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Increased glomerular matrix metalloproteinase activity in murine lupus nephritis

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Lupus nephritis is associated with thickening of the glomerular basement membrane. Here we measured expression of proteins involved in extracellular matrix turnover in kidneys of lupus-prone mice of the NZBxNZW F1 (B/W) strain before the onset of the disease until the development of proteinuria. Expression of the major isoforms of glomerular basement collagen IV (α3/α4/α5) was unchanged throughout disease progression. Collagen IV α1 and α2, however, were highly upregulated at the proteinuric stage while collagen IV α6 was increased at all time points compared to normal mice. There was increased expression of matrix metalloproteinase-2 and -9, their protein inhibitors TIMP-1 and -2 and the metalloproteinase-9 stabilizing protein lipocalin-2 in kidneys of nephritic lupus-prone mice. When proteinuria appeared we found an increased net glomerular gelatinolytic activity. These studies suggest that matrix metalloproteinases contribute to extracellular matrix expansion and proteinuria by altering matrix composition.

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Systemic lupus erythematosus is a systemic autoimmune disease characterized by chronic inflammatory processes associated with deposition of immune complexes in different organs. The glomerulus is one of the sites most seriously affected, with tissue damage progressing to end-stage renal disease in as much as 30% of patients. Although various functional disturbances within tissues have been implicated in the pathogenesis of systemic lupus erythematosus, details of the initiating events and accompanying detrimental processes on both systemic and end-organ levels remain largely unknown.

The histological presentation and patterns of glomerular injury in lupus nephritis are heterogeneous, with varying involvement of mesangial and vascular elements. An invariant finding, however, is the accumulation of immune complexes within glomerular membranes, which is often accompanied by an apparent expansion of vascular and/or mesangial extracellular matrices as the disease progresses. The causes underlying the observed glomerular basement membrane (GBM) thickening are unknown, but it has been speculated that increased synthesis or decreased turnover of extracellular matrix constituents such as collagen IV might be contributing factors.[1–3] On the basis of gene expression and immunohistochemical data, previous studies have reported increases in collagen IV in mice with chronic graft-versus-host disease—another model of lupus nephritis.[4,5]

Autoantibodies against dsDNA are considered to be an important factor in the evolution of lupus nephritis. Several nephritogenic anti-dsDNA antibodies appear to bind DNA in the form of nucleosomes, and extracellular chromatin has been found to colocalize with such antibodies *in situ*.[6,7] Recent data from our group demonstrated considerable affinity of nucleosomes for collagen IV.[8] Within this context, increased production or reduced degradation of collagen IV might theoretically contribute to accumulation of extracellular chromatin, thus maintaining or aggravating autoantibody deposition. We therefore aimed to look at the changes occurring in extracellular matrix composition and turnover around the time of appearance of glomerular immune complexes and the onset of nephritis.

Matrix metalloproteinases (MMPs) represent a family of endopeptidases that play a key role in the turnover of extracellular matrix proteins, and altered expression of

MMPs is believed to be involved in the development of several glomerulopathies.[9,10] MMP-2 and MMP-9, referred to as the gelatinases, have collagen IV among their main substrates, and increased gene expression of these particular MMPs has been especially implicated in glomerulonephritis, including both human[10] and murine[11] lupus nephritis. However, natural inhibitors of MMP activity, the tissue inhibitors of metalloproteinases (TIMPs), are also differentially expressed in nephritis,[12–14] complicating the interpretation of net effects on *in vivo* proteolytic activity.

We have followed the time course of the development of nephritis in a mouse model of lupus nephritis, NZBxNZW F1 (B/W) mice, analyzing gene expression patterns for collagen IV, MMP-2, MMP-9, and TIMP 1–4 within the kidneys at prenephritic stages and at the onset of proteinuria. On the basis of findings of altered levels of expression of MMP-2, MMP-9, TIMP-1, and TIMP-2, we examined the net effect *in vivo* on renal gelatinolytic activity utilizing an *in situ* zymography assay.

RESULTS

The B/W mouse is an extensively studied model of systemic lupus erythematosus,[15] and mice of this breed develop anti-dsDNA antibodies from a relatively early age.[16] We detected no anti-dsDNA antibodies in serum from young B/W mice or BALB/c controls. Moderate increase in the titer was first observed in the 20-week-old (w.o.) B/W, with a further increase toward development of proteinuria (Figure 1a).

