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How partial C7 deficiency with chronic and recurrent bacterial infections can mimic total C7 deficiency: temporary restoration of host C7 levels following plasma transfusion

R. WÜRZNER,*‡ A. E. PLATONOV,§ V. B. BELOBORODOV,§ A. I. PEREVERZEV,§ I. V. VERSHININA,§ B. A. FERNIE,‡ M. J. HOBART,‡ P. J. LACHMANN‡ & A. ORREN†‡ ‡*Molecular Immunopathology Unit, Medical Research Council Centre, Cambridge, UK, and* §*Central Institute of Epidemiology, Moscow, Russia*

SUMMARY

An apparently completely complement C7-deficient patient with refractory otitis media and two episodes of meningococcal disease was given therapeutic plasma transfusions in 1992 and 1994. Following these transfusions unexpected changes were found in C7 levels. Immediately after transfusion the serum C7 levels failed to rise to the expected levels but then rose to 5–10% of the normal mean during the next 5 days and remained at that level for more than 2 weeks before eventually returning to zero. The patient's DNA genotyped C7 M, and therefore C7 N donor plasma was selected for the second transfusion to allow identification of the source of the C7 circulating post-transfusion. This C7 phenotyped C7 M, demonstrating it to be of recipient origin. Therefore, the apparently completely C7-deficient patient was able to secrete some C7. By a combination of DNA typing and isoelectric focusing of the C7 appearing after transfusion, it was demonstrated that the patient was heterozygous for combined subtotal C6/C7 deficiency (inherited from his father) and a different, so far uncharacterized, subtotal C7 deficiency (inherited from his mother). The low amount of C7 secreted appeared to be constantly consumed, probably by generation of C5b6 as a result of his chronic infection. He had been shown to have circulating C5b6 most of the time, and thus only when sufficient exogenous C7 was given to consume the free C5b6 did his own C7 appear in circulation.

INTRODUCTION

Complement component C7 is a central protein of the terminal complement cascade, necessary for the formation of the functionally active terminal complement complex. Deficiencies have been described for all terminal components.[1] All have been found to be associated with increased susceptibility to neisserial infections, particularly meningococcal infections.[2,3]

There are few reports of non-neisserial infections in subjects with terminal complement deficiency, demonstrating that for most bacterial infections opsonization by C4 and C3 is sufficient complement activation to serve immunity. However, it is known that antibiotics synergize with complement in elimination of infections and therefore both antibiotics and complement replacement have been used in treating patients.[4] There are also few reports on the consequences of blood transfusions given to terminal complement-deficient patients. In general, blood or plasma transfusion of deficient individuals leads to partial restoration of that particular component and thus of complement haemolytic activity, but is followed by a rapid exponential decrease. In the case of C6, replacement serum levels were detectable for only 1 week.[5,6] There are possible therapeutic benefits of reconstitution, which can be life saving,[5,7] but also possible detrimental effects such as the provocation of endotoxin release.[6] In addition, anti-complement component antibodies have been found in several transfused terminal component (C9 and C6)-deficient patients,[8,9] although no adverse reactions have yet been communicated.

We have recently reported on the paradoxical reconstitution of complement activity following plasma transfusion of a patient with an apparent complete deficiency of C7.[10] The expected immediate partial reconstitution followed by exponential decay was not observed. Instead, the concentration of functionally active C7 was initially very low and then rose to levels which exceeded the possible concentration that could derive from the amount of transfused C7.[10] Furthermore,

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Abbreviations: IEF, isoelectric focusing; mAb, monoclonal antibody.

*Present address and correspondence: Dr R. Würzner, Institut für Hygiene, University of Innsbruck, Fritz Pregl Strasse 3, A-6020 Innsbruck, Austria.

