Case report

Terminal complement component deficiencies and rheumatic disease: development of a rheumatic syndrome and anticomplementary activity in a patient with complete C6 deficiency

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SUMMARY Hereditary deficiencies of early complement components have usually been associated with the development of rheumatic diseases like systemic lupus erythematosus (SLE), while terminal component deficiency is well known to predispose to recurrent neisserial infection. In contrast, only recently have patients been reported with rheumatic disease and hereditary deficiency of a terminal component. The clinical syndrome in these patients has been characterised as 'SLE-like'. We describe here a third patient with complete C6 deficiency and systemic rheumatic illness characterised by fever, anaemia, lymphadenopathy, hepatosplenomegaly, episcleritis, and asymmetric arthritis. After blood transfusion her serum became anticomplementary; IgG antibody to human C6 was found to be the cause of anticomplementary activity. Persistent absence of C6 in this patient and production of anti-C6 antibody after antigenic challenge indicate hereditary C6 deficiency. This case supports an association between hereditary deficiency of a terminal complement protein and the development of systemic rheumatic disease.

Key words: hereditary complement (C6) deficiency, rheumatic illness, post-transfusion anti-C6 antibody.

Hereditary deficiencies of complement proteins involved in classical pathway activation (C1q, C1r, C1s, C2, C4, and C1 INH) have been associated with the development of rheumatic diseases, especially systemic lupus erythematosus (SLE) or an SLE-like syndrome.¹⁻³ While infections may be increased in some patients with these deficiencies, individuals with hereditary deficiency of C3 or C3b inactivator (I) are particularly at risk for severe bacterial infection.¹⁻³ Hereditary deficiency of a terminal complement protein (C5, C6, C7, C8, C9) has also been associated with bacterial sepsis; however,

severe infections in these persons are usually caused by *Neisseria meningitidis* or *N. gonorrhoeae*.¹⁻³ Several recent reports indicate that a terminal complement deficiency may also be associated with rheumatic illness.¹¹⁻¹⁶ Zeitz *et al.* noted SLE or an SLE-like syndrome in 7% of individuals with hereditary C5, C7, C8, or C9 deficiency.¹⁶ Tedesco *et al.* reported complete C6 deficiency in association with an illness suggestive of SLE,¹⁴ and Trapp *et al.* described discoid lupus erythematosus and Sjögren's syndrome in a patient with no detectable C6.¹⁵

We report here an additional patient with C6 deficiency and a rheumatic syndrome. This case provides further evidence that lack of an intact terminal complement pathway may predispose to the development of a rheumatic disease like SLE. In

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addition, several unique clinical features were identified, including serological anticomplementary activity caused by IgG antibody to C6.

Case report

A 60-year-old woman was referred for evaluation of an undiagnosed systemic illness. As a child she had experienced recurrent episodes of fever, usually in summer. Three siblings had died in the first year of life, one of documented pneumonia. Between the ages of 10 and 25 years the patient had recurrent folliculitis of the eyelids. At age 36 she developed a large left psoas abscess. On surgical drainage only *Staphylococcus albus* was cultured; *N. gonorrhoeae* was not identified. At age 41 recurrent migratory polyarthralgias began and affected both large and small joints in an asymmetric pattern. Ankle pain and swelling were noted on one occasion. At age 43 aseptic meningitis was diagnosed. At age 44 a two to three month episode of fever, lymphadenopathy, anaemia, granulocytopenia, and lymphocytosis was observed, and subacute arthritis of the left wrist and knee was noted. These clinical signs and symptoms resolved spontaneously. During the next 12 years she experienced intermittent fever, arthralgia, and mild anaemia. Initially, frank arthritis occurred infrequently, was subacute, lasted several days to weeks, and affected primarily the right knee, wrist, and shoulder. By age 56 joint symptoms had increased in duration and severity and subsequently were persistent. At age 56 the patient developed acute bronchopneumonia, and two months later, acute non-bacterial episcleritis. Ten weeks after episcleritis worsening anaemia (packed cell volume 25-30%), fever, hepatomegaly, and lymphadenopathy were observed. Acute otitis media occurred subsequently and resolved on antibiotic therapy. During this illness low titre cryoglobulins were transiently present, and IgG and IgA increased to 33.6 g/l (386 IU/ml) and 5.1 g/l (304 IU/ml) from previously normal levels. Systemic manifestations improved spontaneously in three to five months.