Transmission electron microscopy (TEM) analysis of B/W glomeruli revealed normal membranes in 4 and 8 w.o. mice (Figure 1b and c). Whereas only focal membrane irregularities were found in the 20 w.o. B/W mice (Figure 1d), dramatic changes involving thickening of capillary membranes and mesangial matrix along with the presence of electron-dense structures and fused podocyte processes were seen in the proteinuric animals (Figure 1e). These electron-dense structures, typical of lupus nephritis, have been shown to colocalize with autoantibody deposits.[7,17]

Alterations in ECM composition during development of nephritis

Kidney morphology was examined at the age of 4, 8, and 20 weeks as well as at the onset of proteinuria in both B/W and age-matched BALB/c controls. There was no significant matrix expansion in young, nonproteinuric mice as determined by hematoxylin and eosin or periodic acid schiff (PAS) staining (Figure 2a), whereas mild expansion and increased cellularity was seen in the 20 w.o. B/W mice (Figure 2b). At the onset of proteinuria, the animals showed significant expansion of the mesangial matrix and disruption of glomerular architecture (Figure 2c). No such changes were found in the age-matched BALB/c controls (Figure 2d).

Immunofluorescence staining against collagen IV reveals expansion of perivascular and mesangial collagen matrix at proteinuria compared with prenephritic mice (Figure 2e and f), consistent with histological and TEM findings of matrix expansion.

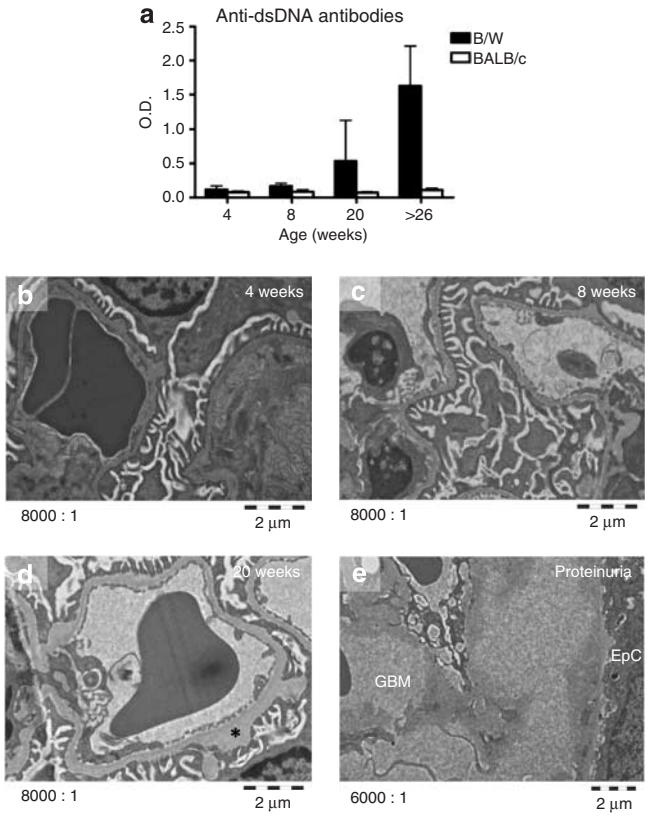

Figure 1 | Serum anti-dsDNA autoantibodies and TEM of (NZBxNZW)F1 kidneys. (**a**) Antibodies against calf thymus dsDNA in sera of B/W and BALB/c mice were detected at end point by ELISA. Data are presented as mean ± s.e.m., $n = 5$. (**b** and **c**) TEM analysis revealed no major alterations in the capillary membranes in the (**b**) 4 and (**c**) 8 w.o. B/W mice. (**d**) Focal irregular membrane changes (*) were seen in 20 w.o. B/W. (**e**) Proteinuric B/W showed dramatic thickening of capillary membranes (GBM) with fused processes of epithelial cells (EpC) and electron dense structures.

The main collagen IV isoform present in the GBM consists of trimers of chains α3-α5, organized into α3/α4/α5(IV) heteropolymeric fibers.[18] mRNA expression of collagen IV α4 remained relatively stable through all the observed time points in both B/W and BALB/c kidneys (Figure 2g). Similar trends were seen for collagen IV α3 and α5 (data not shown). At the same time, expression of the remaining collagen IV chains, α1, α2, and α6, was significantly altered in the lupus prone mice. There was an age-related downregulation of collagen IV α1 and α2 mRNA levels in the BALB/c mice. In the B/W mice, collagen IV α1 and α2 mRNA levels followed the same pattern up to 20 weeks of age but then became significantly upregulated at the proteinuric stage compared with the 20 w.o. group (Figure 2h and i, $P < 0.05$). Expression of collagen IV α6 was much higher in the B/W mice compared with their BALB/c counterparts at all time points studied (Figure 2j, $P < 0.01$). mRNA expression of the collagen chains was also examined on microdissected glomeruli from prenephritic and nephritic mice, confirming that the alterations in collagen IV expression are of glomerular origin (Figure 2g–j, insets).