate and prolong inflammation, increase phagocytosis, promote recruitment of other immune cells, and ultimately resolve the injury. The ensuing sections will review known contributions of macrophages in each step of injury and resolution.

Initial macrophage response during injury

The current understanding of macrophage function during AKI has mainly been through studies in several types of murine AKI models including ischemia reperfusion (IR), nephrotoxin, rhabdomyolysis, and unilateral urinary obstruction. IR-AKI, one of the most widely used injury models, is performed by blocking blood flow to the kidney, which results in endothelial cells displaying signs of vasoconstriction expressing Endothelin-1, Angiotensin II, thromboxane A2, and adenosine [11, 12], which stimulate leukocyte migration to the kidneys by increasing expression of ICAM-1 [13]. In turn, the lack of oxygen results in the neighboring RTECs to express DAMPs and Hypoxia-inducible-factors (HIFs) [14]. The increased vascular rarefaction results in leukocyte migration and subsequent inflammation within 24 hours [15]. In nephrotoxic AKI, RTECs are directly targeted. A widely used chemotherapeutic reagent, cisplatin, directly damages RTECs by entering cells via transporters such as Ctr1 and OCT2, and causes cell death through DNA damage [16]. An *in vitro* study found cisplatin treatment of murine peritoneal macrophages significantly increased pro-inflammatory cytokines and NO expression via MAPK pathway [17]. While this may explain the severity of injury associated with cisplatin-AKI, whether this is true *in vivo* has not yet been proven. Current literature has not compare differences in macrophage responses among various models of AKI.

With the onset of injury, damaged RTECs release DAMPs and pathogen-associated molecular patterns (PAMPs), which act as an initial injury signal to sentinel immune cells within the tissue, such as resident macrophages and dendritic cells. Macrophages recognize the initial damage signals through pattern recognition receptors (PRRs), a family of receptors that recognize DAMPs and PAMPs. Recognition of DAMPs/PAMPs via PRRs results in downstream stimulation of macrophage phagocytosis, phagolysosomes maturation, antigen presentation, and production of the pro-inflammatory cytokine, tumor necrosis factor-alpha (TNFα) [18]. After the initial injury response, resident macrophages further prolong inflammation by recruiting other leukocytes to the site of injury. Among those recruited are: neutrophils, bone marrow derived monocytes and macrophages, and lymphocytes. Macrophage expressed chemokines and cytokines target different stages of leukocyte migration to increase recruitment. For example, TNFα, IL-1β, and histamines target endothelial cells to increase expression of trafficking molecules (selectins, integrin ligands), whereas chemokines CXC1, CXCL2, and CCL2, directly recruit neutrophils to extravasate from the circulatory system into the interstitium [19]. The initial inflammatory macrophage events are subsequently followed by modulation and then inhibition of the

inflammatory response. These phenotypic changes occur via tissue-specific, complex pathways and will be discussed in the ensuing sections.

Macrophage polarization

During injury, macrophages acquire a spectrum of phenotypes—from highly inflammatory at the beginning of recruitment to highly reparative towards the resolution of the injury. Pro-inflammatory (classically-activated/M1) macrophages are the first responders to injury. These macrophages phagocytose cellular debris and secrete the cytotoxic reagents such as nitric oxide synthase (NOS) and reactive oxygen species (ROS), which induce mitochondrial damage and apoptosis [20]. The inflammatory milieu is reversed by the infiltration of cells that promote a reparative microenvironment by secreting anti-inflammatory cytokines. These cells include pro-reparative (alternatively activated/M2) macrophages, CD4+ and CD8+ T-cells, and regulatory T-cells that predominate between days 3 and 5 post-injury in rodent models of AKI [21]. While macrophage polarization occurs as a spectrum, for the purposes of simplicity in this review we will refer to the subtypes of activated macrophages as: M1 and M2 macrophages, but acknowledge that this phenomenon occurs as a broad range of subtypes.

