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Properties of a low molecular weight complement component C6 found in human subjects with subtotal C6 deficiency

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SUMMARY

A sensitive ELISA assay was used to quantitate serum complement component C6 concentrations. Levels in the range 0·3–3 μg/ml were measured in samples from eight individuals (four separate pedigrees) and two subjects with subtotal combined C6/C7 deficiency who have been reported previously. We defined C6 levels in this range as subtotal C6 deficiency (C6SD). In contrast, C6 deficiency with levels below 0·03 μg/ml was defined as C6Q0. C6Q0 has been found in 29 unrelated cases which have already been reported. Investigations of the properties of the C6 found in the C6SD subjects showed it to be haemolytically active and able to incorporate into the terminal complement complex. The protein had a relative molecular weight (M_r) of approximately 86% of normal C6 and this M_r was identical to that of the C6 of one combined deficient subject. The M_r of the C6 of the other combined deficient subject was previously estimated as 79% of the M_r of normal C6.[1] Isoelectric focusing (IEF) analysis with band development by haemolytic overlay revealed that all C6SD samples produced an identical weak C6 band pattern anodal to normal C6A bands. The C7 IEF patterns of the two combined deficient subjects were identical, and the C6 IEF patterns of both were identical to those of the C6SD subjects. Thus the C6 of the combined deficient subjects is probably the same abnormal protein found in the C6SD individuals. None of the C6SD or combined deficient subjects have had meningococcal disease and it may be that low C6 levels afford some protection.

INTRODUCTION

C6 is one of the terminal complement components. These comprise the components C5 through to C9 which, following activation of C5, can combine to form a multi-molecular complex which is capable of penetrating cellular membranes and causing cellular lysis. It has been recognized for some time that deficiencies of late-acting components in man are associated with susceptibility to *Neisserial* infections, particularly *N. meningitidis* infections.[2] We have reported that C6 deficiency (C6D) occurs with what appears to be an unusually high frequency in the Western Cape, South Africa.[3,4] The serum C6 levels in these studies were measured by a functional haemolytic assay. Recently we have published the results of an ELISA assay of serum C6 levels in which many of the original South African (SA) sera were re-investigated.[5] All known SA C6D patients, except two, were found to have either no detectable C6 or levels below 0·03 μg/ml. Levels below 0·03 μg/ml we now refer to as C6Q0. However, subtotal deficiency with levels of 0·5 and 0·37 μg/ml were found in two SA sisters in a family we now call family A. That family is the only SA family in which the proband was not ascertained because of meningococcal disease. The other SA family reported here we call family B and this family was ascertained because a child in the family is C6Q0 and presented with meningococcal meningitis; it is, however, her mother who has subtotal deficiency and a C6 level of 0·79 μg/ml.[5] Lachmann and co-workers[1] first reported subtotal C6 deficiency in a patient, WFH, who also had subtotal C7 deficiency (which we will refer to here as C7SD). Our previous report[5] details C6 levels between 0·3 and 3·0 μg/ml in three other C6D patients including one combined C6/C7 deficiency reported previously.[6] We provisionally call C6 levels in this range subtotal C6 deficiency (C6SD), and we now describe some of the properties of the C6 found in these various patients and show that the protein appears similar in all these individuals. In the cases presented here none of the affected persons has a history of having ever suffered from meningococcal disease. However, one patient reported earlier with a C6 level of 4·8 μg/ml (MB-04)[5] died from the disease but unfortunately none of his serum is available for further investigation.

Abbreviations: C6D, complement component C6 deficiency; C6Q0, C6 quantitatively zero; C6SD, C6 subtotal deficiency; C7SD, C7 subtotal deficiency; IEF, isoelectric focusing; NHS, normal human serum; SA, South Africa; TCC, terminal complement complex.

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MATERIALS AND METHODS

Affected individuals

Most of the patients presented here have previously been reported as having C6 deficiency and where practical we have used identification codes used in previous publications. However, as we have investigated the two SA families A and B in detail we refer to members of these families according to their places in the respective family trees.

