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Macrophages: Contributors to Allograft Dysfunction, Repair or Innocent Bystanders?

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Abstract

Purpose of this review—Macrophages are members of the innate immune response. However, their role in the adaptive immune response is not known. The purpose of this review is to highlight our current understanding of macrophage structure and function and how they may participate in allograft injury.

Recent Findings—Studies in acute kidney injury models identify macrophages as key mediators of inflammatory injury while more recent studies indicate that they may play a reparative role, depending on phenotype—M1 or M2 type macrophages. Mregs, generated in vitro, appear to have immune suppressive abilities and a unique phenotype. In solid organ transplant, the emphasis of studies has been on acute or chronic injury. These data are derived from animal models using depletion of macrophages or antagonizing their activation and inflammatory responses. The relative contribution of macrophage phenotype in transplantation has not been explored.

Summary—These studies suggest that macrophages play an injurious role in acute cellular allograft rejection, as well as in chronic injury. Infiltration of an allograft with macrophages is also associated with worse graft function and poor prognosis. Further studies are needed to understand the mechanisms of macrophage mediated injury, explore their potential reparative role and determine if they or their functional products are biomarkers of poor graft outcomes.

Keywords

macrophage; kidney transplant; rejection; antibody; inflammation

INTRODUCTION

Macrophages are key elements of the innate immune response that are differentiated from circulating peripheral mononuclear cells that migrate into tissues as residents or during inflammatory responses. Macrophages are frequently classified as M1 or classically activated macrophages and M2 for alternatively activated macrophages. The former arise in response to T-cell produced IFN- γ and TNF- α produced by antigen presenting cells (APCs) involved in microbial killing. M2 alternatively activated macrophages develop in response

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to IL-4 produced by T cells or granulocytes and may be involved in injury-repair. Recent studies in mouse kidney injury models suggest they may be responsible for fibrosis. A growing number of studies demonstrate accumulation of macrophages in injured allografts, both in acute settings as well as in chronic injury models. Regulatory macrophages have also been recently identified. The role of these cells and their capability of responding in the adaptive immune response will be the subject of this review.

The Macrophage—Origins and Function of a Multidisciplinary Cell

Macrophages are derived from peripheral blood mononuclear cells, and differentiate in response based on specific cytokine signals. Macrophages are classified as M1 or classically activated or M2 for alternatively activated [reviewed in [1]. M1 macrophages differentiate when exposed to IFN γ produced by T cells or natural killer (NK) cells in concert with TNF α produced by antigen presenting cells that have been activated through their Toll like receptors (TLRs). Additionally, TLR signaling that results in TNF α and IFN β secretion can also activate M1 cells. These cells are further distinguished by their expression of high levels of inducible nitric oxide synthase 2 (iNOS). Following activation, M1 cells produce IL-1, IL-6, and IL-23, potent proinflammatory cytokines that can induce tissue inflammation and may also result in activation of Th17 cells. Finally, these macrophages are responsible for killing a variety of pathogens including bacteria, viruses and parasites such as *Leishmania*. Phenotypically, they are F4/80⁺CD11c⁻MR⁻ [reviewed in [1].

Alternatively activated macrophages (M2) are activated by IL-4, which is produced by basophils and mast cells in response to tissue injury as well as chitin, a polysaccharide polymer found in some parasites and fungi. IL-4 may also be produced via Th2 T cells in concert with IL-13. IL-4 stimulates arginase activity, which converts arginine to ornithine, leading to enhanced collagen production, and wound healing. M2 macrophages may also be characterized by expression of mannose receptor and IL-10 production. Exposure *in vitro* of undifferentiated macrophages with IL-4 and IL-13 results in extracellular matrix production and the absence of intracellular pathogen killing. Moreover, M2 cells produce chitinase and chitinase like proteins whose roles may include pathogen killing or matrix reorganization. In contrast to M1, these cells tend to be F4/80⁺CD11c⁻MR⁺ [reviewed in [1].

Recent investigations now suggest a third phenotype of macrophages, the so called regulatory macrophage or Mreg. These cells were identified following *in vitro* stimulation of mouse macrophages with high density immune complexes plus TLR ligands, resulting in the production of IL-10 but not IL-12 [reviewed in [2*]. Similar results could be obtained following exposure to other stimulants including adenosine, apoptotic cells, cAMP analogues, TGF β , and prostaglandin E2 with coincident TLR activation. These cells appear to have distinct biochemical and functional markers from either M1 or M2 macrophages including the expression of CCL1, lack dependence of STAT 6 activation, and lack of arginase expression. Interestingly, adoptive transfer of these cells results in an amelioration of the inflammatory responses in mice following LPS treatment or in the experimental autoimmune encephalitis (EAE) [2*] and in a mouse chronic colitis model [3]. The potential benefit of these cells *in vivo* is discussed below.

