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Autoantibodies against monomeric C-reactive protein in sera from patients with lupus nephritis are associated with disease activity and renal tubulointerstitial lesions

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ABSTRACT

Serum levels of C-reactive protein (CRP) often remain low despite high disease activity in systemic lupus erythematosus (SLE). Sera from 96 patients with renal biopsy-proven active lupus nephritis, 24 of 96 patients in remission, and 49 patients with SLE with negative urinalysis (nonrenal SLE) was collected. Immunoglobulin G autoantibodies against monomeric CRP (mCRP) were screened by enzyme-linked immunosorbent assay with purified human CRP. Associations with clinical features, pathological data, and laboratory findings were investigated. The prevalence of mCRP autoantibodies in active lupus nephritis (57/96, 59.4%) was significantly higher than that in patients with SLE without clinical evidence of kidney involvement (20/49, 40.8%, $p = 0.034$). For the 13 patients with positive mCRP autoantibodies and sequential sera, their positive mCRP autoantibodies in active phase turned negative in remission (13/13, 100%). Patients with mCRP autoantibodies had significantly higher SLEDAI scores than patients without mCRP autoantibodies (18.3 ± 5.2 vs 15.8 ± 4.0, $p = 0.013$), who were more likely to experience acute renal failure (14/55 vs 2/33, $p = 0.022$), oral ulcer (15/57 vs 3/39, $p = 0.022$), and delayed activated partial thromboplastin time (18/52 vs 2/38, $p = 0.001$). Positive correlations between levels of mCRP autoantibodies and semiquantitative scores of renal histologic features were first observed in lupus nephritis as follows: interstitial inflammation ($r = 0.328$), tubular atrophy($r = 0.276$), interstitial fibrosis ($r = 0.211$), and chronicity index score ($r = 0.243$). Autoantibodies against mCRP are prevalent in patients with lupus nephritis and are associated with disease activity and renal tubulointerstitial lesions.

1. Introduction

Systemic lupus erythematosus (SLE) is a multisystem disease characterized by multiple autoantibody production and complement-fixing immune complex deposition that result in tissue damage [1]. Up to now, over 155 different autoantibodies have been detected in SLE [2,3]; however, whether these antibodies are predictive [4], pathogenic [5], protective [6], or organ-specific is yet to be determined. Lupus nephritis is the most common cause of secondary glomerulonephritis in the Chinese population [7]. However, only a few autoantibodies acting as biomarkers for lupus nephritis have been reported that seemed to be associated with disease activity , such as anti-C1q autoantibodies [8], anti-DNA autoantibodies [9], and anti-α-actinin-binding antibodies [10].

Plasma C reactive protein (CRP) is predominantly produced by hepatocytes, and monomeric CRP (mCRP) is produced partly by neurons [11], lymphocytes [12], smooth muscle cells [13], alveolar macrophages [14], and tubular epithelial cells in the kidney [15]. CRP belongs to the pentraxin family of calcium-dependent ligand-binding plasma proteins, which is composed of five identical non-glycosylated polypeptide subunits, each with 206 amino acid residues. Under certain conditions such as altered pH, high urea, or low calcium concentration, native CRP dissociates irreversibly into monomers [16], which undergo conformational rearrangement resulting in the expression of a distinct isomer with distinct antigenic and physiochemical characteristics [17].

Native CRP has numerous biological functions, such as pro- and antiinflammatory effects. mCRP has a lower isoelectric point than native CRP and is considered a tissue- or cell-based form of the acute phase protein with less known properties and biological effects [18–22]. The median concentration of CRP is 0.8 mg/l in healthy young adult blood donors [23]. However, following an acute-phase stimulus, values of CRP may increase from less than 50 mg/l to more than 500 mg/l. It is not known why SLE fails to elicit major CRP production, particularly if SLE were present without serositis, despite evident inflammation and tissue damage, al-

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though the level of CRP did not markedly increase during bacterial infections [24,25]. Preliminary studies demonstrated that autoantibodies against mCRP exist in sera from patients with SLE [26,27], especially in patients with kidney involvement. Autoantibody levels were associated with disease activity [28–30], indicating that these autoantibodies may have biological functions of pathogenic interest.

We detected anti-mCRP autoantibodies in sera from patients with lupus nephritis, patients with SLE with negative urinalysis (nonrenal SLE [NR-SLE]), and patients with lupus nephritis in the remission phase. The association between anti-mCRP antibodies and disease activity and the clinical and histopathologic features of lupus nephritis were evaluated.

2. Subjects and methods

2.1. Patients and sera

Sera from 96 patients with active lupus nephritis with complete pathological data, consecutively admitted to our hospital between January 2000 and August 2006, were collected upon presentation. Eighty-three were female and 13 were male, with a mean age of 35 ± 11 years. Sera were also collected from 24 of the 96 patients first presenting with active disease and then going into remission during follow-up with a mean duration 858.3 ± 446.4 days and from 49 patients with NR-SLE with a mean age of 33 ± 14 (40 females and 8 males). Sera were obtained from peripheral blood on the day of renal biopsy or at presentation before immunosuppressive treatment in patients in the active phase. All patients fulfilled the 1997 American College of Rheumatology revised criteria for SLE [31]. Complete remission was defined as urinary protein excretion less than 0.3 g per day, with normal urinary sediment (RBC < 5/HP, WBC < 5/HP), normal serum albumin concentration, and normal renal function. The detailed clinical and pathologic data were retrospectively reviewed. Informed consent was obtained for blood sampling.

