Clinical Kidney Journal

ckj

OXFORD

Leading European Nephrology

Clinical Kidney Journal, 2016, vol. 9, no. 6, 765–771

doi: 10.1093/ckj/sfw096
Advance Access Publication Date: 5 October 2016
CKJ Review

CKJ REVIEW

Macrophage in chronic kidney disease

Roser Guiteras[1], Maria Flaquer[1] and Josep M. Cruzado[1,2]

[1]Experimental Nephrology, Departament de Ciències Clíniques, Universitat de Barcelona, Institut d'Investigació biomèdica de Bellvitge (IDIBELL), Hospitalet de Llobregat, Barcelona, Spain and [2]Nephrology Department, Hospital Universitari de Bellvitge, Hospitalet de Llobregat, Barcelona, Spain

Correspondence and offprint requests to: Roser Guiteras; E-mail: rguiteras@idibell.cat

Abstract

Chronic kidney disease (CKD) has become a major health problem worldwide. This review describes the role of macrophages in CKD and highlights the importance of anti-inflammatory M2 macrophage activation in both renal fibrosis and wound healing processes. Furthermore, the mechanisms by which M2 macrophages induce renal repair and regeneration are still under debate and currently demand more attention. The M1/M2 macrophage balance is related to the renal microenvironment and could influence CKD progression. In fact, an inflammatory renal environment and M2 plasticity can be the major hurdles to establishing macrophage cell-based therapies in CKD. M2 macrophage cell-based therapy is promising if the M2 phenotype remains stable and is 'fixed' by *in vitro* manipulation. However, a greater understanding of phenotype polarization is still required. Moreover, better strategies and targets to induce reparative macrophages *in vivo* should guide future investigations in order to abate kidney diseases.

Key words: alternatively activated macrophages, end-stage renal failure, phenotype polarization, therapeutic strategy

Macrophage origin and heterogeneity

Macrophages belong to the mononuclear phagocytic system (MPS) [1, 2] and comprise a heterogeneous population of cells that have the capacity to perform a wide range of critical functions [3]. They play an important role in tissue homeostasis and immune responses in normal and diseased kidneys [2, 4]. Macrophages are present in all tissues and originate from common myeloid progenitor cells in the bone marrow [1, 5] under the influence of colony-stimulating factor 1 (CSF-1) [6]. Monocyte development sequentially gives rise to monoblasts, pro-monocytes and finally monocytes, which are released from the bone marrow into the bloodstream [7]. Monocytes then migrate from the blood to the injured tissue and replenish tissue macrophage numbers, especially during inflammation. Macrophages are crucial components of innate immunity; their main function is to clear the interstitial environment of extraneous cellular material [7] and also to generate an adaptive immune response by serving as antigen-presenting cells (APCs) and by recruiting other immune cells such as lymphocytes [5, 8].

Macrophages are divided into different subpopulations based on their functionality and anatomical location, e.g. Kupffer cells, Langerhans cells and microglial cells [9]. They are defined as tissue-resident phagocytic cells that contribute to critical roles in homeostasis, surveillance and tissue injury and repair [5, 10]. In the tissue injury scenario, blood monocytes are recruited to the site of damage and undergo differentiation in response to microenvironment signals to which they are exposed [11]. Therefore, macrophage infiltration in the kidney is a common feature of chronic kidney disease (CKD) in humans, and the correlation between the degree of macrophage infiltration and the severity of renal injury suggests an effector function for macrophages [10]. Hence, during CKD, resident and infiltrating macrophages undergo a range of activation responses such as phagocytosis and production of pro-inflammatory cytokines and toxic metabolites [6].

Received: May 19, 2016. **Accepted:** August 22, 2016

CKD is becoming one of the most important health problems worldwide [12] and greatly affects patients' quality of life [13]. There is broad agreement that all primary causes of CKD share a common pathogenic pathway of progressive injury resulting from the destructive consequences of scarring (fibrosis) [14]. The development of CKD is characterized by an accumulation of extracellular matrix (ECM) proteins in the glomerulus and interstitium [13], which is thought to be promoted by an aberrant wound healing response involving tubular epithelial cells (TECs), myofibroblasts, fibrocytes and immune cells, among others, thus leading to progressive fibrosis in the kidney and loss of viable nephrons [15].