The proband of family A (II-5) presented with post-streptococcal nephritis at the age of 11 years and routine complement studies revealed absence of total haemolytic complement activity and no antigenic C6 was found on rocket electrophoresis[7] of her serum. Two of her sisters (reported as OP-26 and OP-27)[5] were found to be similarly affected. None gave a history of meningococcal disease but all three were followed clinically in case such disease should develop. They were not put on penicillin prophylaxis.[4]

Family B is the other SA family. Subject II-2 in this family was reported previously as OP-6.[5] Her C6 was investigated only because she was the mother of a C6Q0 patient who had suffered meningococcal infection and who had been found to have total C6 deficiency.[5] However, II-2 was found to be C6SD and has no history of meningococcal disease. She has two siblings who have similar negative histories and low C6 levels.

All six SA patients are Cape Coloureds and of mixed Caucasoid, Southern African and Cape Malay extraction.[8]

Subject WU-01 was identified in Germany when, as a medical student aged 25 years, he performed a total complement assay on his own serum.[9] Absence of C6 was confirmed by a lack of serum bactericidal activity in a bactericidal assay and its restoration by the addition of purified C6.[5] WU-01 is healthy and has no history of any serious infections.

Subject WFH was diagnosed in the U.K. a number of years ago as having combined C6/C7 deficiency.[1] He died at the age of 70 and had no history of meningococcal infections or undue susceptibility to other infections. Unfortunately extremely little of his serum was available for our investigations.

Patient CJ was diagnosed as combined C6/C7 deficient at the age of 21 years in the U.K.[6] She is white and has no history of meningococcal disease. Although she is referred to by Morgan and co-workers as CJ,[6] we have previously referred to her as MO-01.[5]

Reinitz and co-workers[10] first reported patient RE-01 after C6 deficiency was diagnosed at the age of 50 years when she presented in the U.S.A. with arthritis. She was found to have antinuclear antibodies but had no particular susceptibility to infection.

Investigations of serum complement

Serum samples used for haemolytic complement assays were stored at -70° and when transported were kept frozen on dry ice. Samples for the ELISA assays were stored at -20°. However C6 antigenic activity is relatively stable[5] and when necessary samples for the ELISA assay were preserved with 0·1% sodium azide and sent through the post. Nevertheless we report here results of recent assays, usually on fresh samples that were sent on dry ice, for almost all the C6-deficient patients. We have indicated any results that might have been affected by conditions of transport. Except for the SDS-PAGE all assays were performed on unconcentrated serum samples. Concentration of serum samples was carried out by 40% saturated ammonium sulphate precipitation.[1] The concentrated sample was adjusted to 10% of the original volume with phosphate-buffered saline (PBS). Complement activation in serum was generated by treatment with boiled baker's yeast.[5]

Antigenic C6 activity and determination of the formation of the terminal complement complex (TCC) was measured by ELISA assays based on monoclonal antibodies, and C7 was quantitated with a polyclonal antibody-based ELISA.[5]

Haemolytic C6 activity was determined in agarose gels as described previously[1] using the C6 indicator gel with enhanced sensitivity developed for mouse C6[11] with the modification that a monoclonal rat anti-sheep red cell antibody was used to sensitize the sheep red cells.

SDS-PAGE analysis[12] of human C6 was carried out in $16 \times 17 \times 0{\cdot}1$ cm 8% polyacrylamide gels. Serum samples [1·5 μl/track for native or concentrated normal human serum (NHS) or 20 μl/track for concentrated or native C6-deficient samples] were incubated in high SDS loading buffer (20% glycerol, 1·275 mM Tris/HCl, pH 6·5, 5% SDS) for 45 min at 37° before loading. Gels were run at 10 mA for 17 hr. C6 bands were localized by electroblotting the proteins onto nitrocellulose[13] and immunoprobing the blot with biotinylated monoclonal mouse anti-human C6.[5] Bands were developed with streptavidin–horseradish–peroxidase (Sigma Chemical Co., St Louis, MO) together with 4-chloro-1-naphthol as the peroxidase substrate.[5]

Analytical isoelectric focusing (IEF) gels for C6 and C7 allotyping were run in 5% acrylamide, with LKB ampholytes pH 5–7 (Pharmacia LKB, Bromma, Sweden) as described previously.[15] Samples were loaded as indicated in the legends. C6 allotyping bands were developed either with the haemolytic C6 overlay indicator system or by electroblotting and probing with rabbit anti-rabbit C6 followed by ^{125}I-labelled sheep anti-rabbit IgG and autoradiography.[11] For C7 allotyping the IEF gels were electroblotted and the bands were developed by probing the blot with *N*-biotinyl-penicillamine conjugated[16] goat anti-human C7 followed by ^{125}I-labelled streptavidin and autoradiography.

