M2 macrophage in Fibrosis and kidney graft rejection

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Abstract

Commonly macrophages are categorized into M1 and M2 subsets. M1 macrophages are defined as inflammatory cells and may contribute to tissue injury, while M2 macrophages play a central role in tissue remodeling and control of immune responses. It seems that the existence of these cells in graft location is effective for control of inflammation and improvement of transplantation consequences. Although, the relative contribution of M2 cells in organ transplantation is not clear, the accumulation of these cells in acute and chronic injury models of transplantation was shown. In some cases, the depletion of M2 cells lead to amelioration of disease; however in other causes, skewing the response in M2 cells lead to augmentation of graft fibrosis and worsening of graft condition. In spite of these findings, the benefits of such strategies and their implications in human disease are not clearly understood. The purpose of this mini-review is to highlight the role of alternatively activated M2-type macrophages in the improvement or reduction of the kidney transplantation outcome.

Keywords: M2 macrophages, Fibrosis, kidney, Graft rejection
Introduction

Macrophages are derived from circulating peripheral mononuclear cells which enter into tissues and become residential during normal leucocyte re-circulation or during inflammatory responses (1). In human being, the main monocyte populations are composed of classical, intermediate and non-classical subsets. Classical monocytes account for 90-95% of circulating monocytes that migrate to different tissues and are well known as CD14\(^{hi}\) CD16\(^{-}\) CD64\(^{-}\) CCR2\(^{hi}\) CX3CR1\(^{low}\) CD62L\(^{+}\) monocytes. These cells are characterised by their phagocytic and microbicidal activity and low pro-inflammatory cytokine production. Intermediate monocytes are minor sub-population with CD16\(^{+}\) CD14\(^{hi}\) CCR2\(^{low}\) CX3CR1\(^{hi}\) CD16\(^{-}\) CD64\(^{+}\) characteristics and show pro-inflammatory-activity and produce tumour necrosis factor alpha (TNF-\(\alpha\)) (in response to LPS) as well as interleukin-1 beta (IL-1\(\beta\)) and IL-6. The CD14\(^{low}\) CD16\(^{hi}\) CCR2\(^{low}\) CX3CR1\(^{hi}\) CD64\(^{+}\) cell subsets which are known as non-classical monocytes comprise 5-10% of the total macrophages and show anti-inflammatory activity and constitutively produce IL-1RA (2-4). All types of macrophages, as differentiated monocytes, play role in immune surveillance and could sense a wide spectrum of microbial antigenic patterns, immune complexes, apoptotic or necrotic cells and various mediators secreted from other cells (5-8). They become activated in response to several PAMPs, which allow them to phagocytose and eliminate pathogens, maintaining tissue integrity in an immunomodulatory manner (9, 10). Recently, an experimental model has been developed to define the complex mechanism of polarization and activation of macrophages towards the classical (M1) or the alternative (M2) state (9, 11). Bacterial cell wall components, lipoproteins, intracellular pathogens and cytokines such as TNF-\(\alpha\) and interferon gamma (IFN-\(\gamma\)) may induce the M1 activation pathway. The main features of M1 macrophages are the secretion of inflammatory cytokines and production of nitric oxide (NO) following receptor-ligand interaction (12-14). In contrast, activation of M2 cells is induced by cytokines such as tumour growth factor beta (TGF-\(\beta\)), macrophage colony stimulating factor (MCSF), IL-4, IL-13, IL-10, as well as immune complexes and complement activation by products and organisms such as parasites, fungi and apoptotic cells (12). The anti-inflammatory M2 macrophages stimulate angiogenesis, promote cell proliferation and reduce apoptosis by secreting regeneration stimulating factors (15). Regarding to the role of M2-derived products in promotion of tissue repair; it seems that the ex vivo modulation of macrophages to M2 phenotype may be applied for suppression of immune response and promotion of tissue remodelling in kidney transplantation (16).