Between the ages of 57 and 60 years joint involvement was prominent. At a minimum arthralgia was present almost daily, usually in an asymmetric migratory pattern. Sustained periods (two to six months) of non-deforming arthritis were also observed. At age 57 a subacute flare involved the right wrist, left elbow, left knee, and was accompanied by episcleritis, fever, and erythema nodosum. The patient responded quickly to treatment, but arthritis and episcleritis relapsed when prednisone was tapered to 20 mg/day. She was treated with ibuprofen, hydroxychloroquine, and prednisone, with resolution of episcleritis and some improve-

ment in arthritis. For two years joint involvement was minimal to moderate; however, transient Achilles tendinitis and perichondritis of the left external ear occurred despite continuous drug therapy. At age 60 arthritis in the right wrist flared acutely and severely. No bacteria, calcium pyrophosphate or sodium urate crystals were seen, and fewer than 200 cells/mm³ ($0.2 \times 10^9/l$) were found in synovial fluid. X-rays of the right wrist showed only osteoporosis and narrowing of the radiocarpal joint, with irregularity of the ulnar styloid process. X-rays of other joints showed only osteoarthritis of the knees and lumbar spine. Tests for serum rheumatoid factor and multiple other autoantibodies were negative. Erythrocyte sedimentation rate rose and fell, consonant with arthritis activity. Whole complement activity was undetectable.

One month later an atherosclerotic aortic aneurysm (type III) was diagnosed and surgically bypassed. However, an embolic stroke during the operation caused a dense right hemiparesis, and within 10 weeks signs and symptoms of arthritis in the right wrist and knee disappeared. Eight weeks after intraoperative blood transfusion the patient's serum was found to be anticomplementary, i.e., addition of a small amount of her serum to pooled normal human serum (NHS) completely abolished the haemolytic activity of NHS against sensitised sheep erythrocytes. No such activity was present in the patient's serum before blood transfusion. Anticomplementary activity was still present 12 months after surgery, though at only 10% of the level first observed. Fourteen months after surgery another large aneurysm was found below the bypass graft. The patient died during attempts to bypass the second aneurysm. Permission for autopsy was denied.

Materials and methods

Serum samples obtained on three separate occasions before the initial bypass procedure were available for study. Serum and plasma samples were obtained every two months after operation. For experiments which required normal human serum (NHS) a pool of 16 healthy donors was used. All samples were tested fresh or stored in 500 µl aliquots at -80°C until used. Pure C6 when required was obtained from Behring Diagnostics, La Jolla, California and was diluted to 60 mg/l with phosphate-buffered saline, pH 7.3, ionic strength 0.15.

Whole complement activity (CH₅₀) was determined as previously described.¹⁷ Individual complement components were measured functionally as described by Borsos and Rapp¹⁸ and as recommended by Cordis Corp., Miami, Florida. Func-

tionally pure human components and intermediate complexes were obtained from Cordis Corp. C3, C4 and B were also measured by radial immunodiffusion (Kallestad Laboratories, Austin, Texas). Goat antihuman C6 (IgG fraction) was used in Ouchterlony immunodiffusion experiments to detect the possible presence of immunoreactive but functionless C6. C1 inhibitor (C1 INH) function was measured by the method of Levy and Lepow.¹⁹ Immune complexes were tested for by fluid-phase ¹²⁵I-C1q binding assay.²⁰

Serum samples obtained two months after the patient's initial surgery contained the first and most potent anticomplementary activity (ACA) observed. The IgG fraction (IgG Fx) from this serum was isolated by passing a 2.0 ml aliquot of serum through a 15 ml column of diethylaminoethyl DEAE-Affigel blue (Bio-Rad Laboratories, Richmond, California) equilibrated with 0.02 M potassium phosphate buffer, pH 7.9. The IgG Fx was collected in 20 ml of equilibrating buffer and concentrated to 15 g/l (172 IU/ml). Immunelectrophoresis against goat anti-whole human serum showed that the IgG Fx contained only polyclonal IgG and moderate amounts of transferrin. A normal IgG Fx was prepared from NHS in an identical manner and used as a control in studies to identify the cause of anticomplementary activity in the patient's serum.