C7-M/N allotyping distinguishes C7 allotypes via the reaction pattern of the monoclonal mouse anti-C7 antibody (WU 4-15) and was carried out as described previously.[17]

RESULTS

Quantitation of C6, C7 and TCC by ELISA

Table 1 gives the results of C6- and C7-ELISA assays on sera from affected C6SD individuals and the two C6Q0 children from family B. The C6 levels and TCC levels before and after complement activation with baker's yeast are tabulated. Formation of the TCC was assumed when a rise in TCC level and a fall in the levels of the individual, non-incorporated components occurred in the treated serum. Examination of Table 1 shows that this indeed happened for all samples tested except the C6Q0 samples. The C6 and C7 levels need to be compared with normal C6 and C7 levels (published means $\pm$ SD, 45 ± 16 and 90 ± 36 μg/ml respectively; and ranges 20–80 and 30–180 μg/ml respectively).[5] As indicated above, C6 levels in patients classified as C6Q0 were $< 0{\cdot}03$ μg/ml.

Table 1. C6, C7 and TCC levels in sera from C6SD and C6Q0 subjects

Subject	Deficiency	C6 level (μg/ml)		Haemolytically active C6	C7 level (μg/ml)	TCC (μg/ml)	
		Before	After			Before	After
Family A/II-1	C6SD	0·68	0·10	Yes	53	0·29	1·70
Family A/II-3	C6SD	0·79	0·12	Yes	43	0·27	1·70
Family A/II-5	C6SD	0·68	0·27	Yes	54	0·34	1·41
Family B/II-2	C6SD	0·75	0·16	Yes	73	0·27	1·69
Family B/II-4*	C6SD	0·57	0·2	Yes	82	0·44	1·55
Family B/II-6*	C6SD	0·8	0·2	Yes	97	0·69	1·99
Family B/III-1	C6Q0	0·0	0·0	No	109	0·0	0·0
Family B/III-3	C6Q0	0·0	0·0	No	142	0·0	0·0
WU-01	C6SD	0·64	0·16	Yes	131	1·08	2·64
RE-01*	C6SD	0·61	0·32	Yes	143	0·72	1·00
WFH	C6SD/C7SD	1·7	ND	Yes (ref. 1)	4·6	ND†	ND
CJ	C6SD/C7SD	2·00	0·86	Yes	4·4	1·23	2·20

The table gives C6 and TCC concentrations in sera from subjects before and after complement activation by yeast treatment (indicated in the table by before and after). The C7 concentrations in untreated sera and haemolytic C6 activity are also indicated. * Indicates samples that travelled by post. The C6 and C7 levels need to be compared with normal C6 and C7 levels (published means ± SD, 45 ± 16 and 90 ± 36 μg/ml respectively; and ranges 20–80 and 30–180 μg/ml respectively).[5] † ND, not done.