Contributions of M1 and M2 in Renal Disease

Recent studies in rodent models of ischemia reperfusion renal injury have shed some light on the potential contribution of macrophages in this injury. In these models, IRI results in a strong influx of lymphocytes, macrophages, and neutrophils in response to the expression of proinflammatory cytokines and chemokines including IL-6, IL-8 and monocyte chemoattractant protein 1 (MCP-1). These cells can mediate injury in the context of reactive oxygen species, creating a pro-apoptotic environment. Indeed, accumulating evidence

suggests that depletion of neutrophils, lymphocytes or blocking pro-inflammatory cytokines ameliorates this acute injury (reviewed in [4]).

However, the role of macrophages is more complex. Macrophages accumulate early, in the first hour of reperfusion, mediated by CCR2 and CX3CR1 expression, with a distinct F4/80^{low}Ly6C^{high}GR1⁺CX3CR1^{low} “inflamed” phenotype [5] producing IL-6, IL-1 and TNF- α . Depletion of these cells using liposomal clodronate indicates the detrimental contribution of these cells in this model [6]. The complexity of macrophage responses in this model is further demonstrated by disruption of repair mechanisms following ischemia once macrophages are depleted by either clodronate [7], or using transgenic mice expressing human diphtheria toxin receptor in CD11b⁺ macrophages [8]. In both these cases, the depletion of macrophages impaired recovery in these injury models.

The heterogeneity of macrophage infiltration modulating not only injury, necrosis and apoptosis but also repair and cell proliferation may lie in the functional phenotypes of the infiltrating cells. Recent studies by Lee et al. clearly highlight a temporal relationship between M1 and M2 phenotypes in the course of ischemic renal injury [9*]. Within the first 24h of reperfusion, M1 infiltrating cells predominate while at 5–7 days, M2 phenotype is predominant. Moreover, macrophages display plasticity with the ability to convert from one phenotype to the other, mediated in part by the tubular epithelial cell microenvironment. Interestingly, adoptive transfer of macrophages primed to IFN- γ (M1) exacerbated injury while those primed to the M2 phenotype lacked this response. These studies suggest that macrophages play an important role in the immediate injury response following reperfusion but then transition to a “trophic phenotype” to support tubular epithelium repair.

Unilateral urinary outlet obstruction (UO) is another animal model of inflammatory renal injury with kidney fibrosis and interstitial inflammation. In this model, which has been performed in a variety of species including rodents, complete clamping of one ureter results in reduced renal blood flow and diminished glomerular filtration rate within 24 hours. Over the course of several days, hydronephrosis develops, with an interstitial inflammatory cell infiltrate and resultant tubular epithelial cell apoptosis and necrosis [reviewed in [10]]. In this disease model, the interstitium is infiltrated by classically activated M1 macrophages, releasing TGF β and TNF α . These cytokines may either activate renal fibroblasts to become myofibroblasts and/or mediate epithelial mesenchymal transformation of tubular epithelial cells. Moreover, infiltrating hematopoietic stem cells and pericytes may differentiate into fibroblasts. All together, these processes result in extracellular matrix deposition and/or a reduction in matrix degradation [reviewed in [10]]. Coincident production of angiotensin II by activated monocytes stimulates NF- κ B with enhanced production of reactive oxygen species; the overall effect is that of worsening epithelial cell injury. While M2 macrophages in this disease model may be present, their role is currently speculative, perhaps contributing through cell survival signals and modulation of apoptosis [10]. Recently, blockade of microRNA 21 (miR21) resulted in an attenuation of renal fibrosis and a reduction in macrophage infiltration, with an associated decrease in production of profibrotic factors, such as TGF- β 1. This study demonstrates a novel strategy to ameliorate renal fibrosis that could be further explored in other fibrotic injury models [11*].

Macrophages in Organ Transplant Inflammation

While the M1/M2 differentiation is intriguing, the relative contribution of these cells in organ transplantation is not known. Moreover, it is not known whether the ability to skew a response in one direction or the other may have benefit. To date a number of studies have indicated the accumulation of macrophages in both acute and chronic injury models. In some cases, the depletion of these cells led to an amelioration of disease. However, the benefit of such a strategy and the implications in human disease is not known.