M1 macrophages

Recruited macrophages become activated by LPS, IFNγ, and granulocyte monocyte-colony stimulating factor (GM-CSF) released from the damaged microenvironment [22]. In the kidney, M1 specific cytokines increase in expression within the first 24 hours post IR-AKI and significantly decrease at 3 days post injury (dpi) [21]. Upon activation, they secrete inflammatory cytokines such as IL-1β, TNFα, IL-12, IL-18, and IL-23 [2] (Fig. 1). As a result, the site of injury continues to gain other inflammatory cells, including T helper cells [22]. The M1 macrophages also secrete molecules for destruction of pathogenic particles such as nitric oxide generated by inducible nitric oxide synthase (iNOS). Once pathogens or damaged cells are cleared, a rapid change in macrophage polarization is necessary to stop further damage to surrounding cells. Prolonged activation of inflammatory macrophages has negative effects on injury recovery, since the released cytotoxic agents do not discriminate self from pathogenic particles [23, 24]. Various experimental models have concluded that prolonged inflammatory macrophage activation imposes negative consequences in injury resolution due to extensive inflammation. A study in the cardiovascular field reported that atherosclerotic lesions with a higher number of inflammatory macrophages correlated with a higher likelihood for sudden major cardiovascular ischemia [25]. In the kidney, perdurance of M1 macrophages contributes to a worsened outcome after renal ischemia. Clodronate-induced depletion of all macrophages and subsequent transplantation of IFNγ stimulated M1 macrophages prior to renal ischemia resulted in more severe tubular damage [21]. Depletion of macrophages before injury reduced blood urea nitrogen levels and post-injury histological markers of tubular injury, whereas depletion during reparative stages resulted in a significant increase in injury markers [26]. Taken together, these studies suggest that while the initial inflammatory response from macrophages is necessary for removal of damaged and pathogenic particles, prolonged inflammatory activity results in further tissue damage, ultimately inhibiting the reparative phase of injury resolution.

M2 macrophages

M2 macrophages do not become activated until later in the initial injury phase. In IR-AKI, an increase in M2 markers is observed at 3 dpi, peaking in expression at 7 dpi [21]. M2 macrophages originate from newly recruited monocytes dispatched from the circulatory system, as well as initially recruited M1 macrophages [27]. The phenotypic conversion from monocytes and M1 macrophages to M2 macrophages requires specific cytokine stimulation and subsequent transcriptional changes. M2 macrophages are activated by macrophage colony stimulating factor (M-CSF), IL-4, IL-10, IL-13, and TGF-β [22]. Upon activation, M2 macrophages express mannose receptor (MR), which recognizes and downregulates high levels of inflammatory glycoproteins previously produced by the inflammatory response [28]. M2 macrophages also produce Arginase, an enzyme necessary to produce ornithine and polyamine, building blocks for extracellular matrix architecture. Furthermore, M2 macrophages secrete resolvins, lipoxins, TGFβ, and matrix metalloproteinases that target and cleave chemokines and chemoattractants, resulting in inhibition of inflammatory immune cell activity [19](Fig. 1). Over the past decade, M2 macrophages have been categorized into four subtypes based on their *in vitro* upstream activators and downstream gene expression patterns [29]. M2a are activated by IL-4, IL-13, M2b are activated by IL-1, LPS, M2c are activated by IL-10 and TGFβ, and glucocorticoids, and M2d are activated by IL-6 and adenosine.

Researchers have examined M2 macrophage ability to curtail inflammation as well as for their inherent reparative capacity. Saha *et al.* demonstrated that macrophages are crucial for normal repair after an acute injury to the intestines [30]. The post-injury intestinal stem cells required macrophage-derived extracellular vesicles for increased proliferation and repopulation of damaged cells and they found that the Wnt ligands, Wnt5a, Wnt6, and Wnt9a, are critically important factors mediating this effect. In lung injury, mitogen activated protein kinase1/2 inhibition resulted in an increased M2 population, leading to better recovery weight and increased macrophage efferocytosis of inflammatory cells [31]. An AKI study demonstrated that a macrophage-derived Wnt is required for normal kidney repair. Lin *et al.* reported that kidney specific macrophages secrete Wnt7b, a canonical Wnt ligand [32]. In depleting kidney-specific Wnt7b, the study showed this Wnt is necessary for improved tubular repair, and reduced fibrosis by bypassing G2/M cell cycle arrest. Finally, genetic ablation of a pathway required for M2 polarization, IRAK-M, resulted in increased M1 macrophages during AKI. Early injury response did not vary between wild type and *IRAK-M*$^{-/-}$, but in the long-term, the deletion resulted in increased fibrosis and an inability to regenerate kidney mass [33].

