Macrophage heterogeneity in renal inflammation

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**Introduction**

Macrophages evolved to maintain and restore tissue integrity. They originate from bone marrow-derived precursors and traffic through tissues where they have essential roles in remodelling during fetal development; in host defence against infection and tumours; and in wound healing [1]. Macrophages also mediate injury in immune-mediated diseases including glomerulonephritis, and this aspect of their function together with their role in combating infection have tended to overshadow their involvement in tissue repair. Understanding how macrophage function adapts to the needs of particular microenvironments is a principal challenge for inflammatory cell biologists. Importantly, learning to manipulate macrophage function to promote their reparative properties would be a powerful therapeutic tool, a point emphasized by the recognition that many viruses, parasites and tumour cells have evolved to redirect macrophage function to promote their survival [2]. Our purpose here is to review briefly what is known about the role of macrophages in renal injury; to describe recent advances in understanding of macrophage activation; and to show that manipulation of macrophage function can have profound effects on the intensity of glomerular inflammation.

**Macrophages and renal injury**

In 1972, Shigamatsu identified macrophages in inflamed glomeruli by electron microscopy in patients...
with focal necrotizing and crescentic nephritis. These observations were confirmed and greatly extended by Atkins' group and subsequently by many others when the appropriate monoclonal antibodies became generally available [3]. Macrophages infiltrate the renal parenchyma in all types of renal injury, and their number correlates with the intensity of inflammation. It was assumed that the infiltrating macrophages caused the injury, and this was confirmed in experimentally induced nephritis both by depletion studies [4] and, more recently, by repletion with intravenously injected bone marrow-derived macrophages (BMDMs) [5].

The decisive nature of these experiments gave rise to the idea that renal macrophages were uniformly activated to cause tissue injury. However, this is a gross oversimplification, and recent studies of nephritis demonstrate marked macrophage heterogeneity depending on the nature of the injury and location within the kidney. Rastaldi et al. [6] have shown that glomerular macrophages in antineutrophil cytoplasmic autoantibody (ANCA)-positive vasculitis expressed different activation markers from those in patients with cryoglobulinaemic glomerulonephritis, and Segerer et al. [7] showed that glomerular and interstitial macrophages express different chemokine receptors both in severe proliferative glomerulonephritis and in renal transplant rejection. Recent studies of nephrotoxic nephritis (NTN) in genetically modified mice demonstrate that the nature of the macrophage infiltrate has profound functional consequences. Mice genetically deficient for either chemokine receptor 1 (CCR1) or 2 (CCR2) have less macrophage infiltration but develop more severe glomerulonephritis; interestingly, the CCR2-deficient mice initially show a reduction of injury but, by day 7, injury has become considerably worse than in wild-type controls [8,9]. This emphasizes the need to understand much more about how macrophages function in vivo in the kidney.

**Macrophage activation**

Relatively small numbers of resident macrophages are found in normal tissues including the kidney, and it has long been known that resident macrophages isolated from different anatomical sites differ in function, presumably because of adaptive responses to the local microenvironment [10]. Injury causes the rapid recruitment of large numbers of additional macrophages—commonly called inflammatory macrophages. The nature of inflammatory macrophages came under intense scrutiny in the 1960s when it became apparent that elicited macrophages in non-immune injury displayed increased pro-inflammatory properties but that stimulation by T-cell products [principally interferon-γ (IFN-γ)] was needed to ‘activate’ them to destroy intracellular parasites and tumours. Macrophage activation by IFN-γ has been studied in detail and induces ‘classical’ activated macrophages which are histotoxic and responsible for killing microorganisms [11] (Table 1). Recent studies have identified >1000 genes whose function is altered by IFN-γ, with roughly equal numbers being induced and suppressed [12]. Cytokines released from T helper 2 (Th2) cells, such as interleukin-4 (IL-4) and IL-13, induce ‘alternative’ macrophage activation (Table 1). Useful markers of alternative macrophage activation include expression of mannose receptor, FcyRII inhibitory receptor, major histocompatibility (MHC) class II molecules and selected chemokines, as well as new gene products that have been discovered by gene expression studies [11]. Mosser et al. coined the term ‘type II activated macrophage’ to describe a third type of activation; these macrophages secrete IL-10 and drive Th2-like responses following a switch in phenotype induced by FcyRI ligation [13]. The immunoregulatory cytokines IL-10 and transforming growth factor-β (TGF-β) play an important role in dampening macrophage activation. Knockout mice lacking either of these two cytokines have increased susceptibility to inflammatory disease [14].

