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Paradoxical reconstitution of complement activity following plasma transfusion of an individual with deficiency of the seventh component of complement

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SUMMARY

A subject deficient in the seventh component of complement (C7) was plasmapheresed with 660 ml C7-sufficient plasma. The expected reconstitution of C7 activity, followed by exponential decay, was not observed. During day 1, serum haemolytic C7 and total haemolytic complement were undetectable and C7 levels were very low by C7 ELISA. However, low levels of circulating fluid phase terminal complement complex (TCC) were detected. On day 2 about μg C7/ml serum was detected and this rose to 6 μg/ml by day 17. Functional complement activity was also present. At day 28 the serum C7 and total haemolytic complement had dropped to pretransfusion levels. A low level of C5b6 was present in pretransfusion serum and this increased markedly immediately following transfusion when the patient's serum also acquired C7 consuming activity. Throughout the study low levels of anti-C7 antibodies were present but there was no evidence that antibody was directly responsible for the C7 consumption. Nevertheless antibody–antigen interactions could have generated circulating C5b6. C5b6 has been shown previously to have the capacity to inhibit C7 activity *in vitro*. Investigations of the C7 circulating on days 2–17 demonstrated normal molecular weight, functionally active C7. The donor sera and the recirculating C7 allotyped C7–1 by isoelectric focusing; however, the recirculating C7 showed additional weak bands with C7 functional activity, suggesting a possible genetic or acquired abnormality. Although the disappearance of C7 immediately post-transfusion may be explained by the presence of C5b6, there is no satisfactory explanation for the rising C7 levels on days 2–17 and we cannot exclude temporary C7 secretion by the patient.

INTRODUCTION

There are few reports on the consequences of blood transfusions given to patients suffering genetic deficiency of one of the terminal complement components. The terminal complement components comprise the components C5 through to C9 and each is necessary for the formation of the functionally active membrane attack complex. Complete and subtotal deficiency have been described for several terminal components[1,2] and the response to replacement may very well be different in those patients who have very low levels of an antigenically normal protein compared to those with an apparently complete deficiency. Terminal component deficiencies have been found to be associated with increased susceptibility to *Neisserial* infections, particularly meningococcal infections.[3,4]

In general, blood or plasma transfusion of deficient individuals leads to temporary partial restoration of the total haemolytic complement activity and also the serum activity associated with the deficient component; subsequently activity decreases exponentially. The half-life for C8 is about 28 hr,[5] and following transfusions of C6-deficient (C6D) patients, highest C6 levels were observed within 24 hr of transfusion, but C6 could not be detected 1 week after the final administrations.[6,7] There may be therapeutic benefits of giving normal plasma transfusions to terminal complement component-deficient individuals during severe meningococcal infection. Results of transfusions in a patient deficient in the seventh component of complement (C7D)[8] and in a C6D patient[6] have indicated that

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Abbreviations: C7, seventh component of complement; C56, C5b6 complex; C6D, deficient in the sixth component of complement; C7D, deficient in the seventh component of complement; CFD, complement fixing diluent; IEF, isoelectric focusing; NHS, normal human serum; PAGE, polyacrylamide gel electrophoresis; TCC, terminal complement complex.

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transfusions could be life saving. Nevertheless, blood transfusions should be considered carefully as detrimental effects such as an increased endotoxin release[7] may also be possible. Furthermore, meningococcal disease in individuals with terminal complement component deficiency usually responds well to treatment with antibiotics[1] and transfusions are unnecessary.

The short half-life of transfused complement components and the possibility of generating antibodies in deficient individuals precludes the general use of replacement transfusions as prophylactic therapy between episodes of meningococcal infection. Anti-complement component antibodies have been found in several transfused terminal component (C9 and C6)-deficient patients,[9,10] although no adverse reactions have yet been reported.

In the case reported here a transfusion of normal plasma was used to provide C7 as a supplement to antibiotic treatment of a chronic non-neisserial infection in a C7D subject. This patient was one of 31 terminal complement component-deficient individuals identified in Russia;[11] he had suffered chronic otitis media which had remained refractory to antibiotic and surgical treatment over a period of 17 years. The clinical results of the plasma therapy will be reported elsewhere. In this report we demonstrate the unexpected kinetics of serum complement and C7 activity following the plasmapheresis.