In preliminary experiments ACA in the patient's serum had been identified as a dose-dependent decrease in normal complement activity after addition of her serum to NHS. To detect ACA in the patient's isolated IgG Fx a modification of the CH₅₀ assay was used: a 10 µl aliquot of IgG Fx diluted 1:10 in veronal-buffered saline containing 0.1% gelatin, 0.01 M Mg²⁺, and 0.002 M Ca²⁺ (GVBS²⁺) was added to 15 µl of NHS at 4°C. Complement activity in this mixture was measured by its ability to lyse sheep erythrocytes (E) sensitised with rabbit IgM haemolysin (EA). In these experiments the IgG Fx-NHS mixture was combined with 1.0 ml EA (2.5 × 10⁸/ml (2.5 × 10¹¹/l)) and 3.0 ml GVBS²⁺, incubated at 30°C for 60 minutes, and the extent of haemolysis determined by spectrophotometry at 541 nm. ACA was measured as a fall in the percentage haemolysis relative to haemolysis produced by a 15 µl aliquot of NHS that had not been exposed to the patient's IgG Fx. This assay was then used to study the effect of anti-immunoglobulin on the anticomplementary activity of IgG Fx: increasing amounts of rabbit antihuman IgG (DAKO, Accurate Chemical and Scientific Corp., Westbury, New York) were added to 10 µl of a 1:10 dilution of IgG Fx (final volume 20–40 µl), incubated at 37°C for 15 minutes, and centrifuged at 13 000 g for 10 minutes

(DAKO titre – 900 µg IgG neutralised by 1.0 ml anti-IgG). The supernatant fluid was then incubated with 15 µl of NHS at 30°C for 60 minutes, centrifuged, and haemolytic activity in the final supernatant fluid was determined as described above.

To confirm that the patient's IgG Fx inhibited C function specifically, the IgG Fx was added in 10–20 µl aliquots to 50 µl aliquots of NHS. Haemolytic titrations of C6 activity in these mixtures were carried out after incubation at 37°C for 20 min.

IMMUNODIFFUSION STUDIES OF THE PATIENT'S IgG Fx

Immunoreactivity of the patient's serum, plasma and IgG Fx against NHS or pure C6 was initially studied by immunodiffusion in Immuno-Tec II 2% agarose plates, pH 8.6, ionic strength (µ) 0.06 (Behring Diagnostics, La Jolla, California). Subsequent experiments were done in 1% SeaPlaque LGT agarose (Marine Colloids, Rockland, Maine) in 0.013 M barbital buffer, pH 7.4, at ionic strengths 0.02–0.15, adjusted by addition of NaCl. In parallel experiments agarose plates at each ionic strength were made 3% for polyethylene glycol (PEG-6000, Sigma Chemicals, St. Louis, Missouri, 63178). 10 µl of reagent was used per well. The reaction of NHS or pure C6 with goat antihuman C6 (gift of C. Davis, MD, Dept of Pediatrics, Case Western Reserve University, Cleveland, Ohio) served as a positive control. Experiments were performed to block reactions between NHS and goat anti-C6 by incubating 50 µl NHS with 10 µl undiluted IgG Fx before immunodiffusion studies with anti-C6. These experiments were extended by preincubating IgG Fx with rabbit antihuman IgG before it was added to NHS in the blocking experiments just described.

IGG Fx AND COMPLEMENT ACTIVATION

To test for complement activation by the patient's IgG Fx 10 µl of IgG Fx diluted 1:10 in GVBS²⁺ plus 50 µl of NHS were incubated at 30°C for 60 minutes. An equal volume of 22% PEG was added (final PEG 11% w/v).²¹ After thorough mixing, incubation at 4°C for two hours, and centrifugation at 13 000 g for 20 minutes the supernatant was tested for C3 and C3d by radial and Ouchterlony immunodiffusion respectively with C3 radial immunodiffusion plates (Kallestad) and rabbit antihuman C3d (DAKO). Zymosan-treated NHS (37°C, 45 minutes) and NHS incubated with heat-aggregated IgG (30°C, 60 minutes) served as positive controls.

