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Genetic analysis of complement C1s deficiency associated with systemic lupus erythematosus highlights alternative splicing of normal *C1s* gene

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Abstract

Deficiencies of complement proteins of the classical pathway are strongly associated with the development of autoimmune diseases. Deficiency of C1r has been observed to occur concomitantly with deficiency in C1s and 9 out of 15 reported cases presented systemic lupus erythematosus (SLE). Here, we describe a family in which all four children are deficient in C1s but only two of them developed SLE. Hemolytic activity mediated by the alternative and the lectin pathways were normal, but classical pathway activation was absent in all children's sera. C1s was undetectable, while in the parents' sera it was lower than in the normal controls. The levels of C1r observed in the siblings and parents sera were lower than in the control, while the concentrations of other complement proteins (C3, C4, MBL and MASP-2) were normal in all family members. Impairment of C1s synthesis was observed in the patients' fibroblasts when analyzed by confocal microscopy. We show that all four siblings are homozygous for a mutation at position 938 in exon 6 of the C1s cDNA that creates a premature stop codon. Our investigations led us to reveal the presence of previously uncharacterized splice variants of C1s mRNA transcripts in normal human cells. These variants are derived from the skipping of exon 3 and from the use of an alternative 3′ splice site within intron 1 which increases the size of exon 2 by 87 nucleotides.

Keywords: Complement; Human; Immunodeficiency diseases; Autoimmunity; Systemic lupus erythematosus

1. Introduction

The classical pathway of the complement system is activated mainly by the binding of C1q to IgM or IgG bound to specific epitopes. C1q interacts with two distinct serine proteases, C1r and C1s, to form the C1 complex ($C1qC1r_2C1s_2$). C1r and C1s are glycoproteins of approximately 85 kDa. They are activated by cleavage into A (58 kDa) and B (27 kDa) chains (Kusumoto et al., 1988). After C1 binding to the immune complex, C1r auto-activates and then, cleaves C1s, which in turn cleaves C4 (into C4a and C4b) (Patrick et al., 1970) and C2 (into C2a and C2b) (Thielens et al., 1982) to form the classical pathway C3 convertase (C4b2a). The classical pathway will not be activated if any of the three sub-components C1q, C1r or C1s are absent or non-functional. So far, a worldwide total of 57 individuals have been reported with C1 deficiencies (Dragon-Durey et al., 2001; Pickering et al., 2000). They are highly susceptible to infections by microorganisms and have greater risk in developing autoimmune diseases such as systemic lupus erythematosus (SLE) (Pickering et al., 2000).

It has been demonstrated that C1r and C1s are expressed in the liver, the kidney and cells of the nervous system (Kusumoto et al.,

Abbreviations: SLE, systemic lupus erythematosus; MBL, mannose-binding lectin; MASP, MBL-associated serine protease.

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1988), in fibroblasts (Reid and Solomon, 1977), in macrophages (Muller et al., 1978) in chondrocytes (Nakagawa et al., 1997) and in monocytes (Chevailler et al., 1994). The concentrations of C1r and C1s in normal adult serum are 48 and 34 μg/ml, respectively. C1r, having only one known substrate (C1s) is the most selective serine protease known, while C1s may play roles outside the complement system such as cleaving the insulin-like growth factor-binding protein-5 which regulates insulin-like growth Factor I (Busby et al., 2000). C1s is also reported to cleave type I and type II collagen (Yamaguchi et al., 1990) and MHC class I antigens (Eriksson and Nissen, 1990).

C1r and C1s are highly homologous in domain structure and function, and both *C1r* (11 kb) and *C1s* (10.5 kb) genes contain 12 exons (Nakagawa et al., 2003; Tosi et al., 1989). The human genes are located next to one another in a tail-to-tail arrangement (Kusumoto et al., 1988) in the p13 region of the short arm of the chromosome 12 (Nguyen et al., 1988). Tosi et al. (1987) demonstrated the existence of three different C1s mRNA transcripts, however the specific molecular differences between these transcripts have not been described. Possibly because of proximity of these genes, deficiency of C1s is quite often associated with the concomitant lack of C1r. Only three out of fifteen cases of deficiencies in C1r and C1s have been investigated at the molecular level (Dragon-Durey et al., 2001; Endo et al., 1999; Inoue et al., 1998), all of which presented mutations in *C1s*, but not in *C1r*.

In the present study, we report the first Brazilian cases of C1s deficiency with concomitantly low levels of C1r. Of the four deficient siblings, two had developed SLE. We describe the molecular basis of this deficiency and demonstrate the existence of specific and previously uncharacterized splice variants of C1s mRNA transcripts in normal human cells.

