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CASE REPORT

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Behçet's disease associated with complement component 9 (C9) deficiency

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Abstract Behçet's disease is a multisystem inflammatory disorder with unknown etiology. It has been shown that the titer of plasma complement component 9 (C9) is a good indicator of the disease activity. Therefore, the involvement of C9 in the pathogenesis of Behçet's disease has been suggested. We report a case of Behçet's disease associated with complete C9 deficiency (C9D) carrying the homozygous nonsense mutation at Arg-95 of C9 (R95X). The patient presented the typical characteristics of Behçet's disease, such as uveitis, recurrent oral aphthae and genital ulcers, and arthritis, suggesting that C9 does not play an essential role in the pathogenesis of Behçet's disease.

Key words Behçet's disease · Complement component 9 (C9) · Complement deficiency

Introduction

Behçet's disease is a multisystem inflammatory disorder with unknown etiology which is characterized by recurrent aphthous oral and genital ulcers, uveitis, cutaneous manifestations such as erythema nodosum, and arthritis. Acute-phase proteins, C-reactive protein (CRP), and complement component 9 (C9) have been shown to be significantly increased in Behçet's disease.[1–3] Subsequently, plasma C9 was shown to be a better indicator for disease activity of Behçet's disease by using an immunoradiometric assay with two monoclonal antibodies.[4] The significance of these findings in the pathogenesis of Behçet's disease is not clear, but there might be a positive contribution by C9 in the pathogenesis of Behçet's disease. The involvement of the membrane attack complex in the lysis of the affected cells and recurrent oral ulcers has also been suggested. In fact, deposition of complement as well as IgG and IgM have been demonstrated in the biopsies of the aphthous regions from patients with Behçet's disease.[5]

We here report a case of Behçet's disease associated with complete deficiency of plasma C9. Genetic analysis of the C9 deficiency revealed that the patient was homozygous for the nonsense mutation at Arg-95 (R95X), which is the most common mutation of C9 deficiency (C9D) in Japan.[6] The patient presented with the typical phenotype of Behçet's disease including oral and genital ulcers, uveitis, and arthritis. The involvement of C9 in the pathogenesis of Behçet's disease is discussed.

Case report

In February 1991, a 53-year-old woman was referred to the rheumatology branch of Kyushu University Hospital complaining of polyarthralgia and fogginess of vision. The patient had been suffering from recurrent painful oral aphthae since her early twenties. Genital ulcers had occasionally developed since her late twenties. The visual disturbance began in July 1990, and was diagnosed as bilateral uveitis by her ophthalmologist. Combined treatment with topical and oral applications of prednisolone was effective for her visual symptoms. She then gradually developed polyarthralgia, which was not relieved by the administration of loxoprofen, a nonsteroid anti-inflammatory drug (NSAID). She was admitted to this hospital in April 1991.

On admission, a physical examination revealed that her visual acuity was impaired to 20/200 in both eyes. Both of her knee joints, ankle joints, elbow joints, and wrist joints, as well as proximal interphalangial (PIP) and metacarpal (MC) joints were tender but not swollen. Cutaneous lesions,

