Glomerular deposition of α₂-macroglobulin in glomerular diseases

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Abstract

Background. α₂-macroglobulin (α₂ M) is a glycoprotein involved in delivery of growth factors, regulation of matrix degrading enzymes and modulation of fibrinolysis factors, all of which are considered as important pathogenic mechanisms of glomerular injury. However, the role of α₂ M in glomerular disease has not been extensively studied. The amount, frequency and local distribution of α₂ M in diseased glomeruli are similarly undetermined.

Methods. Two hundred and fifty renal biopsy cases with glomerular disease were collected. The glomerular deposition of α₂ M was surveyed with immunofluorescence-microscopy and intraglomerular localization of α₂ M was assessed by immunoelectron-microscopy. To clarify the relationship between circulatory concentration and local deposition of α₂ M, serum samples were collected at time of biopsy and α₂ M was determined using radial immunodiffusion assay.

Results. The amount and frequency of local deposition of α₂ M in glomeruli varied from disease to disease, and the average positive rate was approximately 20%. Patients with minimal-change nephrotic syndrome and IgM nephropathy not only had the highest mean serum α₂ M concentration but also exhibited higher frequency of glomerular deposition of α₂ M (25.9 and 30% respectively). The local deposition of α₂ M revealed by optical and electron-microscopy may not be directly related to the high serum level of α₂ M. The deposited α₂ M was observed to associate with electron-dense deposits, mesangial matrix and mesangial cells.

Conclusions. To our knowledge, this is the first report that reveals the ultrastructural distribution of α₂ M in glomerular disease. The relatively selective deposition of α₂ M in some glomerular diseases strongly indicates that α₂ M may play an active role in the modulation of local inflammatory reaction and tissue repair in these glomerular diseases.

Key words: α₂-macroglobulin; glomerular disease; immunohistochemistry

Introduction

Human α₂-macroglobulin (α₂ M), a 720 kDa glycoprotein, mediates complex physiological and pathological activities through its functional role as binding, carrier, and targeting protein [1]. α₂ M interacts with two major groups of peptides, proteinases and cytokines, to exert important modulatory effects on inflammation, immunity, and tissue repair [2]. The major source of circulatory α₂ M is hepatocytes; however, other cells such as macrophages, fibroblasts, astrocytes, Sertoli cells, adrenocortical cells and certain types of tumour cell synthesize and secrete α₂ M [1]. α₂ M can also be modified to form a receptor-recognized molecule. The receptor has been identified as low-density-lipoprotein-receptor-related protein (LRP), and is responsible for the clearance of activated α₂ M [1,2]. The receptor-mediated endocytosis of α₂ M also plays a role in antigen presentation and intracellular signaling [3,4]. The local synthesis and uptake of α₂ M in specific tissue may imply a compartmentalized function of α₂ M that is independent of circulatory α₂ M [3]. The pathogenic role of α₂ M in glomerular disease has not been extensively studied. As α₂ M is involved in delivery of growth factors, regulation of matrix-degrading enzymes and modulation of fibrinolysis factors, all of which are considered as important pathogenic mechanisms of glomerular injury, it is logical to speculate on the possibility of a close connection between α₂ M and the response of glomerular tissue to injury. The recent identification of LRP on glomerular mesangial cells further suggests the existence of α₂ M delivery/clearance system in glomerular tissue [3]. Local deposition of α₂ M in glomeruli and variations of circulatory levels of α₂ M have been reported in patients with renal disease, especially those associated with nephrotic syndrome [6–9]. However, the effects of elevated serum α₂ M on the amount, frequency, and distribution of α₂ M in glomerular tissue have not been determined.

Subjects and methods

This study collected 250 renal biopsy cases with glomerular disease from Veterans General Hospital, Taipei. Cases
without adequate biopsy specimen for routine histological examination, immunofluorescence staining, or electron-microscope evaluation were excluded. Routine immunofluorescence staining was performed using FITC-conjugated rabbit anti-human antibodies (IgG, IgM, IgA, C1q, C3 and C4: 1:20 dilution, Dako). 188 cases had additional immunofluorescence staining for α2M (FITC-conjugated goat anti-human α2-macroglobulin: 1:10 dilution, Kent Labs). Six cases which were biopsied for renal tumour and had no evidence of glomerulonephritis were used as negative control.

The specificity of antibody for immunofluorescence staining for α2M was confirmed with Western blot analysis using normal human serum (1:20 dilution) and purified human α2M (Sigma) as positive controls.

