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The proinflammatory role of hyaluronan–CD44 interactions in renal injury

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Introduction

Numerous proinflammatory pathways that lead to mononuclear leukocyte invasion have been described in renal disease. These include upregulation of cytokines with ensuing activation of adhesion molecules, stimulation of chemokines with subsequent chemotaxis, and activation of the complement and coagulation cascades that promote leukocyte activation. Recently, we have shown that the enhanced accumulation of the matrix molecule hyaluronan (HA) in the cortical renal interstitium is linked to these classical proinflammatory events in the kidney. Here we discuss the consequences resulting from the interaction of HA with its specific cell surface receptor CD44 on renal parenchymal cells.

Hyaluronan synthesis and degradation in the kidney

HA is an important component of the extracellular matrix. It is composed of endless linear repeats of the disaccharide unit (*N*-acetyl- -glucosamine [$\beta 1 \rightarrow 4$] -glucuronic acid [$\beta 1 \rightarrow 3$]) [1,2]. HA is synthesized by specific HA synthases (HAS) and is extruded into the extracellular space by these plasma membrane enzymes as a high-molecular-weight molecule [3]. HA is cleaved into fragments of low and intermediate molecular weight by specific hyaluronidases (Hyal), or *via* the action of oxygen free radicals, peroxynitrites, and UV irradiation [4,5].

HA is not present in the cortex of the adult kidney, but it is found in the medullary and papillary interstitium, where it plays a role in the urinary concentrating process [6,7]. In contrast, a pronounced accumulation of HA occurs in various interstitial and glomerular disease states, including ischaemic injury [8], allograft rejection [9], interstitial nephritis [10], anti-GBM nephritis [11,12], lupus nephritis, and others. For example, we have recently found HA deposition in the cortical tubulointerstitial space at sites of tubular injury and in glomerular crescents in mice with lupus nephritis [13].

HA is found in the serum, and levels of circulating HA are elevated in patients with chronic renal failure [14]. In the kidney HA appears to be synthesized locally *via* specific HAS. We have analysed the expression of HAS in the kidney and found that the enzymes HAS1 and HAS2 are constitutively expressed in normal mouse kidney. HAS2 but not HAS1 expression is enhanced at the mRNA level in autoimmune MRL-*Fas*^{lpr} mice, suggesting that HAS2 could mediate the enhanced deposition of HA in the tubulointerstitial compartment in autoimmune renal injury [13].

The degradation of HA is achieved *via* cellular uptake and lysosomal degradation by specific enzymes in an acid cellular compartment. Examining the expression of these *Hyal* genes, we found that several of these enzymes are constitutively expressed in the normal kidney. Transcript levels of *Hyal* genes are not elevated in autoimmune lupus mice, suggesting that the *Hyal* enzymes play a role in the normal turnover of HA by the kidney [15]. The mechanisms of extracellular HA cleavage have not been defined but could involve additional *Hyal* enzymes or the action of oxygen free radicals and peroxynitrites which are generated in an inflammatory environment.

As HA deposition is important in renal injury, we have used an in-vitro approach to examine the biological behaviour of HA. Using defined CD44-positive tubular epithelial cells (TEC) we could demonstrate that HA is synthesized by these cells upon stimulation with the proinflammatory cytokines TNF-α and IFN-γ [13]. We also found that HAS and *Hyal* genes are constitutively expressed, suggesting that HA synthesis as well as HA degradation occurs in these cells [13,15]. Addition of exogenous HA has a growth-retarding e ect, demonstrating that HA can influence cell proliferation and/or di erentiation of tubular epithelial cells [16].

The hyaluronan-receptor CD44 is upregulated in inflammatory renal diseases

The major HA receptor is CD44, a 90-kDa cell-surface molecule that occurs in multiple isoforms generated by alternative mRNA splicing and variable glycosylation. In the normal kidney, CD44 is only found on interstitial dendritic cells and passenger leukocytes [17,18]. In contrast, CD44 is markedly enhanced in inflammatory renal diseases, particularly on tubular epithelial cells and in glomerular crescents [12]. We found a marked upregulation of CD44 on renal proximal tubular epithelial cells in MRL-*Fas*^{lpr} mice with lupus nephritis and in CBA-*kdkd* mice with interstitial nephritis at sites where HA

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accumulation is abundant, suggesting in-vivo interaction between HA and CD44 [10,17].

