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Biochemical analysis of the epitope specificities of anti-C1q autoantibodies accompanying human lupus nephritis reveals them as a dynamic population in the course of the disease

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ABSTRACT

We analyzed the epitope specificities of the polyclonal anti-C1q antibodies, present in human LN sera, searching to deduce the structural characteristics of C1q associated with its transition to an autoantigen. We screened 78 serum samples from LN patients distributed in three clinical groups – non-active, moderately active and severely active. We found three classes of C1q autoepitopes: (a) neo-epitopes, exposed upon immobilization due to conformational changes; (b) epitopes formed by sequences that are brought together by the conformation of the whole molecule; (c) cryptic epitopes that become exposed only after fragmentation of C1q. The latter suggest that the immunogen involved in the initiation of anti-C1q autoantibodies might be an extrinsic molecule that shares some degree of structural similarity to C1q. None of the tested epitope specificities was associated with active LN. We found a prevalence of anti-gC1q antibodies among the non-active LN patients suggesting that they might be the fraction of the polyclonal anti-C1q, preceding the initiation of autoimmunity to C1q, or alternatively, preceding LN flare.

1. Introduction

It has been long since C1q was identified as one of the multiple autoantigens targeted by the autoantibodies [1] that are developed in the course of human autoimmune diseases like systemic lupus erythematosus (SLE), rheumatoid arthritis (RA), mixed connective tissue disease, Felty's syndrome, rheumatoid vasculitis, polyarteitis nodosa, polychondritis, Sjögren's syndrome, etc. [2–5]. Yet the immunogenic stimulus that gives rise to the formation of anti-C1q antibodies is still unknown. It has become clear by plenty of experimental data that C1q turns into an autoantigen upon immobilization [6–8], an event that occurs well outside the context of pathology whenever C1q interacts with any of its various ligands. It is assumed that the process of immobilization brings about the exposure of neo-epitopes which are recognized by the anti-C1q antibodies.

The human C1q molecule (460 kDa, 18 polypeptide chains: 6A, 6B, and 6C) is folded in a complex structure containing an N-terminal collagen-like stalk, designated collagen-like region (CLR), and six globular "heads" (gC1q) composed of the C-terminal halves of one A (ghA), one B (ghB), and one C chain (ghC). This biochemical structure underlies a modular organization [9] of the gC1q domain, being composed of three structurally and functionally independent modules – ghA, ghB and ghC, and retaining multivalency in the form of a heterotrimer. The heterotrimeric organization thus offers functional flexibility and versatility of ligand recognition to the whole C1q molecule [10]. However, this complex structure can also underline complex antigenic properties in terms of different epitopes for the anti-C1q autoantibodies to recognize and bind.

The first reported C1q autoepitopes were within CLR [11–15]. Later gC1q was also proved to contain autoepitopes [16,17]. We addressed the issue of the putative autoimmunogenicity of C1q by analyzing the epitope characteristics of anti-C1q autoantibodies present in lupus nephritis (LN) patients as the part of SLE patients whose clinical status is featured by the presence of anti-C1q [18–29]. There is also evidence that the increased serum levels of anti-C1q autoantibodies are prognostic for LN flares [2,4,16,30–36]. So we were interested as well to find out whether particular types of epitope specificities of these polyclonal anti-C1q autoantibodies were associated with a high degree of clinical activity of LN in a

Abbreviations: SLE, systemic lupus erythematosus; LN, lupus nephritis; gC1q, globular region of human C1q; gh, globular head fragment; CLR, collagen-like region.

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Table 1
General information for the LN patients.

Sex	Phase		
	Non-active (remission)	Moderately active (partial remission)	Severely active
Females	26/78	19/78	23/78
Males	7/78	3/78	0/78
Number of samples	33	22	23

search of an epitope-specific biomarker for an up-coming flare of LN.

2. Materials and methods

2.1. Buffers

The following buffers were used: sodium carbonate buffer (SC) [0.035 M $NaHCO_3$, 0.015 M Na_2CO_3, pH 9.6]; phosphate buffered saline (PBS) [0.01 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.145 M NaCl, pH 7.4]; PBS containing 0.1% Tween 20 (TPBS); phosphate buffered saline with higher salt concentration (PBS/0.75) [0.01 M Na_2HPO_4, 0.01 M NaH_2PO_4, 0.75 M NaCl, pH 7.4]; alkaline phosphatase buffer (AP) [100 mM Tris–HCl, containing 100 mM NaCl and 5 mM $MgCl_2$, pH 9.6]; elution buffer (EB) [0.1 M Gly-HCl buffer, pH 2.8].