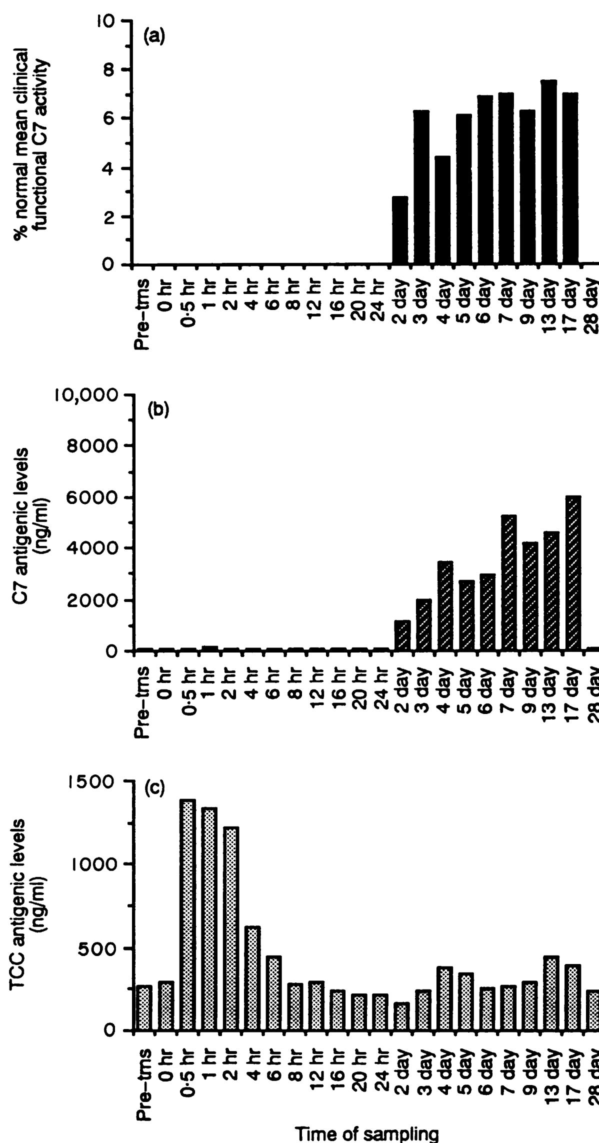

Figure 1. Results of C7 assays and the TCC assay on the sequential samples. (a) Clinical functional C7 activity as a percentage of the mean normal functional C7 activity. (b) Antigenic C7 levels as determined by ELISA. (c) Antigenic TCC levels as determined by ELISA. Time 0 is the time of commencement of the plasmapheresis.

MATERIALS AND METHODS

Patient

Past medical history. The patient was a male of 20 years, weight 71 kg. He had experienced two episodes of meningococcal infection (1978 and 1990). In 1990 he was diagnosed as C7D. He has been suffering chronic otitis media since the age of 10 months with frequent acute exacerbations, and he has repeatedly received hospital treatment. In 1991 an extensive ear operation was done and he also received a course of a cephalosporin (kefzol). Nevertheless the drainage of pus and growth of both *Staphylococcus aureus* and *Bacterioides faecalis* continued. The bacterial cultures were sensitive to cephalosporins and kefazol treatment did produce temporary clearing, but growth recurred 4 months later. Unfortunately, the patient could not recall if he had ever received a blood transfusion.

Treatment and clinical observations

A plasmapheresis was performed in the intensive care unit of Moscow Infectious Hospital in an attempt to clear the chronic infection. Approximately 660 ml of patient's plasma was replaced by 660 ml of fresh plasma (approximately half from each of two complement-sufficient donors and including 25% anticoagulant). After 3 hr, therapy by cefamezin was started (i.v., 4 g daily, 12 days). After 70 hr solcoseril (Solco, Basel, Switzerland) was given i.m. to stimulate the local repairing process in the middle ear. Heart and respiratory rates, systolic and diastolic blood pressure, and body temperature were monitored for 12 hr; these were normal and stable. Starting from 1 month prior to plasmapheresis, sequential serum samples were obtained at the times indicated in Fig. 1. Samples were stored at -70° and transported to Cambridge on dry-ice.

Immunological investigations

Determinations of total serum haemolytic complement activity (CH50) were done using the standard method of Mayer[12] with minor modifications.[11] C1, C2, C3, C4, C7 and C8 haemolytic activity was evaluated by adding serum depleted of the particular complement component to the studied serum . The functional C7 assay done in this manner will be referred to as the clinical functional C7 assay in order to distinguish it from the sensitive functional C7 assay (see below).

Levels of C7, C6 and the fluid phase terminal complement complex (TCC) were determined using specific monoclonal antibodies in ELISA assays.[13]

Levels of the C5b6 complex (C56) were measured using a functional haemolytic assay in an agarose gel.[14] Briefly, the 1% agarose gel contained 0·5% guinea-pig erythrocytes, 10 mM EDTA in phosphate-buffered saline and 0·75% normal human serum (NHS). Five-microlitre samples were inoculated into the

wells and the plate was incubated at 4° overnight and then at 37° for 2 hr. Three standards of partially purified C56 and a C56 euglobulin control were assayed. Diameters of the haemolytic rings produced by the C56-positive samples were measured and areas of haemolysis calculated.