Alternatively activated macrophages

The concept of alternative activated macrophages was defined in the early 1990s and the nomination of ‘alternatives’ were derived from induction of this cells by IL-4 rather than by IFN-\(\gamma\) (17, 18). The M2 macrophages turn off the damaging mechanisms of immune system activities by production of anti-inflammatory cytokines. M2 is the phenotype of resident tissue macrophages and can be further activated by IL-4. In addition to their excellent phagocytic capacity, M2 macrophages produce high levels of IL-10, TGF-\(\beta\) and low levels of IL-12 (19). Moreover, M2 macrophages produce angiogenic and extracellular matrix (ECM) components which are involved in tissue turnover (20). The M2 macrophages also clear
damage-associated molecular patterns (DAMPs) and apoptotic cells which regulate inflammatory responses and promote wound healing and tissue repair (21). Despite the anti-inflammatory and regulatory effects of M2 macrophages, they can induce allergic inflammation and promote growth of tumours (22, 23). The current classification of M2 macrophages is based on type of stimuli and the gene transcription or protein expression of M2 markers. M2 macrophages could be subdivided into M2a, M2b, M2c and M2d types based on their stimulatory source of activation (24, 25) (26, 27). IL-4 and IL-13 are the main stimuli for the induction of M2a activation (Figure 1), while bacterial lipopolysaccharides (LPS) and immune complexes may stimulate M2b cells. Moreover, glucocorticoids and TGF-β stimulate M2c cells and IL-6 and adenosines are needed for activation of M2d cells (28). Recently, classification of the M2 macrophages was performed based on the activation of stimulatory factors. In this subdivision, the M2a group is called M(IL-4) and M2b is called M(IC), and the M2c includes M(IL-10), M(GC+TGF-β) and M(GC). Given that a wide range of other signals is needed to induce M2 macrophage activation, this classification still fails to cover M2 subdivision. In addition, the in vivo translation of these M2 subdivisions is difficult (12). Moreover, tissues may contain mixed macrophage populations with a spectrum of activation states (29).

**FIGURE1.** Macrophage polarization. Classically activated (M1) and alternatively activated (M2) phenotypes. TNF-α, tumor necrosis factor-α; TGF-β, transforming growth factor-beta; MR (CD206), mannose receptor; NO, nitric oxide; ROS, reactive oxygen species; SR, scavenger receptor; VEGF, vascular endothelial growth factor; GCs, glucocorticoids; ICs, immune complexes; IL1-ra, IL-1 receptor antagonist; TLR, Toll Like Receptor.
Clinical signs, such as interstitial fibrosis, tubular atrophy and glomerulosclerosis occur during chronic renal allograft failure (30). Among these, fibrosis is the hallmark pathological feature that develops before occurrence of any significant clinical dysfunction (31). Inflammatory processes such as cell-mediated/ antibody-mediated rejection, or non-immune events such as hypertension and ischaemia reperfusion injuries are common causes of fibrosis in transplanted tissues (32, 33). Recent studies in the rodent models of ischaemia reperfusion of renal injury have shown that a strong influx of neutrophils, macrophages and lymphocytes occur in response to pro-inflammatory cytokines, moreover, expression of chemokines such as IL-6, IL-8 and monocyte chemotactic protein 1 (MCP-1) is elevated (34-36). Production of reactive oxygen species (ROS) and creating a pro-apoptotic environment by recruited cells can mediate injury in ischaemia reperfusion model (37); While, blocking of pro-inflammatory cytokines or depleting of neutrophils and lymphocytes may hinder acute injury (38). Some studies show that CCL2 and CCL3 are crucial chemokines for recruitment of macrophages (39, 40). Th2 cytokines (IL-4, IL-13) promote M2 macrophages phenotype and, subsequently, inflammatory fibrosis can be induced by TGF-β secreted from M2 macrophages (41, 42).

Recently, Lee et al. showed that in the course of ischaemic renal injury the infiltration of macrophages has a particular pattern. So that within the first 24h the M1 phenotype is predominant, while five to seven days after reperfusion the M2 phenotype surmount the others (43). Some studies suggest that following reperfusion, macrophages may play role in the immediate injury response but these cells may then transit to a ‘trophic phenotype’ to support tubular epithelium repair. There is another animal model of inflammatory renal injury with kidney fibrosis and interstitial inflammation (43, 44). In the unilateral urinary outlet obstruction (UUO) model that completes clamping of one ureter, this results in reduced renal blood flow and reduced glomerular filtration rate within 24h. In this model, M2 macrophages may be present and contribute through modulation of apoptosis and cell survival signals (43). Recently, studies have shown blockade of microRNA 21 (miR21) leads to reduction in macrophage infiltration and production of pro-fibrotic factors, such as TGF-β1, with an attenuation in renal fibrosis (44).

In some studies, significant correlation between interstitial fibrosis and accumulation of CD68 + CD163+ M2-type macrophages have been identified by immunostaining. Therefore, amelioration of acute and chronic injury in experimental models following the depletion of macrophages has indicated the role of these cells in the injury models (45, 46). However, the implications of such strategies in human disease are not known.