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such as pustules and erythema nodosum, were not observed. Pathergy to needle prick was negative. Neurological examination showed no abnormal findings. Laboratory studies showed slight leukocytopenia (white blood cell count of 3500/μl; stab 1%, seg 48%, eosino 3%, lymph 37%, mono 11%) with normal hemoglobin and platelet count. The leukocyte count was constant at around the lower limit of the normal value during the admission period. A bone marrow study showed a nucleated cell count (NCC) of 142000/μl, a megakaryocyte count of 62.5/μl, and a myeloid/erythroid ratio of 1.48 with normal granulopoiesis. The reason for the mild leukocytepenia was not clear. Liver function and renal function were normal on admission, but the titers of transaminases (AST and ALT) showed occasional slight elevation just above the upper limit. She had no history of blood transfusion. Antibodies for hepatitis virus type B and type C were negative. The serum albumin level was 4.2 g/dl (normal range 4.0–5.0) and the cholesterol level was 157 mg/dl (normal range 120–150). A hepaplastin test was normal. Abdominal ultrasonography showed a bright liver and mild splenomegaly. The value of the erythrocyte sedimentation rate (ESR) was 20 mm/h and 49 mm/2 h. Immunoglobulin titers were all within the normal range. The titers of antibodies against streptococcus were within the normal range. Assays for CRP, antinuclear antibody (ANA), and the rheumatoid factor were all negative. Serum concentration of complement components C3 and C4 were within the normal range, 58 mg/dl (normal range 38–96) and 28 mg/dl (normal range 11–46). However, serum total hemolytic complement activity (CH50) had decreased to 14 U/ml (normal range 30–50), which at the time was thought to be cold activation of complement. HLA type was A26, AW33, B44, B15, CW3. Roentgenograms of her joints and bone scintigraphy were normal, but Ga scintigraphy showed a slight elevation of uptake in her bilateral knees and ankles. A diagnosis of Behçet's disease was made.

In addition to loxoprofen, the administration of 0.5 mg/day colchicine was started, which was later increased to 1 mg/day. The patient got some relief from her symptoms and was discharged from hospital in June 1991. The patient then attended the outpatient clinic once a month. She had been complaining of recurrent oral aphthae and genital ulcers, but CRP was always negative. Since the CH50 level of her sera was always between 10 and 20 U/ml, complement component 9 (C9) deficiency was suspected and the serum titer of C9 was studied. The C9 concentration was <0.5 mg/dl (normal range 2.7–7.3). Further examination to detect the molecular basis for the C9 deficiency (C9D) was undertaken. DNA was purified from the peripheral blood mononuclear cells (PBMCs) as described.[7] Polymerase chain reaction (PCR)/single-strand conformation polymorphism (SSCP) was performed for exon 4 of the C9 gene because 90% of Japanese C9D is caused by a nonsense mutation of Arg at amino acid residue 95 in the exon 4 (R95X).[6] As shown in Fig. 1a, the PCR product of this patient (lane 3) displayed two bands migrating differently from those of a normal control (lane 1). The migration pattern of the present case was identical with that of a C9D subject carrying homozygous R95X (lane 2). The subject had already been shown to carry the heterozygous R95X mutation, as displayed in the mixed pattern of a homozygous C9D subject and a normal control (lane 4). Subsequent nucleotide sequencing confirmed that our patient was carrying homozygous R95X mutation in exon 4 of the C9 gene, which had caused the complete loss of serum C9 (Fig. 1b).

