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Macrophages and dendritic cells: what is the difference?

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Segerer *et al.* report the expression and localization of macrophage and dendritic-cell markers in human renal biopsies and indicate that both cell types express CD68, findings that resonate with murine studies. The functional and phenotypic distinction between macrophages and dendritic cells is discussed.

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Introduction

Macrophages and dendritic cells are key players in many renal diseases, such that modulation of their function holds therapeutic promise. Until recently they have been regarded as relatively discrete cell types, with macrophages being a key component of the innate immune system while dendritic cells interface with the adaptive immune system and modulate immune responses. During disease, dendritic cells may initiate autoimmune responses and stimulate T cells with resultant macrophage activation inducing significant tissue damage. There are, however, additional complexities, including the involvement of macrophages in tissue homeostasis and repair and the tolerogenic actions of dendritic cells under normal circumstances and during disease states; these functions may act to prevent or limit tissue injury as well as promote the resolution process. Such a model regards macrophages and dendritic cells as clearly separated in terms of cellular function while occupying overlapping anatomical sites in peripheral tissues and the reticuloendothelial system. In practice, distinguishing between macrophages and dendritic cells has relied on the use of cell-surface markers thought to be specific to either cell. The progressive refinement and increasing number of available markers have served to complicate rather than simplify our understanding of the renal mononuclear phagocyte system, and this is highlighted in the study by Segerer *et al.*[1] (this issue).

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Dendritic-cell markers in human disease

Segerer *et al.*[1] examined a variety of contemporary cell markers in 55 human renal biopsies, including normal tissue, non-proliferative glomerulonephritis (focal segmental glomerulosclerosis, membranous nephropathy, and minimal-change disease), and proliferative glomerulonephritis (necrotizing and lupus glomerulonephritis). The cell markers used were CD68 (macrosialin; conventionally regarded as a macrophage marker), dendritic cell-specific ICAM-3-grabbing nonintegrin (DC-SIGN; a marker of myeloid dendritic cells), blood dendritic-cell antigen-2 (BDCA-2; a marker of plasmacytoid dendritic cells), S-100 (expressed by Langerhans cells and dendritic cells), and langerin (expressed by Langerhans cells). DC-SIGN$^+$ cells (myeloid dendritic cells) coexpressed CD68 and were found in abundance in the tubulointerstitium but were intriguingly absent in glomeruli. Woltman *et al.*[2] also noted limited glomerular numbers of DC-SIGN$^+$ cells in IgA nephropathy. CD68$^+$/DC-SIGN$^-$ cells (macrophages) were found in significant numbers in the glomeruli of patients with proliferative nephritis but also infiltrated the tubulointerstitium. BDCA-2$^+$ plasmacytoid dendritic cells were present in the tubulointerstitium and tended to form follicular aggregates, whereas S-100$^+$ or langerin$^+$ dendritic cells were rare. Langerin$^+$ dendritic cells were, however, intimately associated with the collecting ducts, with this localization implying a potential sentinel function for ascending infections.

The specificity of cell markers

This study raises a number of important issues that will need to be taken into account by investigators in the future. For example, the cell-surface marker CD68 does not distinguish between macrophages and dendritic cells (Figure 1). This resonates with recent murine studies indicating that the F4/80 marker detects both macrophages and dendritic cells that are resident within or infiltrating the kidney.[3] Indeed, the F4/80 knockout mouse exhibits defective generation of antigen-specific regulatory T cells in an ocular model of inflammation, indicating that F4/80$^+$ cells exert important immunoregulatory actions.[4] Although dendritic-cell markers exist, it is also noteworthy that both macrophages and dendritic cells can express major histocompatibility complex class II. We may need to reassess our usage of names given to cells such as macrophages or dendritic cells despite the fact that they have a long history. For example, the resident sentinel cell of the lung is CD11c$^+$ but is generally referred to as an alveolar macrophage! Although such 'names' are useful, it may be best to simply additionally describe cells by the particular set of markers expressed together with their purported function. Also, the findings of previously published studies may need to be reappraised in the light of the nonspecificity of some cell-surface markers, such as CD68 and F4/80.

