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Combined total deficiency of C7 and C4B with systemic lupus erythematosus (SLE)

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SUMMARY

The first inherited combined total deficiency of C7 and C4B complement components associated with SLE is described in a young female. Functional C7 assays showed a homozygous C7 deficiency in the propositus and her sister, and an heterozygous one in their parents. C4 molecular analyses showed that both the propositus and her mother had two HLA haplotypes carrying only C4A-specific DNA sequences and a normal C4 gene number. Thus, only C4A proteins could be expressed, with resultant normal C4 serum levels. The coexistence of a combined complete C7 and C4B deficiency may therefore abrogate essential functions of the complement cascade presumably related to immune complex handling and solubilization despite an excess of circulating C4A. These findings challenge the putative pathophysiological roles of C4A and C4B and stress the need to perform both functional assays and C4 allotyping in patients with autoimmune pathology and low haemolytic activity without low serum levels of a classical pathway complement component.

Keywords systemic lupus erythematosus complement deficiency C4 allotyping DNA

INTRODUCTION

Inborn complement deficiency states of the early acting classical pathway components predispose to autoimmune immune complex diseases affecting the connective tissue, such as SLE, while deficiencies of late-acting components predispose to severe or recurrent pyogenic infections, mainly neisserial (reviewed in [1]). However, individuals having selective early-acting complement deficiencies (like complete C4B deficiency, present in about 2% of the white population) may conversely develop pyogenic infections [2] and those with late-acting complement deficiencies (like C7 deficiency, present in about 0·01% of the population) may have autoimmune manifestations [3]. Individuals with any complement deficiency, including C4B and C7, may also be completely healthy [4]. Thus, additional genetic or environmental factors may explain the heterogeneous clinical manifestations of complement deficiencies.

In addition, combined inherited deficiencies of late-acting or regulatory complement components with C4A and C4B deficiency were reported in severe pyogenic infections [5–7]. Such associations suggest a summatory contribution of C4 to the clinical manifestations of complement terminal pathway dysfunctions. In the present work we report immunochemical, functional and molecular studies of the second C7 and C4B complete deficiency so far reported, but in this case associated with SLE instead of a severe infection [5]. These findings question fundamental knowledge about the contribution of early and late-acting complement components to the development of autoimmune pathology.

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MATERIALS AND METHODS

Case report

A 32-year-old woman had a history of proliferative mesangial glomerulonephritis in childhood; this disease was apparently healed at the age of 11. At age 14 epilepsy was diagnosed and treated with hydantoin. Simultaneously with her first pregnancy she presented with skin and joint lesions and a treatment with salicylates was established. After 9 months her symptomatology recurred, a malar erythema associated with sun exposure developed, and since then she has been treated with corticosteroids. No history of repeated infectious episodes was recorded. Laboratory investigations showed a positive rheumatoid factor (145 U/ml) by nephelometry (Array, Beckman, Fullerton, CA) and anti-nuclear antibodies in high titres (1/2560) with both homogeneous and peripheral staining patterns. Other autoantibodies (anti-smooth muscle, anti-mitochondrial, anti-reticulin and anti-native DNA) were negative. A non-

detectable haemolytic complement activity (CH50 < 50 U/ml) led to a detailed study of the complement cascade, described below. Following current standard criteria [8], the patient was diagnosed as having SLE.

Immunochemical and functional complement studies

The propositus, one sister and both parents were studied (see pedigree in Fig. 1). Individual complement components (C1q, C1r, C1s, C2, C5, C6, C7, C8, C9, factors I and H) were screened by Ouchterlony double diffusion using commercial antibodies (Atlantic Antibodies, USA; Serotec, Oxford, UK). C3 and C4 levels were measured by nephelometry (Array, Beckman). The presence of C7 protein was also analysed by rocket electrophoresis as described [9] and by immunoblotting and isoelectric focusing at pH 5–7 [10] with specific anti-C7 serum. CH-50 haemolytic test was carried out by standard methods. Functional C4 and C2 activities were tested using C4-deficient guinea pig serum and a human C2-deficient serum. Functional C7 activity was determined by a modification of the method of Nelson *et al.* [11]. The ability to recover haemolytic activity was tested using 100 U of functional C7 (Cordis, Miami, FL). In other family members and controls, C7 activity was tested using 200 μl serial dilutions of sera plus 200 μl of sensitized erythrocytes (adjusted at 10^8 cells/ml) incubated with 200 μl of a 1/20 diluted propositus serum (the lowest serum dilution allowing 100% reconstitution of C7 complement haemolytic activity when 100 U functional C7 were added) in a standard quantitative haemolytic assay.