Results

Whole complement activity (CH₅₀) was totally absent in multiple serum samples from the patient

before and after blood transfusion. As shown in Table 1 all complement components measured functionally and/or immunochemically were normal except C6, which was undetectable by haemolytic assay and immunodiffusion studies against goat antihuman C6. Immune complexes were not detected in samples obtained before or several months after bypass surgery.

Although ACA was not detectable in serum samples before blood transfusion, it was consistently present in all samples tested after transfusion. Maximal ACA was detected in serum obtained two months after surgery and it gradually diminished during the following year. This observation suggested that the patient had developed antibody to C6. To investigate this possibility IgG was isolated from serum containing ACA, and studies were performed to determine its effect on complement activity.

EFFECT OF IgG Fx ON COMPLEMENT FUNCTION

In the assay system used 10 µl of a 1:10 dilution of the patient's IgG Fx completely inhibited haemolytic activity in 10 µl of NHS; with 15 µl of NHS 70–80% inhibition was achieved. In contrast, an IgG Fx isolated from NHS by DEAE-Affigel blue did not inhibit haemolysis when it was added to NHS. Initial incubation of the patient's IgG Fx with rabbit antihuman IgG blocked anticomplementary activity in IgG Fx and restored haemolysis in a dose-dependent manner. A representative experiment is shown in Fig. 1. In experiments which studied the effect of IgG Fx on C6 function IgG Fx added to NHS caused a 50–98% fall in C6 haemolytic activity proportional to the amount added. These experiments showed that ACA is associated with the IgG fraction in the patient's serum and suggested that ACA is due to antibody to C6.

Table 1 Complement components by haemolytic assay and radial immunodiffusion*

CH ₅₀ (100±20 U/ml)	0	C6 (76.6±17×10 ³ U/ml)	0
C1 INH (6±2 U/ml)	5	C7 (80.6±27×10 ³ U/ml)	90 200
C1 (190±44×10 ³ U/ml)	177 600	C8 (150±40×10 ³ U/ml)	150 400
C2 (15.6±6.1×10 ³ U/ml)	19 000	C9 (43±14×10 ³ U/ml)	55 000
C3 (8.1±2.6×10 ³ U/ml)	5 700	C3' (126±50 mg/dl)	156
C4 (133±46×10 ³ U/ml)	120 200	C4' (30±14 mg/dl)	50
C5 (54.8±24×10 ³ U/ml)	52 400	B ² (21±9 mg/dl)	24
		Immune complexes:	none detected

* Normal range in parentheses: mean±SD.

° By radial immunodiffusion.

Fig. 1 Reversal of anticomplementary activity of IgG Fx after preincubation with antihuman IgG. (A) By definition, 100% haemolysis was produced by mixing 15 µl normal human serum (NHS) with 1.0 ml sensitised sheep erythrocytes (EA) (2.5×10^8 /ml) (2.5×10^{11} /l) and 3.0 ml buffer at 30°C for 60 min. (B) Percentage lysis after 10 µl of the patient's IgG fraction (1:10 dilution) was incubated with NHS, EA, and buffer. (C) Percentage lysis after 10 µl IgG Fx was first preincubated (37°C, 30 min) with 15 µl antihuman IgG (DAKO) before incubation with NHS, EA, and buffer. (D) Preincubation with 25 µl anti-IgG. (E) Preincubation with 30 µl anti-IgG.