†Present address: Department of Immunology, University College Hospital, Galway, Ireland.

instead of a steady decline this concentration remained constant for more than 2 weeks before it dropped to the very low pretransfusion level. No satisfactory explanation was given but a number of hypotheses concerning the source of the C7 were put forward, including the suggestion that the C7 might have been of donor origin and hidden or somehow protected in the host.[10] Antibodies, however, appeared to play no functional role in this case.[10]

When the patient required a second transfusion we used the opportunity to try to trace the source of the C7 that we expected might appear a few days after the transfusion, as seen after the 1992 plasmapheresis. To do this we selected a donor with a C7 phenotype/genotype different from the genotype of the C7-deficient recipient. We used the C7 M/N polymorphism,[11] which allows determination and quantification of serum C7 allotypes in C7-sufficient individuals. The opportunity to assess the contribution of different sources towards a combined pool via the C7 M/N ratios has proved to be very useful in assessing the contribution of the liver towards C7 synthesis.[12] However, the principal reason for making use of the C7 M/N polymorphism was that the DNA base change responsible for the phenotypic differences is known[13] and thus we were able to genotype DNA independently of, but in perfect correlation with, a C7 M/N phenotype.[13] This was essential because the C7 deficiency of the recipient precluded accurate C7 M/N protein phenotyping.

Here we report the results of the second plasma transfusion, which was performed using an appropriate blood donor selected because he was homozygous for a C7 M/N type different from that of the patient.

MATERIALS AND METHODS

C7-deficient patient

The C7-deficient patient was a white male from Russia. He had a history of recurrent meningococcal disease and also suffered chronic otitis media, which had remained refractory to antibiotic and surgical treatment over a period of 17 years. Details of his past medical history are reported elsewhere.[10] In 1992 he was plasmapheresed with 660 ml C7-sufficient plasma as supplement to antibiotic treatment. Because of the significant clinical improvement he was transfused again in 1994 (with 500 ml plasma from a single donor) after another relapse of otitis media with continous drainage of pus from the ear.

DNA and serum samples

DNA was obtained from the C7-deficient patient, his healthy mother and half-brother (same mother), and prepared by an adaptation of the method of Jeffreys[14] as detailed elsewhere.[15] Serum samples were obtained from 22 healthy Russian blood donors, from the C7-deficient patient before and at several time-points after the transfusion, and from his mother and half-brother. The sera were stored at $-70°$ until use.

Immunological investigations

Functional assays for total (CH50) and C7-specific (C7-50) haemolytic complement activity were performed according to standard protocols as detailed elsewhere.[16] Quantification of C7 was carried out using an enzyme-linked immunosorbent assay (ELISA) based on a polyclonal anti-C7 antibody (ELISA P).[17] C7 isoelectric focusing (IEF) typing was performed by haemolytic overlay.[18] C7 M/N phenotyping (protein typing) is based on the reactivity of an allospecific monoclonal antibody (mAb) to the gene products of the *C7*M* allele. In an ELISA assay this mAb, WU 4-15, is used for the plate-coating step (ELISA M) and recognizes all C7 molecules produced by homozygous C7 M individuals and half of the C7 molecules in C7 MN subjects, as the reaction with C7 N molecules is extremely weak. ELISA M/ELISA P ratios between 0·75 and 1·25 identify C7 M individuals, whereas ratios between 0·35 and 0·65, or less than 0·02, identify C7 MN or C7 N subjects, respectively.[11]

Molecular analyses

C7 M/N genotyping and analysis of the C6 intron 15, 5′ boundary sequence were performed as detailed elsewhere.[13,15] Briefly, genomic DNA sequences were amplified using flanking primers in the polymerase chain reaction. The amplified DNA was ligated into M13mp8 and sequenced by the dideoxy chain-termination method. For C7, Thr-565 (ACA) determines C7 M and Pro-565 (CCA) C7 N.[13] The normal C6 exon 15/intron 15 boundary is GTAG/gtaa, whereas the defective 5′ splice donor site of combined subtotal C6/C7-deficient subjects reads GTAG/gcaa.[19]

RESULTS

Assessment of the C7 M/N genotype of the C7-deficient patient and his relatives

By sequencing part of the DNA, we determined that both C7 genes of the C7-deficient patient genotyped *C7*M*. No difference from the normal C7 gene sequence was observed within this area of 152 bp, excluding the remote possibility that