For immunoelectron-microscope localization of α2M in glomeruli, a post-embedding immunohistochemical method was used. Renal biopsy specimens were fixed in freshy prepared 4% paraformaldehyde in phosphate-buffered saline (PBS) at 4°C. The specimens were dehydrated in an alcohol series (50, 70, 95, 100%) at 4°C and subsequently embedded in a hydrophilic resin (Unicryl, British BioCell) which was polymerized with UV light at −20°C. A total of nine cases showing positive immunofluorescence staining of α2M in glomeruli had renal biopsy tissue embedded in Unicryl resin. The thin sections were cut from these blocks and collected on nickel grids. The sections were sequentially incubated for 10 min with 1% bovine serum albumin (BSA), for 1 h with specific goat anti-human α2M antibody (1:20 dilution, Chemicon) and for 1 h with gold-conjugated rabbit anti-goat antibody (10 gm, 1:20 dilution, British BioCell). The gold-labelled sections were counterstained with uranyl acetate and lead citrate and viewed under a Zeiss electron microscope (EM 10C) at 60 kV. The specificity of primary antibody for immunoelectron-microscopy was confirmed with Western blot analysis using normal human serum (1:20 dilution) and purified human α2M (Sigma) as positive controls.

One hundred and fifteen serum samples obtained on the day of biopsy were divided into small aliquots and refrigerated at −70°C. α2M in serum was measured by radial immunodiffusion method (radial immunodiffusion kit, Serotec). Fourteen normal serum samples were donated by healthy adults (7 females and 7 males). All the serum samples were analysed in duplicate.

### Results

**Deposition of α2M in glomeruli with glomerular disease**

Immunofluorescence staining for α2M was performed on 188 cases to yield 13 groups of glomerular diseases: amyloidosis (AMY), diffuse crescentic glomerulonephritis (DCG), diabetic glomerulonephropathy (DMG), fibrillary glomerulonephritis (FGP), focal segmental glomerulosclerosis (FSG), IgA nephritis (IgA), IgM nephritis (IgM), lupus glomerulonephritis (LPG), minimal-change nephrotic syndrome (MCN), membranous glomerulonephritis (MGN), membranoproliferative glomerulonephritis (MPN), glomerulonephritis associated with Schönlein–Hénoch purpura (SHP), transplant glomerulopathy (TGP).

The number and staining intensity of positive cases are listed in Table 1. The staining patterns are shown in Figure 1. All normal controls showed negative staining of glomeruli. LPG, AMY, and DMG showed non-specific staining consistent with the locations of sclerosis or hyaline deposits (Figure 1G, 1H, and 1I).

### Table 1. The frequency and intensity of α2M deposition in glomerular disease. The immunofluorescence intensity is defined as trace (tr), slight (+), moderate (+ +) and strong (+ ++ +). % indicates the percentage of positive cases in each group. The trace staining does not count as positive. AMY, amyloidosis; DCG, diffuse crescentic glomerulonephritis; DMG, diabetic glomerulonephropathy; FGP, fibrillary glomerulonephritis; FSG, focal segmental glomerulosclerosis; IGA, IgA nephritis; IgM nephritis; LPG, lupus glomerulonephritis; MCN, minimal-change nephrotic syndrome; MGN, membranous glomerulonephritis; MPN, membranoproliferative glomerulonephritis; SHP, glomerulonephritis associated with Schönlein–Hénoch purpura; TGP, transplant glomerulopathy.

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<th>Group</th>
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The positive cases in minimal-change–focal-sclerosing complex (MCN, IGM, and FSG) had very similar staining pattern that was diffuse, global mesangial with occasionally segmental linear deposits (Figure 1B and 1C). MCN and IGM had higher positive rate (25.9 and 30%, Table 1) and intensity (Figure 1C) compared with FSG (10%, Table 1) (Figure 1B). IGA also showed mesangial pattern of staining in positive cases (Figure 1D). Two of three cases of FGP showed positive staining of α2M, though the intensity was low and the distribution was non-specific (Figure 1E). Only one case of TGP displayed moderate peripheral capillary and segmental mesangial staining (Figure 1F).

The positive rate of MGN was 19% (Table 1) and the staining was performed on nine cases (MGN 3 cases, FSG 2 cases, IGA 1 case) with positive serum samples obtained on the day of biopsy were divided into small aliquots and refrigerated at −70°C. α2M in serum was measured by radial immunodiffusion method (radial immunodiffusion kit, Serotec). Fourteen normal serum samples were donated by healthy adults (7 females and 7 males). All the serum samples were analysed in duplicate.

The monospecificity of primary antibody for immunoelectron-microscopy was confirmed with Western blot analysis using normal human serum and purified human α2M as antigenic sources. The antibody showed monospecific reactivity toward 185 kDa protein in both sources of proteins (not shown). The ultrastructural analysis revealed localization of α2M molecules in electron-dense deposits in MGN and IGA (Figure 2A, 2B, 2C).
The column scattered distribution of serum $\alpha_2$M by glomerular disease is displayed in Figure 3. The mean serum level of pooled patients in all 12 groups (3159 ± 120 mg/L, $n=115$) was significant higher than that of healthy subjects (2040 ± 63 mg/L, $n=14$, Student $t$ test $P<0.001$). The mean serum levels of individual groups were also numerically higher than that of healthy control group (Figure 3). Statistical analysis further revealed significant elevation of serum $\alpha_2$M in AMY, DMG, FSG, IGA, IGM, LPG, MCN, and MGN (Student $t$ test $P<0.05$). The elevation of serum levels in DCG and TGP showed no statistical significance (student $t$ test $P=0.07$ and 0.09 respectively). The small sample in MPG and SHP made the statistical analysis invalid. No serum sample of FGP was collected.

