

Complement factor 2 deficiency: a clinical and serological family study

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Abstract

Inherited complement deficiencies are associated with a variety of connective tissue diseases. A family with inherited deficiency of complement factor 2 (C2) is described in which two family members with homozygous C2 deficiency developed cutaneous vasculitis and sicca syndrome. The other family members had heterozygous C2 deficiency and each member had the HLA-A25, B18, DR2 (w15) haplotype. The mother had seropositive rheumatoid arthritis. Further studies showed the presence of cryoglobulins, antibodies against endothelial cells, and anticardiolipin antibodies.

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Complement factor 2 (C2) deficiency is the most common inherited complement deficiency. In white populations heterozygous C2 deficiency occurs in 1-2% and homozygous deficiency in 1 in 10 000 subjects.^{1 2} Agnello described the occurrence of autoimmune or lymphomatous diseases in 61% of patients with homozygous and 13% of patients with heterozygous C2 deficiency;¹ systemic/discoid lupus erythematosus, lupus-like disease, Henoch-Schönlein purpura, or polymyositis are the most commonly described diseases.³ We describe here two family members with homozygous C2 deficiency and three other members with heterozygous C2 deficiency.

Case reports

CASE NO 1

A 24 year old white woman presented with an eight year history of recurrent sore throats associated with fevers, loin pain, haematuria, arthritis of the wrists and ankles, and vasculitic rashes on her legs, buttocks, and elbows (fig 1). Schirmer's test showed less than 2 mm of wetness (normal >15 mm). Full blood count and plasma viscosity measurements were normal and the erythrocyte sedimentation rate (ESR) was 71 mm/in the first hour (Westergren). Renal function was normal but a biopsy sample showed a mild mesangial proliferative glomerulonephritis with IgA deposition. A minor salivary gland biopsy sample showed a mild non-specific chronic inflammatory infiltrate. Skin biopsy showed leucocytoclastic vasculitis. Serum IgA concentrations were increased at 4.2 g/l (normal 0.5-2.0 g/l). Serology, including antinuclear antibodies, antibodies to double stranded DNA, antibodies to extractable nuclear antigen,

neutrophil cytoplasmic antibody levels, antibodies to streptolysin O, and viral serology including hepatitis A and B were negative or normal. Immune complex concentrations were increased at 147 mg IgG/l, (normal <49). Rheumatoid factor was positive with a Rose-Waaler titre of >1/4096. IgG anticardiolipin antibodies were positive at 14.4 U/ml (normal <5.0). Cryoglobulins were detected with a cryocrit of 2%. The precipitate dissolved completely on warming to 37°C and reappeared on cooling to 4°C. Immunofixation of the cryoprotein showed the major component to be polyclonal IgG λ (fig 2).

Total haemolytic complement pathway concentrations were undetectable but alternate pathway, C1q, C1r, C1s, C3, and C4 concentrations were normal. Concentrations of C2 were undetectable, compatible with homozygous C2 deficiency.

Her disease flared intermittently and the rheumatoid factor, immune complex, and cryoprecipitate concentrations followed the disease activity. Treatment with prednisolone by mouth (5-10 mg daily), azathioprine (100 mg

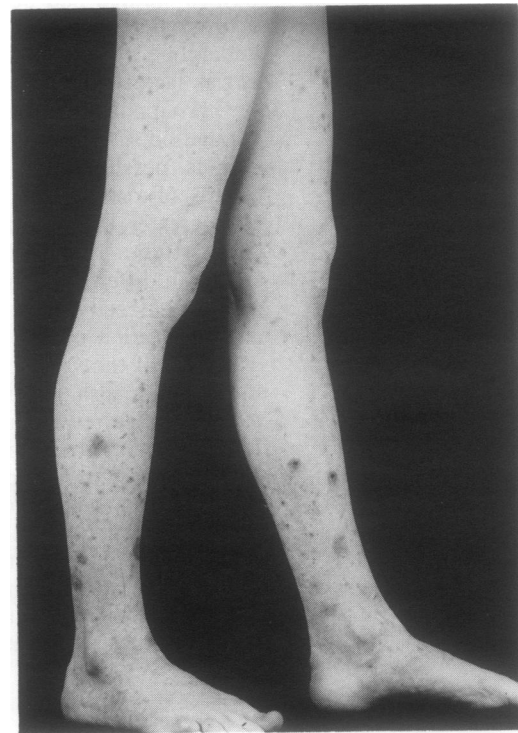


Figure 1 Clinical distribution of vasculitic purpuric rash in the proband. The rash started as small raised maculopapular lesions, some of which progressed to necrosis and ulceration followed by healing.