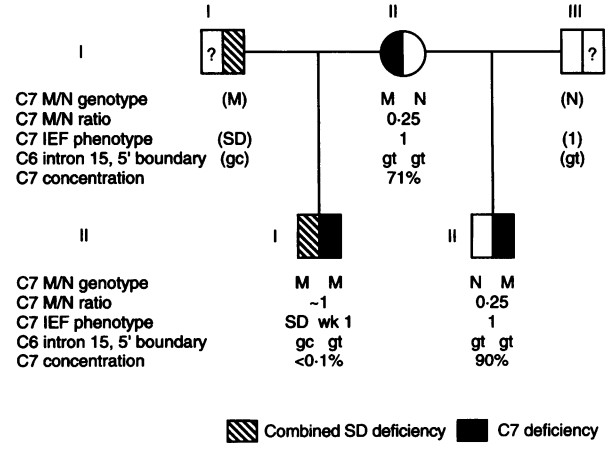

Figure 1. Family tree of the C7-deficient patient. C7 M/N genotype,[13] C7 M/N ratio,[11] C7 IEF phenotype,[18] C6 intron 15, 5′ boundary sequence[15,19] and C7 concentration[17] are listed; ( ), deduced results, subject not available for study; ~1, C7 M/N ratio around 1 but C7 concentrations too low to allow accurate C7 M/N typing; SD, subtotal C7 deficiency IEF phenotype as seen in homozygous combined subtotal C6/C7-deficient subjects;[21] wk1, weak C7 1 IEF phenotype; gc, mutated C6 intron 15, 5′ boundary sequence as seen in homozygous combined subtotal C6/C7-deficient subjects; gt, normal C6 intron 15, 5′ boundary sequence.

the mutation responsible for his deficiency lies within this region. Both the mother and the brother were found to be heterozygous C7 MN (Fig. 1).

Assessment of C7 M/N phenotypes, C7 IEF patterns and C7 concentrations of the C7-deficient patient, his relatives, and possible blood donors

Over a period of 4 years the patient was always found to have less than 100 ng C7/ml (less than 0·1% of the normal mean)[17] in both C7 ELISA. Therefore, the C7 concentrations were too low to allow accurate C7 M/N or IEF phenotyping. The C7 concentrations of the mother and the half-brother were between 70% and 90% of the normal mean, the C7 IEF was indistinguishable from a normal C7 1, and the C7 M/N ratios were 0·25 for both subjects (seven different quantifications each) (Fig. 1). As both subjects were genotyped C7 MN, their *C7*M* allele must be hypomorphic to generate such a low C7 M/N ratio. Furthermore, the index case must also have inherited this hypomorphic *C7*M* allele because genotyping, phenotyping and C7 quantification excluded the presence of a normal *C7*N* allele.

The two blood donors of the 1992 plasmapheresis were retrospectively typed C7 M (ratio 0·95) and C7 MN (ratio 0·62). As the patient was genotyped C7 M, we looked for a C7 N blood donor for a possible second transfusion in order to obtain informative post-transfusion samples.

Among 22 Russian blood donors 15 were C7 M, six were C7 MN and one was C7 N, which is approximately the expected distribution of C7 M/N allotypes in European white people.[11] The C7 N subject was selected as donor for the second transfusion reported here.

Quantification of C7 and assessment of C7 M/N phenotypes in serum samples obtained after transfusion

Both C7-specific (C7–50) and total complement haemolytic (CH50) activity of several pretransfusion samples of the C7-deficient patient were below the detection limit of the assays and remained undetectable for the first 36 hr after the transfusion. After day 2 there was a marked increase in functional activity using both assays, with activities on days 3–24 of greater than 8% (peak 16% on day 12) of the normal C7 haemolytic activity, and of greater than 58% (peak 78% on day 12) of the normal total complement haemolytic activity. The activity in both assays then declined and was undetectable again after 33 days.

C7 quantification by ELISA P showed essentially the same profile, but a small C7 peak was detected for about 2 hr after the transfusion (Fig. 2a). Double measurements on three different days (with an interassay variation of less than 20%) confirmed that the small peak, which was about twice as high as the baseline level and more than a magnitude higher than the concentrations found in other C7-deficient patients,[17] was real. Subsequently, the C7 level dropped again to the very low pretransfusion level of below 100 ng/ml, and remained low for the next 40 hr, after which the marked increase started, rising to a maximum level of 11 μg/ml (almost 12% of the normal mean) between days 7 and 12 (Fig. 2b). Interestingly, the profile was very similar to that of the 1992 plasmapheresis, although the recent C7 concentrations were higher and the pretransfusion level was reached later (33 days versus 28 days). The amount of C7 present at day 20 was on both occasions calculated to be more than the amount administered (40 mg) and approximately 100 times higher than expected when the amounts of fluids transfused and the total intra- and extracellular volume of the recipient were taken into account, and a half-life of 91 hr was assumed for the transfused C7 (details of calculation not shown). This half-life was determined in a different C7-deficient subject[20] who was transfused with 200 ml fresh plasma. In that case no C7 was synthesized as a result of the transfusion within the 5-day observation period after the transfusion.[20]