Following recruitment to the damaged kidney, macrophages can be broadly classified into two different subtypes, depending on their local microenvironment: classically activated (M1) and alternatively activated (M2) macrophages [10, 14, 16]. Although they have been strongly associated with tissue injury [17], they also have a critical role in both host defence and tissue repair [18, 19]. Pro-inflammatory M1 macrophages are produced by exposure to interferon (IFN)-γ or lipopolysaccharide (LPS) and are considered pro-inflammatory due to their capacity to release certain cytokines such as interleukin (IL)-1, IL-6 and tumour necrosis factor (TNF)-α [20]. In contrast, M2 macrophages have anti-inflammatory functions and express arginase, mannose receptor and IL-10, among others (Table 1) [10, 20]. Alternatively activated M2 macrophages can be further categorized into three subgroups: M2a induced by IL-4 and/or IL-13, which display a wound-healing (tissue repair) role; M2b induced by immune complexes and M2c, with anti-inflammatory effects and induced by IL-10, transforming growth factor (TGF)-β or glucocorticoids [7, 21]. Thus, macrophages are highly heterogeneous cells that exhibit distinct phenotypes and functionality in response to microenvironment stimuli, leading to the previously described classification system [5, 10]. Although extensive *in vitro* studies have supported the phenotype classification, this does not necessarily reflect their true phenotypes *in vivo* [5].

Pro-inflammatory M1 macrophages in CKD

Early phases of CKD trigger a remarkable infiltrate of immune cells, firstly neutrophils, natural killer (NK) cells and T helper (Th)1/17 cells, followed soon after by M1 macrophages [5]. At sites of tissue injury, the interstitial microenvironment is dominated by pathogen-associated molecular patterns (PAMPs) such as adenosine triphosphate (ATP), high mobility group box 1 (HMGB-1) and uric acid, derived from damage-associated molecular patterns (DAMPs) released by necrotic cells [5, 21–24]. During toxic, infectious or traumatic injuries, PAMPs activate resident macrophages as well as parenchymal cells via innate pattern recognition receptors (PRRs) [25, 26], thus leading to the secretion of pro-inflammatory cytokines and providing defence against pathogens and also a functional barrier to prevent further pathogen entries [5, 27, 28]. Inflammation kills host cells at the site of infection, which causes some non-specific collateral tissue damage [26, 29]. In sterile kidney injury, PAMPs are often absent, and DAMPs mostly drive the infiltration of inflammatory macrophages into the sterile kidney [5, 30, 31].

Macrophages detect endogenous danger signals via toll-like receptors (TLRs) [21, 22, 30, 32], intracellular PRRs and the IL-1 receptor (IL-1R) through the adaptor molecule myeloid differentiation primary response gene 88 (MyD88) [33]. Thus, a TLR ligand acting in an MyD88-dependent manner will induce TNF transcription, which can act in conjunction with IFN-γ in an autocrine manner to activate the macrophage population [7]. IFN-γ is released by NK cells in response to stress and infections, which can prime macrophages to secrete pro-inflammatory cytokines [34]. The combination of these two signals results in a macrophage population with enhanced microbicidal effects as well as in increased production of pro-inflammatory cytokines (IL-6, IL-1 and TNF-α), superoxide anions and oxygen and nitrogen radicals. The two signals together also promote cytotoxic adaptive immunity by upregulating major histocompatibility complex class II (MHC II) in conjunction with co-stimulatory molecules (CD40, CD80 and CD86) [35, 36]. Mulay *et al.* [26] support a theory in which renal injury and inflammation are reciprocally enhanced in an autoamplification loop, referred to as necroinflammation. Cell necrosis releases DAMPs and alarmins that activate infiltrating monocytes via TLRs towards a pro-inflammatory phenotype. Thus, infiltrating macrophages, in turn, further contribute to necroinflammation due to the secretion of numerous pro-inflammatory cytokines [26]; therefore, inhibiting activated pro-inflammatory macrophages would prevent immunopathology in injured kidneys.