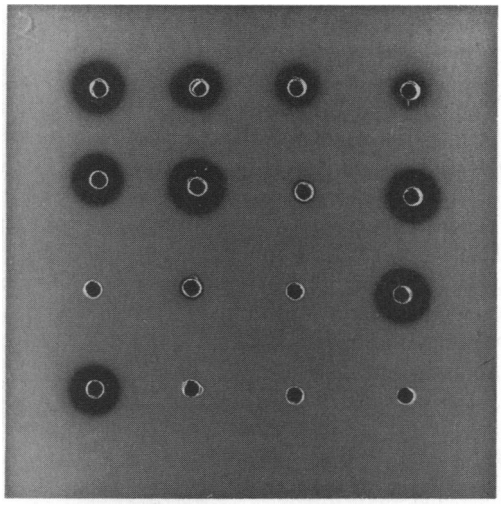

Figure 1. C6 functional haemolytic assay. Five-microlitre serum samples were inoculated in each well from left to right as follows: row 1 (i) normal human serum (NHS), (ii) NHS 1/2, (iii) NHS 1/4, (iv) NHS 1/8; row 2 (i) family A/I-1, (ii) family A/I-2, (iii) family A/II-5, (iv) obligate heterozygous C6Q0/C6A; row 3 (i) family B/III-1 (ii) family B/II-2, (iii) family B/III-3, (iv) family B/II-7; row 4 (i) family B/III-2, (ii–iv) empty. For method see text. After overnight incubation at 4° normal haemolytic rings were found around the following samples: row 1 (all samples but with decreasing size); row 2 (i, ii and iv); row 3 (iv); and row 4 (i). Very tiny, but discernible rings were seen round the two C6SD samples [row 2 (iii) and row 3 (ii)]. No haemolysis at all was seen round the C6Q0 samples [row 3 (i) and iii)].

Measurement of C6 haemolytic activity

Serum samples from C6-deficient individuals were inoculated into wells in C6 indicator gels. A representative gel is shown in Fig. 1 and this shows that whereas no ring at all was visible round the C6Q0 samples, very small rings did develop around the C6SD samples. This assay was done on samples from all C6SD subjects and from CJ (although not all are illustrated), but not from WFH (his serum had been previously shown to have weak C6 activity)[1] and Table 1 presents the results together with the results of the ELISA assays. In all these samples small amounts of functionally active C6 were found.

Family studies

Figure 2 depicts the family trees of families A and B showing the results of the C6 and C7 assays and allotyping including C7-M/N typing. In family B two children of II-2 had no detectable C6 by either ELISA or the functional assay and it is therefore apparent that II-2 herself must be heterozygous C6SD/C6Q0 whereas her husband II-7 is heterozygous C6A/C6Q0. Although C7 levels are low in II-2 it is not possible to deduce that a C7-deficiency gene is present in family B. In family A the C6 genotypes cannot be deduced because there are no homozygous C6Q0 members. The C6SD gene product can be detected only in the presence of a second C6SD gene or a C6Q0 gene which means one or the other of these must be present, as well as a C6SD gene, in all affected family members. Since we have found many more families in the Cape with C6Q0 than C6SD, it seems probable that in family A either I-1 or I-2 carries this gene rather than the C6SD gene. Also, the low C7 levels in a number of the members of family A suggest that there may be segregation of a C7-deficiency (or low) gene in addition to the C6 gene defect(s). If this is true then the data in Fig. 2a are compatible with a C7-deficiency gene segregating in association with one of the maternal C6-deficiency genes.

Studies of the abnormal C6

SDS–PAGE studies

Concentrated and unconcentrated serum samples from normal, C6Q0, C6SD and C6SD/C7SD individuals were run on SDS–

