Invited Comment

Necrotizing-crescentic glomerulonephritis in ANCA-associated vasculitis: the role of monocytes

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Introduction

ANCA-associated renal vasculitis (Wegener’s granulomatosis, Microscopic polyarteritis and its renal limited variant) are morphologically characterized by two major glomerular lesions: necrosis of the tuft and extracapillary proliferation, the intensity and degree of which are widely variable, ranging from focal and segmental to massive and diffuse (Figure 1) [1–4].

Mesangial and endocapillary proliferation are usually mild, and undamaged glomeruli and non-necrotic parts of the glomerular tuft appear to be normal. Interstitial leukocyte infiltration is usually intense with frequent periglomerular localization and concomitant rupture of Bowman’s capsule, making it difficult to distinguish between glomerular and interstitial lesions (Figure 1) [5–7]. In some cases periglomerular infiltrates are massive giving a picture of granuloma-like reaction with accumulation of epithelioid cells and sometimes also giant cells [8].

Monocyte-macrophages are the basic cell types of the mononuclear phagocyte system and play a central role in inflammatory processes, acting directly or in concert with other arms of the immune system. According to previous studies, both in human and experimental models and according to our personal experience, we firmly believe that monocyte-macrophages are involved in the lesion of renal vasculitis both at glomerular (crescent formation) and interstitial level, playing a crucial role in the inflammatory process and in further progression of renal damage.

In this comment we discuss the presence, mechanisms of recruitment, and activity of monocyte-macrophages in renal vasculitis.

Presence of monocyte-macrophages in renal vasculitis

Although the composition of cellular crescents still remains a controversial issue, 20 years ago Atkins et al. [9] cultured isolated glomeruli from patients with crescentic glomerulonephritis and demonstrated for the first time the presence of macrophages in these lesions. Many groups then confirmed this feature. In 1985 our group identified a great number of macrophages in crescentic lesions in renal biopsies of vasculitis by histochemistry [10]. During the same period, studies performed on experimental models, using both electron microscopy and histochemistry, also documented the presence of monocytes-macrophages in the crescents [11,12].

The recent availability of monoclonal antibodies directed against leukocyte populations provided an elegant technique to confirm the presence of a great number of macrophages at the glomerular level. Such a study was performed by our group [7] in 15 cases of ANCA-associated renal vasculitis. We were able to precisely identify the accumulation of monocyte-macrophages in areas of necrotizing-extracapillary lesion (Figure 2), and their massive participation to periglomerular infiltrates and granulomatous reactions (Figure 3). Conversely, very few cells in crescents were positive for cytokeratins, a good marker for epithelial cells, confirming that in these cases monocyte-macrophages prevail.

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whereas VCAM-1 expression is confined to Bowman’s capsule epithelial cells and is completely negative in the normal tuft.

For this reason, although we observed increased ICAM-1 expression in all glomeruli of patients with renal vasculitis, *de novo* tuft expression of VCAM-1 is more important in our opinion. In fact, we found VCAM-1 only in glomeruli with necrotizing-extracapillary damage. The molecule was present not only in crescents, but also in well-delineated areas of the tuft exactly corresponding to necrotizing lesions seen by light microscopy and to segmental fibrinogen deposition observed by immunofluorescence. Mononuclear phagocytes within these areas were also strongly positive for VLA-4, the integrin receptor of VCAM-1. In our experience VCAM-1 expression in the glomerular tuft is a peculiar feature of the forms of glomerulonephritis which are characterized by tuft necrosis, such as anti-GBM nephritis, Henoch–Schönlein purpura, and some forms of endocarditis. Consequently, it was important to investigate whether synthesis of VCAM-1 could be documented in these areas. In effect, VCAM-1 mRNA, investigated by *in situ* hybridization, was present in the same areas, confirming *de novo* synthesis and expression of this adhesion molecule by endothelial cells and probably also by some macrophages (Figure 4).

**Monocyte-macrophages in necrotizing-crescentic renal injury**

It is well known that after migration into a tissue monocytes turn into macrophages. Subsequently, depending on the particular microenvironment, they are activated, are capable of more effective phagocytosis and produce a wide variety of proinflammatory products which mediate macrophage induced tissue

One mechanism of macrophage accumulation within Bowman’s space is migration of monocytes from the glomerular capillary bed, a process that involves chemotactic molecules and cell–matrix and cell–cell adhesion interactions.

Recent studies have demonstrated that a variety of chemotactic factors, produced by both infiltrating and resident kidney cells can lead to monocyte adhesion and subsequent migration. In particular, an important role for chemokines and especially for monocyte chemoattractant protein-1 (MCP-1) and RANTES was reported both in experimental nephritis and human crescentic glomerulonephritis [13–17].

Among adhesion molecules, ICAM-1 and VCAM-1 are fundamental because they promote firm leukocyte adhesion to the endothelium [18]. Many recent studies demonstrated a good correlation between their expression and the number of leukocytes present in glomeruli and interstitium, respectively [19]. In normal human glomeruli ICAM-1 is present at the endothelial level, whereas VCAM-1 expression is confined to Bowman’s capsule epithelial cells and is completely negative in the normal tuft.