Sera from 60 healthy subjects matched for gender and age were collected and used as normal controls. All sera were stored at −20°C until use.

2.2. Detection of mCRP autoantibodies by enzyme-linked immunosorbent assay

Human CRP (Sigma, St. Louis, MO) was diluted to 5.6 μg/ml with 0.05 mol/l bicarbonate buffer, pH 9.6, and coated onto the wells of one half of a polystyrene microtiter plate (Costar, Corning, NY). The wells in the other half were coated with bicarbonate buffer alone to act as antigen-free wells. Incubation was carried out for 1 hour at 37°C. Free binding sites were blocked for another hour at 37°C with phosphate-buffered saline containing 0.1% (vol/vol) Tween 20 (PBST), containing 1% (10 mg/ml) bovine serum albumin. Sera were diluted to 1:50 in PBST–bovine serum albumin. The volumes of this step and subsequent steps were 100 μl and all incubations were carried out at 37°C for 1 hour. The plates were washed three times with PBST. The binding was detected by horseradish peroxidase-conjugated goat antihuman immunoglobulin G antibodies at a dilution of 1/5000 (Gibco BRL, Grand Island, NY). The horseradish peroxidase substrate *o*-phenylenediamine was used at 0.4 mg/ml in 0.1 mmol/l citrate phosphate buffer (pH 5.0). The reaction was stopped by the addition of 1.0 mol/l H_2SO_4 and results were recorded as the net OD 490 nm (average value of antigen wells minus average value of antigen-free wells). The binding of the known positive control serum was 100% and the bindings of the tested sera were expressed as a percentage of a known positive sample. The cutoff value was set as the mean + 2SD of the 60 healthy blood donors.

2.3. Statistical analysis

Statistical analysis included the use of the statistical software SPSS 10.0 (SPSS, Chicago, IL). Quantitative data were expressed as means ± SD and medians with ranges (25th percentile, 75th percentile). For comparison between patients and controls and comparison of clinical features and pathological data of patients, the Student *t* test, one-way analysis of variance, χ^2 test, logistic analysis, and Spearman's correlation were used. Statistical significance was considered $p < 0.05$.

3. Results

3.1. Prevalence of autoantibodies against mCRP in patients with lupus nephritis

Autoantibodies against mCRP were measured in sera from 96 patients with active lupus nephritis, 24 patients in remission, and 49 patients with NR-SLE. The cutoff value of the enzyme-linked immunosorbent assay was 17%. As illustrated in Figure 1, autoantibodies against mCRP from the positive control serum could be tittered up to 1:800.

The mCRP autoantibodies were detected in 57/96 patients (59.3%) with active lupus nephritis, which was significantly higher than that in patients with SLE without clinical evidence of renal involvement (20/49, 40.8%; $p = 0.034$). For the 24 patients with sequential sera, their positive mCRP autoantibodies in active phase turned negative in remission (13/24, 54.2%). The other 11 patients without mCRP autoantibodies in the active phase had mCRP autoantibodies that remained negative in remission (Figure 2). None of the 60 healthy controls were positive for mCRP autoantibodies.

3.2. Clinical association of mCRP autoantibodies

Patients with mCRP autoantibodies had significantly higher SLEDAI scores than patients without mCRP autoantibodies (18.3 ± 5.2 vs 15.8 ± 4.0, $p = 0.013$) in the active lupus nephritis group (Figure 3). Patients with mCRP autoantibodies were more likely to suffer from acute renal failure (14/55 vs 2/33, $p = 0.022$), oral ulcer (15/58 vs 3/38, $p = 0.022$), and delayed activated partial thromboplastin time (APTT; 18/52 vs 2/38, $p = 0.001$; Table 1). Delayed APTT was significantly different between the two groups of patients after permutation correction ($p = 0.0136$). Levels of mCRP autoantibodies did not correlate with serum levels of CRP ($p = 0.195$).

3.3. Pathological association of mCRP autoantibodies

Positive correlations between the levels of anti-mCRP autoantibodies and semiquantitative scores of renal histological features were observed in patients with lupus nephritis. Semiquantitative scores of renal histologic features were explored to evaluate the

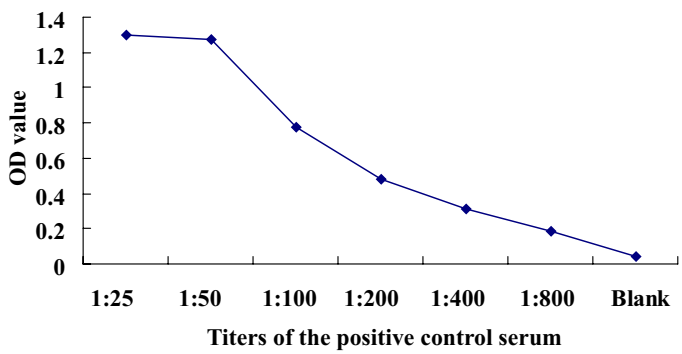

Fig. 1. Binding curve of mCRP autoantibodies of the positive control serum from a patient with active lupus nephritis. The horizontal axis indicates titers of the known positive control serum and the vertical axis indicates the net OD value of mCRP autoantibodies.