Discussion

In our survey of 188 cases of glomerular disease, the immunofluorescence data for $\alpha_2$M deposition showed variation of positive rate and staining pattern in different groups of glomerular disease. The total positive rate was about 20%. Patients with minimal-change nephrotic syndrome and IgM nephropathy not only had the highest mean serum $\alpha_2$M concentration but also exhibited higher frequency of glomerular deposition of $\alpha_2$M (25.9 and 30% respectively), suggesting that $\alpha_2$M should play a significant role in the pathophysiology of the diseases related to minimal-change nephrotic syndrome. The elevated circulating $\alpha_2$M levels noted in our study encompass not only glomerular diseases associated with nephrotic syndrome but also other types of glomerular disease. Our results are compatible with a previous report of elevated serum $\alpha_2$M levels in patients with glomerulonephritis [7]. Thenephritis deposition of $\alpha_2$M in the glomeruli may not be directly related to the high serum level of $\alpha_2$M since many cases in our study with markedly elevated serum $\alpha_2$M had no glomerular deposition of $\alpha_2$M.

In our series, $\alpha_2$M deposition in certain types of glomerular lesions seemed non-specific and may be attributed to local leakage and trapping of plasma protein. These lesions included amyloidotic glomerulopathy and fibrillary glomerulonephritis with extensive deposition, diabetic glomerulopathy with extensive insudative and sclerotic lesions and active lupus glomerulonephritis with segmental necrosis and sclerosis. On the contrary, the depositions in minimal-change nephrotic syndrome, IgM nephritis and focal segmental glomerulosclerosis were more specific and concentrated in the mesangial and paramesangial regions. The ultrastructural localization of $\alpha_2$M in the glomeruli with these diseases further confirms the presence of $\alpha_2$M molecules in mesangial and paramesangial regions. The association of gold particles with the cell surfaces rather than the cytoplasm of mesangial cells strongly implies the existence of receptor-mediated $\alpha_2$M delivery/clearance system in glomerular mesangium. The mesangial $\alpha_2$M may be either produced by mesangial cells or taken up by mesangial cells from blood plasma.
cells or released from blood circulation. Our observation that the $\alpha_2$M in mesangial matrix predominantly distributed in areas facing capillary lumen suggests a release of these macromolecules from blood circulation in response to the local pathological process in this area. The localization of $\alpha_2$M in subendothelial electron-lucent deposits is consistent with the paramesangial immunofluorescence staining of $\alpha_2$M in FSG or MCN, and its deposition may be related to the hyperfiltration mechanism as described in primary FSG [10]. In MGN and IGA, the localization of $\alpha_2$M in electron-dense deposits may be related to the local immunological process. It has been demonstrated that TGF-β1 could accumulate within electron-dense deposits found in membranous nephropathy and IgA nephritis [11]. This finding, together with the fact that $\alpha_2$M is the major binding protein of many cytokines including TGF-β, implies that the deposition of $\alpha_2$M in electron-dense deposits may represent local $\alpha_2$M-cytokine delivery/clearance system [2,12]. The visceral epithelial cells have phagocytotic properties and have shown to internalize macromolecules through receptor- or non-receptor-mediated endocytosis [13,14]. The presence of $\alpha_2$M in visceral epithelial cells may result from non-receptor-mediated endocytosis since visceral epithelial cells lack $\alpha_2$M receptor [5,15].

Although the pathogenetic mechanism of local deposition of $\alpha_2$M is not completely clear at present

Fig. 2. The ultrastructural localization of $\alpha_2$M in glomeruli using postembedding immunogold (10 nm) staining method. A, The localization of $\alpha_2$M (arrows) in subepithelial electron-dense deposits in a glomerulus with membranous glomerulonephritis. B, Labelling of lysosomal structures (arrows) in visceral epithelial cells of a glomerulus with membranous glomerulonephritis. C, The localization of $\alpha_2$M in mesangial matrix (arrowheads) and on mesangial cell process (arrows) in a glomerulus with minimal-change nephrotic syndrome. Some gold particles are also distributed alongside of the cell surfaces of mesangial cell (asterisks). D, The localization of $\alpha_2$M (arrows) in subendothelial electron-lucent deposits in a glomerulus with focal segmental glomerulosclerosis. E, The localization of $\alpha_2$M in electron-dense deposits in a glomerulus with IgA nephritis (arrow). F, The control section without incubation with primary antibody shows negative labelling of gold particles. A–E, $\times 61,065$; F, $\times 48,852$. 

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time, the demonstration that glomerular deposition of $\alpha_2$M in patient with steroid-refractory nephrotic syndrome [6] and the effect of rised serum $\alpha_2$M on severity of proteinuria in experimental nephrotic nephritis [16] further implies the potential clinical significance of this macromolecule in certain types of glomerulonephritis.

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