A C7 consumption assay was modified from that described by Nemerow *et al.*[15] In that assay purified C7, or NHS containing C7, was incubated with C7D serum and the ability of the C7D serum to remove C7 was its C7-consuming activity. Briefly, for our assay, the sample taken 4 hr after transfusion was first titrated in order to determine the ratio of C7D serum to NHS required to consume haemolytically active C7 from NHS. Different dilutions of this sample were preincubated at 37° for 30 min with 1% NHS in complement-fixing diluent (CFD) (Oxoid, Basingstoke, U.K.). The resultant samples were assayed for C7 activity. The 4-hr sample, at 20% concentration, completely inhibited the C7 activity of 1% NHS. The C7 consuming activities in other sequential samples were assayed by a similar preincubation at 20% sample and 1% NHS and again the resultant samples were assayed for C7 activity.

The sensitive C7 haemolytic assay was done in a final reaction volume of 210 μl CFD in round-bottomed microtitre plates. Reactants were pretitrated to produce a sensitive assay in which the control wells with C7 in a final concentration of 0·001 NHS produced approximately 100% haemolysis but 0·0005 NHS or less produced incomplete lysis. Sheep erythrocytes were sensitized with a rat anti-sheep erythrocyte monoclonal antibody (SO16; produced in the Cambridge laboratory) and added at a final concentration of 0·5%. As a complement source, human C7D serum was added at 0·5%; the use of C7D serum was possible because it was at too low a concentration to exhibit C7 consuming activity.[15] Three other C7D patients, who were not ill at the time of venesection, provided C7D sera. The microtitre plates were incubated at 37° for 90 min, spun briefly and 100-μl supernatant samples harvested. The optical density at 405 nm was determined using an ELISA reader (BioRad, Hemel Hempstead, U.K.). Complete haemolysis was produced with distilled water and background haemolysis was determined by incubating sensitized erythrocytes in CFD. Haemolysis in the test wells was expressed as a percentage of the complete haemolysis. The inhibition of C7 activity was calculated from results obtained for the 0·001 dilution of NHS because this was the greatest dilution producing approximately 100% haemolysis.

C7 and C6 isoelectric focusing (IEF) allotypes were analysed in 5% polyacrylamide gels with 5% ampholytes, range pH 5–7, as previously described.[16] The C7 and C6 IEF band patterns were visualized by specific haemolytic overlays in agarose gels.

The C7 in a number of the sequential samples was analysed by SDS–polyacrylamide gel electrophoresis (PAGE).[17] The C7 was either visualized by immunoblotting and probing with polyclonal biotinylated goat anti-C7 and ^{125}I-labelled streptavidin, followed by autoradiography,[2] or washing in 0·25% Triton X-100 and application to C7 agarose indicator gel.[18]

The samples obtained at 0 hr, 48 hr and 28 days and the sera from the two donors were investigated for the presence of anti-C7 antibodies using an ELISA assay. This was performed essentially as described for the direct version of the antigen-specific ELISA[19] using 100 ng purified human C7 (Quidel, La Jolla, CA) adsorbed to wells of microtitre plates, and peroxidase-labelled anti-human IgG (Sigma, Poole, U.K.) at a 0·05 dilution. The quantification was standardized using serial dilutions of purified human IgG.

The ability of antibodies to inhibit functionally active C7 was investigated by determining antibody inhibition of TCC formation *in vitro*, using an ELISA method detailed elsewhere.[20] Briefly, IgG was purified from the pretransfusion sample by caprylic (octanoic) acid precipitation[21] and this preparation was added at a 10–40-fold molar excess over C7 to NHS from three different normal blood donors. The reaction mixtures were complement activated using baker's yeast and the resulting TCC generation was quantified and the inhibition evaluated.

RESULTS

Clinical observations

The patient was comfortable during the transfusion. The flow of pus from the middle ear had decreased by day 3 and by day 7 it had stopped. Bacterial cultures showed substantial growth and *S. aureus* and *B. faecalis* were isolated before and 1 day after plasmapheresis. However, growth had decreased by day 2, and was practically absent during days 9–17. Otorhinolaryngological examinations at day 10 and 1 month revealed the partial renewal of the destroyed epithelium in the middle ear.