These M1 macrophages promote Th1 and Th17 responses and are therefore involved in initiating and sustaining inflammatory processes [7, 37]. In inflammation in mice, Ly6C^{high} monocytes are recruited and differentiated into M1 macrophages that express CC-chemokine receptor 2 (CCR-2), thus responding to CC-chemokine ligand 2 (CCL-2), an important chemokine required for monocyte/macrophage recruitment to damaged sites [1, 38]. Pro-inflammatory macrophages also release matrix metalloproteinases (MMPs) to enable their migration through basement membranes and interstitial ECM networks [5, 39]. LPS/IFN-γ-activated M1 macrophages induce renal fibrosis by secretion of MMP-9, which increase tubular cell ECM transition via the β-catenin pathway [5]. The transcription factor IRF5 also seems to play a key role in M1 macrophage polarization, suggesting that inhibiting IRF5 might be useful for chronic macrophage-induced inflammation [40].

The pathogenic role of macrophages has been demonstrated by depletion of kidney-resident macrophages with liposomal clodronate (LC) in different types of experimental kidney disease

Table 1. Distinct macrophage populations

Macrophage phenotype	Stimulation	Effect	Marker expression
M1	IFN-γ, TNF-α, LPS, GM-GSF	Pro-inflammatory	CD86, CD80, MHC II, Ly6C^{hi}, TLR2, TLR4
M2a	IL-4 and/or IL-13	Profibrotic	MR/CD206, MHC II, Arg-1
M2b	IC + LPS	Immunoregulation	CD86, MHC II
M2c	IL-10, TGF-β, apoptotic cells, glucocorticoids	Anti-inflammatory	MR/CD206, B7-H4, TLR1, TLR8

Adapted from Anders and Ryu [21], Cao *et al.* [5] and Martinez *et al.* [50].

Depending on the microenvironment, macrophages can differentiate into specific populations with distinct functions.

GM-CSF, granulocyte macrophage colony-stimulating factor; IC, immune complexes; IL, interleukin; IFN, interferon; LPS, lipopolysaccharide; MHC II, major histocompatibility complex class II; TLR, toll-like receptor; TNF, tumour necrosis factor.

[41–43]. However, increasing evidence from extensive studies shows that macrophages also play a reparative role during disease progression.

Anti-inflammatory M2 macrophages in CKD

Macrophages that secrete anti-inflammatory cytokines and promote wound healing and tissue remodelling have been referred to as alternatively activated macrophages (AAMs) [5, 10], also called M2. The mechanisms by which kidney-resident M1 macrophages switch to an anti-inflammatory M2 phenotype are still not well understood. Detailed studies in which renal injury resolves have identified that macrophages undergo a phenotypic change during recovery and this confers a protective and reparative role [44–46]. Moreover, depletion of macrophages during this phase delays recovery, indicating a functional role for macrophages in renal repair [44]. Macrophages modulated *ex vivo* with IL-4 and/or IL-13 (M2a) express high levels of mannose receptor (CD206), produce anti-inflammatory IL-10 and have immunoregulatory functions [8, 47]. These cells secrete components of the ECM and therefore their main functions seem to be associated with wound healing and tissue remodelling and repair [7, 10]. Martinez *et al.* [48] demonstrated that when ECM clearance is deficient due to defects in the engulfment of dying cells, functional markers of renal injury, such as serum creatinine, blood urea nitrogen and proteinuria, progressively increase. This process causes persistent inflammation and consequently an increase in the M1:M2 ratio. Another category of AAMs is the M2b macrophages, which represents crosstalk with B cells. This category induces IL-10 secretion in addition to upregulating antigen presentation and promoting Th2 responses [49]. Both M2a and M2b macrophages have an immunoregulatory role through downregulation of IL-12, IL-6 and TNF [7]. Moreover, M2c macrophages are induced by IL-10, TGF-β and glucocorticoids [50]; this subset is known to exhibit anti-inflammatory cytokine production and suppressive functions *in vitro*, and they are often referred to as deactivated [21]. Similarly to classically activated macrophages, wound healing macrophages can develop in response to innate and adaptive signals [7]. IL-4 is one of the first signals released during tissue injury by basophils and mast cells, among others, and this early IL-4 production converts resident macrophages into a population reprogrammed to promote wound healing [7].