These studies demonstrate the diversity of macrophage responses when exposed to different stimuli and show that ligation of individual macrophage receptors alters the expression of large numbers of genes, with some being up- and some downregulated. Therefore, simultaneous exposure to more than one activating signal (e.g. when macrophages infiltrate inflamed tissue) would be expected to amplify some responses whilst attenuating others. The challenge now is to understand how macrophages integrate these multiple and possibly contradictory signals and produce a coordinated response that facilitates restoration of tissue integrity.

**Table 1. Macrophage activation states**

<table>
<thead>
<tr>
<th>Activating signal</th>
<th>Classical</th>
<th>Alternative</th>
<th>Type II</th>
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<tbody>
<tr>
<td>Function</td>
<td>IFN-γ</td>
<td>IL-4, IL-13</td>
<td>Toll-like receptor ligation</td>
</tr>
<tr>
<td>Secretory products</td>
<td>Pro-inflammatory</td>
<td>Regulatory</td>
<td>IgG complexes</td>
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<td>Biological markers</td>
<td>TNF, IL-12</td>
<td>IL-1RA, IL-10</td>
<td>Immunosuppression</td>
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<td></td>
<td>MHC class II</td>
<td>Mannose receptor</td>
<td>IL-10, TNF, IL-6</td>
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<td></td>
<td>CD86</td>
<td>Scavenger receptor</td>
<td>Tissue repair</td>
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<tr>
<td></td>
<td></td>
<td>CD23</td>
<td>IL-10, TNF, IL-6</td>
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</tbody>
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**Classical activated macrophages**

- **IFN-γ** secretory products include TNF, IL-12; biological markers include CD86, MHC class II.

**Alternative activated macrophages**

- **IL-4, IL-13** secretory products include IL-1RA, IL-10; biological markers include CD23, scavenger receptor.

**Type II activated macrophages**

- **Toll-like receptor ligation** secretes IL-10, TNF, IL-6; biological markers include mannose receptor.
Macrophage programming

This issue was first addressed systematically by Riches, who analysed the responses of uncommitted murine BMDMs after simultaneous or sequential exposure to IFN-γ, tumour necrosis factor-α (TNF-α) and TGF-β [15]. We have extended these observations by examining a wider range of stimuli including the anti-inflammatory cytokines IL-4 and IL-10 and additional macrophage functions including uptake of apoptotic cells [16]. A number of conclusions can be drawn from these experiments: (i) macrophages develop coordinated sets of non-overlapping and mutually exclusive properties when exposed to specific cytokines; (ii) macrophage function (at least under these experimental conditions) is determined by the initial cytokine contact which induces ‘programmed’ unresponsiveness to subsequent cytokines; and (iii) there is a hierarchy when macrophages are exposed to two cytokines simultaneously, e.g. IFN-γ dominates over TNF-α and TGF-β. Macrophage programming is clearly an important but not the only mechanism by which macrophages integrate complex signals. This is exemplified by recent studies analysing the effects of different cytokines on chemokine receptor expression by human monocytes. These show that macrophage functions can be controlled hierarchically in that one stimulus dominates over another independent from the order in which they are given, i.e. IL-10-mediated upregulation of CCR1 in human monocytes is completely abrogated by pre- and post-treatment with TGF-β. Interestingly, the hierarchy is reversed in the control of CCR-2 expression where IL-10 dominates over TGF-β [A. Schuette, A. Dempster, A.J. Rees and L.P. Erwig, unpublished observations]. Thus, macrophage functions are tightly regulated and specific macrophage properties are the consequence of aggregated, programmed or hierarchical responses, and the governing rules are increasingly understood.

Analysis of experimental models of nephritis shows that similar principles apply to macrophage activation in vivo. Accordingly, macrophages purified from normal rat glomeruli behave as though uncommitted, and can be activated ex vivo in different ways by IFN-γ, IL-4 and TGF-β [17]. Interestingly, resident retinal macrophages are different and behave as though already programmed by TGF-β, which is in keeping with their maturation in the TGF-β-rich environment of the eye [18]. Furthermore, infusion of TGF-β induces a similar phenotype in resident glomerular macrophages [19]. In contrast, macrophages infiltrating glomeruli of rats with NTN are invariably activated by IFN-γ. They generate large amounts of nitric oxide that cannot be suppressed by IL-4 or TGF-β [17]. Thus it appears that in vivo as in vitro, macrophages are programmed and activation by IFN-γ dominates hierarchically over that by TGF-β when exposed simultaneously.