In renal fibrogenesis, a T-helper2 (TH2)-prone inflammatory milieu promotes M2 macrophages recruitment and activation in a MyD88-dependent manner (Figure 2). However, TLR2, TLR4 and MyD88 knockout (KO) mice demonstrates reduced TH2 cytokine production, lower M2 macrophage infiltration (IL-10+ and CD206+ CD11b high cells), and decreased fibrosis formation with an improved renal function. Moreover, in this model, absence of IL-4 was associated with better renal function, and in IL-13 and TGF-β levels led to a decrease in arginase activity and fibrosis formation, compared with IL-12 KO and wild-type (W-T) animals (47).
FIGURE 2. Fibrosis formation mediated by MYD88 Signaling. In renal fibrogenesis, a T helper2 (TH2)-prone inflammatory milieu promote M2 macrophages recruitment and activation in a MyD88-dependent manner. TLR4 signaling enhances TGF-β signaling in renal tubular cells. TGF-β accompanying MMPs trigger fibrogenesis.

In mice, protection of acute kidney injury from renal function impairment and severe tubular injury was demonstrated during in vivo infusion of mesenchymal stem cells (MSCs), accompanied by a time-dependent increase in CD206-positive M2 macrophage infiltration. In addition, in vitro co-culture of macrophages with MSCs resulted in an anti-inflammatory M2 phenotype, with an increased expression of CD206 and secretion of IL-10 (48). Moreover, human mesenchymal stem cells promote regeneration ischaemia-reperfusion injury via alteration of the phenotypes of macrophages and supporting the transition from tubule injury to tubule repair (48, 49).

Migration of human umbilical cord-derived stromal cells (HUCSCs) into injured kidneys improves renal function by reduction of the infiltration of macrophages in the mouse model of renal IRI. Also, increase in M2-like macrophages was shown during repair, which is beneficial
for an accelerated recovery. Thus, HUCSCs are effective in ameliorating mouse renal IRI possibly by skewing of the infiltrated macrophages into the M2 phenotype (50). Injection of amniotic fluid stem cells in treated mice resulted in delayed progression of renal fibrosis and an increase in levels of IL1-RII, typical of an M2 macrophage phenotype. The presence of M2 macrophages led to promotion of tissue remodelling versus progression of fibrosis (51).

Renal fibrosis usually predisposes end-stage renal disease (ESRD). Studies have reported an important correlation between TGF-β1 and bone morphogenic protein 7 (BMP7) in the epithelial-to-mesenchymal transition (EMT) of renal tubular epithelial cells during chronic renal injury. Unlike BMP7, TGF-β1 induces renal fibrosis by promotion of the epithelial-to-mesenchymal transition. After renal injury in a mouse unilateral ureteral obstruction (UUO) model, the recruited macrophages are polarised to a M2 subtype. Released high levels of TGF-β1 by M2 macrophages suppress BMP7 and enhance EMT-induced renal fibrosis. Depletion of M2 macrophages, but not of M1 macrophages, inhibits EMT and subsequently the renal fibrosis. Also, adoptive transplantation of M2 macrophages deteriorated renal fibrosis in the UUO renal fibrosis model (52)]. As mentioned above, IL-4/IL-13-induced M2a macrophages are tissue repairers. This function depends on upregulation of TGF-β and arginase, as well as their ability to produce certain ECM components (53-55). TGF-β produced by IL-4-activated M2a cells is a powerful activator of fibroblast collagen production and partly responsible for collagen production and enhancement of fibroblast proliferation (54, 56, 57). Rodent M2a macrophages also produce arginase, which has been suggested to be important for tissue repair (53, 58). Produced polyamines, such as proline via arginase-arginine pathway, resulted in cell proliferation and collagen synthetase (59). M2 Macrophages promoting fibroblast collagen synthesis via arginase and TGF-β, these cells are capable of synthesising Extracellular Matrix (ECM) components, including fibronectin, collagen type VI and βIG-H3 (60, 61)]. The βig-h3 is a TGF-β-induced ECM protein that induces the secretion of MMPs (matrix metalloproteinases) (62).