To date, the diverse roles of macrophages in *in vivo* studies are still not fully understood, although it is generally known that macrophages eagerly participate in the clearance of apoptotic and necrotic cells in injury resolution and tissue remodelling [5, 51, 52].

Persistent M2 macrophages are associated with fibrosis

Macrophage depletion via anti-macrophage serum or LC nearly always reduced persistent inflammation and also the subsequent development of fibrosis [17, 21]. Nevertheless, renal fibrosis may not only be triggered by pro-inflammatory M1 macrophages, but instead by insufficient epithelial healing or by profibrotic M2 macrophages and fibrocytes [21]. In the face of ongoing damage, sustained M2 macrophage infiltration may result in constant production of several wound healing growth factors [3], and what initially begins as a reparative mechanism may subsequently become harmful. In fact, persistence of the wound healing process could be pathological, resulting in irreversible fibrosis and progressive kidney tissue destruction [3]. On the other hand, M2 macrophages may help to resolve inflammation through high endocytic clearance capacities and the production of trophic factors that promote angiogenesis and mediate wound healing producing ECM [53, 54]. For instance, *in vitro* cytokines such as IL-4 and IL-13 further promote the M2 phenotype, which predominantly releases fibronectin 1 (FN-1) and other ECM molecules that could contribute directly to renal fibrosis [55, 56]. Likewise, Kim *et al.* [57] recently demonstrated that M2 macrophages play a more important role than M1 macrophages in the development of fibrosis in an *in vitro* cisplatin-treated culture. It is important to note that these studies were performed *in vitro*, and the role of macrophages as a contributing factor in the development of renal fibrosis *in vivo* remains under discussion.

Members of the TGF-β superfamily are the most extensively studied growth factors derived from macrophages, which are mainly associated with an M2-like phenotype [58], among other cell types such as TECs and myofibroblasts. Within the kidney, macrophage-derived TGF-β may promote fibrosis by paracrine activation of matrix-producing myofibroblasts [59]. Unilateral ureteral obstruction (UUO) is a well-characterized model to investigate the factors that contribute to renal fibrosis [5]. Therefore, many experimental approaches have been studied using this fibrosis model. For instance, Braga *et al.* [60] demonstrated that M2-phenotype macrophages contribute to renal fibrosis in an MyD88-dependent manner and through TLR signalling pathways. Moreover, galectin-3, a nuclear M2 marker, has been shown to be produced by kidney-resident macrophages and to enhance renal fibrosis in UUO [61]. López-Guisa *et al.* [62] also demonstrated that macrophages expressing the marker mannose receptor-2 (Mrc2) displayed a fibrosis-attenuating role in UUO [62], as mice deficient in Mrc2 exhibited worsened renal fibrosis. Indeed, to study the macrophage-specific role of TGF-β1 in the development of renal fibrosis, Huen *et al.* [63] developed mice with a homozygous deletion of TGF-β1 in myeloid lineage cells and demonstrated that despite TGF-β1 mRNA reduction and the prevention of downstream Smad activation, interstitial fibrosis and tubular injury were not significantly different after UUO compared with the control UUO group. Thus, they suggested that specifically targeting myeloid TGF-β1 may not be sufficient to combat the progression of renal fibrosis. Several studies targeting TGF-β1 have highlighted the complex role of cytokine in both injury and wound repair processes, showing that further research is necessary to clarify the functional impact of this complex. Taken together, renal fibrosis may not only be triggered by pro-inflammatory M1 macrophages, but M2 macrophages could also contribute somehow to the development of fibrosis and progressive fibrotic scarring [3].

