Cellular Immunology 291 (2014) 58–64

Contents lists available at ScienceDirect

Cellular Immunology

journal homepage: www.elsevier.com/locate/ycimm

Macrophages in renal transplantation: Roles and therapeutic implications

Tony Kwan, Huiling Wu, Steven J. Chadban *

Department of Renal Medicine, Royal Prince Alfred Hospital, Sydney, Australia
Collaborative Transplant Research Group, Sydney Medical School, The University of Sydney, Sydney, Australia

ARTICLE INFO

Article history:
Received 5 February 2014
Accepted 27 May 2014
Available online 14 June 2014

Keywords:
Macrophages
Innate immunity
Renal transplantation

ABSTRACT

The presence of macrophages within transplanted renal allografts has been appreciated for some time, whereby macrophages were viewed primarily as participants in the process of cell-mediated allograft rejection. Recent insights into macrophage biology have greatly expanded our conceptual understanding of the multiple roles of macrophages within the allograft. Distinct macrophage subsets are present within the kidney and these sub-serve discrete functions in promoting and attenuating inflammation, immune modulation and tissue repair. Unraveling the complex roles macrophages play in transplantation will allow identification of potential therapeutic targets to prevent and treat allograft rejection and maximize graft longevity.

Introduction

Renal transplantation remains the optimal form of renal replacement therapy for those with end-stage renal failure. Despite technical and pharmacological breakthroughs that have improved short-term allograft outcomes, graft and patient attrition rates in the long term remain unchanged [1]. During the lifetime of an allograft it is vulnerable to numerous injurious processes that challenge its longevity. All transplanted organs undergo a period of ischemia–reperfusion injury (IRI) during the organ retrieval, preservation and transplantation procedures. As a consequence of this injury, an innate immune response is triggered, inflammatory cells are recruited within the allograft and parenchymal cells are also activated. The resulting inflammatory microenvironment has two key consequences: (a) allograft damage and repair; and (b) promotion of an adaptive allo-immune response, which may cause acute rejection. Whilst the vast majority of grafts survive IRI and acute rejection, ongoing risks of chronic rejection, recurrent disease and non-immune organ injury persist for the remainder of its life [2].

This review will focus on the role of macrophage/monocytes as mediators of allograft injury. Previous work on the pathogenesis of allograft rejection has focused on adaptive immunity and the role of T lymphocytes in this process is well documented [3]. The increasing awareness of antibody-mediated rejection has provided additional insights into the role of B lymphocytes and plasma cells [4]. Macrophages have long been recognized within the graft during IRI, acute and chronic rejection [5]. Traditionally, these cells were viewed as contributors to T-cell mediated processes such as acute rejection, recruited into the graft under the influence of T cell derived chemokines to promote inflammation, cause tissue injury and act as antigen presenting cells (APCs) [6]. The more recent discovery of Toll-like receptors (TLRs) and their essential role as innate activators of macrophages during organ IRI has led to a growing appreciation of the role of macrophages and innate immunity in allograft responses and highlighted the importance of innate-adaptive cross-talk in the development of adaptive immune responses. In this brief review, we aim to provide an overview of monocyte/macrophage biology in the context of their potential roles in contributing to allograft damage.

A summary of monocyte and macrophage subsets

Monocyte and macrophage biology has recently been reviewed elsewhere in depth [5,7,8]. In brief, circulating monocytes arise from hematopoietic stem cells in the bone marrow. These stem cells subsequently undergo commitment to the myeloid lineage and pass through several stages of differentiation (the granulocyte/macrophage progenitor (GMP) and the macrophage/DC progenitor (MDP)) that incrementally restrict developmental potential. Both the growth factor macrophage colony-stimulating factor (M-CSF) and the transcription factor PU.1 are required for

* Corresponding author at: Renal Medicine, Royal Prince Alfred Hospital, Missenden Road, Camperdown, NSW 2050, Australia. Fax: +61 2 9515 6329.
E-mail address: steve.chadban@sswahs.nsw.gov.au (S.J. Chadban).

http://dx.doi.org/10.1016/j.cellimm.2014.05.009
0008-8749/

this process. The MDPs subsequently give rise to conventional dendritic cells (cDC), macrophages and monocytes.

Phenotypic heterogeneity in both murine [9] and human monocytes [10] has been described. The murine monocyte subsets are classified by their expression of Ly6C. Ly6C$^+$ monocytes also express high levels of the chemokine receptor CCR2 and low levels of the fractalkine receptor CX_3CR1 ($CCR2^{high}CX_3CR1^{low}$). These monocytes circulate in the blood and are selectively recruited to sites of active inflammation and produce high levels of TNF-α and IL-1β. Ly6C$^+$ monocytes also have the capacity to differentiate into M1-type macrophages (discussed below) when they have migrated into inflamed tissue. Thus, the Ly6C$^+$ subset are also called inflammatory monocytes on account of their numerous pro-inflammatory roles. In contrast, the Ly6C$^-$ monocytes are $CCR2^{low}CX_3CR1^{high}$ and are usually found patrolling the vascular endothelium. They are termed "resident" monocytes because they are thought to maintain the population of resident tissue macrophages and dendritic cells. They also have the capacity to differentiate into M2 macrophages, and thus are involved in tissue healing and repair after the initial inflammatory process resolves.