IMMUNOPRECIPITATION EXPERIMENTS

To show that the patient's IgG Fx contained antibody to C6 the following experiments were performed. Reaction of NHS or pure C6 with goat antihuman C6 in 2% agarose gels (μ 0.04, pH 8.6) gave identical single lines of precipitation. Under these conditions no precipitin line formed between NHS or C6 and the patient's plasma, serum or IgG Fx in amounts varying from 2×10^{-5} g/l to 30 g/l. However, incubation of IgG Fx with NHS completely eliminated the subsequent precipitin reaction between NHS and anti-C6. This precipitation was restored when IgG Fx was first incubated with rabbit anti-IgG before incubating it with NHS. Heating at 56°C for 60 minutes did not change the behaviour of IgG Fx in these immunodiffusion studies. When immunodiffusion was studied in 1% agarose gels (μ 0.05–0.15, pH 7.4) the only precipitin reaction observed was between goat antihuman C6 and normal human serum or C6. This reaction was stronger at lower ionic strength. At μ 0.02 additional faint reactions were observed between the patient's plasma, serum, or IgG Fx and NHS or C6. (The concentration [IgG] in these experiments was: patient's serum 14 g/l (161 IU/ml); patient's plasma 14 g/l (161 IU/ml); patient's IgG Fx 7.5 g/l (86 IU/ml). These reactions were not increased by incubation overnight at 4°C. In contrast, in 1% gels containing 3% PEG identical precipitin lines formed in reactions between the patient's IgG Fx, serum, or plasma and pure C6 or NHS at all ionic strengths tested (μ 0.02–0.15). No reactions occurred between NHS, C6, and/or normal IgG Fx under these conditions. The intensity of precipitation increased with lower ionic strengths (optimal μ 0.05–0.08). Precipitation also intensified with incubation at 4°C. On rewarming to 25°C precipitin lines became fainter and occasionally disappeared, particularly at higher ionic strength in reactions involving NHS as the source of C6.

STUDIES TO DETECT COMPLEMENT

ACTIVATION OF C6/ANTI-C6 COMPLEXES

The experiments described above show that anti-C6 antibody was present in the patient's serum. This antibody could be anticomplementary by binding to C6 and preventing formation of the membrane attack complex, C5–9. However, C6/anti-C6 immune complexes (IC) might also contribute to ACA by activating the classical complement pathway. To test this possibility NHS was incubated with IgG Fx, precipitated with PEG, and the supernatant fluid tested for C3d. NHS used in these experiments had been fresh frozen at -80°C and contained no C3d after treatment with 11% PEG as determined by

C3d (DAKO). Similarly, incubation of NHS with the patient's IgG Fx produced no detectable C3d by Ouchterlony analysis. In contrast, both alternative and classical pathway activation by zymosan and aggregated IgG respectively generated C3d and a significant fall in C3 protein concentration. Negative tests for IC and normal C3, C4, and B levels in post-transfusion sera provided additional evidence that ACA in this patient did not involve complement-fixing IC, whether or not C6-containing complexes were present.

Discussion

In this patient signs and symptoms indicating the presence of a systemic illness were observed for over 20 years. Her possible diagnoses included chronic or recurrent infections, lymphoma, SLE, rheumatoid arthritis, and/or undifferentiated connective tissue disease. The most striking laboratory finding was total absence of C6, and a complement deficiency-associated rheumatic syndrome was considered. Although selection or ascertainment bias makes interpretation difficult,²² in the context of previous reports^{14 15} this case supports a relationship between C6 deficiency and the development of a rheumatic disease.

As the third reported individual with complete C6 deficiency and a rheumatic illness this patient's syndrome is at some variance with other descriptions of systemic rheumatic illness and deficiency of a terminal component: (a) it is less clearly SLE or SLE-like, and tests for autoantibodies were repeatedly negative; (b) the arthritis was asymmetric and affected larger joints; (c) x-rays of the right wrist showed joint narrowing and juxta-articular osteoporosis; (d) recurrent fever, lymphadenopathy, and hepatosplenomegaly were prominent; and (e) several severe bacterial infections were reported, but none was caused by the neisseria species. The development of anticomplementary activity (ACA) after transfusion is another unique finding in this patient and appears to be caused by polyclonal IgG antibody to C6. While whole complement activity (CH_{50}) and C6 were consistently absent, ACA was found only in sera collected after blood transfusion and was still present 12 months later. In the absence of other family members for study, persistent total absence of C6 and the development of antibody to C6 after antigenic challenge strongly support a genetic complement deficiency in this patient. Unlike IgG antibody to human C6 raised in other species (rabbit, goat) the antibody in this patient did not readily precipitate C6 in immunodiffusion experiments. Its detection required agarose gels which contained 3% poly-

ethylene glycol and was facilitated by lowered ionic strength and cold incubation. However, it readily blocked the reaction between C6 contained in NHS and goat antihuman C6. Reversal of this inhibition by anti-IgG confirmed that the inhibitor was IgG.