Further studies in experimental animal models of CKD, such as diabetic nephropathy, undergo suggested that, at later disease stages, macrophages confer a shift towards chronic activation of the M2 phenotype [64, 65], which leads to glomerulosclerosis, tubular interstitial fibrosis and eventually organ failure [65, 66]. Thus, distinct macrophage subsets can coexist in kidney tissue and certain subsets can predominate at different disease stages, from the beginning of kidney damage to the recovery phase [5]. Shen *et al.* [67] demonstrated that phagocytosis by M1 macrophages to remove dead cells may only function in the early stages of UUO, and M2 macrophages appeared to be the major cell type in advanced stages. These results are in agreement with those reported by Cao *et al.* [5], who described how, in CKD, due to progressive and persistent injury and inflammation, M1 macrophages persistently remained at sites of tissue injury and consequently reduced numbers of M2-phenotype macrophages were recruited into the kidney. Therefore, M2 macrophages either

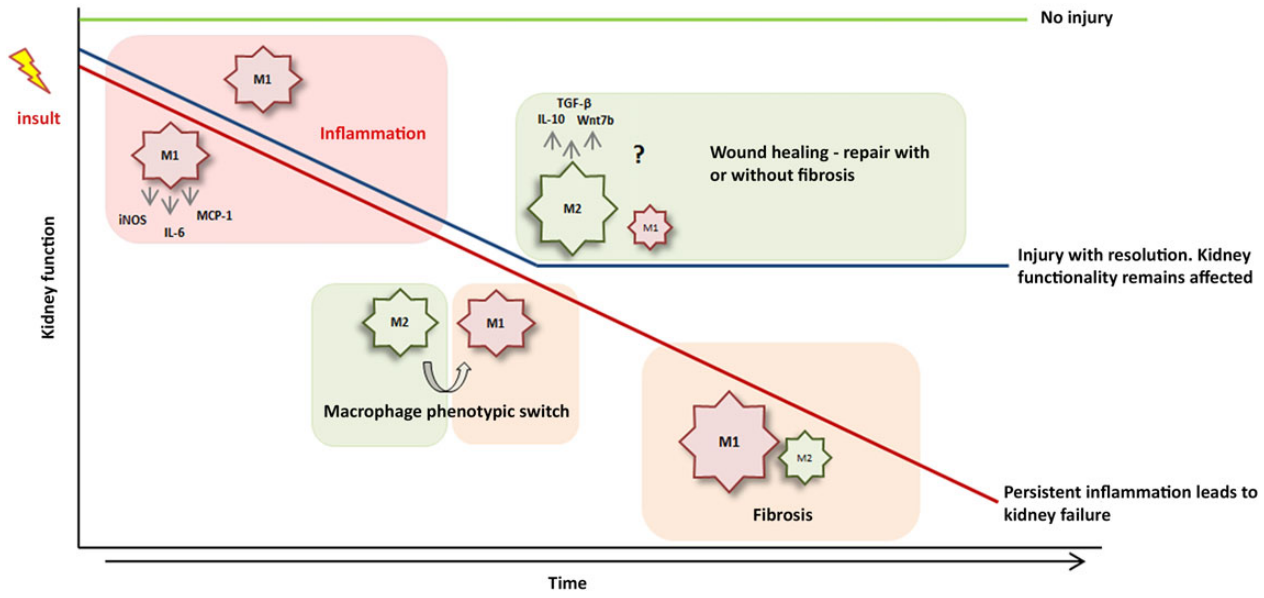

Fig. 1. M1/M2 macrophage balance depending on chronic kidney disease (CKD) progression. Renal function varies over time, depending on the type of injury, the persistence and severity of the damage and the reparative ability of the kidney. In the early stages of CKD, pro-inflammatory macrophages (M1) infiltrate the injury site and release pro-inflammatory cytokines, which promote an inflammatory state. If the injury resolves, renal function as well as renal mass ameliorate, depending on damage severity and duration. Macrophages also switch to an anti-inflammatory (M2) phenotype, leading to a wound healing phase that may involve tissue fibrosis. However, if there is no injury resolution, M1 macrophages persist at injured sites and there is a decrease in the number of M2 macrophages, which could also subsequently undergo a phenotypic switch to M1. The continuous release of profibrotic and inflammatory factors promote renal fibrosis, leading to renal failure.

coexist in small numbers or are absent because of permanent inflammation. Taken together, the mechanisms regulating the distinct functions of M2 macrophages during tissue repair or fibrosis remain largely unclear (Figure 1).