Macrophages have long been identified in allograft biopsies of kidney transplants in both animal models and in humans [reviewed in [12]. While macrophages are passengers in the donor organ, their presence declines over time in the early post-transplant period [reviewed in [13]. However, in the setting of acute injury such as allograft rejection, macrophages may account for anywhere from 38–60% of infiltrating leukocytes in rejecting kidney biopsies as detected by immunohistochemical staining [14]. Monoclonal antibody against CD68, an intracellular lysosome associated glycoprotein, is the most commonly used antibody to detect human macrophages. These cells may accumulate and be actively recruited into the rejection organ by the chemokine MCP-1, a potent chemotractant. Indeed, blockade of MCP-1 has led to prolonged islet allograft survival although the mechanism for this response appears to be infiltration by mononuclear cells expressing the inhibitory receptor, programmed death-1 (PD-1) [15]. Once in the allograft, macrophage proliferation may occur and contribute to the accumulation of these cells. M-CSF is a key regulator of this process which may be expressed by renal tubular epithelial cells [16]; blockade of this factor reduces the extent of infiltrate and severity of the rejection response [17].

Location of Intragraft Macrophage Infiltrates: Clues to Function?

Within the kidney, macrophages may accumulate in the interstitium, as well as in perivascular regions and within glomeruli. Interstitial infiltration during acute cellular rejection may be an independent predictor of worse outcome [18]. Tubulitis is less common but has been reported, particularly following lymphocyte depletion in the absence of maintenance immunosuppression during episodes of abnormal allograft function [19]. While cell mediated rejection has been the primary context associated with macrophage infiltration, there are a number of recent observations associating macrophage infiltration with antibody mediated rejection (AMR). These include the association of interstitial macrophage infiltrates more commonly in biopsies with positive C4d staining in peritubular capillaries compared to those that lacked C4d staining [20] and identifying a greater proportion of macrophages to T lymphocytes in peritubular capillaries in biopsies with criteria for AMR [21].

Transplant glomerulitis, a potential forerunner to glomerulopathy, has been frequently associated with macrophage infiltrates in greater proportion than T cells and may be further associated with episodes of AMR [12]. Similarly, macrophages have been identified in biopsies with acute vascular rejection, with endothelialitis or intimal arteritis. These infiltrates may be facilitated in the context of AMR by the macrophage Fc receptors such as CD64 and CD32 engaging endothelial bound antibody, as well as via complement split products C3a and C5a, both potent chemotractants. However, more recent data in humans, albeit in a small sample size, suggest that macrophage infiltration in the intima of arteries alone is not associated with antibody mediated injury nor is there any worsened impact on allograft outcome [22]. Further study is needed to understand the potential function and prognosis of macrophage infiltration in the vasculature.

Form and Function

The functional nature of macrophages in allografts remains incompletely understood. Recent data using surveillance/protocol biopsies of kidney allografts have demonstrated that Banff criteria for acute cellular rejection may be met in 10–30% of biopsies, in the absence of any functional change in serum creatinine [23], so called “subclinical rejection”. This dissociation between infiltration and graft function suggest that lymphocytes may not be the sole or primary force underpinning allograft dysfunction. Indeed, analysis of 78 consecutive recipients of kidney allografts with histological acute cellular rejection. While the extent of change in renal function was associated with the degree of cellular infiltration, monocyte infiltration, not lymphocyte infiltration, was quantitatively associated with this functional

change as measured by serum creatinine [24]. Moreover, the presence of CX3CR1 positive macrophages in acute rejection biopsies is similarly associated with poor steroid response to treatment and worse clinical outcomes at 1 year [25*]. Relevant to the pathological assessment of rejection, lymphocytes, not monocytes, are the only cells that are included in the histologic criteria of acute rejection [26*]; thus further understanding of the contribution of these cells will have significant clinical implications.

How macrophages mediate injury in acute rejection is not clear. *In vitro*, macrophages have the capability of presenting antigen to T cells and this may be facilitated by heat shock protein 60 which may be upregulated in allografts [27]. More significantly, the production of proinflammatory cytokines such as IL-1 and TNF- α have been associated with acute rejection as well as macrophage produced IL-18 [13]. Direct tissue damage may occur via reactive oxygen species or eicosanoids.