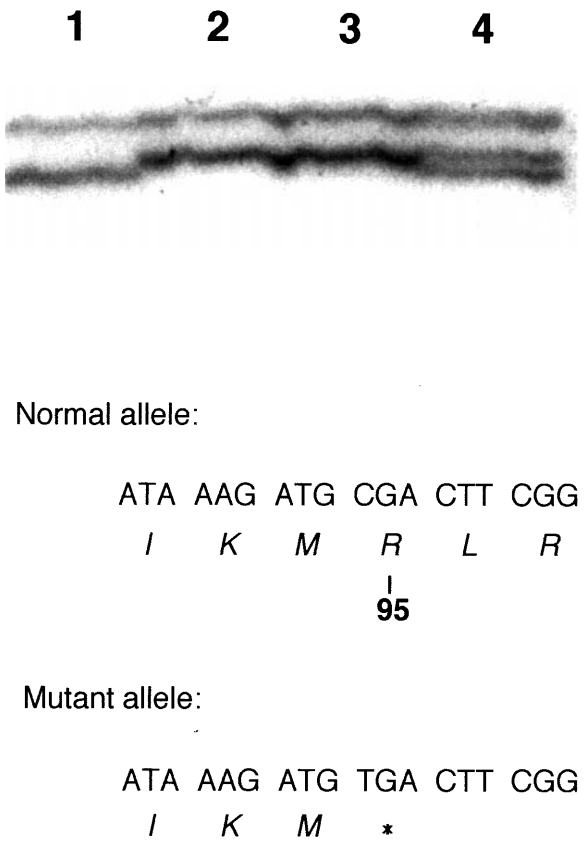

Fig. 1. a PCR/SSCP analysis of the patient. PCR products amplified from genomic DNA using exon 4-specific primers were electrophoresed on 5% polyacrylamide gel containing 5% glycerol at 25°C. Lane 1 is a normal control. Lane 2 is a C9D subject homozygous for the R95X mutation in the C9 gene, and lane 4 is a C9D subject heterozygous for the R95X mutation. Both have been reported previously.[6] Lane 3 is our patient carrying both Behçet's disease and C9D. **b** Nucleotide sequences and deduced amino acid sequence (*one-letter code, italic*) around Arg at amino acid residue 95 (Arg-95). The C to T transition of the first nucleotide of the codon for Arg-95 resulted in the generation of a stop codon (*) and the truncation of the C9 protein

Discussion

Inherited C9D is one of the most common genetic disorders in Japan, with an incidence of one homozygote in 1000, whereas only a few C9D cases have been reported in Caucasians.[8,9] Although C9D subjects are usually healthy, they carry a significantly higher risk of developing meningococcal meningitis than normal controls.[10] The genetic bases of Japanese C9D have recently been clarified by our group in

their studies of 10 unrelated C9D subjects.[6] Of 20 unrelated null alleles studied, 18 (90%) were carrying C to T transition of the first nucleotide of the codon CGA for Arg at amino acid residue 95 (Arg^{95}). The mutation generates a stop codon (R95X), resulting in the truncation of C9 protein. Another putative molecular defect was the amino acid substitution from Cys to Tyr at amino acid residue 507 (C507Y), which was observed in one allele (5%), and which causes the disruption of the intramolecular disulfide bond, while the abnormality of the remaining one allele was not determined. Our case was carrying the homozygous mutant allele, R95X, which is frequently identified in Japanese C9D.

A deficiency of the early components of complement system is frequently associated with systemic lupus erythematosus,[11] but the association of autoimmune diseases with the deficiency of late components of complement, including C9D, is rarely described. We here report the first case of Behçet's disease associated with C9D. This case was a typical type of Behçet's disease, since the patient presented with uveitis and recurrent oral and genital ulcers.

There has been some debate about whether C9 is directly involved in the pathogenesis of Behçet's disease or is induced nonspecifically as a result of inflammation, because plasma C9 levels have been reported to be a good indicator of disease activity.[4] Our case provided some insights into this discussion. Because the patient developed typical features of Behçet's disease in the absence of plasma C9, C9 might not be essential for the inflammation in Behçet's disease. This is in contrast to the case of paroxysmal nocturnal hemoglobinuria (PNH). PNH is an acquired hemolytic disease in which erythrocytes as well as other cells are incapable of expressing glycosylphosphatidylinositol (GPI)-anchored complement regulatory proteins such as decay accelerating factor (DAF) and homologous restriction factor (CD59), resulting in the uncontrolled deposition of membrane attack complex on erythrocytes and hemolysis.[12] In a patient affected by both PNH and C9D, there were no episodes of massive spontaneous hemolysis,[13] suggesting the essential role of C9 in hemolysis of erythrocytes in vivo. Several cases of C9D have been reported to be associated with autoimmune disorders, such as systemic lupus erythematosus (SLE).[14,15] However, considering that C9D is a common genetic disorder in Japan and is not associated with increased risk of infection except in the case of meningococcal meningitis, the involvement of C9D in the pathogenesis of immunological disorders might not be significant.

Our patient is the first case where Behçet's disease was associated with C9D. Although C9 has been implicated in the pathogenesis of Behçet's disease, typical symptoms of Behçet's disease, including recurrent oral and genital aphthae and uveitis, were present in this case. Therefore, it is likely that C9 is not positively involved in the development of Behçet's disease.

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