A requirement for readouts of cell function

Although descriptive studies are often informative, it can be difficult to determine whether the cells within the tissue are directly involved in mediating the disease process or simply reflect a response to the tissue injury. This

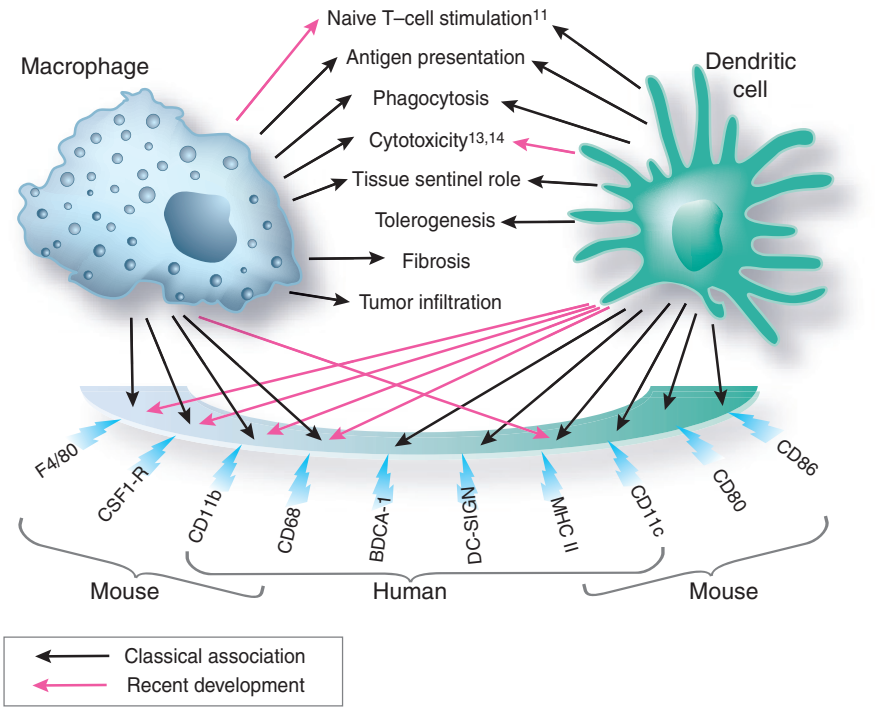

Figure 1 | Recent advances in the study of both functional characteristics and surface markers of the cells of the mononuclear phagocyte system have led to increasing overlap between what is considered a 'macrophage' and a 'dendritic cell'. Abbreviations: CSF1-R, colony-stimulating factor 1 receptor; BDCA-1, blood dendritic-cell antigen-1; DC-SIGN, dendritic cell-specific ICAM-3-grabbing nonintegrin; MHC II, major histocompatibility complex class II.

is a key area, as the leukocyte population within the diseased kidney will be heterogeneous and contain both pro-inflammatory and reparative cells. Thus, additional interrogation will be required to determine the functional phenotype of infiltrating or resident cells. Various markers that reflect the state of macrophage activation, such as expression of inducible nitric oxide synthase, mannose receptor, arginase, FIZZ1 (found in inflammatory zone 1), and so on, have been described, and these may be informative (reviewed by Gordon[5]). In experimental studies the infiltrating leukocyte population may be negatively or positively immunopurified following enzymatic dissociation of inflamed kidneys. The nature of these isolated cells can then be examined by approaches such as flow cytometry, quantitative real-time PCR, microarray, and proteomics. Such an approach is not so readily feasible for human tissue, but laser capture microscopy and subsequent analysis of gene expression signatures may provide insights into the phenotype of leukocytes present within diseased kidneys.

Experimental models of cell ablation

How can we explore the role of macrophages and dendritic cells in experimental models of renal inflammation? The administration of liposomal clodronate has long been used as a means to deplete 'macrophages,' but we have found that renal resident F4/80$^+$/CD11c$^+$ dendritic cells are also reduced by this treatment. Indeed, any experimental manipulation that reduces circulating monocyte numbers will have an effect on the development of inflammatory myeloid dendritic cells, as these cells are derived from recruited monocytes.[6] Transgenic conditional cell-ablation models using the restricted expression of the human diphtheria toxin receptor (DTR) have been developed and have proved useful. Various mice have already been generated, including the CD11b-DTR mouse,[7] the CD11c-DTR mouse, and a langerin-DTR mouse.[8] Further conditional depletion systems may become feasible as murine homologues of markers such as DC-SIGN and so on are found. Although such studies undoubtedly generate useful information, there are caveats to the interpretation of such models. For example, the induction of widespread apoptosis is not a 'neutral' event from the immunological and inflammatory perspective, as apoptotic cells may exert anti-inflammatory effects on both macrophages and dendritic cells.