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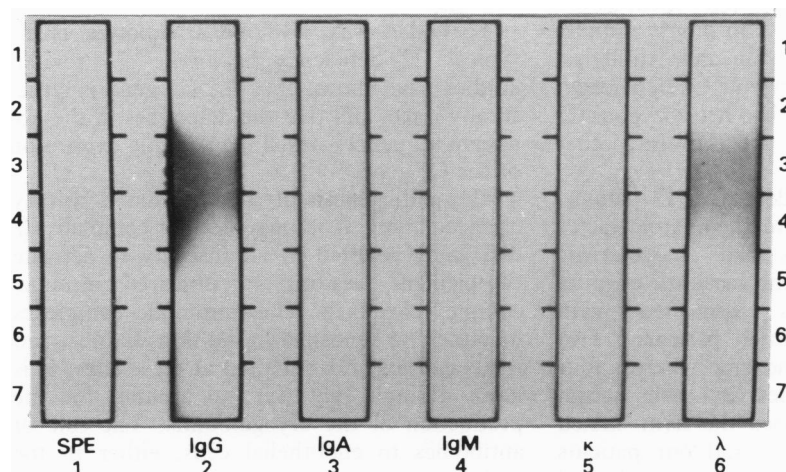


Figure 2 Immunofixation showing a broad band that was mainly IgG λ . SPE=serum protein electrophoresis of normal controls.

daily), and regular plasma exchange using fresh frozen plasma reduced the frequency of the flares.

CASE NO 2

At the age of 30 years the proband's brother noticed dry eyes, a purpuric rash on his legs, transient joint pain, and occasional abdominal pain especially after exercise or alcohol ingestion. Schirmer's test showed less than 5 mm wetness. The rash was maculopapular, similar to his sister's rash, and a biopsy sample showed a leucocytoclastic vasculitis with IgA deposition. A cryoprecipitate of 1% was found which disappeared on warming to 37°C and reappeared on cooling to 4°C. A full blood count and biochemistry were normal and the ESR was 16 mm in the first hour. Serum immunoglobulin concentrations, antinuclear antibodies, antibodies to double stranded DNA, and antibodies to extractable nuclear antigens were normal or negative. Rheumatoid factor was positive with a Rose-Waaler titre of 1/64 and immune complexes were increased at 371 mg IgG/l. Total haemolytic complement concentrations were 3% and C2 was undetectable, compatible with homozygous C2 deficiency. All other complement component concentrations were normal. IgG anticardiolipin

antibodies were positive at 10.5 U/ml. He continues to have occasional episodes of rash and arthralgias, but is not receiving treatment.

Family study

The other three adult family members were traced and tissue typing and complement studies were performed. The two homozygous C2 deficient siblings described here who had cutaneous vasculitis were homozygous for the HLA-A25, B18, DR2 (w15) haplotype. Their parents and another brother were noted to have heterozygous C2 deficiency and were also heterozygous for the HLA-A25, B18, DR2 (w15) haplotype. The heterozygous brother and father were well and had no clinical findings of note. The mother had seropositive erosive rheumatoid arthritis (RA) and was receiving low dose prednisolone and non-steroidal anti-inflammatory drugs (NSAIDs). The C4 loci were analysed by restriction fragment length polymorphism and these studies showed that the father and the heterozygous C2 brother had a haplotype consisting of one C4A gene and a duplicated C4B locus. Restriction fragment length polymorphism analysis also showed that the C2 gene in the homozygous C2 deficient patients was normal. The table summarises these findings.

Further laboratory studies

The finding of low levels of anticardiolipin antibodies suggested binding of the cryoprecipitate to cardiolipin giving a false positive result. The assay was therefore repeated with the serum warmed to 37°C to dissolve the cryoprecipitate. The sample was then cooled to 4°C and centrifuged at 5000 g to remove the cryoprecipitate and the assay was repeated. In serum samples from the proband and her brother the anticardiolipin antibody reactivity disappeared following the removal of the cryoprecipitate. None of the other family members had anticardiolipin antibodies.

Serum samples from all the family members were also tested for antibodies against endothelial cells using cultured endothelial cells in a cellular enzyme linked immunosorbent assay (ELISA) as described previously.⁴ The proband had a binding index of 69% for antibodies against endothelial cells and her homozygous C2 deficient brother had a binding index of 48% (normal <40%). After removal of the cryoprecipitate as described earlier, there was a reduction in the binding index to 56 and 36% respectively. All the heterozygous C2 deficient family members were negative for antibodies against endothelial cells.

The three heterozygous family members did not have cryoglobulins in their serum samples and were negative for rheumatoid factor, except for the mother.

Discussion

The index patient had vasculitic purpura, IgA mesangial nephritis, arthritis, and sicca syndrome. Her brother also had vasculitic purpura

Class I, II, and III specificities. HLA-A and B were defined serologically and DR and C4 were determined by restriction fragment length polymorphism analysis. C4 polymorphisms are described in terms of restriction fragment lengths: each number (7, 6, 5.4) represents the fragment length in kilobases and corresponds to the different C4 genes. Thus 7 kb=C4A and 6 and 5.4 kb=C4B genes

	C2 concentrations (% of normal)	HLA-A	HLA-B	C4	HLA-DR
Mother	50	25 3	18 60	7-5.4 7-5.4	2 (w15) 3
Father	50	3 25	14 18	7-6-6 7-5.4	6 (w14) 2 (w15)
Brother	50	3 25	14 18	7-6-6 7-5.4	6 (w14) 2 (w15)
Brother	0	25 25	18 18	7-5.4 7-5.4	2 (w15) 2 (w15)
Sister	0	25 25	18 18	7-5.4 7-5.4	2 (w15) 2 (w15)

with IgA deposition in the skin biopsy sample, sicca syndrome, abdominal pain, and arthralgias. These patients had homozygous C2 deficiency. The other family members had heterozygous C2 deficiency and were well apart from the mother who had RA.