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To better understand the role of accumulated macrophages in the inflammatory process and in the progression of vasculitic damage, we studied the expression of activation markers of monocyte-macrophages in biopsy samples of patients with vasculitis. In areas of monocyte-macrophage accumulation we found strong upregulation of MHC class II antigens (a clear sign of macrophage activation) and intense staining for the 27E10 antigen. This antigen is expressed by monocyte-macrophages only in acute inflammatory lesions and is not found in normal tissue or in chronic inflammation (Figure 5).

Although few studies have been performed in human glomerulonephritis [21], studies in experimental models and cell cultures have provided evidence that Interleukin-1 (IL-1) and TNF-α are the most effective cytokines [22–25] among cytokines and growth factors produced by activated macrophages. They share many biological activities including upregulation of leukocyte adhesion molecule expression, induction of many other cytokines and expression of the inducible form of nitric oxide synthase [26].

Blockade of endogenous IL-1 and TNF-α by receptor antagonists prevents glomerular injury in many experimental nephritides [27–30].

In our biopsies staining for IL-1 and TNF-α was present in areas of macrophage accumulation, both in glomeruli and interstitium. It was particularly intense in granulomatous lesions, confirming the presence and the role of these pro-inflammatory agents in human renal vasculitis (Figure 6).

According to recent experiments, accumulation of macrophages can also originate from their local proliferation in addition to their recruitment from the blood [31,32]. Moreover, Yang et al. [33] have shown in human glomerulonephritis that macrophage proliferation was restricted to areas of severe damage (glomerular segmental proliferative lesions and crescents) suggesting that local macrophage proliferation is an important mechanism for amplifying injury in these kind of lesion.

We were able to confirm these findings in our cases of renal vasculitis. By double staining, many monocyte-macrophages were positive for proliferation markers PCNA and MIB-1. They were preferentially localized in crescents and granulomatous lesions where macrophage proliferation is likely to favour a further increase of inflammatory processes (Figure 7).

It is known that monocyte-macrophages play also an important role in the repair of inflammatory processes, in which their ability to secrete TGF-β is certainly crucial for collagen deposition. Repeat biopsies in our patients with renal vasculitis showed that, despite timely and adequate immunosuppressive treatment, necrotizing-extracapillary damage evolves and commonly leads to the development of fibrocellular and fibrous crescents. This phenomenon is particularly evident in cases of focal segmental necrotizing lesions. Here second biopsies show well delineated segmental areas of sclerosis as consequence of previous localized active lesions (Figure 8).
The monocyte-macrophages that are present in tissue seem to be acutely activated and show marked upregulation of MHC class II antigens and intense positivity for the 27E10 antigen. They are also able to produce a variety of cytokines and growth factors, such as IL-1 and TNFα that amplify the inflammatory response leading to fibrotic kidney damage. Moreover, confirming previous experimental data, we have also demonstrated local macrophage proliferation within Bowman’s space. The final result of all these mechanisms of injury is progression of lesions to fibrocellular and fibrous crescents.

Better understanding of the inflammatory mechanisms involved in human necrotizing-crescentic glomerulonephritis is crucial for future therapeutic strategies. In animal models of crescentic glomerulonephritis specific immunosuppressive drugs have been already tested with good success in an effort to modulate or suppress glomerular crescent formation.

Conclusions

Many experimental data, and our experience in human biopsies, suggest that in renal vasculitis extracapillary lesions are mainly characterized by accumulation of activated monocyte-macrophages that play a crucial role in inflammatory process and in further progression of renal damage.

The cell composition of crescents (epithelial cells versus monocyte-macrophages) has varied in different studies and remains a controversial issue. We believe that in renal vasculitis acute endothelial damage causes a marked inflammatory reaction, with recruitment of large numbers of leukocytes, mainly monocytes, not only into Bowman’s space but also into the interstitium, particularly in the periglomerular space. This strong inflammatory reaction can cause rupture of Bowman’s capsule with further monocyte-macrophages accumulation in the extracapillary lesion.

Such lesions are mainly observed in ANCA-associated renal vasculitis, but can also be present in other forms of necrotizing/extracapillary glomerulonephritis such as anti-GBM disease, Henoch–Schönlein purpura and some cases of Berger’s disease.

On the other hand, acute or chronic immune complex glomerulonephritis, i.e. post-streptococcal GN and membrano-proliferative GN, is characterized by prevalent intracapillary proliferation without obvious necrosis of the tuft. Small gaps in the glomerular basement membrane, frequently demonstrated by electron microscopy, can allow extravasation of fibrin and inflammatory cells into Bowman’s space that trigger proliferation of the parietal epithelial cells of Bowman’s capsule, without damage to its integrity [34–37]. In such cases we have commonly found a strong cytokertatin positivity, confirming the epithelial nature of the crescentic lesion [4].

In contrast, in cases of necrotizing-crescentic vasculitis we have always found a massive monocyte infiltration that appears to be especially VCAM-1 mediated.

References

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