**BIG-H3** promotes the adhesion and migration of monocytes, fibroblasts and keratinocytes and increases fibroblast collagen production (63-65). Perhaps the strongest evidence for the early-M1/late-M2 paradigm and for switching from a pro- to an anti-inflammatory phenotype is the pattern of pro-inflammatory cytokine production by tissue-repair macrophages. For instance, in cases of skeletal muscle and skin injury, M1 activator IFN-γ is upregulated rapidly and is required for proper healing of these tissues (66, 67). In models of renal disease, including allograft injury, glomerulonephritis and interstitial fibrosis modulating macrophage phenotype and function, have been reported to reduce renal injury (68-72). In mice with chronic inflammatory renal disease, injection of ex vivo manipulated splenic macrophages (stimulated with IL-4/IL-) can reduce renal injury and facilitate repair by downregulating inflammatory cytokine and chemokine expression of the host infiltrating macrophages (15, 72). In models of glomerular disease, the protective effect of the transplanted macrophages was associated with M2 skewing of host macrophages (73, 74). The transfer of macrophages transduced with an NF-κB inhibitor into a rat model of nephrotoxic nephritis, resulted in a reduction in iNOS and MHC class II expression in glomeruli. However, injection of ant-inflammatory macrophages consisted of only 15% of the glomerular macrophages; they could significantly reduce activation of host macrophages and glomerular infiltration that resulted in the attenuation of renal injury (73).

Transplantation of renal allografts is an established treatment for patients with chronic renal failure (75). Studies on acute kidney injury models show that macrophages are key mediators of
the onset inflammatory injury; however, more recent studies indicate a reparative role depending on macrophage phenotype. Study of the mouse kidney allografts and kidney acute tubular necrosis demonstrated that expression of activation markers of alternative macrophage in allografts is associated with molecules that induce development of M2 macrophage phenotype including IL4, IL13 and inhibin A. In this regard, increased expression of alternative macrophage activation markers and inhibin was shown in kidneys of the patients with acute tubular necrosis injured by ischaemia/reperfusion (76). Despite IFN-γ activated macrophages, kidneys undergoing T-cell-mediated rejection progressively occur in the presence of alternative activation phenotype-dependent IL4 and IL13. Similar to acute tubular necrosis, high alternative macrophage activation transcription levels in rejecting allografts are strongly associated with parenchymal deterioration (36, 76). Defects in IFN-γ, or MHC class I products, develop accelerated necrosis in mouse kidney allografts (77). IFN-γ-induced donor class I products deliver inhibitory signals to host inflammatory cells via perforin and granzymes (78). Microarrays analysis cleared that, compared to wild-type (W-T) allografts, class I-deficient allografts (class I-suppressed transcripts [CISTs]) and IFN-γ-deficient allografts (IFN-γ-suppressed transcripts [GSTs]), were associated with alternative macrophage activation markers such as macrophage mannose receptor 1 (Mrc1), arginase 1 (Arg1) and macrophage elastase (Mmp12) (78).

**CCR5**

The chemokine (C-C motif) receptor 5 (CCR5) deficiency mice (Ccr5−/− C57BL/6), as kidney recipients, show a significant reduction in glomerular damage, vascular rejection, tubulointerstitial inflammation and numbers of CD4+, CD8+, CD11c+ and alpha smooth muscle actin (α-SMA)+ cells seven and 42 days after transplantation. In the recipient mice, unlike mRNA expression of Th2-associated markers, Th1-associated markers decreased during the time (79). In addition to these findings, markers of M2 macrophages (arginase 1, chitinase 3-like 3, resistin-like alpha, mannose receptor), were strongly upregulated (mRNA and/or protein level) only in allografts Ccr5−/− recipients. Ccr5− deficiency shifted intragraft immune responses during the chronic phase towards the Th2 type and led to accumulation of M2 macrophages. Hence, CCR5 deficiency promotes monocyte polarisation to alternative macrophage activation (79).

**Liver X receptors (LXR)-α, β**

Liver X receptors (LXR)-α, β regulate intracellular cholesterol homeostasis and inhibit inflammatory gene expression (80). In the F344-LEW rat kidney transplant model, (LXR)-α, β-agonist GW3965 significantly improved function and morphology of rat kidney allografts by substantial reduction of mononuclear cell infiltrate and fibrosis. In vitro, LXR activation by GW3965 increases the induction of alternative activation of bone marrow-derived macrophages (BMDMs) by IL-4/IL-13. This finding suggests an additional mechanism by LXRs to prevent graft damage. This results reveals the role of macrophage LXRs in allograft rejection and prevention of fibrosis in acute and chronic organ transplantation(81, 82).