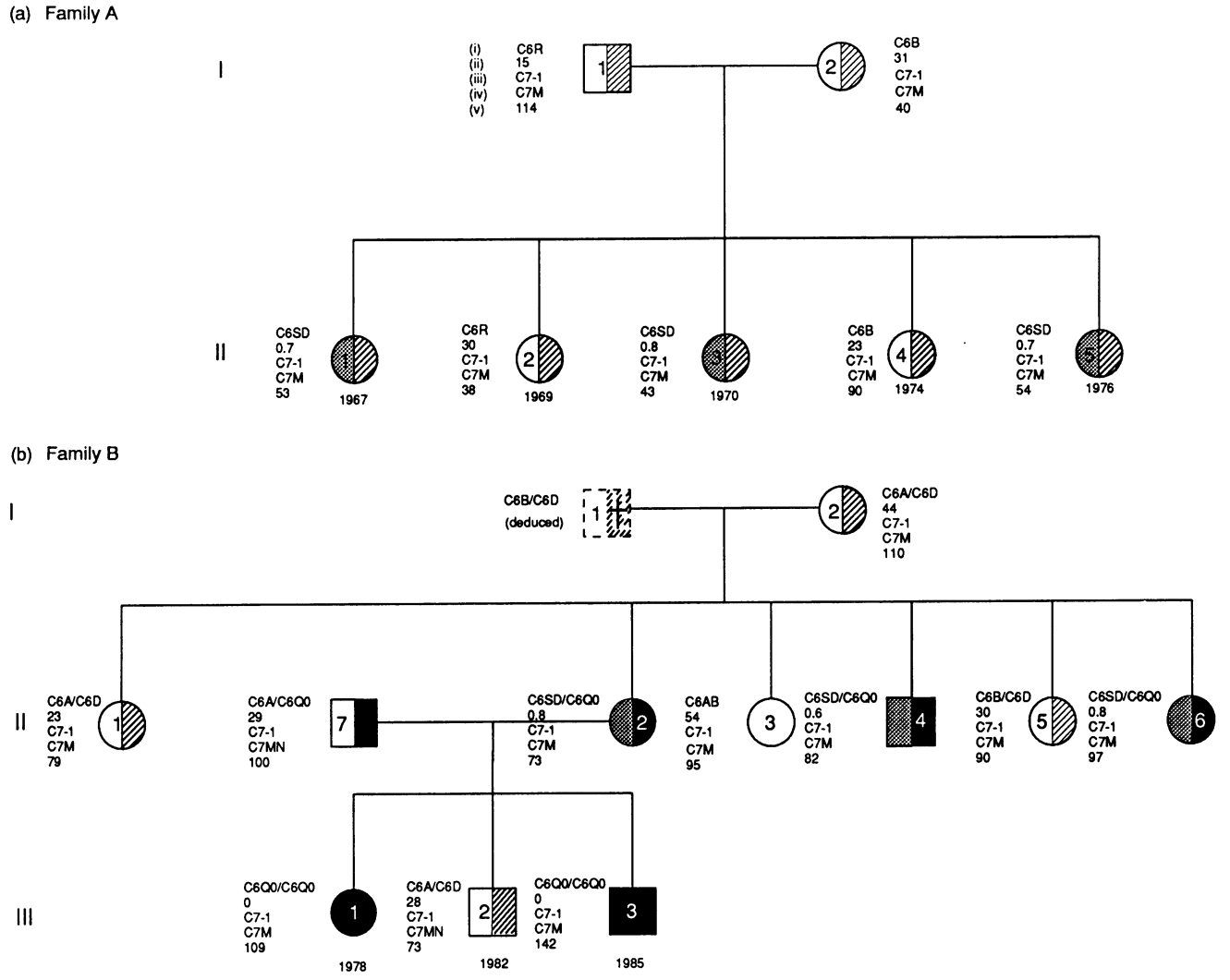

Figure 2. Family trees for families A and B. (a) Family A showing C6 and C7 phenotypes, C6 and C7 levels in μg/ml. C6 genotypes have been partially deduced from the data. However in this family in most instances it is not possible to distinguish the C6Q0 from the C6SD genotypes and accordingly these genotypes are indicated as C6D. (b) Family B showing C6 and C7 phenotypes, C6 and C7 levels, and the C6 genotypes where we have been able to deduce them from the data we have available. However, as in family A it is not always possible to distinguish C6SD from C6Q0 genotypes. (i) C6 IEF allotype (R = rare, SD = subtotal deficiency, Q0 = total deficiency); (ii) serum C6 level in μg/ml; (iii) C7 IEF allotype; (iv) C7-M/N allotype; (v) serum C7 level in μg/ml. C6 normal (□); C6 SD (▨); C6D, i.e. C6Q0 or C6SD (▨); C6Q0 (■).

PAGE gels and the bands developed as described. Figure 3 shows that a concentrated NHS sample produced C6 bands associated with a calculated molecular weight (M_r) of 99 kDa; sera from C6SD individuals gave rise to very much weaker C6 bands that represented a protein with a slightly lower molecular weight (85 kDa) calculated to be 86% of the M_r of normal C6, and no bands were detected in the tracks of samples from the C6Q0 individuals. The samples we assayed in this manner were from family A II-1, II-3 and II-5 and from family B II-2 and WU-01, CJ and RE-01. Thus samples from all propositi except WFH were assayed. In each case a weak band of identical mobility indicating C6 of the lower M_r was found. What we cannot be certain of is whether normal serum also gives rise to weak bands associated with this low M_r C6. The concentrated samples loaded per track had approximately 100 times the C6 and 15 times the protein compared to the 1·5-μl normal human serum (NHS) that we would routinely run on such a gel. The concentrated NHS did produce some weak bands below the main C6 band and interpretation is difficult. However, it is clear that the concentrated C6Q0 serum gives rise to none of these bands.