Complement assays

Total haemolytic complement. There was complete absence of classical haemolytic activity as measured by the CH50 during the first 24 hr after plasmapheresis (Table 1). At 48 hr the CH50 approached normal and was in the normal range on days 4–17; on day 28 it was again below the detection limit. The activities of C1, C2, C3, C4, C6 and C8 were not changed significantly by the plasmapheresis and were within the normal ranges as tested at 4 hr, 24 hr, 9 days and 28 days post-transfusion (data not shown). Nevertheless, C1 and C6 were found to be elevated by 1·65-fold 4 hr after the transfusion, whereas C8 was found to have decreased to 70% of the pretransfusion level. The possibility that the antibiotic or anticoagulant used, acted as complement inhibitors can be excluded, because they had no effect on complement haemolytic activity *in vitro* (data not shown).

C7 assays. Results of the clinical functional C7 assay showed that the kinetics of C7 activity were similar to the kinetics of the CH50 (Table 1). By ELISA, C7 concentrations were in the range of 20–60 ng/ml before the transfusion and remained near that level during the first 24 hr of therapy. Nevertheless, there was a slight increase in C7 concentrations in the immediate post-transfusion samples (Fig. 1 and Table 1). On day 2, however, there was a 10-fold increase in C7 levels to 1 μg/ml. From day 2 until day 17 the levels gradually increased to 6 μg/ml, approximately 7% of the mean for normal individuals (90 μg/ml).[13] No further samples were taken until day 28 when the C7 level had fallen to approximately 100 ng/ml.

C7 consumption, C56 assay, TCC assay. Selected sequential samples were tested for their ability to consume normal C7. The sensitive C7 functional assay was used to determine the residual C7 activity. Figure 2 shows the haemolysis obtained with dilutions of NHS and the C7 activity remaining after incubation

Table 1. Results of complement assays indicating time of sampling, together with data of serum CH50, C7 levels, C56 haemolytic activity, the ability of the sample to produce inhibition of C7 activity in NHS, and the TCC levels

Time of sampling	CH50 units	Clinical functional C7 assay (% control mean)	C7 ELISA (ng/ml)	% consumption of C7 haemolytic activity*	Haemolytic C56 (area mm^2)	TCC (ng/ml)
Pre-transfusion	<2†	<0·2	35	22	13	ND
0 hr	<2	<0·2	61	13	13	297
0·5 hr	<2	<0·2	112	81	39	1380
1 hr	<2	<0·2	126	100	44	1340
2 hr	<2	<0·2	95	94	50	1216
4 hr	<2	<0·2	62	91	44	620
6 hr	<2	<0·2	68	95	44	442
8 hr	<2	<0·2	47	97	33	276
12 hr	<2	<0·2	41	41	20	296
16 hr	<2	<0·2	48	ND	16	244
20 hr	<2	<0·2	44	ND	16	221
24 hr	<2	<0·2	53	11	16	220
48 hr	19	2·8	1141	9	0†	169
4 days	30	4·4	3428	9	ND	380
9 days	42	6·3	4153	8	ND	295
13 days	56	7·5	4554	ND	ND	447
17 days	57	7·0	6022	12	0	398
28 days	<2	<0·2	92	5	0	241
Donor A	46	54	102,000	ND	0	396‡
Donor B	80	115	123,000	ND	0	157‡

* The C7 consumption was calculated by determining the inhibition of C7-dependent haemolysis of NHS diluted to 0·001 in CFD.
† Below the detection limit of the assay.
‡ Indicates that the TCC assay was done on a plasma sample, all other TCC assays were done on serum samples.
Means for normal serum samples: CH50 47 units, C7 ELISA 90,000 ng/ml, TCC ELISA 7270 ng/ml (serum) and <600 ng/ml (plasma).
ND, not done.

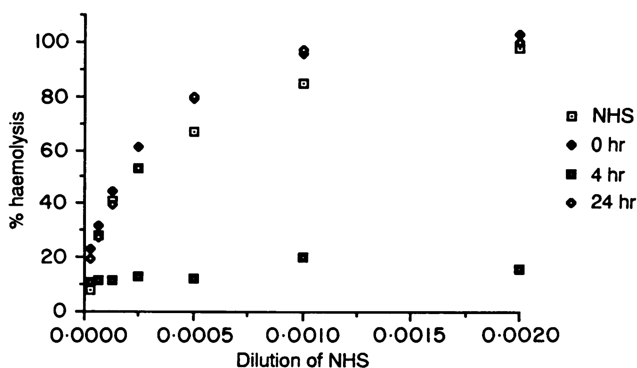

Figure 2. C7-sensitive functional assay demonstrating C7 consumption activity in the sample taken at 4 hr. The serum samples were obtained from the patient at the times indicated; they were incubated at a final concentration of 20% sample with 1% NHS at 37° for 30 min. The resultant samples were titrated for residual functional C7 activity and this was compared to the activity of C7 in NHS that had been diluted in CFD. Dilution indicates the final dilution of NHS in the wells, and percentage haemolysis is haemolysis expressed as a percentage of the result obtained for total haemolysis.

