IMMUNOHISTOCHEMICAL STUDY OF VITRONECTIN AND MEMBRANE ATTACK COMPLEX IN NORMAL AND DISEASED RENAL TISSUES

Jun Wada, Hirofumi Makino, Kenichi Shikata, Shigeru Morioka, Zensuke Ota
Third Department of Internal Medicine, Okayama University Medical School, Okayama 700, Japan

ABSTRACT

The localization of vitronectin (VN) was studied in comparison with membrane attack complex (MAC) by indirect immunofluorescence in normal human kidneys and in renal biopsy specimens from patients with various types of glomerulonephritis. In normal kidneys, VN was stained in arteriole walls, capillary walls, mesangium and tubular basement membrane. MAC was negative in the cortical kidney. In glomerulonephritis, VN and MAC were co-localized in glomerular immune deposits. VN and MAC were also co-localized in the glomerulosclerotic region, and the intensity of the staining increased as the sclerosis advanced. The intercellular staining of VN within cellular crescents and between infiltrating cells in the interstitium were observed. This study suggested the important role of VN in the progression of glomerulosclerosis, crescent formation and infiltration of inflammatory cells in the interstitium.

Key words; vitronectin (S-protein), membrane attack complex (MAC), C5b-9, glomerulonephritis, glomerulosclerosis

INTRODUCTION

Vitronectin (VN) exists mainly in human serum in two molecular forms with molecular weights of 75,000 and 65,000. Like fibronectin, VN is also
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localized in the extracellular matrices of various tissues (1). VN is the glycoprotein that has many functions such as regulation of the complement system and coagulation system and promotion of cell attachment (2,3). MAC (C5b-9) has been immunohistochemically reported to be located in renal tissues from patients with renal diseases (4,5). In experimental models, MAC plays important pathogenic roles (6). To clarify the mechanism of progression of glomerulonephritis, we immunohistochemically investigated the presence and location of VN and MAC in normal human kidneys as well as renal biopsy specimens from patients with glomerulonephritis.

PATIENTS AND METHODS

Normal portions of human kidneys (N=5) were obtained from the unaffected part in nephrectomies for renal tumor. Renal biopsy specimens were obtained from 39 patients with IgA nephropathy (N=19), membranous nephropathy (MN)(N=8), minimal change nephrotic syndrome (MCNS)(N=6), diffuse proliferative lupus nephritis (DPLN)(N=4) and nephrosclerosis (N=2). Indirect immunofluorescence was performed with 1) polyclonal rabbit anti-human vitronectin antibody (Telios, California, U.S.A.), 2) monoclonal mouse anti-human MAC antibody (Quidel, California, U.S.A.) as primary antibodies and 3) Fluorescein isothiocyanate (FITC) conjugated goat anti-rabbit IgG antibody (Cappel, Pennsylvania, U.S.A.), 4) FITC conjugated goat anti-mouse IgG antibody (Tago, California, U.S.A.) as second antibodies. Renal sections were also stained with FITC conjugated anti-human IgG, IgA, IgM, C3, Clq, and fibrin antibody (Cappel, Pennsylvania, U.S.A.).

RESULTS

In normal human kidneys, VN was weakly stained along the capillary wall
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either in a linear pattern or granularly in the mesangial area. VN was also stained along the tubular basement membrane (TBM), Bowman's capsule and vessel walls of arteries and arterioles. MAC was not stained in the glomerulus or tubulus. In some normal human kidneys, MAC was detected in the vessel walls of the interstitium. In the vessel walls, VN and MAC co-existed but neither C3 nor immunoglobulins were stained (Fig.1-a,b). In IgA nephropathy, VN was stained intensely in the expanded mesangial area. MAC was also stained granularly in the mesangial area. VN and MAC were co-localized with IgA and C3. MAC was localized exclusively in immune deposits, but in addition to these areas, VN was stained in the mesangium and along the capillary wall (Fig.2-a,b). In the MN, VN was stained intensely along the capillary walls granularly in addition to a linear pattern, and MAC was also detected along the capillary walls. VN and MAC were co-localized with IgG and C3 (Fig.3-a,b). In MCNS, VN and MAC had the same localization as in normal human kidneys. In DPLN, VN and MAC were stained strongly in immune deposits of the mesangium, capillary wall, TBM and vessels. VN was also stained in the intercellular space of cellular crescents and along Bowman's capsule (Fig.4-a,b). In sclerotic glomeruli, VN was strongly stained in sclerotic portions and fibrous crescents. In these areas, MAC was also stained. VN was also detected in cases with severe tubulointerstitial lesions around the sclerotic glomeruli, intensely along the TBM and in intercellular matrices of infiltrating inflammatory cells (Fig.5-a,b).

DISCUSSION

In this study, the distribution of VN and MAC and intensity were evaluated in normal renal tissues and in glomerulonephritis. Concerning the
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origin of VN, there is no evidence for the in situ synthesis of VN by the renal resident cells in this study, but vitronectin receptor is reported to exist in glomerular epithelial cells, endothelial cells and mesangial cells (9). This evidence suggested that VN plays a role in cell attachment in renal tissues. Terminal complement components, C5b, C6, C7 and C8 self-assemble within the membrane of the attacked cells and initiate the polymerization of C9 leading to the formation of the circular lytic pore (7). VN tightly associates with C5b-7, and prevents insertion into cell membrane (8). The studies in human and experimental models suggested pathogenic roles of MAC in the induction of glomerular lesions (4-6). In immune-mediated glomerulonephritis, we confirmed that VN and MAC are components of immune deposits as shown in previous studies (10-13). Because VN and MAC are co-localized with C3, C5b-9 may be locally formed by complement activation and then complexed to VN. But the presence of SC5b-9 (S-protein C5b-9 complex) in the plasma of patients with lupus nephritis (14) and other immune-mediated diseases raises the possibility of trapping from the circulation. The significance of VN and MAC in immune deposits remains unknown. But VN may play a role in the regulation of glomerular injury caused by MAC. In glomerulosclerosis, the intensity and localization of VN are related to the severity of sclerosis. Inter cellular staining of VN in cellular crescents and interstitial infiltrating cells suggested in situ synthesis by fibroblasts or macrophages. The possibility of trapping of VN and MAC from plasma to sclerotic lesions cannot be excluded. But this study suggests that VN may play a significant role in the progression of glomerulosclerosis.
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Fig. 5-a

Fig. Immunofluorescent micrographs stained for vitronectin (VN) and MAC. In normal human kidneys, VN was stained in the glomerulus (Fig.1-a) and MAC was not stained (Fig.1-b). In IgA nephropathy, VN (Fig.2-a) and MAC (Fig.2-b) were stained in the mesangial area. In membranous nephropathy, VN (Fig.3-a) and MAC (Fig.3-b) were stained granularly in the capillary loop. In lupus nephritis, VN was stained in the intercellular space of the cellular crescents (Fig.4-a). MAC was stained strongly in immune deposits (Fig.4-b). In sclerotic glomeruli, VN(Fig.5-a) and MAC(Fig.5-b) co-existed. (magnification x300)

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