Experimentally manipulating macrophage polarization

Studies have begun to focus on macrophage polarization as a means to enhance regeneration in post-AKI models (Table 1). In various experimental models of AKI, increased M2 macrophage polarization has been found to enhance numerous functional outcomes [24, 34]. Treating co-cultures of rodent RTECs and undifferentiated monocytes with GM-CSF stimulated monocytes to convert to M2 macrophages by elevating STAT5 activity [24]. *In vivo* inhibition of GM-CSF improved RTEC proliferation in post-AKI mice but the study did not examine effects of the treatment on longer-term pathological outcomes (i.e. fibrosis and

progression to chronic kidney disease). Another pathway implicated in macrophage polarization is retinoic acid (RA) signaling, a critical pathway for kidney development and in AKI for reducing injury and fibrosis [34]. Chiba *et al.* demonstrated that locally synthesized RA simultaneously reduces M1 macrophages and activates RA signaling in RTECs. When RA signaling is genetically blocked in RTECs, M2 macrophage markers significantly decreased, suggesting that RA is part of an RTEC/macrophage crosstalk pathway that regulates macrophage polarization. Other studies have shown erythropoietin (EPO), a hormone widely known for its role in hematopoietic differentiation, suppressed inflammatory cytokine expression in rodent kidneys [35, 36]. EPO decreases CCL7 expression in macrophages, thereby limiting RTEC apoptosis. EPO also influenced macrophage polarization by increasing JAK2 and STAT3 activity, resulting in a significantly higher number of peritubular M2 macrophages [36]. Studies have focused on utilizing bone-marrow derived mesenchymal stem cells (MSC) to affect macrophage polarization. It is speculated that MSCs increase M2 macrophage polarization both *in vivo* and *in vitro* via MSC-secreted trophic factors, particularly IL-10 [37, 38].

Macrophage polarization in human AKI

To translate rodent AKI studies to clinical therapies, the pathways responsible for macrophage polarization need to be conserved in human AKI. It has been reported that an increase in the number of macrophages is seen in human patients with AKI [39, 40]. Analysis of human kidney biopsies with acute tubular injury during the early repair phase showed that 75% of the recruited macrophages expressed the M2 macrophage marker, CD163. Further structural analysis demonstrated that $CD163^{+}$ macrophages closely adhered to the basement membrane of damaged tubular cells whereas the M1 or $CD163^{-}$ macrophages did not show specific cellular contact [41]. However, the effect of M1 and M2 macrophages in patients with AKI is complex. In patients with macroscopic hematuria induced-AKI, the incomplete recovery group had increased M2 (CD163+) macrophages in the kidney. However, patients with leptospirosis-induced AKI showed an increased number of M1 (HLA-DR+) macrophages (biopsied at week4) [39]. The opposing results can possibly be attributed to lack of biopsied samples and the inherent variability in injury severity and outcome. Other human studies have conducted prophylactic administration of EPO and Pentoxifylline in patients undergoing cardiac surgery, a procedure with a high risk of inducing AKI [42–46]. In these studies, positive outcomes were associated with decreased inflammatory markers, including lower urinary NGAL, TNF α, IL-6, and C-reactive protein [42, 46]. This was accompanied by a significant decrease in the number of leukocytes, though M1/M2 polarization changes were not investigated. Overall, these studies suggest that suppression of the early inflammatory response is beneficial to high-risk patients.

To date, only a handful of human clinical trials have focused on chemically manipulating macrophage infiltration or polarization to enhance repair of acute injuries. Recombinant human (rh) GM-CSF, a pro-polarization cytokine, has been utilized for promoting an increased M2 population in humans. Severe burn victims treated with rhGM-CSF displayed significantly improved healing rates [47, 48]. However, the studies did not follow up with histological analysis to demonstrate that increased repair is a direct result of increased M2 polarization. Others have used drugs that target pathways important for macrophage

recruitment to reduce fibrosis. Angiotensin converting enzyme (ACE) inhibitors have been shown to lower Monocyte chemotactic protein-1 (MCP-1), minimizing fibrosis and improving renal function in a mouse model of diabetic nephropathy [49] [50]. In a human clinical study, ACE inhibitor treatment in patients with diabetic nephropathy resulted in decreased urinary levels of MCP-1 as well as increased renal function [51]. Aside from amelioration of acute injuries, macrophage polarization can be manipulated to achieve other clinical goals, such as immunosuppression in transplant patients. Mercalli *et al.* demonstrated that Rapamycin, normally administered to transplantation patients to suppress T helper cells, simultaneously drives changes in macrophage polarization dynamics both *in vitro* and *in vivo*. [52].