M2 macrophage cell-based therapy for renal repair and regeneration

The kidney has limited regenerative capacity. Therefore, interventions such as gene and cell-based therapies are being extensively developed as an alternative treatment modality for the prevention of progression to end-stage renal failure [68]. Nevertheless, there are very few therapies that induce renal repair in chronic nephropathies [69]. For instance, Flaquer *et al.* [70] demonstrated that hepatocyte growth factor (HGF) gene therapy was able to enhance the amount of bone marrow–derived cells in the diabetic kidney and showed that these cells located around the glomeruli were mainly M2 macrophages. Thus, rodent models are valuable for studying macrophage phenotypes in the context of CKD [71].

Among potential strategies, macrophage cell-based therapy provides a contrast of promising [18] and discordant results at the same time [54, 72, 73]. Macrophages modulated *ex vivo* and displaying an anti-inflammatory or reparatory activity have been used as cell-based therapy in a number of mouse models of CKD (Table 2). M2 macrophages must meet at least two conditions if they are to be used as a therapeutic tool *in vivo*: first, the ability to reach the injured tissue or organ, and second, a stable phenotype [10]. These two conditions are particularly important, since infused macrophages may be harmful if they switch from an anti-inflammatory to a pro-inflammatory phenotype.

Professor David Harris's group has extensively investigated the effects of infusing *in vitro*–generated M2 macrophages in rodent models of both acute kidney failure and CKD [18, 54, 72]. Using a mouse model of adriamycin nephropathy (AN), they demonstrated that a single intravenous injection of 1×10^6 macrophages generated by splenic CD11b$^+$ cells and exposed to IL-10 and TGF-β (M2c) provided increased protection against renal structural and functional injury compared with IL-4/IL-13-exposed macrophages (M2a) [72]. These IL-10/TGF-β-modulated M2 macrophages (M2c) expressed high levels of B7-H4, whereas IL-4/IL-13-modulated macrophages (M2a) did not. Thus, these authors attributed the greater potency of M2c macrophages to the expression of the co-stimulation molecule B7-H4, which suppresses T cell proliferation and induces regulatory T cells both *in vitro* and *in vivo*. However, they also demonstrated that infused M2c macrophages did change their phenotype during the disease course, although not towards a distinct M1 phenotype [72, 74]. In a severe combined immunodeficient (SCID) mouse model of AN, the infusion of M2 macrophages isolated from the spleen and modulated *ex vivo* by IL-4 and IL-13 was associated with an amelioration of renal injury [54]. In contrast, a report by Cao *et al.* [18] showed that using the same mouse model but with M2 macrophages isolated from the bone marrow (BM-M2 macrophages) failed to reduce proteinuria and to preserve renal function, owing to a change in macrophage phenotype. Therefore, these studies demonstrated that transfused BM-M2 macrophages lost their suppressive function *in vivo* due to their proliferation, whereas splenic M2 macrophages (SP-M2) were protective because they did not proliferate. The enhanced proliferation of BM-M2 macrophages can be explained by their increased expression of macrophage colony-stimulating factor (M-CSF) receptor, in comparison with SP-M2 macrophages [18], which could be partly prevented by blocking CSF-1-mediated signalling. Transfer of SP-M2 macrophages seems to protect against renal injury, whereas transfer of BM-M2 macrophages appears to promote renal fibrosis [18, 75]. However, the bone marrow, rather than

Table 2. Effects of macrophage cell-based therapy in different mouse models of CKD

