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Anti-C1q autoantibodies from active lupus nephritis patients could inhibit the clearance of apoptotic cells and complement classical pathway activation mediated by C1q *in vitro*

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ABSTRACT

Anti-C1q antibodies are prevalent in patients with active lupus nephritis and were found to be closely associated with renal involvement and predictive for a flare of nephritis. However, the pathogenesis of anti-C1q antibodies involved in human lupus nephritis remains unclear. C1q, which plays a key role in apoptotic cell and immune complex removal, is a very important functional molecule in the pathogenesis of SLE. The aim of this study was to investigate the influence of anti-C1q autoantibodies from active lupus nephritis patients on the bio-functions of C1q *in vitro*. We purified IgG autoantibodies against C1q from lupus nephritis patients, and found that they could recognize C1q bound on early apoptotic cells at 30 μg/ml, and could significantly decrease the phagocytosis by macrophages of early apoptotic cells opsonized by 50 μg/ml C1q in comparison with normal IgG. Levels of circulating immune complexes of the ten patients were measured by a circulating immune complexes (CIC)-C1q Enzyme Immunoassay Kit. Anti-C1q autoantibodies affinity purified by microtiter plates could significantly inhibit the deposition of C3c on CIC-C1q in a dose dependent manner in comparison with IgG from 10 healthy blood donors. The binding of opsonized immune complexes to RBCs was significantly inhibited by anti-C1q autoantibodies purified by microtiter plates in a dose dependent manner. Our observations suggest that serum anti-C1q autoantibodies from active lupus nephritis patients could interfere with some biological function of C1q *in vitro*.

Introduction

Systemic lupus erythematosus (SLE) is the prototype of autoimmune disease and is characterized by the production of a variety of autoantibodies. Of the various major organ manifestations of SLE, lupus nephritis continues to be a principal cause of morbidity and mortality (Cervera et al. 2003). It was proposed that the development of glomerulonephritis in patients with SLE was correlated with the presence of some specific nephritogenic autoantibodies. Anti-C1q antibodies were prevalent in patients with active lupus nephritis (Sinico et al. 2005; Marto et al. 2005; Fang et al. 2009; Trendelenburg et al. 2006; Yang et al. 2012) and were found to be closely associated with renal involvement and predictive for a flare of nephritis (Marto et al. 2005).

Animal studies indicated that immune deposition of C1q and anti-C1q antibodies in the kidney was dependent on the presence of glomerular IgG (Trouw et al. 2003a,b, 2004a). A further study indicated that anti-C1q autoantibodies deposited in glomeruli but were only pathogenic in combination with glomerular C1q-containing immune complexes (Trouw et al. 2004b). However, there still lacks direct evidence of how anti-C1q antibodies involves in the pathogenesis of human lupus nephritis.

C1q, the first protein of the classical complement pathway, is a very important functional molecule in the pathogenesis of SLE. More than 90% of the homozygous C1q-deficient human subjects developed SLE-like features, and hereditary C1q deficiency is the strongest known single genetic risk factor for the

Abbreviations: SLE, systemic lupus erythematosus; RBC, red blood cell; NET, neutrophil extracellular trap; UVC, ultraviolet C; PI, propidium iodide; CIC, circulating immune complexes; AP, alkaline phosphatase; 7-AAD, 7-aminoactinomycin D; CR1, complement receptor 1; CLR, collagen-like region.

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development of SLE (Botto and Walport 2002; Pickering et al. 2000). C1q is composed of a collagenous portion and globular heads. It can engage a broad range of ligands, including immunoglobulins, prion protein, lipid A, β-amyloid fibrils, lipopolysaccharides (LPS), phospholipids (PL), DNA, apoptotic cells and some acute phase reactants (Gaboriaud et al. 2004). Recent studies showed that C1q could also exert other immuno-regulatory properties, including limiting the differentiation of monocytes into dendritic cells (Son et al. 2012), immune complex-induced interferon-α production in plasmacytoid dendritic cells (Lood et al. 2009), and participating in neutrophil extracellular trap (NET) degradation (Elkon and Santer 2012). Thus, C1q has been shown to be involved in the key processes of activation of classical complement pathway to clean immune complexes, neutralization of pathogens, maintenance of immune tolerance via clearance of early apoptotic cells, cell adhesion, etc. (Kishore and Reid 2000; Kishore et al. 2004). Previous studies suggested that apoptotic cell debris was the source of autoantigens in SLE (Muñoz et al. 2010). Apoptotic cells could become antigenic and drive autoimmunity with the occurrence of autoantibodies (Urbonaviciute et al. 2008). Rapid removal of early apoptotic cells by phagocytes prevents the release of their intracellular contents and is essential to reduce inappropriate inflammation and avoid autoimmune disorders (Savill et al. 2002). So the improper clearance of early apoptotic cells might play a central role in the development of SLE. On the other hand, immune complexes deposition in kidney was thought to be associated with lupus nephritis. The activation of classical complement pathway plays an important role in the course of immune complexes clearance and renal injury induced by immune complexes. We assumed that anti-C1q autoantibodies might be involved in the pathogenesis of lupus nephritis through interfering the above biological functions of C1q, which should be further investigated.