Zebrafish as a model organism for AKI and the innate immune response

It is well established that early kidney development and function are conserved between zebrafish and mammals [53, 54]. Zebrafish models also recapitulate the pathophysiology of injury and repair typically observed in mammalian models of AKI [55, 56]. At the onset of injury, both zebrafish and mammalian RTECs loses cell polarity, increase expression of injury markers (i.e. KIM-1), and undergo apoptosis and necrosis [34, 57–59]. Additionally, the larval zebrafish appears to possess a relatively robust capacity for renal repair. A nephrotoxic model of AKI in larval zebrafish responds to injury with increased proliferation and reactivation of developmental genes, as evidenced by increased RA signaling and Pax2a positive RTECs [34, 57, 60, 61]. With conserved mechanisms in development and repair, paired with the power of high-throughput screening, zebrafish studies have elucidated various signaling pathways and identified therapeutic compounds with the potential to improve repair [57, 62–64].

Aside from conserved kidney function, zebrafish leukocyte differentiation and function are also comparable to mammalian models. Mammalian and zebrafish HSCs share conserved mechanism of myelopoesis during embryonic development. As in mammals, there are two waves of HSC migration and differentiation in the zebrafish, termed primitive and definitive myelopoiesis. Primitive myelopoiesis occurs between 12–48 hours post-fertilization (hpf), when precursor cells initiate differentiation and produce mature cells from mesodermal tissue along the notochord [65]. Definitive myelopoiesis occurs when HSCs migrate and seed the anterior segment of the pronephros. The migrated HSCs remain as the definitive pool of self-renewing cells that continue to produce mature leukocytes from 96 hpf to adulthood [65, 66]. There are key regulatory factors that are conserved between mammalian and zebrafish macrophage differentiation. For example, *Pu.1*, *Spi-b*, and *Irf8* are required for macrophage development and differentiation in both models [67]. While conservation of mammalian and zebrafish myelopoiesis is well outlined, zebrafish macrophage polarization is relatively a new area of study. To date, few *in situ* studies for zebrafish M1 and M2 macrophage markers have been conducted.

The field has generated several versatile transgenic tools to observe neutrophil and macrophage response. For example, *Tg(lyzC:egfp)* and *Tg(mpeg1:egfp)* allows visualization of neutrophil and macrophages *in vivo*, respectively [68, 69]. *Tg(mpeg1:dendra2)* allows photo-conversion of macrophages to trace their fate at desired time points, and

Tg(mpeg1:gal4/UAS:ntr-mcherry) allows genetic ablation of macrophages [70]. These lines have been used to address the roles of macrophages in repair after fin and liver regeneration [71, 72]. A recent study has shown live imaging of macrophage polarization in fin amputation, using transgenic reporters for *tnfa* (M1 marker) and macrophage expressed 1 (*mpeg1)* [73]. Nguyen-Chi *et al.* visualized by *in vivo* imaging activation and subsequent repression of TNFα in macrophages recruited to the site of injury. TNFα+ macrophage recruitment was observed as early as 1 hour post amputation, while TNFα-macrophage recruitment occurred at approximately 25 hours post amputation. The convenience of generating transgenic lines paired with imaging tools resulted in tracking macrophages *in vivo*, enabling characterization of M1/M2 velocity, contact frequency, and morphological changes [73]. Taken together, the zebrafish larva has the potential to serve as a good model organism to elucidate the effect of macrophage polarization during AKI [34].