2.2. Patients

Forty-three Bulgarian patients with previously diagnosed biopsy-proven LN who have attended for a period of two and a half years the Nephrology Clinic of University Hospital "Queen Giovanna" of the Medical University, Sofia, Bulgaria, have been enrolled in this study. The cohort consisted of 38 females (88.4%), average age 43.1 years, ranging from 24 to 73, and 5 males (11.6%), average age 32.5 years, ranging from 26 to 41. The patients were distributed in three groups according to the degree of the clinical activity of LN. The patients in complete remission were defined as non-active (na), the patients with partial remission – as moderately active (ma), and the patients with LN flare – as severely active (sa) (Table 1). Complete remission was defined as urinary protein excretion <0.5 g/day, normal urinary sediment (RBC < 5/HP, WBC < 5/HP), normal serum albumin and normal renal function. Patient's partial remission was defined as having an improvement in any of the following items: a decrease of serum creatinine to <130 mol/L for patients with a baseline serum creatinine level ≥130 mol/L but ≤260 mol/L); a decrease of serum creatinine by >50% for patients with a baseline serum creatinine level >260 mol/L; a decrease of urinary protein excretion by >50% and <3.0 g/day, with a serum albumin level ≥30 g/L and stable renal function.

During the follow-up period 18 of the patients have attended the Nephrology Clinic more than once. Multiple changes in the clinical activity of LN were observed in 8 of them (Table 2) and have been monitored by collecting serum samples. The time gaps between serum sampling referring to one individual varied from approximately 1 and half to 19 months.

2.3. Sera

Sera from patients with lupus nephritis were obtained from peripheral blood. For the duration of the follow-up 78 serum samples were collected. All sera were stored at −20 °C until use.

Pooled sera from 56 healthy donors were used as a control [17].

Table 2
Schematic presentation of the analyzed LN serum samples.

43 Patients (78 serum samples)		
25 Patients (with single serum samples)	18 Patients (with multiple serum samples)	
	8 Patients (with changes in the clinical activity of LN)	10 Patients (without changes in the clinical activity of LN)
p2na, p3na, p4na, p5na, p10na, p11na, p12na, p13na, p15na, p16na, p17na, p19na, p20na, p21na, p22na, p23na	p7s1na, p7s2na, p7s3ma, p7s4na p8s1ma, p8s2na, p8s3na p14s1na, p14s2ma p24s1sa, p24s2sa, p24s3ma, p24s4na, p24s5ma p25s1sa, p25s2ma, p25s3na p26s1ma, p26s2sa p32s1sa, p32s2ma, p32s3ma p34s1ma, p34s2ma, p34s3ma, p34s4sa, p34s5sa	p1s1na, p1s2na p6s1na, p6s2na p9s1na, p9s2na, p9s3na p18s1na, p18s2na p28s1ma, p28s2ma, p28s3ma, p28s4ma p29s1ma, p29s2ma p36s1sa, p36s2sa, p36s3sa, p36s4sa, p36s5sa p38s1sa, p38s2sa p42s1sa, p42s2sa p43s1sa, p43s2sa
p27ma, p30ma, p31ma, p33ma		
p35sa, p37sa, p39sa, p40sa, p41sa		

2.4. Expression and purification of the recombinant globular head regions of the three chains of C1q

The recombinant C1q globular head regions designated ghA, ghB and ghC were expressed as fusion proteins with MBP in *Escherichia coli* BL21(DE3) and purified, as described previously [17].

2.5. Preparation of IgG from pooled autoimmune LN sera

Aliquots from all LN sera were pooled, dialyzed overnight against PBS and subjected to affinity chromatography on HiTrap Protein-G (Amersham). The bound IgG was eluted with EB in 1 mL fractions which were immediately neutralized with an appropriate amount of 1.5 M Tris, pH 8.8. All aliquots of IgG containing fractions were pooled and dialyzed extensively against PBS. This IgG preparation was labeled "IgG/LN".

2.6. Pepsin digestion of native C1q for producing of CLR

The CLR was produced according to Reid [37]. Briefly, the native C1q (Sigma–Aldrich) (1 mg/mL) was dialyzed against 100 mM-sodium acetate buffer (pH 4.45) and was incubated with pepsin (Sigma) (the protein/enzyme ratio is 30:1) at 37 °C for 24 h. CLR was purified by molecular-sieve chromatography using Superose 12 column (Pharmacia).

2.7. ELISA assays for the recognition of C1q, CLR, ghA, ghB and ghC by autoantibodies from LN patients' sera

Microtiter wells were coated with test-antigens in SC buffer for 1 h at 37 °C, blocked with 1% BSA for another 1 h at 37 °C and then incubated overnight at 4 °C with the control, or with sera of LN patients, diluted 1:100 in PBS/0.75. The immobilized anti-C1q antibodies were detected by rabbit polyclonal anti-human IgG-alkaline phosphatase conjugate (IgG-AP) (DAKO). The enzyme reaction was carried out with *p*-nitro phenyl phosphate dissolved in AP. The absorbance was read at 405 nm. Following each period