In this study, we aimed to investigate the influence of anti-C1q autoantibodies, affinity purified from sera of patients with active lupus nephritis, on the bio-functions of C1q, including the phagocytosis of C1q opsonized apoptotic cells by macrophages, complement-mediated binding of immune complexes to normal human erythrocytes and activation of the complement classical pathway, *in vitro*.

Materials and methods

Patients

Sera from 10 patients with active lupus nephritis with complete clinical data, and plasma exchanges from 3 out of the same 10 patients were collected upon their presentation. They fulfilled the 1997 American College of Rheumatology revised criteria for SLE (Hochberg 1997), and were confirmed serum C1q autoantibodies positive in our previous study (Fang et al. 2009). C1q levels of all the patients were also measured in our previous study (Tan et al. 2013). The clinical characteristics of the 10 patients were shown in Table 1.

Informed consent was obtained for blood sampling from each patient. The research was in compliance with the Declaration of Helsinki. Ethical approval was obtained for this study.

Affinity-purified IgG from 10 healthy blood donors were used as normal controls.

Preparation of anti-C1q autoantibodies from sera of lupus nephritis patients by microtiter plates

According to a previously described method (Radanova et al. 2012) with mild modification, microtiter plates (Costar, Corning, NY) were coated with 5 μg/ml of purified human C1q in 0.05 mol/L bicarbonate buffer (pH 9.6) for 1 h at 37 °C. After blocking with 1% BSA, the wells were incubated overnight at 4 °C with tested sera, diluted 1:50 in PBS/0.5 M NaCl (0.01 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.75 M NaCl, pH 7.4). After washing, bound antibodies were eluted with 50 μl/well of 0.1 M glycine–HCl, pH 2.8 (1 min incubation). The glycine buffer in the wells was immediately neutralized using 10 μl of 1.5 M Tris, pH 8.8 buffer. Aliquots of eluted anti-C1q autoantibodies were pooled and dialyzed extensively against PBS/0.75 M NaCl. The protein concentrations of eluted autoantibodies were determined spectrophotometrically (OD280).

Preparation of affinity-purified anti-C1q autoantibodies from plasma exchanges of patients with active lupus nephritis

1.0 mg of purified human C1q (Sigma, St. Louis, MO) was coupled to 1 ml hydrochloric acid-activated Sepharose 4B gel (GE Healthcare, Piscataway, NJ) with 0.1 mol/L $NaHCO_3$ and 0.5 mol/L NaCl (pH 8.3) as coupling buffer at room temperature for 2 h, and blocked with 0.2 mol/L glycine (pH 8.0) at room temperature for 2 h. IgG fractions were purified by protein G affinity column (GE Healthcare, Piscataway, NJ) with 0.01 mol/L phosphate-buffered saline (PBS, pH 7.4) as starting buffer, and 0.1 mol/L glycine (pH 2.7) as eluting buffer at a flow rate of 1 ml/min at room temperature. IgG was eluted and neutralized to pH 7.0 by 2 mol/L Tris–HCl (pH 9.0) immediately, and dialyzed against PBS (PBS + 0.5 M NaCl). Total IgG fractions from plasma exchanges of lupus nephritis patients were applied to the affinity column coupled with C1q with 0.01 mol/L PBS (PBS + 0.5 M NaCl) (pH 7.4) as starting buffer, and 0.05 mol/L glycine, 0.5 mol/L NaCl (pH 2.7) as eluting buffer at a flow rate of 0.1 ml/min at room temperature. Anti-C1q autoantibodies were eluted, neutralized to pH 7.0, concentrated by Vivaspin 20 (Sartorius, Gottingen, Germany), and dialyzed against PBS.

Measurement of the titer of purified anti-C1q autoantibodies by ELISA

Microtitre wells (Costar, Corning, NY) were coated with 5 μg/ml of human C1q (Calbiochem, Merck, Germany) in 0.05 mol/L bicarbonate buffer (pH 9.6) over night at 4 °C. After washed three times with PBS containing 0.1% Tween-20, the plates were blocked with 1% BSA in PBS at 37 °C for 1 h. Then microtiter wells were incubated with purified anti-C1q autoantibodies, or total IgG fractions from the same patients with a serial two-fold dilution from 10 μg/ml in PBS/0.75NaCl (0.01 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.75 M NaCl, pH 7.4) at 37 °C for 1 h. Alkaline phosphatase-conjugated anti-human IgG (Calbiochem, Darmstadt, Germany), diluted at 1/5000, was used as detection antibodies. The P-nitrophenyl phosphate (pNPP, 1 mg/ml; Sigma) was used in substrate buffer (1.0 M diethanolamine and 0.5 mM $MgCl_2$ (pH 9.8)). Optical density (OD) was measured at 405 nm.

Cell culture

The human T cell line, Jurkat, and promonocytic cell line, THP-1, (American Type Culture Collection) were cultured in RPMI 1640 supplemented with 10% fetal bovine serum at 37 °C in 5% CO_2. Cultures were established by centrifugation with subsequent resuspension at 2×10^5 cells/mL. Cells were passaged when the cell concentration reached 8×10^5 cells/mL every 2–3 days. THP-1 monocytes (2×10^5 cells/well) were stimulated with 160 nM phorbol 12- myristate 13-acetate (PMA; Sigma, St. Louis, MO) for 24–48 h in 24-well plates to induce a macrophage phenotype (Tsuchiya et al. 1982).