Towards the future

For the past several decades, the field has elucidated the pathways required to polarize macrophages. However, among the multiple pathways outlined throughout this review, it is still unclear how the identified pathways interact with one another to ultimately drive polarization in injury settings. One reason for the shortcoming may be that specific cellular mechanisms driving macrophage polarization could vary in each organ or injury model. In order to implement macrophage polarization as a clinical immunotherapy, the effects of macrophages in different injury types must be specifically outlined as follows: 1. What specific pathways are required for macrophage polarization in each injury model and tissue; 2. What signaling molecules are secreted by M1 and M2 under each condition; 3. What are the downstream effects of M1 and M2 signaling molecules in various cell types that reside in the organ; and 4. Will pro-polarization drugs have any off-target effects? With a more comprehensive understanding of the immune response the field of regenerative medicine may be able to take advantage of reparative powers of the immune system.

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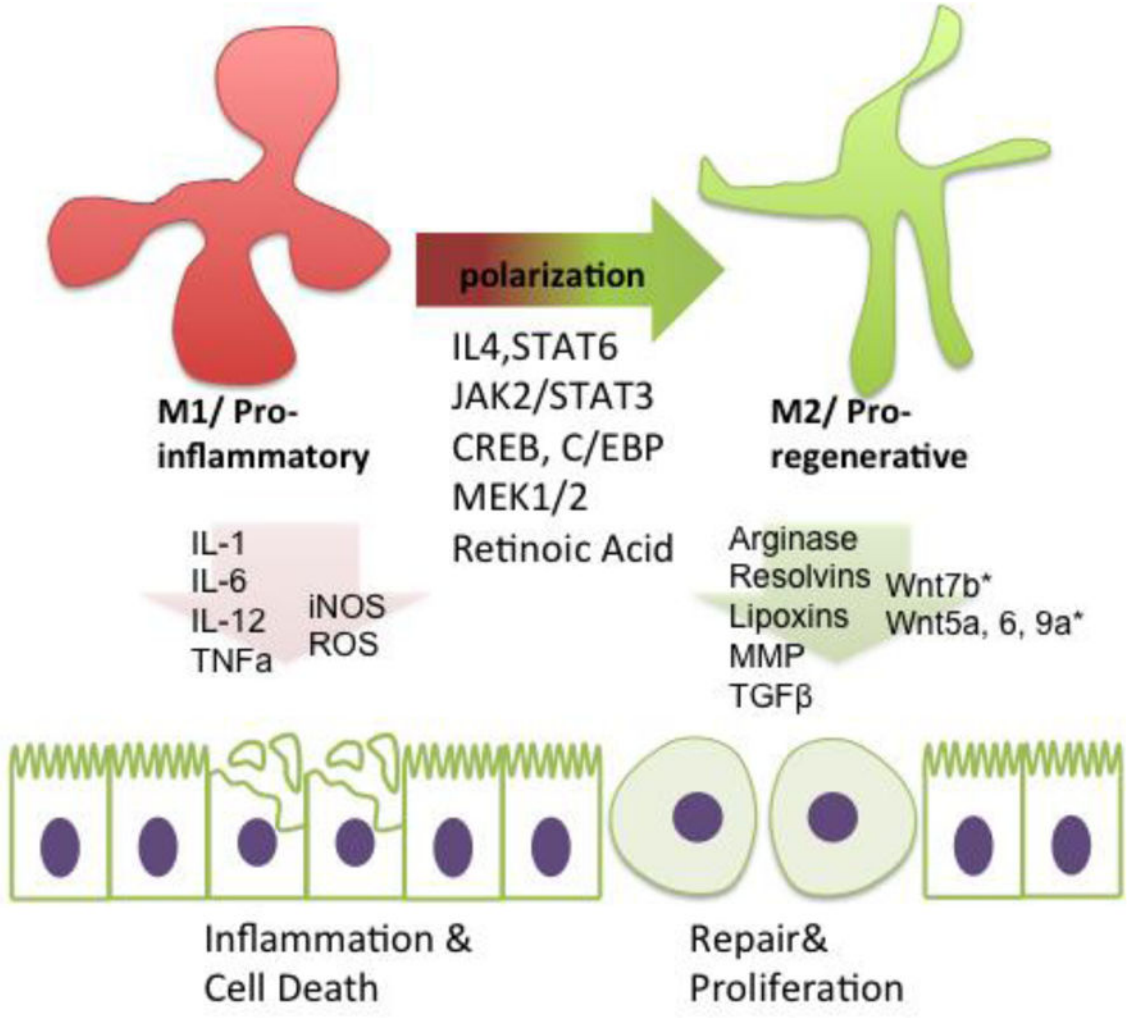