with the patient samples taken at 0 hr, 4 hr and 24 hr. Only the 4-hr sample was capable of marked C7 consuming activity and this sample removed almost all the functional C7 available under the conditions of the incubation. Results of the assays of C7 consuming activity done on the sequential samples at the 0·001 NHS dilution point are shown in Table 1. ELISA assays confirmed that incubation of NHS with the patients 4-hr sample led to the removal of antigenic C7 (data not shown).

Results of the C56 and TCC assays are shown in Table 1. We were unable to express the C56 levels as protein levels because we did not have a pure C56 standard available. However, we ran a partially purified standard and confirmed a linear relationship between the amount of C56 in the sample and the area of the haemolytic rings. C56 activity was present in very low amounts pretransfusion and at 0 hr, whereas highest levels were found from 0·5 hr until 8 hr and C56 was undetectable from 48 hr onwards. C56 activity in three NHS samples and the three other C7D subjects, who were not ill at the time of venesection, were all below the limit of detection. The times of highest C56 activity coincided with the times the serum samples had the ability to consume C7 activity.

The assays for TCC in serum measure TCC generated *in vivo* as well as that generated during clotting. The highest TCC levels in the serum samples were in those taken from 0·5 hr until 2 hr (Table 1 and Fig. 1). Levels subsequently dropped markedly and

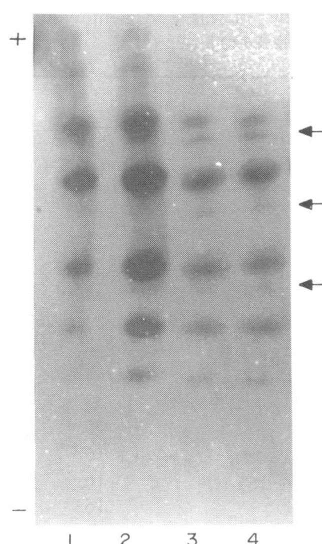

Figure 3. C7 isoelectric focusing band patterns. The pH range was 5–7 and the anode was at the top. The bands were developed with C7 functional overlay and visualized after 18 hr incubation at room temperature. Samples were: NHS (track 1), donor B (track 2), day 7 sample (track 3), day 17 sample (track 4). All four samples allotyped C7-1, however, in tracks 3 and 4 additional weaker bands, just cathodal to the main bands can be visualized (arrows). At no stage were equivalent bands seen in tracks 1 or 2.

stayed low despite the increase in available C7 from 48 hr onwards.

Anti-C7 antibody levels and inhibition of TCC formation. The 0 hr, day 2 and day 28 serum samples were found to contain 3·5, 3·5 and 3·6 μg/ml of anti-C7 IgG antibody, respectively. The two donor sera had background levels of 0·5 and 0·8 μg/ml. IgG prepared from the pretransfusion sample was, however, completely unable to inhibit TCC formation in the three samples of NHS treated with zymosan *in vitro* (data not shown).

Properties of C7 and C6 in the donors and the post-transfusion samples. C7 and C6 typing of donor sera by IEF showed them both to be C7-1, one was C6 A and the other C6 B. C7 IEF analysis of the samples taken on days 2, 7 and 17 revealed a normal C7-1 band pattern. The day 7 and day 17 samples gave rise to moderately strong C7-1 bands and in addition produced an unusual extra set of cathodal weak bands appearing later than the normal C7-1 bands. These occurred close to the normal C7-1 bands and produced an appearance of 'doubling' (Fig. 3). The patient's pretransfusion serum typed C6 A. A normal C6 A band pattern was produced by samples taken at 0, 0·5, 1 and 20 hr and days 2,7, 17 and 28, but the 1-hr sample produced additional weak C6 B bands.

Results of SDS-PAGE, electroblotting and immunoprobing showed the presence of a single band, with a molecular weight identical to normal C7, in the day 9 and day 17 serum samples; no C7 band was observed in the 1 and 12 hr samples, nor in the day 28 sample. No evidence of an abnormally sized C7 was seen. SDS-PAGE analysis, followed by a functional C7 indicator overlay,[18] revealed normal molecular weight, functionally active C7 in the day 7 and day 17 samples but not in the pretransfusion sample.