The next question is whether macrophages infiltrating a nephritic glomerulus can be pre-programmed and rendered unresponsive to activation by IFN-γ by an anti-inflammatory cytokine. This has been addressed by systemic administration of IL-4 and TGF-β to rats before induction of NTN. In both cases, the treatment failed to prevent the infiltrating macrophages adopting an IFN-γ-activated state [17,19]. Interestingly, the TGF-β-rich environment of the eye also fails to prevent macrophages from being programmed by IFN-γ in experimental autoimmune uveoretinitis [18].

Recent studies in the anti-Thy 1.1 model of nephritis have shown that infiltration itself does not always induce programming, and revealed that in the right conditions, macrophage commitment occurs within minutes of localization. These experiments also show that not all macrophages entering a particular environment are programmed in the same way. In a two-shot model of Thy 1.1 nephritis, macrophages infiltrating glomeruli 1 h after the second dose of anti-Thy 1.1 antibody were strikingly heterogeneous in that 30% of the cells were programmed by TGF-β and 70% by IFN-γ [20]. Thus all macrophages infiltrating an appropriate environment become programmed shortly after localization; and macrophages infiltrating glomeruli at the same time can be programmed in different ways. It strongly suggests that commitment to a particular programme is determined by the properties of the tissue which the macrophages infiltrate and that the programme the cells adopt is a stochastic event critically dependent on the local microenvironment.

Effect of genetically modified macrophages on renal inflammation

The studies outlined emphasize the constraints of manipulating macrophage function within inflammatory foci. An effective approach to achieve this has been to transduce macrophages to express specific transgenes that promote their reparative functions. This has the attractive advantage of examining how effective macrophage manipulation could be as a therapeutic strategy.

Macrophages transduced by recombinant adenovirus to express IL-1 receptor antagonist (IL-1ra) injected into rats with NTN [21] or mice with unilateral ureteric obstruction [22] attenuated inflammation and reduced glomerular and interstitial macrophage infiltration. We have studied the effects of macrophages transfected to express anti-inflammatory cytokines IL-4, IL-10 and TGF-β. The rat alveolar macrophage cell line NR8383 was transfected with adenovirus to express IL-4. The cells localized to glomeruli of rats with NTN, produced the cytokine in vivo and reduced the level of albuminuria, histological markers of glomerular inflammation and macrophage infiltration [23]. In contrast, injection of NR8383 cells transfected to express active TGF-β had limited impact on the
development of renal injury in rats with NTN (unpublished data). To prevent any alloimmune response, we subsequently utilized rat BMDMs transduced with adenovirus expressing IL-10. These cells localized preferentially to inflamed glomeruli in NTN and produced a profound reduction in degree of albuminuria and conventional markers of histological glomerular damage. In addition, there was a decrease in glomerular macrophage infiltration and expression of their activation markers MHC class II and ED3 [24].

The injection of IL-4- and IL-10-expressing macrophages into the renal artery results in highly effective localization to the glomeruli of the injected kidney and very low numbers in the contralateral kidney. Despite this, we have found that injection into a single kidney attenuates the development of inflammation in the contralateral kidney. This implies that the expression of cytokine-expressing macrophages at one inflammatory site can decrease injury at separate foci of inflammation. This contralateral effect could not be elicited by systemic injection of cytokine-expressing macrophages and the relevant cytokines could not be detected in the serum. Given the importance of effective glomerular localization in achieving this effect, the most likely explanation is that high concentrations of anti-inflammatory cytokine alter the properties of other infiltrating inflammatory cells, in particular macrophages, which then traffic to regional lymph nodes and downregulate the subsequent development of the immune response. Thus local manipulation of macrophage function provides a powerful tool to control glomerular inflammation and systemic immune response.

Conclusion

Recent advances in our understanding of macrophage activation in vitro and in vivo have led to a major reevaluation of the role of macrophages in inflammatory renal disease. Macrophages can no longer be regarded as cells that solely cause injury, but rather as promising targets for therapeutic intervention for repair and restoration of normal renal architecture and function.

Conflict of interest statement. None declared.

References

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