HLA, BF, C2 and C4 typing

All family members were HLA typed for class I, II and III antigens as previously reported [10,12,13].

HLA class III molecular studies

Genomic DNA digestion (*Taq*I, *Sst*I, *Bgl*II), Southern blot analysis and hybridization with BF (pFB3b), C2 (pG850), 21-hydroxylase or CYP21 (pC21/3c) and C4 probes (pAT-A) were performed as described [13]. Specific C4d analysis was done after *Nla*IV and *Eco*O109 digestion and hybridization with a C4d-specific probe (Pb) as described [14]. All probes were kindly provided by Dr R. D. Campbell.

RESULTS

Immunochemical and functional studies

Individual complement components in all family members were always detected for all (C1q, C1r, C1s, C2, C3, C4, C5, C6, C7, C8, C9, factors I and H) except for C7 proteins in the propositus and her sister. Table 1 shows immunochemical levels and functional activity of C7, C4 and C2 proteins. The propositus and her (so far) healthy sister also lacked any C7 detectable protein by using rocket electrophoresis (Table 1) and immunoblotting with anti-C7 serum (not shown). No C7 *in vitro* haemolytic activity was observed in both sisters, although their total haemolytic activity (CH50) could be restored to 100% by the addition of functional C7 protein. C4 haemolytic activity was markedly low in the propositus and in her mother. C2 haemolytic activity was always within the normal range.

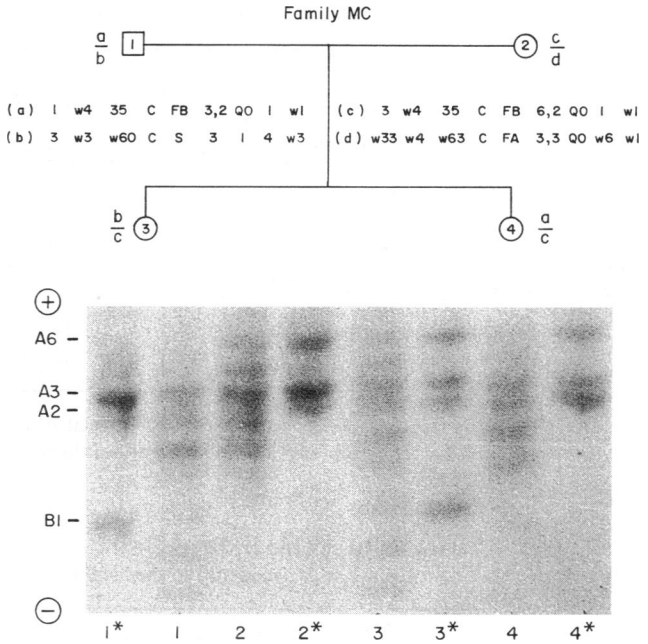

Fig. 1. Pedigree of the family MC (No. 4 is the propositus), carrying C7 and C4B deficiencies (sequence of data: HLA-A, C, B, C2, BF, C4A, C4B, HLA-DR, DQ). C4 allotypes obtained after electrophoresis and immunofixation in samples treated only with neuraminidase or also with carboxipeptidase (*).

HLA typing and C4 molecular analysis

Figure 1 shows the family HLA genotypes, including BF, C2 and C4 allotypes, and the C4 protein typing pattern. The propositus carries two C4BQ0-bearing haplotypes, with distinct C4A duplications (3,2 and 6,2). The mother is also C4BQ0 homozygous, whilst the father and the sister are C4BQ0 heterozygous. DNA analysis of class III genes showed identical C2, BF, C4 and CYP21 restriction fragment length polymorphisms (RFLPs) and gene copy number of both propositus haplotypes (Table 2). All three C4BQ0-bearing haplotypes carry long C4 genes at locus I and II, hence excluding any gene deletions.

To analyse further the molecular basis of the C4 deficiency in this family, major Rodgers and Chido determinants, and consequently C4A and C4B isotypes of each haplotype, were analysed by means of a C4d-specific genomic probe and RFLP analysis. Figure 2 and Table 2 show that C4B-specific Chido sequences Ch+4 and Ch+1 were only present in haplotype b, corresponding to the C4B1 gene, carried by father and sister of the propositus. These findings prove that the propositus has four C4 genes of the C4A isotype.