**TGF-β**
Biopsies of cases with non-rejected kidneys (NRK), acute rejection (AR) and chronic allograft nephropathy (CAN), for the expression of TGFβ-1 and TNF-α and the proportion of macrophages and eosinophils in the development of interstitial fibrosis (28) and graft atherosclerosis, showed the proportion of M2 macrophages that produced TGF-β was 10%–40% of the infiltrated cells in the specimen (83). Recent studies suggested M2 macrophages are involved in the progression of chronic kidney allograft injury (CAI). The results of biopsies identified a significant increase in interstitial fibrosis with accumulation of CD68+ CD163+ M2-type macrophages in children undergoing transplantation diagnosed with CAI. Localisation of M2 macrophages frequently induces interstitial fibrosis by type I collagen deposition and accumulation of α-smooth muscle actin (α-SMA) + myofibroblasts (84).

**transglutaminas**
In the rat renal allografts (Fischer-344 to Lewis) model of chronic allograft injury (CAI), leukocytes accumulated in blood vessels express transglutaminase (Tgm2), a multifunctional protein that is an established marker of M2 macrophages that are involved in acute and chronic graft rejection. After the resolution of acute rejection, leukocytic Tgm2 levels are lower (85). Ischaemia-reperfusion (IR) injury is one of the causes of acute and chronic graft rejection (86).

**Kidney injury molecule-1**
Using mesenchymal stem cells (MSCs) after IR reduces proximal tubule kidney injury molecule-1 (KIM-1) expression by inducing an anti-inflammatory ‘M2’ phenotype gene expression. MSCs are home to injured kidneys and promote repair, which may be mediated by their ability to promote M2 macrophage polarisation (87).

**Conclusion**
Macrophages are a critical component of an immune system that contributes to repair and regeneration in numerous tissues, but may also contribute to chronic tissue damage and fibrosis. A broad spectrum of functional macrophages phenotypes can be produced by different stimuli and this distinct population likely contributes to the contradictory roles of macrophages in tissue injury, fibrosis and regeneration. An undeniable role of macrophages in the worst late transplant outcome and allograft rejection has been proven in many studies. In more detail, their pathophysiology in injury models of the kidney has provided implications for the management of solid organ allografts. Current clinical assessment of cellular rejection suggests M1 and M2 macrophages have a distinct role in transplantation. M1 cells mainly contribute to tissue injury and graft rejection by producing pro-inflammatory cytokines and chemokines. However, M2 macrophages produce anti-inflammatory cytokines and play a central role in the modulation of immune responses, angiogenesis, tissue remodelling and repair. In many studies, accumulation of M2 macrophages has been shown in acute and chronic injury models. In some cases, amelioration of disease has reported that depletion of M2 cells and in other cases, the presence of M2 cells, has led to an increase in graft fibrosis and inferiority of graft condition. Furthermore, human clinical studies have shown M2 cells improve graft outcome by amelioration of renal
allograft inflammation. However, other studies have reported M2 macrophages produce mediators, such as TGF-β (tumour growth factor-β), VEGF (vascular endothelial growth factor) and MMPs (matrix metalloproteinases) that increase renal fibrosis and graft rejection. In addition, TH2 cytokines (IL-4, IL-10 and IL-13) skew monocyte to M2 cells, thereby these cells are predictive of fibrosis and graft rejection in a mechanism in which innate immune response is triggered in a MyD88-dependent pathway. Clearly, additional studies are needed to identify the exact mechanism of injury and determine if this may be an important therapeutic pathway in humans. Therefore, according to current studies, tissue injury or repair by M2 macrophages exhibits complex and heterogeneous phenotypes. There is no conclusive evidence about the role of these cells in graft outcome and many open questions remain in this regard. Whereas M2 cells are known to promote cell proliferation, angiogenesis, wound debridement, matrix remodelling and tissue-repair, it remains to be seen about the mechanisms by which these M2 macrophages accomplish these functions. The actual factors that regulate M2 cells phenotype during tissue repair are unknown, whereas many potential modulators of M2 macrophages activation have been identified in vitro. It is not clear whether different phenotypes of macrophages during the kidney inflammatory are derived from distinct migrated blood-monocyte populations or from existing wound M2 macrophages that change in phenotype. Furthermore, rodent and human M2 macrophages maybe exhibit dissimilar responses to in vitro activation, and it remains to be determined whether tissue injury and repair are a species-dependent difference in M2 macrophages function during in vivo. Finally, regulation of the tissue-repair environment and M2 macrophages phenotype will provide insight into novel therapies based on manipulating the function of M2 cells to promote tissue repair and improve graft outcome. This review identified double-edged effects of M2 macrophages in the formation of renal fibrosis and renal allograft function. Further studies are needed for more precise identification of the role of M2 macrophages in renal allograft outcome. Thereby, for better renal outcomes and decreased fibrosis formation, therapeutic strategies should be accompanied by the modulation of the TH2 and M2 cells.

Disclosure
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