Directly after the transfusion of C7 N plasma (ratio 0·02), the C7 M/N ratio was found to be 0·2 (Fig. 2a) and coincided with the appearance of the small peak in C7 concentration, detected by ELISA P (Fig. 2a). Although this calculation was done on quite low C7 concentrations, and thus must be regarded with caution, this is much too high a ratio to represent only the C7 N of the donor plasma. Indeed, it strongly suggests the presence of both C7 M and C7 N molecules. During the hours after transfusion the C7 M/N ratio appeared to increase steadily but these determinations were also carried out on very

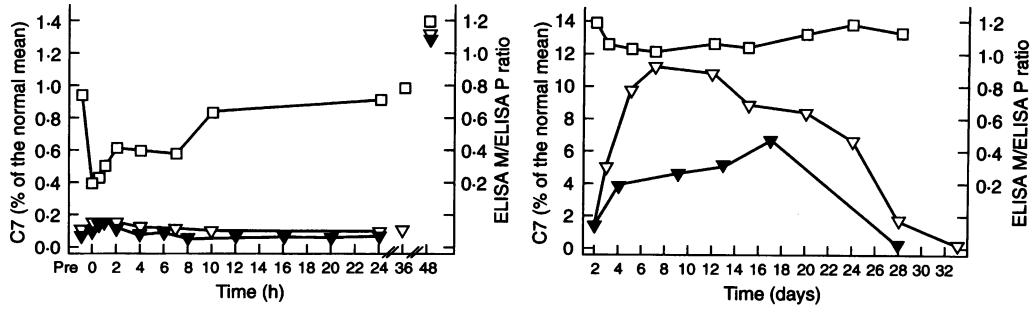

Figure 2. Post-transfusional C7 concentrations and C7 M/N allotypes. Time of sampling is indicated: (a) time period in hours from 1 hr before to 48 hr after transfusion; (b) time period in days from day 2 to day 33 after transfusion. C7 concentration measured by ELISA P, detecting all C7 phenotypes, (1994 transfusion, open triangles; 1992 plasmapheresis, filled triangles) as a percentage of the normal mean, and C7 M/N ratios calculated from ELISA M and ELISA P results (open squares). Please note that the quantifications for 48 hr = 2 days is in both (a) and (b) for better comparison. C7 M/N ratios can be readily compared (ratios between 0·75 and 1·25 define the C7 M phenotype, ratios between 0·35 and 0·65 the C7 MN phenotype, and ratios below 0·02 the C7 N phenotype) but the intervals on the ordinate for C7 concentrations in (b) represent 10-fold higher C7 concentrations.

low C7 quantifications. The appreciable amount of C7 circulating after day 2, however, could be typed unequivocally and was predominantly, if not exclusively, of C7 M type with ratios between 1·0 and 1·2 (Fig. 2b).

Assessment of the C6 exon 15/intron 15 boundary

The subject was found to be heterozygous for the T > C substitution at the 5′ splice donor site of C6 intron 15. The mother and the half-brother were homozygous for a normal C6 exon 15/intron 15 boundary.

DISCUSSION

A C7-deficient patient suffering from recurrent infections was plasmapheresed in 1992 and transfused with C7-sufficient plasma in 1994. The C7 kinetics were unexpected but similar on both occasions. The use of a donor with a C7 M/N allotype different to that of the recipient for the second transfusion enabled us to identify the origin of the C7 detected in the post-transfusion samples. Immediately after transfusion the serum C7 level was predominantly of donor origin, as expected. However, this C7 disappeared rapidly from the circulation. A similar pattern was observed following the first plasmapheresis, when the disappearance of C7 was associated with (1) development of serum C7 consuming activity, (2) the appearance of circulating C5b6 and (3) the presence of circulating soluble terminal complement complex.[10] In our previous report we suggested that these changes may not have fully explained the complete disappearance of donor C7, and we therefore could not exclude totally the possibility that donor C7 may be 'hidden and protected' somewhere and released later into the circulation. The C7 M/N ratios and the C7 kinetics found in the present study, however, showed that there was no marked contribution of donor C7 towards the C7 circulating after day 2. Thus the patient had the ability to secrete low amounts of C7 and thus, in certain circumstances, to restore haemolytic activity.