2. Patients and methods

2.1. Case report

Patient IV-1 is a 25-year-old female who presented recurrent infections (several episodes of pneumonia, septic arthritis and sinusitis) and SLE manifestations (according to the criteria defined by the American College of Rheumatology (Hochberg, 1997; Tan et al., 1982) since the age of 7: arthritis, proteinuria (>0.5 g/day), positive anti-nuclear (ANA) and anti-ribonucleoprotein (anti-Sm) antibodies. She also presented deposition of IgG and C1q on the glomeruli determined by immunofluorescence. At the present moment, she is well while still being treated with methotrexate (10 mg/week) and prednisone (5 mg/day). Her 24-year-old brother (IV-2) also presented SLE (arthritis, photo-sensibility, positive ANA and anti-Sm antibody) at the age of 13 years. He is also well at the moment and is taking no medication. So far, the other two brothers IV-3 (20-year-old) and IV-4 (10-year-old) are healthy and both have not presented any clinical symptoms of SLE. The parents (II-1 and II-2) are first-degree consanguineous cousins and are both healthy (Fig. 1a). Blood samples and skin biopsies were obtained with the consent of patients and their parents and this study has been approved by our Institutional Ethical Committee.

2.2. Antibodies

Rabbit anti-sheep erythrocyte antiserum was produced as described (Isaac and Mariano, 1988). Goat antibodies against human C1q, C1s, C4, C3, Factor H, Factor I and Factor B were purchased from EMD Biosciences, San Diego, CA, USA. Anti-goat IgG conjugated with alkaline phosphatase (EMD Biosciences), anti-human beta-actin and anti-IgG-fluorescein isothiocyanate (FITC) conjugated (Sigma–Aldrich Co., St. Louis, MO, USA) and anti-human C1r (Serotec, Oxford, UK) were also employed.

2.3. Functional complement assays

Fresh blood samples were withdrawn from controls, patients and their family and kept at 0 °C until centrifugation. The sera were then harvested, aliquoted, stored at −80 °C, and thawed immediately before use. Complement alternative pathway-dependent hemolytic activity (AP50) was measured according to Servais et al. (1991) using rabbit erythrocytes at 1.5×10^8/ml prepared in GVB-EGTA-Mg^{++} [GVB (143 mM NaCl, 1 mM sodium barbital, 2.5 mM barbituric acid, pH 7.2) supplemented with 1 g/l gelatin, 10 mM EGTA (pH 7.4) and 5 mM $MgCl_2$]. To measure complement activity mediated by the classical pathway (CH50) we used 1.5×10^8 sheep erythrocytes/ml suspended in EA buffer: 4 mM sodium barbital, 145 mM NaCl, 0.83 mM $MgCl_2$, 0.25 mM $CaCl_2$, pH 7.2. Cells were then treated with 3% rabbit anti-sheep erythrocyte antiserum (EA) for 30 min at

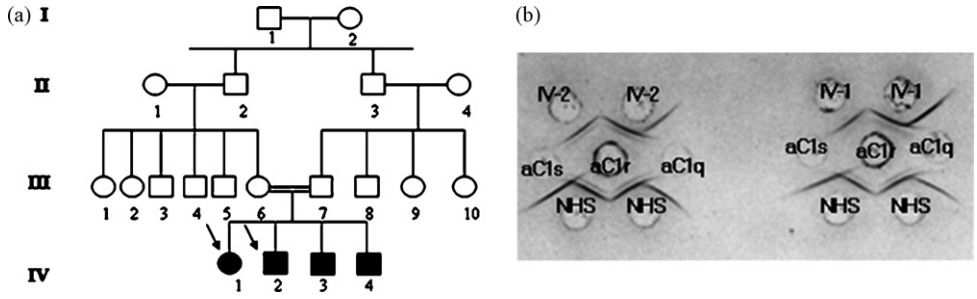

Fig. 1. C1s and C1r deficient family. (a) Heredogram of the family. Squares: male, circles: female. Double line represents consanguineous parents. Roman numbers indicate the generations and Arabic numbers represent each individual. Dark squares and circle represent the C1s/C1r deficient individuals of this family and arrows indicate the SLE affected patients. (b) Analysis of the presence of C1 complex proteins in the patients' sera by double immunodiffusion. IV-1: patient 1; IV-2: patient 2; NHS: normal human serum; center: anti-C1r.