C6 and C7 allotyping

C7 allotyping by IEF revealed that all serum samples from the C6SD individuals, but not WFH and CJ, typed C7-1. The unusual C7 band pattern previously reported for WFH[1] was confirmed and moreover we showed that the C7 band pattern produced by serum from CJ was identical (Fig. 4). Track 2 in Fig. 4 was loaded with serum from a daughter of WFH; this appears to type C7-1. However, there are faint bands below the main C7-1 bands that are weak and partially obscured by the normal bands. It should be noted that the six children of WFH, who all must carry one of the abnormal C7 genes, all appear phenotypically as C7-1.[1] Thus the abnormal band pattern

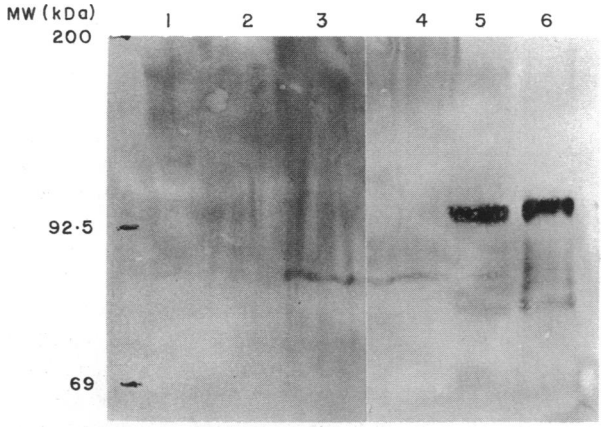

Figure 3. SDS–PAGE and immunoblotting. An 8% SDS–PAGE gel was loaded, run and blotted as described in Materials and Methods. Samples were: lane (1) 20 μl serum family B/II-2 (C6SD); lane (2) 20 μl concentrated serum family B/III-1 (C6Q0); lane (3) 20 μl concentrated serum family B/II-2 (C6SD); lane (4) 20 μl concentrated serum family A/II-5 (C6SD); lane (5) 1·5 μl concentrated serum family A/I-2 [heterozygous C6B/C6D (C6Q0 or C6SD)]; lane (6) 1·5 μl concentrated NHS. The positions of the molecular weight (MW) standards are shown on the left. In lanes (3) and (4) the C6SD bands can be seen; the calculated M_r for this protein is 85 kDa. No band is visible in lane (1) where the unconcentrated C6SD sample was run, nor in lane (2) where the concentrated C6Q0 sample was run.

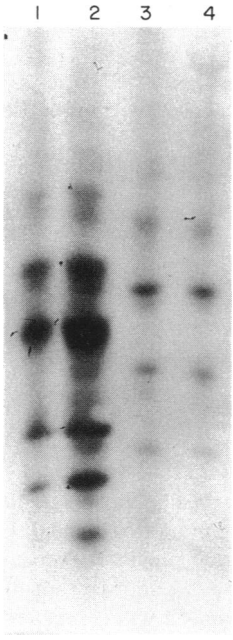

Figure 4. Isoelectric focusing and immunoblotting analysis of C7. The 5% polyacrylamide gel with ampholytes pH range 5–7 was run, blotted and probed as described in Materials and Methods. The anode is at the top. The serum samples loaded were: track (1) 5 μl NHS (C7-1); (2) 5 μl from the daughter of WFH (phenotypically C7-1); (3) 10 μl WFH (C6SD/C7SD); (4) 10 μl CJ(C6SD/C7SD).