In 1966 Meltzer *et al* described 12 patients with cryoglobulinaemia and rheumatoid factor activity.⁵ These patients had characteristic clinical features of lower limb vasculitic purpura with arthralgias/arthritis associated with glomerulonephritis in three patients. Two patients had Sjögren's syndrome whereas four had antinuclear antibodies in their serum samples. These authors used the term 'mixed essential cryoglobulinaemia' and our patients have almost identical clinical and serological features. The finding of polyclonal IgG λ in the proband's cryoprecipitate is unusual. Mixed IgG and IgM κ and λ globulins were the most common components of the cryoglobulins in the original series, though Meltzer *et al* did describe two patients with polyclonal IgG λ in the cryoglobulins.⁵

The two homozygous C2 deficient members with cutaneous vasculitis also had antibodies to endothelial cells. These antibodies have been associated with cutaneous vasculitis and nephritis in other connective tissue diseases, but have not been described with cryoglobulinaemic vasculitis.^{4 6}

The presence of low levels of anticardiolipin antibodies which disappeared on removal of the cryoprecipitate has implications for other patients with cryoglobulins in whom these antibodies may be found. It is possible that anticardiolipin reactivity was present in the serum samples but was confined to the immunoglobulins within the cryoprecipitate. Neither of these patients had any of the clinical features usually associated with anticardiolipin antibodies. Similar considerations apply to patients tested for antibodies to endothelial cells where some of the endothelial reactivity may be due to immunoglobulins within the precipitate. Previous work has shown that anticardiolipin antibodies and antibodies to endothelial cells have distinct specificities.⁷

The HLA-A25, B18, DR2 haplotype is the typical C2 deficiency haplotype.^{8 9} Previous studies have shown that the C2 gene is structurally intact and that the defect lies in the upstream sequences which regulate the expression of the C2 gene.^{10 11}

The pathogenesis of this disorder is worthy of speculation. It is possible that complete C2 deficiency resulted in an inability to activate complement, leading to impaired immune complex clearance. These immune complexes may then be deposited in the skin, kidney, and joints causing inflammation at these sites. It is more difficult, however, to account for the production of the cryoglobulins. The role of antibodies to endothelial cells, either in the pathogenesis or as an epiphenomenon in the development of vasculitis and nephritis, remains to be clarified.

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- 1 Agnello V. Complement deficiency states. *Medicine (Baltimore)* 1978; 57: 1-23.
- 2 Glass D, Raum D, Gibson D, Stillman J S, Schur P H. Inherited deficiency of the second component of complement. *J Clin Invest* 1976; 58: 853-61.
- 3 Schur P H. Inherited complement component abnormalities. *Ann Rev Med* 1986; 37: 333-46.
- 4 D'Cruz D P, Houssiau F A, Ramirez G, et al. Antiendothelial cell antibodies in systemic lupus erythematosus: a potential marker for nephritis and vasculitis. *Clin Exp Immunol* 1991; 85: 254-61.
- 5 Meltzer M, Franklin E C, Elias K, McCluskey R I, Cooper N. Cryoglobulinaemia—a clinical and laboratory study. *Am J Med* 1966; 40: 837-56.
- 6 Heurkens A H M, Hiemstra P S, Lafeber G J M, Daha M R, Breedveld F C. Antiendothelial cell antibodies in patients with rheumatoid arthritis complicated by vasculitis. *Clin Exp Immunol* 1989; 78: 7-12.
- 7 Cervera R, Khamashta M A, Font J, et al. Antiendothelial cell antibodies in patients with the antiphospholipid syndrome. *Autoimmunity* 1991; 11: 1-6.
- 8 Awdeh Z L, Raum D D, Glass D, et al. Complement—human histocompatibility antigen haplotypes in C2 deficiency. *J Clin Invest* 1981; 67: 581-3.
- 9 McCarty D J, Tan E M, Zvaifler N J, Koethe S, Duquesnoy R J. Serologic studies in a family with heterozygous C2 deficiency. *Am J Med* 1981; 71: 945-8.
- 10 Cole F S, Whitehead A S, Auerbach H S, et al. The molecular basis for genetic deficiency of the second component of human complement. *N Engl J Med* 1985; 313: 11-6.
- 11 Pariser K M, Raum D, Berkman E M, Alper C A, Agnello V. Evidence for a silent or null gene in hereditary C2 deficiency. *J Immunol* 1978; 121: 2580-1.