DISCUSSION

The failure to reconstitute complement activity during the first 2 days

The kinetics of serum complement activity observed following the plasmapheresis was unexpected. Firstly there was the only an extremely weak increase in serum complement activity for at least the first 24 hr, and subsequently there was an apparently sudden increase of C7 and total complement activity. After 48 hr the C7 concentrations continued to rise slowly up to day 17, but then fell, so that functional C7 was undetectable by day 28. Previous communications about C8 or C6 activity after transfusion of deficient individuals reported an immediate sharp increase of activity followed by a steady decrease.[5,7]

One explanation for the very low C7 and complement activity in the first 24 hr is that donor C7 is incorporated into a complex and that C7 functional and antigenic activities are hidden. This might be because of the presence of anti-C7 antibodies and formation of antigen–antibody complexes and possibly sequestration of the C7. We were indeed able to detect anti-C7 antibodies. The evaluation of the significance of these antibodies is, however, difficult because we know of no data for levels of anti-C7 antibodies in other C7D patients and the levels we detected were not very much higher than the background levels in the donor sera (3·5 μg/ml against a mean of 0·65μg/ml). Wisnieski *et al.*[10] found highly inhibitory anti-C6 antibody in the serum of a C6D patient who had had a blood transfusion but they did not report actual levels. The anti-C6 antibodies were detected because of the anti-complementary activity of the patient's serum. The anti-C7 antibody levels we measured remained almost constant throughout the study but the assay we used to detect C7 consuming activity failed to demonstrate significant anti-C7 anti-complementary activity except in samples taken between 0·5 hr and 12 hr (Table 1 and Fig. 2). Moreover the anti-C7 antibodies were unable to inhibit the *in vitro* formation of the TCC in NHS. This is borne out by the rise in TCC levels in the patient's samples following the transfusion. Furthermore, it is difficult to explain why antibody capable of removing all C7 activity on day 0 would fail to inhibit C7 activity on day 2. It therefore appears unlikely that the patient's antibodies would have been responsible for the observed immediate, and almost total, *in vivo* removal of the activity of the donor C7.

Another form of complexing could be with inhibitors or other complement components. Sera contain several C567 inhibitors,[20,22] including C8,[23] and it is possible that one of these is responsible for the abolition of complement and C7 activity on the first day. Sera from C7D individuals have been shown to have the ability to inactivate normal C7 *in vitro*.[15,24] Nemerow *et al.*[15] showed that the C56 was very readily generated in their patient's serum and suggested that C56 was responsible for the C7-consuming activity. Our studies with C56 showed that the patient did have low levels of circulating C56 prior to transfusion, although this was insufficient to provide marked C7 consuming activity. Immediately following transfusion there was a sharp rise in the ability of the patient's serum to inhibit C7 activity, with a simultaneous rise in circulating C56. The recognized ability of C56 to inactivate C7[15,24] and the close correlation between the C56 activity and C7 consuming ability leads us to the conclusion that probably the same phenomena

happens *in vivo*. It may be that the C56 was generated because of the presence of anti-C7 antibodies, or because of other transfusion-related antigen–antibody interactions. The role of C56 does not preclude the requirement for additional inhibitors such as vitronectin for the removal of the C7. Milis *et al.* do suggest that vitronectin may be responsible for both C567 inactivation and clearing.[22] Boyer *et al.*[25] reported a 91-hr half-life for transfused labelled C7. However, not all C7D patients have anti-C7 antibodies and it may be that these are necessary to generate sufficient C56 to consume the donor C7. The patient given labelled C7 did have very low levels of circulating C7 and that might have prevented the generation of anti-C7 antibodies.

Although C7 was virtually undetectable immediately following plasmapheresis, some biological effects of donor C7 were observed. Serum samples taken at 0·5–2 hr contained levels of TCC that were elevated in comparison to the 0-hr level (Table 1). The amount of circulating TCC was insufficient to account for the absence of the donor C7; nevertheless, TCC generation does demonstrate the availability of functionally active C7.