DISCUSSION

The study of complement-deficient patients is a major tool for gaining insight into the *in vivo* function of a particular complement component. By these means, the crucial role of the early acting classical pathway complement components in promoting the solubility and clearance of immune complexes [15] and of the late-phase components in host defence against infections [16] has been clearly established. However, several patients have been reported with early or late-acting complement deficiencies who were completely healthy or who had

Table 1. Immunochemical and functional studies of C7, C4 and C2 in the MC family

	Serum concentrations		Hemolytic activities (U/ml)		
Member	C7* (%)	C4 (mg/dl)	C7	C4	C2
1 (Father)	85	24	15551	14000	2173
2 (Mother)	52	39	11365	4000	3333
3 (Sister)	0	23	< 50	14450	2083
4 (Propositus)	0	21	< 50	4400	2000
Normal range	100	14–38	> 18750	> 8717	> 1500
(*n*)	(25)	(225)	(12)	(12)	(12)

* C7 concentration was calculated as a percentage of the height obtained by rocket electrophoresis with a pool of healthy individuals.

Table 2. C2, BF, C4 and 21-OH gene restriction fragments and Rodgers/Chido (Rg/Ch) determinants (see Fig. 2) assigned to the haplotypes of family MC (see pedigree in Fig. 1)

					Restriction fragments (kb)								
							C4-locus I			C4-locus II			
	C2	BF	C4A	C4B	C2 Sst	BF Taq	BglII	TaqI	CYP21A Taq	BglII	TaqI	CYP21B Taq	Rg/Ch
a	C	FB	3, 2	Q0	2·7	4·5	15	7·0	3·2	12	6·0	3·7	Rg1, Ch-1, -4
b	C	S	3	1	2·7	4·5	15	7·0	3·2	12	6·0	3·7	Rg1, Ch1, 4
c	C	FB	6, 2	Q0	2·7	4·5	15	7·0	3·2	12	6·0	3·7	Rg1, Ch-1, -4
d	C	FA	3, 3	Q0	2·4	6·6	4·5	7·0	3·2	15	6·0	3·7	Rg1, Ch-1, -4

clinical manifestations not fitting with those expected [7,17].

These conflicting reports apply also to C7 and C4B complement components. Deficiency of C7 is usually found in patients with adult onset systemic neisserial infections [1]. However, some cases with this deficiency presented with overt SLE [3], scleroderma, rheumatoid arthritis [4] or nephritis [18], entities which may have an autoimmune pathogenesis. Similarly, Schifferli *et al.* [15] reported that about 10% of these and other deficiencies of late-acting complement components were accompanied by immune complex diseases and defects in complement-mediated inhibition of immunoprecipitation. C4B deficiency has also been predominantly found in putative autoimmune diseases like insulin-dependent diabetes mellitus, rheumatoid purpura, vasculitis [4] and IgA nephropathy [19], but also in several types of bacterial infections [2,20]. These heterogeneous clinical pictures are difficult to interpret in the light of the postulated *in vivo* function of C7 and C4B. Indeed, there are several reports of homo- or heterozygous C4A or C4B deficiencies combined with C7 [5], C5 [6] and properdin deficiencies [4] with severe bacterial infections that point towards a summatory role of C4 in host defence against infections. However, a more recent report [21] casts doubt on this view, showing that none of 27 patients with late-acting complement activities and bacterial infections had either C4A or C4B deficiency.

The present report of a combined total C7 and C4B deficiency with SLE symptomatology, including a history of nephritis and a high titre of rheumatoid factor, suggests that both deficient components may also participate in the solubilization and clearance of immune complexes. The fact that other family members having only one of the two deficiencies are completely healthy underlines the possibility that a total lack of both proteins is necessary for the onset of clinical manifestations. Although all other complement components analysed were present, an additional functional impairment of any of them should not be discounted. This was the case with C4, where low C4 haemolytic activity was found in the propositus and her mother in spite of normal C4 serum levels. This result led us to analyse C4 allotypes in all family members, showing unusual C4 protein patterns where all individuals carried at least three allotypes of the C4A isotype.