Tubulointerstitial versus glomerular localization

The study by Segerer *et al.*[1] highlights the differential localization of macrophages and dendritic cells, with dendritic cells appearing to be restricted to the tubulointerstitium. The authors speculate that this may prevent the generation of an immune response to antigens within the glomerulus or might reflect an absence of glomerular lymphatic vessels to allow the egress of dendritic cells to draining lymph nodes. It may, however, be the case that the postglomerular sampling of filtered antigen is more important, and recent work indicates a role for filtered renal antigen in maintaining peripheral tolerance,[9] though the concentrated antigen gains access to draining lymph nodes by dendritic cell-dependent and independent mechanisms. The location of dendritic cells within the tubulointerstitium may represent the optimal location, as they can access filtered antigen, intravascular antigen, and any ascending pathogens with comparative ease.

The mononuclear phagocyte system: 'cellular spectrum'

The work of Segerer *et al.*[1] should be set in the context of the growing body of evidence that demonstrates the plasticity of both functions and surface markers between cells of the mononuclear phagocyte system.[10] Accordingly, it may be more sensible to regard macrophages and dendritic cells as cells that exist on a spectrum (Figure 1) where cells may express variable sets of cell-surface markers and functions. For example, dendritic cells are believed to be important for detecting and reacting to tissue injury or infection as well as regulating immune responses through antigen presentation, T-cell stimulation, or the generation of regulatory T cells. However, CD11c$^-$ macrophages can effectively present antigen and stimulate

T cells[11] and undoubtedly play a role as sentinel cells in various tissues. Also, several investigators have demonstrated that subcapsular splenic macrophages can effectively present antigen to B cells (reviewed by Martinez-Pomares and Gordon[12]). Activated macrophages have been regarded as important mediators of host tissue damage, as they can induce apoptosis through a variety of mechanisms, but recent work has also demonstrated that dendritic cells may express high levels of inducible nitric oxide synthase[13] and induce cell death.[14]

Conclusion

The work by Segerer *et al.*[1] has carefully utilized various cell markers to identify macrophages and dendritic cells within normal and diseased human renal tissue and has demonstrated differential and distinctive spatial localization. The challenge for the future is to understand the nature and function of these various cell types together with the factors that lead to their particular localization within the kidney. This may generate insights into which cells represent the best target for modulating the inflammatory process and augmenting subsequent tissue repair.

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Setting the stage for acute-on-chronic kidney injury

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Acute-on-chronic kidney disease will be familiar to many nephrologists. Hsu *et al.* quantify the risk of acute-on-chronic disease across the stages of preexisting chronic kidney disease. Their study demonstrates the valuable insights that large epidemiological studies can bring to the field of acute kidney injury.

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Several studies have shown that the prevalence of chronic kidney disease (CKD) is increasing,[1] by consensus definitions developed by the Kidney Disease Outcomes Quality Initiative (K/DOQI). These definitions and staging of CKD allow researchers to compare disease prevalence across time and across populations and establish links between CKD and other diseases.[2] For example, it is well established that CKD is a risk factor for cardiovascular disease, and this risk is significant even with mild impairment of kidney function.[3] Hsu and colleagues[4] (this issue) explore the relationship between CKD stage and the risk of developing acute kidney injury (AKI). They demonstrate that even mild chronic impairment of kidney function significantly increases the risk of AKI.

Patients with 'acute-on-chronic' kidney disease should be familiar to most nephrologists. In terms of clinical practice, one of the strengths of the study by Hsu *et al.*[4] is the quantification of the relationship between CKD stage and risk of in-hospital, dialysis-requiring, AKI. The authors studied a large patient group, adults from a Kaiser Permanente cohort in northern California. By definition, this population has health insurance, and we hope future studies include patients without insurance. The staging of 'baseline' CKD was based on outpatient measurements of serum creatinine that predated the index episode of AKI, a significant advantage over inferring baseline creatinine from in-hospital measurements. This strategy allows for a more inclusive and perhaps more accurate view of the acute-on-chronic population. When the incidence of dialysis-requiring AKI was compared across the CKD stages, the authors found that "the propensity to develop in-hospital acute kidney failure is another complication of chronic

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