Properties and function of the C7 circulating between day 2 and day 17

If the C7 circulating from day 2 until day 17 comprised only donor C7, the serum levels maintained over this period were surprisingly high. Figure 1a demonstrates that the kinetics of the C7 activity were not exponential decay and calculating expected levels has little meaning. Nevertheless, the patient received 660 ml normal plasma which would contain approximately 50,000 μg C7 (average C7 concentration about 77 μg/ml with diluents). The patient's plasma volume would be approximately 3 litres but the total extra-cellular fluid diluting the C7 could be as much as 19 litres.[26] The maximum plasma C7 level after equilibration would therefore be between 3 and 17 μg/ml. However, if the 91-hr half-life[25] was correct, by day 17 the plasma C7 would have decreased to approximately 0·05 of the starting value and be at most approximately 1 μg/ml. Thus the plasma C7 level of 6 μg/ml as late as day 17 is very high for donor C7. It possible that the donor C7 was somehow 'hidden and protected' in complexes so that normal decay processes did not apply. This seems unlikely in that C7 appeared in circulation on day 2 and would presumably be subject to decay processes from then onwards. Moreover, we also observed a steady rise in C7 levels from day 2 to day 17. Taken together these observations do suggest the possibility that the patient himself was producing the C7 circulating between day 2 and day 17. This hypothesis does ultimately depend on the patient himself having a C7 gene. The molecular characterization of the defect(s) responsible for the deficiency has not yet been done. However, we did succeed in preparing genomic DNA from his lymphocytes and amplyfying, by the polymerase reaction, a 152 base pair fragment of C7-specific sequence covering most of exon 13 of the C7 gene[27] (data not shown). Thus we have demonstrated that a section of at least one C7 gene is present.

The patient was treated with both antibiotics and complement reconstitution and there was observable clinical improvement within the first few days; the long-term results will be reported elsewhere. It is certainly possible that circulating C7 played a role in the improvement. We investigated some properties of this C7. In all except one respect the C7 circulating in days 2–17 appeared completely normal. The C7 was haemolytically active and had the same molecular weight as normal C7. The only abnormality found in the circulating C7 was the development of an unusual band pattern after IEF analysis (Fig. 3). This pattern appeared like a normal C7–1 pattern with additional, slightly cathodal bands superimposed. We cannot at present comment on whether those arose because of a subtle change in C7 that had been released from some form of complex. The SDS–PAGE experiments would have failed to detect non-covalently complexed C7 and therefore they do not definitively prove the absence of complexes. The unusual IEF band pattern could also have been a manifestation of an abnormal phenotype in the C7 secreted by the patient and this possibility requires further investigation. Recent work on C9 secretion shows that relatively small changes in amino acid sequence can alter intracellular transport and secretion of the protein.[28] The molecular mechanisms responsible for C7 deficiencies are not yet understood and it is possible that certain stimuli such as a plasma transfusion, or the presence of a complete complement cascade, could provide the stimulus required to induce temporary C7 production in this particular deficiency.

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REFERENCES

1. Würzner R., Orren A. & Lachmann P.J. (1992) Inherited deficiencies of the terminal components of human complement. *Immunodefic. Rev.* **3,** 123.
2. Orren A., Würzner R., Potter P.C., Fernie B.A., Coetzee S., Morgan B.P. & Lachmann P.J. (1992) Properties of low molecular weight complement component C6 found in human subjects with subtotal C6 deficiency. *Immunology,* **75,** 10.
3. Ross S.C. & Densen P. (1984) Complement deficiency states and infection: epidemiology, pathogenesis and consequences of neisserial and other infections in an immune deficiency. *Medicine,* **63,** 243.
4. Platonov A.E., Beloborodov V.B. & Vershinina I.V. (1993) Meningococcal disease in patients with late complement component deficiencies: studies in the USSR. *Medicine* (in press).
5. Rao C.P., Minta J.O., Laski B., Alper C.A. & Gelfand E.W. (1985) Inherited C8-beta subunit deficiency in a patient with recurrent meningococcal infections: in vivo functional kinetic analysis of C8. *Clin. exp. Immunol.* **60,** 183.
6. Potter P.C., Frasch C.E., van der Sande W.J.M., Cooper R.C., Patel Y. & Orren A. (1990) Prophylaxis against *Neisseria meningitidis* infections and antibody responses in patients with deficiency of the sixth component of complement. *J. infect. Dis.* **161,** 932.
7. Lehner P.J., Davies K.A., Walport M.J., Cope A.P., Würzner R., Orren A., Morgan B.P. & Cohen J. (1992) Meningoccocal septicaemia in a C6 deficient patient and effects of plasma transfusion on lipopolysaccharide release. *Lancet,* **340,** 1379.
8. Cuadrado Gomez L.M., Jimenez de Diego L., Perez Venegas J.J. & Palau Beato E. (1987) Tratamiento con plasma fresco de la meningitis meningococica en un individuo con deficit de C7. *Med. Clin. (Barc.),* **89,** 69.
9. Inaba S., Okochi K., Fukada K., Kinoshita S., Maeda Y. & Yoshinari M. (1987) The occurrence of precipitating antibodies in transfused Japanese patients with hereditary ninth component of complement deficiency and frequency of C9 deficiency. *Transfusion,* **27,** 475.