Recent studies of human monocyte subsets have expanded our understanding of the complexity in their phenotypic diversity. The original human monocyte subset classification is based on the expression of the low affinity Fcγ receptor CD16. Approximately 85% of circulating monocytes are CD16$^-$ and constitute the "classical" subset. They are similar to murine Ly6C$^+$ monocytes phenotypically in that they are also $CCR2^{high}CX_3CR1^{low}$, but they are functionally distinct to CD16$^+$ monocytes since they produce IL-10 in addition to TNF-α upon LPS stimulation.

The remaining 15% of the monocyte population is CD16$^+$. This is further subdivided based on their expression of the LPS binding cofactor CD14: the "intermediate" subset is $CD14^+CD16^+$ whilst the "non-classical" subset is $CD14^-CD16^{++}$. The non-classical subset is functionally and phenotypically similar to the murine Ly6C$^-$ monocytes. They are found patrolling the vascular endothelium and also respond poorly to LPS stimulation. The intermediate subset has a phenotype between that of the classical and non-classical subsets and originally was thought to represent a transitional stage between these two populations. Wong et al. [11] further clarified the distinct role of this monocyte subset using a combination of gene profiling, flow cytometry and cytokine analysis which revealed that the intermediate subset has strong pro-inflammatory roles and highly expresses genes required for T cell co-stimulation and antigen presentation. They also produce high levels of TNF-α, IL-1 and IL-6 upon LPS stimulation. Thus, this population most closely resembles the "inflammatory" murine monocytes. The disparity between phenotypic and functional correlation of human and murine monocytes highlights the complexities in this field and is an area of active investigation.

Like monocytes, macrophages also demonstrate phenotypic and functional heterogeneity. Macrophages can be derived from in situ proliferation of resident tissue macrophages, or recruited as a result of differentiation from circulating monocytes that have migrated into the tissue. This process is dependent on the growth factor macrophage-colony stimulating factor M-CSF. Indeed, increased M-CSF has been demonstrated in association with increased macrophage and monocyte infiltration in numerous inflammatory kidney diseases [12,13]. Macrophages demonstrate significant plasticity and are able to change their phenotype and function in response to their surrounding microenvironment.

M1 or classically activated macrophages are induced when monocytes are exposed to a combination of IFN-γ, TNF-α and LPS. The M1 phenotype is pro-inflammatory, characterized by secretion of pro-inflammatory cytokines (TNF-α, IFN-γ, IL-12 and IL-1β), enhanced phagocytic activity, and increased production of reactive oxygen species via up-regulation of inducible nitric oxide synthase (iNOS).

M2 or alternatively activated macrophages encompass several phenotypes and are further classified into three subsets. The M2a phenotype is induced upon exposure to IL-4 or IL-13, the M2b phenotype is induced by LPS exposure in the presence of immune complexes, whilst the M2c phenotype comprise of a heterogeneous population that result from exposure to anti-inflammatory mediators such as IL-10, TGF-β and glucocorticoids. M2 macrophages tend to adopt a reparative or immunomodulatory role. They elaborate IL-10, demonstrate reduced phagocytic activity and up-regulate arginase rather than iNOS. The last action directs arginine and its metabolites towards biochemical pathways required for the synthesis of collagen (in particular the synthesis of proline).

Although the M1/M2 classification system serves as a useful starting point to appreciate macrophage function according to propensity to induce or control inflammation, it does not adequately encompass additional diverse roles such as tissue homeostasis and immune regulation. An alternative classification system proposed by Mosser and Edwards [14] categorizes macrophages according to their function, namely: (1) classically-activated macrophages that participate in host defense; (2) wound-healing macrophages involved in tissue fibrosis and repair; and (3) regulatory macrophages responsible for modulation of the immune response. In summary, macrophages are phenotypically plastic cells that can tailor their function to the microenvironment in which they reside. Their functional diversity highlights their potential significance in a wide variety of pathologic processes.

Under the influence of GM-CSF and IL-4, monocytes are capable of differentiating into dendritic cells [15,16]. Like macrophages, subsets of dendritic cells have been described [17]. Both DCs and macrophages are capable of processing and presenting antigen to T cells. However, DCs express high levels of co-stimulatory molecules on their cell surface and thus can effectively present antigen to both naïve and primed T cells. In the context of allograft rejection, it is likely that DCs initiate the process by presenting alloantigen to naïve T cells in the draining lymph nodes [3]. This is subsequently propagated and amplified through recruitment of activated macrophages to the graft which then interact with primed T cells. A certain degree of functional plasticity exists between DCs and macrophages, and differentiation of DCs to macrophages, and vice versa, have been described in vitro. The significance of these findings in vivo remains uncertain and it remains conceptually useful to recognize DCs and macrophages as distinct cell types in the context of allograft pathology.

Macrophages in ischemia–reperfusion injury

The process of organ retrieval, preservation, transportation and implantation by necessity causes IRI to the transplanted organ. The severity of IRI incurred is dependent upon the duration and type of ischemic insult, with warm ischemia imparting greater damage than cold. The clinical impact is a delay in establishment of organ function following implantation: in the context of kidney transplantation, severe IRI causes delayed graft function requiring dialysis. Innate immunity plays a prominent role in mediating this process, particularly the TLRs [18]. TLRs are a family of germline encoded receptors that evolved to recognize molecular motifs that are ubiquitous to pathogenic micro-organisms (termed pathogen-associated molecular patterns or PAMPs). Thirteen TLRs have been characterized to date and their repertoire of ligands is diverse. These include bacterial cell wall components (diacyl- and triacyl-lipopeptides, LPS), flagellin, and genetic material unique to micro-organisms (dsRNA, unmethylated CpG motifs). Of greater relevance