CKD mouse model	Characteristics	Macrophage cell-based therapy	Results	Limitations
Unilateral ureteral obstruction (UUO) [56, 59, 73]	Well-established model with rapid interstitial fibrosis Non-reversible	Bone marrow-derived macrophages (BM-M0) RAW246.7 M2 macrophages	Controversial results: transition into collagen-producing myofibroblasts. At late stage, renal fibrosis is reduced	No renal function data Aggressive fibrosis
Adriamycin-induced nephropathy (AN) [18, 47, 62]	Gradual development of proteinuria, podocyte injury followed by glomerulosclerosis, tubulointerstitial inflammation and fibrosis	Splenocytes (M2a and M2c) Bone marrow-derived macrophages (BM-M2a)	Failed renoprotection of BM-M2a, whereas splenocytes prevented renal injury Reduction of histological and functional injury	The pathophysiology has not been clarified in full detail by the sequence of events, with proteinuria preceding the development of renal fibrotic lesions
Diabetic kidney disease [74]	STZ-induced diabetes Destruction of β-cells and induction of the hyperglycaemic state associated with inflammatory infiltrates	Splenocytes (M0 and M2a)	Amelioration of tubular atrophy, glomerular hypertrophy and interstitial expansion. Degree of interstitial fibrosis, but no effects on renal function	Regeneration of pancreatic islets can occur after STZ treatment Dose-dependent strain

Search criteria: 'macrophage infusion kidney', 'm2 macrophage fibrosis', 'transfused macrophages' and 'alternatively activated macrophages kidney'.

the spleen, represents an accessible source of macrophage precursors for AAM therapy. Therefore, the source of origin of macrophages is another critical issue to bear in mind due to the contradictory outcomes that have been published recently.

Nowadays, the use of anti-inflammatory and regenerative macrophage-derived molecules is increasing. Heme-oxygenase-1 (HO-1), for example, is a protective and anti-inflammatory enzyme upregulated in response to renal injury, whereas its down-regulation is associated with susceptibility to damage [76, 77]. Chen *et al.* [78] demonstrated that sustained overexpression of HO-1 counteracted multiple detrimental renal fibrosis-associated pathological processes in a UUO mouse model. On the other hand, accumulating evidence suggests that TNF-α is involved in diabetic nephropathy progression [79, 80]. Furthermore, Awad *et al.* [80] demonstrated that blockade of TNF-α conferred kidney protection by reducing albuminuria, plasma creatinine, kidney macrophage recruitment and plasma cytokine levels. Therefore, their results suggest that inhibition of TNF-α may be a viable strategy to treat diabetic nephropathy in humans. Moreover, Lin *et al.* [81] found that the Wnt pathway may play an important role in tissue regeneration. They showed that Wnt7b was produced by macrophages and was required to stimulate renal repair and regeneration by acting on injured TECs to promote regeneration of the tubule basement membrane, thereby re-establishing renal function and reducing renal fibrosis.

Therefore, macrophages have been shown to be important in renal repair, wound healing and regeneration processes [5]. However, it is still unknown whether they can promote renal repair processes directly by fusing with other cells or transforming into new ones or indirectly by providing help to other cell types [82, 83].

Macrophages: future challenges

Macrophages have been shown to participate actively in tissue repair. Nevertheless, it is important to note that many intermediate phenotypes and many subpopulations are likely to coexist in the same tissue. Moreover, macrophages do not remain in a specific phenotype due to their cell plasticity; they may revert to a resting state and can be subsequently reactivated, depending on the microenvironment [3]. Therefore, a greater understanding is needed in this field [66]. Macrophage dynamics during the different phases of CKD progression are not fully known, and assessment of the predominant macrophage phenotype may be relevant in terms of defining the type of therapy [21]. AAMs have been demonstrated to be protective in reducing renal injury due to their anti-inflammatory role. However, whether these macrophages could become fibrolytic to reduce renal fibrosis still remains unknown [5]. Anders and Ryu [21] have proposed to classify tissue macrophages according to their predominant roles in different phases of kidney disease: pro-inflammatory, anti-inflammatory, profibrotic and fibrolytic macrophages. However, there is a lack of information regarding macrophage types and their dynamics, plasticity and function in human CKD. Hence, more studies are needed before testing macrophage cell-based therapy in humans, since macrophages represent a spectrum of activated phenotypes rather than discrete stable subpopulations [5, 84]. Therefore, better strategies to induce truly regenerative and reparative macrophages *in vivo* need to be developed.

Acknowledgements

We thank Pau Cruzado-Boix for his assistance with the English language revision.

Conflict of interest statement

None declared.

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