10. WISNIESKI J.J., NAFF G.B., PENSKY J. & SORIN S. B. (1985) Terminal complement component deficiencies and rheumatic disease: development of a rheumatic syndrome and anti-complementary activity in a patient with complete C6 deficiency. *Ann. Rheum. Dis.* **44**, 716.
11. PLATONOV A.E., BELOBORODOV V.B., GABRILOVITCH D.I., KHABAROVA V.V. & SEREBTOVSKAYA L.V. (1992) Immunological evaluation of late complement component deficient individuals. *Clin. immunol. Immunopathol.* **64**, 98.
12. MAYER M.M. (1961) Complement and complement fixation. In: *Experimental Immunochemistry* (eds E. A. Kabat & M. M. Mayer), 2nd edn, p. 133. Charles C. Thomas, Springfield, IL.
13. WÜRZNER R., ORREN A., POTTER P.C., MORGAN B.P., PONARD D., SPÄTH P., BRAI M., SCHULTZE M., HAPPE L. & GÖTZE O. (1991) Functionally active complement proteins C6 and C7 detected in C6- and C7-deficient individuals. *Clin. exp. Immunol.* **83**, 430.
14. LACHMANN P.J. & THOMPSON R.A. (1970) Reactive lysis: the complement-mediated lysis of unsensitized cells. II. The characterization of activated reactor as C56 and the participation of C8 and C9. *J. exp. Med.* **131**, 643.
15. NEMEROW G.R., GEWURZ A. & LINT T.F. (1978) Inherited deficiency of seventh component of complement associated with nephritis. Propensity to formation of C56 and related C7-consuming activity. *J. clin. Invest.* **61**, 1602.
16. HOBART M.J., LACHMANN P.J. & ALPER C.A. (1975) Polymorphism of human C6. In: *Protides of the Biological Fluids. 22nd Colloquium* (ed. H. Peeters), p. 575. Pergamon Press, Oxford.
17. LAEMMLI U.K. (1970) Cleavage of structural protiens during assembly of the head of bacteriophage T4. *Nature*, **227**, 680.
18. ORREN A., LERCH W.H. & DOWDLE E.B. (1983) Functional identification of serum complement components following electrophoresis in polyacrylamide gels containing sodium dodecyl sulphate. *J. immunol. Meth.* **59**, 63.
19. WÜRZNER R., OPPERMANN M., ZIERZ R., BAUMGARTEN H. & GÖTZE O. (1990) Determination of the epitope specificities of monoclonal antibodies using unprocessed supernatants of hybridoma cultures. *J. immunol. Meth.* **126**, 231.
20. WÜRZNER R., SCHULZE M., HAPPE L., FRANZKE A., BIEBER F.A., OPPERMANN M. & GÖTZE O. (1991). Inhibition of terminal complement complex formation and cell lysis by monoclonal antibodies. *Complement Inflamm.* **8**, 328.
21. STEINBUCH M. & AUDRAN R. (1969) The isolation of IgG from mammalian sera with the aid of caprylic acid. *Arch. Biochem. Biophys.* **134**, 279.
22. MILIS L., MORRIS C.A., SHEENAN M.C., CHARLESWORTH J.A. & PUSSEL B.A. (1993) Vitronectin-mediated inhibition of complement: evidence of different binding sites for C5b-7 and C9. *Clin. exp. Immunol.* **92**, 114.
23. NEMEROW G.R., YAMAMOTO K., OSOFSKY S.G. & LINT T.F. (1979) Restriction of complement-mediated membrane damage by the eighth component of complement: a dual role for C8 in the complement attack sequence. *J. Immunol.* **123**, 1245.
24. WELLECK B. & OPFERKUCH W. (1975) A case of deficiency of the seventh component of complement in man. Biological properties of a C7- deficient serum and description of a C7-inactivating principle. *Clin. exp. Immunol.* **19**, 223.
25. BOYER J.T., GALL E.P., NORMAN M.E., NILSSON U.R. & ZIMMERMAN, T.S. (1975) Hereditary deficiency of the seventh component of complement. *J. clin. Invest.* **56**, 905.
26. LAIKEN N.D. & FANESTIL D.D. (1990) Physiology of body fluids. In: *Physiological Basis of Medical Practice* (ed. J. B. West) 12th edn, p. 406. Williams and Wilkins, Baltimore.
27. HOBART M.J., FERNIE B.A & DISCIPIO R.G. (1993) Structure of the human C6 gene. *Biochemistry* **32**, 6198.
28. DUPUIS M., PEITSCH M.C., HAMANN U., STANLEY K.K. & TSCHOPP J. (1993) Mutations in the putative lipid-interaction domain of complement C9 result in defective secretion of the functional protein. *Molec. Immunol.* **30**, 95.