The functional impact of macrophages has been best understood by rodent studies of allograft rejection where macrophage depletion or antagonism was explored. This includes strategies to limit macrophage infiltration including the chemokines receptor antagonist met-RANTES [28], and genetic deletion of CCR1 [29] and CCR5 [30]. These strategies result in improved histological features of rejection and improved graft survival in animal models of rejection. Using DTCM-glutarimide, an inhibitor of macrophage activation via AP-1 was also of benefit using as demonstrated in a mouse cardiac allograft model [31*]. Depletion of macrophages using liposomal clodronate, an encapsulated bisphosphonate that is actively phagocytosed by macrophages inducing their apoptosis, has been successful in altering the rejection responses in a number of models [13]. In a rat kidney allograft model, treatment resulted in significant reductions in circulating and intragraft macrophages, with associated improved allograft function [32]. NOS activity and NO production were reduced, accompanied by a reduction in tubular epithelial injury. However, T cell activation and cytotoxic T cell effector molecules were not affected. Finally, using a transgenic CD11b-DTR mouse that undergoes macrophage depletion conditionally, treatment with diphtheria toxin substantially reduced macrophages infiltration in mouse kidney allografts, with a reduction in arteritis and tubulitis, and reduced NO generation [33]. These studies implicate macrophage cytokine production or other inflammatory molecules as critical participants not only in acute allograft injury.

The recent identification of so called Mregs has led to an initial trial in human recipients of living donor kidney allografts. In this exciting and novel pilot study, donor-derived Mregs were generated *in vitro* and expressed a distinct phenotype of cell surface markers [34*]. Following adoptive transfer, immunosuppression could be dramatically reduced to low dose calcineurin inhibitor monotherapy with peripheral blood gene expression signatures consistent with clinical stability [35**]. Further application of this therapy to a larger test population is being explored.

Macrophages in Chronic Injury

While studies have predominantly focused on macrophages in acute rejection, there are some data regarding macrophage infiltration and function in chronic allograft injury. In models of chronic allograft nephropathy characterized by interstitial fibrosis (IF) and tubular atrophy (TA), macrophages have been shown to accumulate in the damaged kidney, both in mice [36] and in rat models (reviewed in [12]). In human recipients of kidney allografts, the presence of macrophages in an early biopsy was predictive of IF/TA development [37]. Furthermore, targeting macrophage function using an adenoviral vector for TNFRp55-Ig, IL-12p40, and vIL-10 ameliorated the histological features and allograft dysfunction in a rat model of IF/TA [38]. In depletion studies, near-selective ablation of macrophages using intraperitoneal carageenan improved transplant vasculopathy in a mouse cardiac transplant

model, while gadolinium treatment, which inhibits phagocytosis, had no effect on the severity of disease [39]. As already noted, macrophages are part of the inflammatory infiltrate in both interstitium and glomeruli during AMR. Moreover, macrophages have been localized in the intima of vessels with chronic transplant arteriopathy; these cells expressed elevated levels of platelet derived growth factor B, a smooth muscle cells growth factor [40]. Macrophages may also contribute to IF/TA by expressing TGF β and promoting matrix deposition by fibroblast activation or via the promotion of inflammatory cytokines or reactive oxygen species. Whether these cells are of the M2 phenotype that may promote matrix deposition directly remains unknown. Clearly, additional studies are needed to identify the exact mechanism of injury and determine if this may be an important therapeutic pathway in man.

Macrophage Inflammation in Other Solid Organs

While the focus of this review has been predominantly on renal injury and inflammation, the participation of macrophages in other solid organs is also been identified. In pediatric recipients of intestinal transplants, higher counts of CD14⁺CCL5⁺ monocytes in the periphery as well as higher counts of CD14⁺ monocytes in post-perfusion biopsies were found in repeated rejectors compared to non-rejectors, implicating these cells in priming the cellular responses in intestinal transplant rejection [41*]. Similarly, in nonvascularized models of rejection, macrophage depletion results in improved graft survival in rat corneal transplants [42] and in islet xenografts [43;44]. These reports implicate that while there may be microenvironment considerations in the participation of macrophages in allograft injury, uniform mechanisms of injury via proinflammatory cytokines and production of detrimental reactive oxygen species may be nonspecific.

CONCLUSION

Numerous studies have implicated macrophages as a detrimental component of allograft rejection. Their presence has been associated with worsened allograft function and may also be a potential biomarker of worse late transplant outcome. Their pathophysiology has been studied in more detail in other injury models of the kidney which provide implications to the management of solid organ allografts. Current clinical assessment of cellular rejection overlook their participation; moreover, current clinical strategies of immunosuppression are not specific to their function. Further studies are clearly needed to identify the phenotype and role of macrophages in allograft injury.

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Key Points

- Macrophages are key inflammatory mediators of the innate immune response that contribute to acute and chronic allograft injury through a variety of mechanisms
- Due to their pleiotropic nature, macrophages in some models may have some beneficial and reparative functions.
- In animal models of kidney transplantation, using a variety of strategies of depletion or functional antagonism, macrophages have been shown to contribute to both acute and chronic injury.
- Further investigation into the mechanisms of macrophage injury and repair are needed to identify their therapeutic potential.