DNA analysis of the HLA class III region in this family was considered of particular interest given the different molecular basis reported in several C4A and C4B deficiencies (reviewed in [4]). Both isotypes, C4A and C4B, are coded by closely linked genes within the HLA class III region. The C4 region is very unstable, probably due to the 99% nucleotide sequence identity of C4A and C4B, which gives rise to a higher frequency of recombinations leading to several types of deletions and duplications. Thus, C4 gene copy number and size were readily analysed using *Taq*I RFLPs [22] and the isotypic differences between C4A and C4B (resulting from a difference of only four amino acids produced by alteration of five nucleotides within a 17-nucleotide block) were resolved using endonucleases which specifically recognize these changes and subsequent hybridiza-

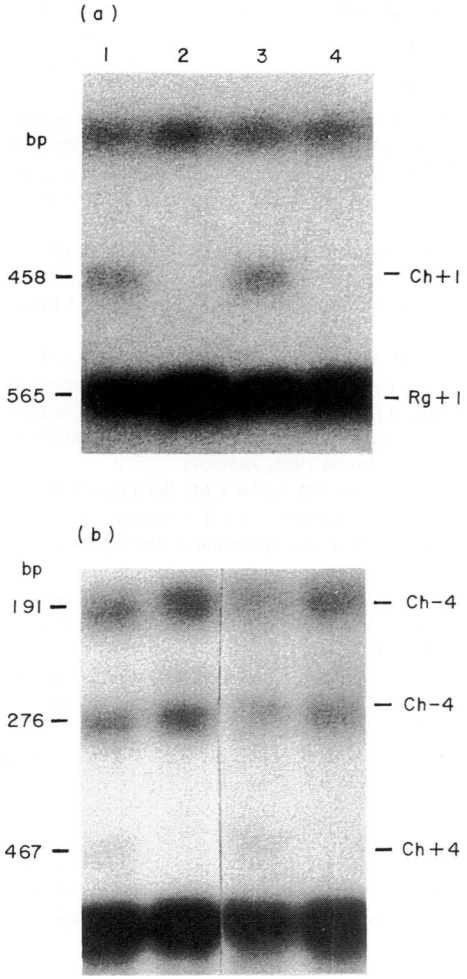

Fig. 2. Restriction fragment analysis of the C4d region from members of family MC (see Fig. 1) using *Eco*O109 (a) and *Nla*IV (b) endonucleases subsequently hybridized with a C4d-specific genomic probe Pb [14]. Fragment sizes in base pairs (bp) on the left as determined by standard molecular weight markers (not shown), corresponding Rg/Ch DNA sequences on the right.

tion with a C4d-specific probe [14]. Both haplotypes of the propositus and her mother had no C4 deletions, carrying four C4 long genes of the C4A isotype.

The finding of a normal C4 serum level with abnormal haemolytic activity both in the propositus and her mother may thus be explained through a gene conversion generated from C4B to C4A [23]. Despite the considerable structural homology, the gene products of the two C4 loci differ in antigenic reactivities, covalent binding affinity to antigens and antibodies and in their haemolytic activities. C4B has a four-fold higher haemolytic activity than C4A, and the C4A6 allotype, also present in both C4B-deficient members, is haemolytically inactive due to its inability to form a functional C5 convertase [24]. It might be speculated that the C4A protein excess might have influenced the lack of pyogenic infections in the propositus, since this was not the case in the first combined C7 and C4B deficiency reported [5].

The history of nephritis in the patient deserves special comment. A deficiency of C7 has been reported in a patient with nephritis and tendency to form the activated $C\overline{56}$ complex [18]; moreover, C4B deficiency is the major genetic risk for IgA nephropathy, having possibly a primary pathogenetical role in this disease [19]. Also, partial deficiency of one or more of the terminal complement components may predispose to membranoproliferative glomerulonephritis [25]. Thus, the combination of both deficiencies in the propositus might have contributed to her recorded proliferative mesangial glomerulonephritis.

This study raises some questions about the present knowledge on C4A and C4B function *in vivo*. C4A is more effective than C4B in inhibiting the precipitation of immune complexes, especially if there is antibody excess, whilst C4B enhances immunoprecipitation in antigen excess [26]. Thus, C4A (but not C4B) has a predominant role in immune complex solubilization, as supported by the prevalence of C4A homo- or heterozygous deficiency in several Caucasoid and black populations with SLE. However, SLE may also develop with C4B deficiency and excess C4A protein (this report); C4B deficiency (included within the HLA-B18, F1C30, DR3 haplotype), but not C4A deficiency, is predominantly found in Spanish SLE patients [27].

In conclusion, the *in vivo* pathophysiological role of C4A and C4B may be more complex than previously thought. This report suggests a complementary effect of the early- and late-acting complement components, in addition to the sequential activation of the cascade, in the solubilization and/or precipitation of the immune complexes. Thus, the possibility of combined deficiencies, especially of C4A or C4B, should be borne in mind when either late or alternative pathway deficient patients develop autoimmune processes like SLE. Furthermore, these findings stress the need to perform both functional assays and C4 allotyping in patients with autoimmune pathology and low haemolytic complement activity without low serum levels of a classical pathway complement component.

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