The subject was able transiently to produce 80% of normal total haemolytic complement activity, with approximately 10% of the normal mean serum C7 concentration. This discrepancy may be explained by a wide security margin allowing sufficient haemolysis at concentrations of only 10% of the normal mean, as shown previously for C7[20] but also for C6.[17]

The C7 circulating after day 2 of the 1992 plasmapheresis appeared completely normal with the exception of an unusual C7 IEF band pattern, consisting of normal C7 1 bands and additional weak bands.[10] These bands are characteristic of the C7 produced by the combined subtotal C6/C7 deficiency.[21] At the molecular level, the patient was found to be heterozygous for the C6 exon 15/intron 15 boundary mutation, previously observed in homozygous form in a case of combined subtotal C6/C7 deficiency[19] and in heterozygous form in two families,[15] one of which carried the combined C6/C7 subtotal deficiency chromosome as determined by DNA marker analyses (and data not shown),[22,23] while the other carried the gene for subtotal deficiency of C6 only. The flanking polymorphic DNA marker data of our patient further supported the heterozygous presence of the combined deficiency haplotype (data not shown). As the IEF pattern indicated that both types of C7 contributed towards the functionally active C7 post-transfusion, two different types of subtotal C7 deficiency genes appeared to be present.

The most plausible explanation for the appearance of host C7 after transfusions is the following. The patient is a compound heterozygote for two defective C7 genes. One is that previously described in the subtotal C6/C7 deficiency haplotype,[15,19,21] which makes a recent mutation unlikely and implies that he has inherited this gene from his father. The other is a so far uncharacterized C7 gene producing low levels of apparently normal C7 M/C7 1 molecules without any associated C6 deficiency. C7 M/N ratios of mother and half-brother indicate both as carriers for the latter, again excluding a *de novo* mutation. The patient also has a chronic bacterial infection, which produces a chronic state of complement activation. That leads to the consumption of the available C7, its apparent total deficiency in plasma and to the intermittent presence of free C5b6. When the patient is transfused with normal C7-sufficient plasma the host's excess C5b6 is consumed by the transfused C7 and complete terminal complement complex is found in the plasma, as detected.[10] The depletion of C5b6 allows the host C7 being synthesized to appear in the plasma and it is only when complement activation produces sufficient excess of C5b6 that C7 again disappears. This assumption, however, implies that in the immediate days after the transfusion the complement activation (probably in part caused by the transfusion itself) is not sufficient to generate enough C5b6 to consume the newly synthesized C7, and thus may indicate a temporary resolution of the chronic inflammation.

This is corroborated by the low concentrations of terminal complement complex on days 2–33 (data not shown), comparable to those found after the previous transfusion,[10] and also clinically, as the deterioration of the disease was stopped and the patient improved markedly. Within the next days after the transfusion the ear draining liquid became clear and sterile under antibiotic therapy and stopped at day 5. This improvement lasted about 6 months, when the inflammatory process started again so that a surgical intervention was recommended but rejected by the patient. Although it is certainly open for debate whether the improvement was a result of C7, other transfused plasma factors, the antibiotics, or a combination of these factors, our data imply that other terminal complement-deficient patients suffering from recurrent infection may also benefit from plasma transfusions.

C7 is unusual among complement components in not being an acute-phase reactant and is therefore not up-regulated by the chronic infection. The effect of plasma transfusion on C7 synthesis in the patient could not be measured readily, and transfusion alone may stimulate C7 synthesis, but there is no need to postulate up-regulation to explain the observed results.

Our conclusion also explains why C7 can be detected in homozygous combined subtotal C6/C7-deficient patients: the low levels of circulating C5b6, caused by the additional homozygous C6 exon 15/intron 15 boundary defect, are insufficient to deplete the low amounts of C7. Thus, the absence of detectable C7 levels in other C7-deficient patients does not necessarily indicate a complete inability to secrete C7.

This may be of importance as, despite the patient's recurrent meningitis and chronic otitis, low concentrations of functionally active C7 molecules may afford some protection against acute infections by microorganisms within a confined space. Furthermore, low systemic C7 levels can be enriched in areas of inflammation where polymorphonuclear leucocytes

may contribute a notable proportion of the C7 present in the inflammatory focus.[24]

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