Mechanisms of renal damage in mixed cryoglobulinaemia nephritis

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Introduction

Mixed cryoglobulinaemia (MC) is a systemic disorder characterized clinically by the typical triad of purpura, arthritis and weakness, and serologically by the presence of cryoprecipitating complexes, formed by a monoclonal or polyclonal rheumatoid factor and polyclonal immunoglobulins. The presence of anti-HCV antibodies and viral mRNA in the serum of MC patients has shed new light on the pathogenesis of the disease. However, the mechanisms leading to the different manifestations of MC are still unknown.

Mechanisms of renal damage

Almost one-third of the patients develop renal involvement, histologically characterized by a membranoproliferative glomerulonephritis with subendothelial deposits. The deposition of circulating immune complexes has been suggested to play a major role in inducing renal damage. However, no correlation has been observed between cryocrit or immune complex levels and the presence and/or severity of renal manifestations. Complement levels are in fact persistently low and the evidence that immune complexes containing viral proteins are a main component of glomerular deposits is scanty. It has been demonstrated that autoantibodies specific either for circulating antigens deposited in the kidney or for renal structural antigens can concentrate in the kidney and cause renal damage [1–3]. Recent evidence suggests that a similar mechanism can cause renal damage in MC. A membranoproliferative nephritis similar to the human cryoglobulinaemic nephritis was recently induced in mice by intravenous injection of solubilized mixed cryoglobulins from patients with nephritis [4]. The monoclonal IgM rheumatoid factor isolated from these cryoglobulins was able, if injected separately, to deposit in glomeruli: this result suggests that the rheumatoid factor reacts with a glomerular antigen and forms in situ immune complexes. The identification of the exact renal antigen targets of autoantibodies from the sera of patients with systemic autoimmune disorders is difficult, because of the variety of autoantibodies present in different sera and the multiple reactivities of a single autoantibody. We studied by immunoblot the reactivity of mixed cryoglobulinemia (MC) sera with kidney extracts [5]. Some of the patient sera reacted with a 48 kDa antigen and a close correlation between the presence of this antibody specificity and renal involvement was observed; in fact, all of the MC patients whose sera reacted with the 48 kDa protein had active nephritis, while antibodies with this specificity were not detected in the sera from MC patients without renal involvement. This observation prompted us to characterize the 48 kDa renal antigen, and sequencing analysis showed it to be identical with the glycolytic enzyme α-enolase [6]. α-Enolase catalyses the dehydration of 2-phosphoglycerate to phosphoenolpyruvate; it is a homodimer formed by the non-covalent association of two polypeptides of 433 amino acids each, which are encoded by a single copy gene. This enzyme is abundant in the kidney and it is also expressed on the cell membrane [7,8].

To evaluate the pathogenicity of anti-enolase antibodies, monoclonal anti-enolase antibodies were obtained from BALB/c mice. A monoclonal antibody has been used to study the distribution of the enzyme in the kidney by immunohistochemistry. The enzyme is highly expressed in tubular cells and almost undetectable in glomeruli. In renal biopsies from SLE patients, the amount of enolase in tubuli is higher. In glomeruli, enolase is expressed in mesangium, in glomerular parietal and visceral epithelium and especially in crescents. BALB/c and SCID mice were injected intraperitoneally with the hybridoma-producing cells. After 1 week, anti-enolase activity was detected in serum. The animals were sacrificed after 3–4 weeks and kidneys were examined. Glomeruli were either normal or showed...
only focal infiltrates in mice injected with a control hybridoma producing IgM anti-DNA antibodies and in mice injected with four hybridomas producing anti-enolase antibodies. Diffuse proliferative lesions were induced by two anti-enolase antibodies. SCID mice injected with these latter hybridomas showed similar lesions. In addition, a third monoclonal induced in SCID diffuse glomerulonephritis and tubulonephritis.

**Conclusion**

In mixed cryoglobulinaemia nephritis, it has been demonstrated that autoantibodies specific either for circulating antigens deposited in the kidney or for renal structural antigens can concentrate in the kidney and cause renal damage. In addition, the presence of the enzyme α-enolase at sites of active renal lesions and the ability of some anti-enolase antibodies to induce renal damage by passive transfer suggest that the immune response to α-enolase may contribute to renal damage in MC.

**References**