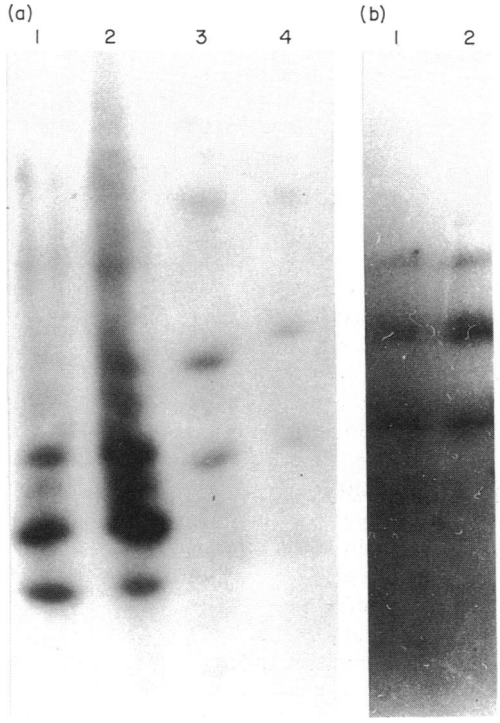

Figure 5. Isoelectric focusing and immunoblotting analysis of C6. (a) The 5% polyacrylamide gel with ampholytes pH range 5–7 was run, blotted and probed as described in Materials and Methods. The anode is at the top. The serum samples loaded were: track (1) 5 μl NHS; (2) 5 μl from the daughter of WFH (genotypically C6A/C6SD); (3) 10 μl WFH (C6SD/C7SD); (4) 10 μl CJ (C6SD/C7SD). (b) The 5% polyacrylamide gel with ampholytes pH range 5–7 was run and overlayed with a C6 indicator gel as described in Materials and Methods. The photograph was taken after approximately 24 hr incubation at room temperature. The serum samples loaded were: track (1) 30 μl family A/II-5 (C6SD); track (2) 30 μl CJ (C6SD/C7SD). For allotypic analysis by C6 functional overlay 10 μl NHS would normally be loaded per track, and development of bands would normally be for 2 hr at room temperature.

cannot be detected with any accuracy in the presence of the normal C7-1 band pattern. The results of the C7-M/N typing for families A and B are given in Fig. 2; the other C6SD samples were all C7 M, plus those from WFH and CJ who had associated C7SD.

Figure 5 shows results of the C6 allotyping gels. In Fig. 5a we show the result of the IEF analysis of sera from WFH and CJ; band development was by electroblotting and immunoprobing. The figure shows that both samples produced identical band patterns with bands that focused slightly anodal to the normal C6 bands. Figure 5b shows the results of an IEF gel that was developed with a C6 haemolytic overlay; again weak bands are visible; these focused slightly anodal to those produced by C6A (not illustrated). The figure shows that the C6 bands produced by CJ serum had the same mobility as those produced by the SA family B II-2 individual. In addition to the samples illustrated we have tested samples from the affected members of family A, the other affected members of family B and individuals WU-01 and RE-01. In each case we found weak functionally active bands with precisely the same mobility; we will, therefore, refer to this C6 allotype as the C6SD allotype. Because the bands are weak they cannot be visualized in the presence of a normal C6

band pattern and therefore C6SD/C6-sufficient heterozygotes cannot be identified by this method.

DISCUSSION

We describe here an abnormal C6 found in a number of individuals who presented in different parts of the world. All the information that we have so far been able to gather on this abnormal protein suggests that it is identical in all the subjects we have investigated. The M_r of the abnormal C6 from all C6SD individuals and one combined C6SD/C7SD patient was estimated and found to be 86% of the M_r of normal C6. This is close to the 79% of normal M_r found for the abnormal C6 from WFH by Lachmann and co-workers[1] and we believe that WFH had the same abnormal protein as the other subjects. This belief is strengthened because IEF analysis of WFH C6 showed it to have the same IEF mobility as that of the C6 from CJ. Moreover the abnormal C6 proteins from all individuals were shown to produce haemolytically active bands on IEF and to be functionally active, in that on yeast treatment of the serum they become incorporated into the TCC.

All the subjects had C6 levels in the range 0·3–3·0 μg/ml. The affected subjects in families A and B, WU-01 and RE-01 have C6 levels approximately half those in WFH and CJ. This may be because the former could be heterozygous C6SD/C6Q0 and the latter homozygous C6SD. Alternatively the only two subjects with C6 levels above 1·0 μg/ml are the two proven combined deficiency patients and this may be a factor.