Thus far the pathophysiological role of HA accumulation and the interaction with CD44 has not been clear. HA is an extracellular polysaccharide with a high water-binding capacity and it has been speculated that is could play a role in the generation of tissue oedema [19]. Furthermore, HA could provide an interstitial matrix along which CD44-positive invading mononuclear cells could easily migrate. New information regarding the biological role of HA has come from a series of experiments which show that fragmented, but not intact, high-molecular-weight HA displays a number of important proinflammatory e ects, including the upregulation of cytokines, chemokines, and adhesion molecules.

Proinflammatory effects of hyaluronan fragments in renal cells

The HA-receptor CD44 is present on many di erent cell types, but not all CD44-positive cells bind HA. To bind HA e ciently, the CD44 molecule has to be in an activated configuration. We have identified a proximal renal tubular cell line that expresses CD44 abundantly and binds fluorescein-conjugated HA constitutively [16]. Since CD44 is markedly upregulated on proximal tubular cells *in vivo*, we have used this tubular epithelial cell line (MCT cells) as a model system to examine the proinflammatory e ects of HA [17].

Using these cells we could demonstrate that fragmented but not intact HA upregulates the expression of the chemokine MCP-1 [20] The e ect of HA is not limited to MCP-1, as RANTES or cytokines such as TN F-α are also stimulated in response to HA (unpublished observation). The HA-stimulated production of MCP-1 can be inhibited with anti-CD44 antibody, suggesting that the e ect is mediated by this HA receptor. McKee *et al.* have similarly shown that HA fragments have a stimulatory e ect on the expression of various chemokines in pulmonary macrophages [21]. HA also promoted the expression of cytokines such as TNF-α and IL-12 by macrophages [22,23]. These data suggest that HA transforms tubular epithelial cells and macrophages into an inflammatory phenotype.

We have recently shown that HA fragments of a defined size upregulate the adhesion molecules ICAM-1 and VCAM-1, suggesting a link between matrix degradation and leukocyte adhesion [24]. High-molecular-weight preparations of HA are without e ect; however, when high-molecular-weight preparations of HA are digested with hyaluronidase, an adhesion-molecule-inducing activity can be elicited. Very small HA molecules such as HA hexamers, which represent the minimal binding motif for CD44, are also without e ect on ICAM-1/VCAM-1 expression. Collectively these data have shown that HA is only active on adhesion molecule expression when the molecules have a defined molecular size. Presumably the upregulation of adhesion molecules occurs only when the CD44 molecules are aggregated. That CD44 is involved in the upregulation of ICAM-1/VCAM-1 could also be shown by cross-linking of CD44 on the cell surface of tubular epithelial cells. This manoeuvre also leads to the upregulation of ICAM-1 and VCAM-1 on tubular epithelial cells [25,26].

Together the in-vitro data suggest that a pro-inflammatory loop exists between the synthesis of HA and the recruitment of mononuclear leukocytes. Accumulation of HA, its breakdown into low-molecular-weight products *via* specific hyaluronidases or oxygen free radicals, and the subsequent stimulation of chemokines, cytokines, and adhesion molecules may represent a co-ordinated inflammatory response that could occur in many renal injury processes.

A number of additional e ects have been described for HA, including the upregulation of iNOS in macrophages and the stimulation of COX-2 and prostaglandins in epithelial cells [27,28]. Thus, HA has a profound activating e ect on numerous inflammatory genes. The activation of most of these genes involves common transcription factors, including NF-κB and AP-1 [24,29]. These transcription factors are induced upon HA stimulation or cross-linking of CD44. Therapeutic interventions that target these transcription factors might prove to be beneficial.

Can the HA/CD44 pathway be influenced by therapeutic manoeuvres?

The true significance of HA fragmentation in the kidney cortex in the context of inflammatory renal diseases remains to be determined. However, given its profound proinflammatory e ects a strategy which targets the HA/CD44 interaction could be beneficial. Using anti-CD44 mAb it could be shown that collagen-induced arthritis was improved in the rat [30]. The application of HA hexamers was also beneficial in rat models of acute and chronic renal allograft rejection [31,32]. *In vitro*, high-molecular-weight HA has anti-inflammatory e ects, probably by competing with the low-molecular-weight fragments at the level of the CD44 molecule. Injection of high-molecular-weight HA is beneficial in inflammatory synovial diseases such as osteoarthritis. More information will be gained in the future from in-vivo studies using mice with targeted disruption of the HAS genes.

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