The mechanism by which anti-C6 IgG inhibited complement activity appears to involve only binding to C6. It is unlikely that C6/anti-C6 immune complexes contributed to ACA by activating complement because incubation of normal serum with the antibody did not decrease C3 concentration or generate C3d.

An association between deficiency of a complement protein and serious infection has been reported for many components of the complement system.¹⁻³ This association is supported by experimental^{9, 22-28} and clinical^{1-9, 29} observations that have recorded the importance of complement in host resistance to infection. The pivotal role of C3b and its cell membrane receptors is shown by the severe recurrent bacterial infections seen in patients with C3 or I deficiency^{1, 3, 24} and in patients who genetically lack C3b receptors or associated proteins.^{24, 30, 31} The importance of terminal components is illustrated by their requirement to lyse certain bacteria and to generate chemotactic factors C5a and C567.^{1-4, 8}

The relationship between hereditary deficiency of an early complement component and rheumatic illness is more difficult to explain. The occurrence of SLE in patients with C2 or C4 deficiency has been attributed to linkage disequilibrium between null alleles of C2 or C4 and abnormal alleles of putative immune response (IR) genes in the HLA region.^{1, 2, 32} Because of tight linkage, null alleles of C2 or C4 and pathologic IR alleles would be inherited as a unit. However, this hypothesis does not explain systemic rheumatic illness observed in patients with deficiencies of C1 INH, C3, C1q, C1r, or C1s, since genes for these proteins are not HLA linked.

If linkage disequilibrium is discounted, at least two direct mechanisms exist by which an early component deficiency might be involved in pathogenesis. The first involves abnormal immune clearance: an otherwise benign microbe could become pathogenetic if not efficiently cleared by antibody and/or complement and the reticuloendothelial system.^{1, 2, 24, 33} A second mechanism could involve a lack of activated complement peptides necessary for an effective and appropriate immune response. For example, products derived from C3 have been shown to modulate the response of different subsets of T cells to in-vitro stimulation.³⁴⁻³⁷ Since both of these mechanisms would depend on intact complement function through C3, it might be expected that

individuals with early classical pathway deficiencies are at greater risk than those with a terminal component deficiency. Observations by Zeitz *et al.* are consistent with this hypothesis. In their review incidence of SLE in early component deficiencies was 20%, versus 7% in those with a terminal component deficiency and compared with an estimated 0.05% incidence overall.¹⁶ While these estimates are undoubtedly too high because of ascertainment bias, they do suggest a difference in incidence of rheumatic illness in *early* versus *terminal* deficiency versus *no* deficiency.

How absence of a terminal component might predispose to rheumatic illness is even less clear. The possible mechanisms described above would not apply, since the activation sequence is intact through C3. Although an intact terminal pathway is required for direct killing of neisseria, there is no current evidence that these components are required for efficient processing of microbes other than neisseria and a few other organisms like salmonella. Furthermore, sera deficient in a terminal component can solubilise immune complexes.¹⁶ Although C5a is known to be a potent chemotactic factor, no data are currently available on the immunoregulatory properties of C5, its products, or any of the other terminal components.

To confirm deficiency of a terminal component as a risk factor for rheumatic disease requires prospective study of asymptomatic complement-deficient individuals matched with appropriate controls. To determine how early or terminal component deficiencies might contribute to pathogenesis will require a much clearer understanding of the interplay between multiple factors, e.g., genetic background, hormonal influences, age, and the quantity and quality of the human immune response. Any hypothesis to explain the role of hereditary complement deficiency in pathogenesis must also account for the majority of deficient individuals who never develop a rheumatic illness.

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