One question which arises about the abnormal C6 is whether this low M_r C6 is present in very low concentrations in normal individuals. Indeed we have no proof that it is not, although we can say fairly categorically that it is not present in C6Q0 patients. Examination of the track with concentrated NHS in Fig. 3 shows the presence of some bands below the strong C6 band whereas nothing is visible in the track where the concentrated C6Q0 sample was run. The presence of such weak bands in the track in which the highly concentrated NHS was run may mean nothing positive but these weak bands do mean we cannot exclude the presence of the C6SD protein. Similarly any of the C6SD bands on IEF would be obscured in the presence of a normal C6 band pattern. If the low M_r C6 is present in normal individuals then it is possible that the C6SD defect results from abnormal gene transcription which is blocked for normal transcription but continues with transcription of the gene for the lower M_r protein. This low M_r protein would normally represent a very small proportion of the total C6 concentration. Orren and co-workers[11] have described a C6 M_r polymorphism in mice in which some inbred strains have two M_r C6 forms and some strains only the lower M_r C6 form. It is possible there are similar mechanisms in men and mice with the difference being that in mice that express both forms there is not such a great differential in the levels of expression.

We present data from two families who carry the C6SD gene. Both these families were found in the Western Cape, South Africa where C6Q0 is relatively common. It is apparent that the diagnosis of C6SD is facilitated by the co-existence of a C6Q0 gene. It is also apparent that both abnormal C6 genes segregated in family B and it is probable that this is also true in family A. We suspect that the co-existence of these two abnormal genes is purely fortuitous. We have now diagnosed homozygous C6Q0 in members of 25 different Cape families including family B but not family A. In contrast we found only two families with C6SD and its seems probable that in neither family did we find a homozygous C6SD subject. None of the C6SD subjects that we report in this paper have suffered *Neisserial* disease and it may be that the small amount of functionally active C6 that they possess is sufficient to render them no more susceptible to these infections than members of the population at large. However, one patient previously reported[5] had a C6 level of 4·8 μg/ml and thus appeared to be C6SD; he died from meningococcal disease and could not be further investigated. As we know nothing about his C6 he is not included in this report. If C6SD patients are indeed less susceptible to meningococcal disease than C6Q0 patients, C6SD patients will be less likely to be ascertained. In the Cape, all C6Q0 patients were ascertained because of meningococcal disease either in themselves or in a sibling and it may be that the apparently greater gene frequency of C6Q0 is an ascertainment artefact. We also have no information about the type of C6 deficiency in patients from the U.S.A. other than RE-01. We do know that many presented with *Neisserial* infection and that many were black.[19,20] Our Cape Coloured patients have a variety of ethnic origins[8] and it is not possible at present to ascribe their C6 genetic deficiency to any particular one of these.

Our previous studies have not found an association of C7 deficiency with C6Q0[3,5] but association of C7SD with C6SD has already been reported in the patients WFH[1] and CJ[6] and association of some form of C6 deficiency with C7 deficiency was suggested in the families reported by Glass and co-workers[21] and Coleman and co-workers.[22] The low C7 levels and the distribution of these low levels in family A (Fig. 2a) suggest that a C7-deficiency gene is segregating in that family. However, normal C7 levels have a wide range and at present it is not possible to diagnose heterozygous C7 deficiency definitively apart from the obligate cases who are first-degree relatives of patients with homozygous C7 deficiency. It is also obviously not possible to deduce from our data whether the putative C7-deficiency gene in family A codes for C7SD similar to that found in WFH and CJ or for a different C7 deficiency. We have found only two SA families with C7 deficiency (one reported by Cooper and co-workers[23]) and although C7 deficiency could occur fortuitously together with a C6 deficiency, in the same way that the two C6 deficiencies co-exist in family B, this seems unlikely. It is unfortunate that IEF analysis of C7 does not allow identification of C7SD in heterozygotes who possess one normal C7 allotype. WU-01 and RE-01 both have a normal C7 level, and thus C6SD is not necessarily associated with low C7 levels.

The work in this paper confirms our previous report[5] that C6 deficiency falls into at least two types (three types were suggested in that report) and examines some properties of the abnormal C6 found in subtotal deficiency. Much more information is obviously needed before we conclude what relationship this protein has to normal C6 and whether or not it is present in normal individuals. What we have clearly shown is that the C6SD protein is haemolytically active and further we speculate that this small amount of protein may provide some protection against meningococcal disease.

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