Figure 1. Various pathways identified to be critical for macrophage polarization from M1 (pro-inflammatory) to M2 (pro-regenerative)

Experimental studies of several pathways have elucidated critical pathways for driving macrophage polarization from M1 to M2. Among them are IL-4/STAT6, JAK2/STAT3, CREB/C/EBP, and Mitogen-activated protein kinase 1/2 (MEK1/2). Each macrophage phenotype has signature expression of certain cytokines and secreted products. M1 macrophages secrete inflammatory cytokines and products and chemoattractants, such as: IL-1, IL-6, IL-12, TNFa, iNOS, and ROS. M2 macrophages secrete anti-inflammatory cytokines and pro-reparative secretions such as: Arginase, Resolvins, Lipoxins, Matrix metalloproteinases, TGFB, and Wnt ligands. *There seems to be tissue-specificity to types of Wnt ligands secreted, specifically Wnt7b in kidney macrophages and Wnt5a, Wnt 6, and 9a in intestinal macrophages.

Table 1

Summary of macrophage polarization studies in kidney injury

Factor tested	Injury type	Major findings	Effect on macrophages	Ref
Relaxin	UUO AKI	Relaxin administration lowers TLR4 expression Increased renal function and attenuated injury and fibrosis	Increased M2 polarization	[74]
PRDX6	LPS AKI	PRDX6 overexpression decreased mortality and renal injury by lowering ROS via decreasing p38 MAPK and JNK.	Overall lower macrophage infiltration	[75]
Gpnmb	IR AKI	Gpnmb is highly expressed in M2. Knocking down Gpnmb results in higher IL-1B and TNFa. Gpmb induces M2 activation via IL-4/STAT6	Required for IL-4/STAT6 dependent M2 activation	[76]
IL-4/IL-13	DT AKI; IR AKI	IL-4/IL-13 activate JAK3/STAT6 Deletion of IL-4/13 results in worsened fibrosis	Required for M2a activation	[77]
EPO	RI AKI	EPO administration increases macrophage polarization reduces macrophage recruitment, increased phenotype switch to M2, repressed M1 and increases Jak2/STAT3/STAT6 pathway	Overall lower macrophage infiltration; higher ratio of M2 activation	[36]
RA	I/R AKI	RA synthesis (Raldh3) is activated in recruited renal macrophages All-Trans Retinoic Acid reduces post-AKI fibrosis RTEC-specific RAR knockdown results reduces M2 polarization in mice	RTEC-specific RA required for M2 activation	[34]
EPO	I/R AKI	EPO administration resulted in increased function Increased expression of Wnt7b, B-Cat, downregulation of miR-21, −214,−210, −199a	EPO lowers renal macrophage infiltration	[78]
CSF-1	I/R AKI	CSF-1 depletion results in reduced M2 polarization, delayed functional, structural recovery, increased fibrosis	Required for M2 polarization	[79]
GM-CSF	I/R AKI	GM-CSF secreted by RTECs increase STAT5 activation and increases RTEC proliferation	Required for M2 polarization	[24]
SOCS-3	RI AKI; I/R AKI	SOCS-3 KO in RTECs resulted in increased proliferation and attenuated injury	Inhibits M2 polarization	[80]
MSC	RI AKI	Increased renal function and injury attenuation function and injury attenuation.	Increases M2 polarization	[37]

UUO - unilateral uretral obstruction

LPS - Lipopolysaccharide

DT - diphtheria toxin

RI - Rhabdomyolysis induced

I/R - Ischemia/reperfusion

PRDX6 - Peroxiredoxin 6

Gpnmb - Glycoprotein non-metastatic melanoma protein b

EPO - Erythropoietin

RA - Retinoic Acid

CSF-1 - Colony Stimulating Factor-1

GM-CSF - Granulocyte-macrophage colony-stimulating factor

SOCS-3 - suppressor of cytokine signaling 3

MSC - Mesenchymal Stem Cells