POLYMORPHISM OF C4 WITH SPECIAL REFERENCE TO THE SEROLOGICAL DETERMINANTS, RODGERS AND CHIDO.

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Introduction

The fourth component of complement (C4) in man has an important central role in the classical pathway of complement activation. It interacts with other complement components (C1, C2, C3) and binds covalently to antibody or antigen on cell surfaces. The highly complex polymorphism is thought to relate to its diversity of function (Porter 1983) and is limited, with one reported exception (Mauff et al. 1983a), to the α chain of the molecule or more precisely to the C4d (α_2) fragment (Tilley et al. 1978, Mevag et al. 1981). Since the C4d fragment contains structures involved in the activation, inactivation and covalent binding of C4 and also 13 of the 15 reported nucleotide sequence differences (Belt et al. 1985), Porter suggests that the α chain is probably on the exterior of the C4 molecule.

There are two closely linked genes, C4A and C4B, which probably arose by duplication and, with genes for two other complement components C2 and Bf, comprise the class III genes of the Major Histocompatibility Complex (MHC) on chromosome 6 in man. The gene order is C2, Bf, C4A, C4B (Carroll et al. 1984 a,b) though the orientation in relation to HLA-B and -DR loci is not known. The term complotype was proposed (Alper et al. 1983) for the class III complement proteins as no cross-over has been detected between them and the extended haplotypes including HLA-B and -DR products show marked linkage disequilibrium (Awdeh et al. 1983). It is clear that the complex polymorphism of C4 has a role to play as a genetic marker particularly in the studies of disease associations and susceptibility (Fielder et al. 1983, Dawkins et al. 1983), but many of the interrelationships of demonstrable polymorphism are not completely resolved so the value in forensic investigations must be rather limited at present.

Each C4 gene, C4A and C4B, has a series of alleles and relatively frequent silent alleles termed A*QO and B*QO. Generally the C4 allotypes have been detected by immunofixation electrophoresis of fresh EDTA plasma after neuraminidase treatment and the recommended nomenclature (Mauff et al. 1983b) is based on this method. Distinction between the C4A and the C4B products can be made by their marked difference in

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haemolytic activity which is useful in classifying the rarer fast C4B products and the slower C4A products whose banding patterns can overlap. There are other differences between C4A and C4B products. The C4A protein is strongly associated with Rg (Rodgers) determinants and C4B with Ch (Chido) determinants (O'Neill et al. 1978a) and an apparent molecular weight difference of 2000 was demonstrated between the heavier C4A and lighter C4B α chains (Roos et al. 1982) which absorb anti-Rg and anti-Ch respectively. A combination of techniques will provide the best definition of the products of the two genes. However the classification into C4A and C4B is becoming less easy with the increase in knowledge of the polymorphism.

Rg and Ch determinants on C4

The Rodgers (Rg) and Chido (Ch) blood groups were described before the association with C4 was known; linkage to HLA had been observed for both and clearly a relationship between them existed (Giles 1977). The frequency of Rg+ and Ch+ did not allow them to be products of alleles at a single locus. This knowledge enabled O'Neill and colleagues to establish Rg and Ch as determinants on C4 and necessitated the proposal that the polymorphism of C4 was controlled by two loci rather than one (O'Neill et al. 1978b).

Patients who lack C4A or C4B and who have received many transfusions of blood may become immunised to determinants on their missing C4 component. The alloantibodies are IgG and do not themselves bind complement in serological tests; they are detected in compatibility tests with normal red cells. Much stronger reactions are obtained with red cells that have been coated with C4. Trypsin treatment of these C4 cells does not affect their reactivity, demonstrating that anti-Rg/Ch are detecting determinants on the C4d fragment (Tilley et al. 1978).

Both anti-Rg and anti-Ch are inhibited by C4 in serum (Middleton & Crookston 1972, Longster & Giles 1976) but two types of inhibition, complete and partial, were described for anti-Rg. Partial inhibition of anti-Ch was reported later (Nordhagen et al. 1980). It was not clear whether partial inhibition represented a quantitative, as the name implied, or qualitative difference, but it was inherited recessively and linked to HLA (Giles 1977). Recent studies (Giles 1985) have shown that all anti-Rg/Ch capable of detecting partial inhibition are polyspecific and that there are separable antibodies which detect two Rg determinants (Rg1,Rg2) and three Ch determinants (Ch1,Ch2,Ch3). All anti-Rg contain anti-Rg1+2 whereas most anti-Ch contain only anti-Ch1 but a few antisera have additional speci-

ficities for Ch2 and Ch3.

Table I Frequency (%) of Rg and Ch phenotypes in the South London donor population (Giles 1984).

Rg phenotype	%	Ch phenotype	%
Rg;1,2	94.5	Ch:1,2,3	87.7
Rg:1,-2	3.2	Ch:1,-2,3	4.9
Rg;-1,-2	2.3	Ch:1,2,-3	3.2
		Ch:-1,-2,-3	4.2

There are three Rg phenotypes and 4 Ch phenotypes; Rg:-1,-2 is usually found with C4A*QO and Ch:-1,-2,-3 is usually with C4B*QO. The Rg:-1,-2 Ch:-1,-2,-3 combined phenotype represents the extremely rare C4 deficient individual not found in random testing. None of the donors (Table I) lacked a determinant for both Rg and Ch, so the frequency of donors lacking one or more determinants was about 18%. There are 12 possible combined Rg/Ch phenotypes of which only two remain undetected (see below).

Two new Ch phenotypes have been observed recently in Rg/Ch typing family material (Giles and colleagues, unpublished observations) which have only one of the three Ch determinants (Table II). These findings all support the concept of multiple determinants and antibody specificities for Rg and Ch.

Table II Eight possible Ch phenotypes.

Ch:1,2,3	Ch:-1,2,3*	Ch:1,-2,-3	Ch:-1,-2,-3
	Ch:1,-2,3	Ch:-1,2,-3	
	Ch:1,2,-3	Ch:-1,-2,3*	

* not detected yet

Association of Rg/Ch phenotypes with C4 allotypes

The Rg:1,-2 and Ch:1,-2,3 phenotypes were strongly associated by Nordhagen and colleagues (1980,1981) with the duplicated C4A haplotype A*3A*2,B*QO (FI) and the C4B haplotype B*2 (M). The Ch:1,2,-3 phenotype (Giles 1984) subdivided the common C4 B*1 allele. Studies of the segregation of rare C4 haplotypes (Rittner et al. 1984b) and the recessive inheritance of partial inhibition (Giles et al. 1984) in families showed that Rg and Ch phenotypes do not have a direct relationship with particular C4 allotypes, but simply that there are strong associations which is inevitable. The absence of two combined Rg/Ch phenotypes, Rg:1,-2 Ch:1,2,-3 and Rg-1,-2 Ch:1,2,-3 can be accounted for in Table III.

The C4 A6 allotype found with the B1 that is Ch:-3 has always been Rg1,2 and the B1 in the A*QO,B*1 haplotype has never lacked Ch3.

Table III
Association of Rg/Ch haplotypes with C4 haplotypes.
(Giles et al. 1984)

1,-2		1,-2,3		1,2,-3	
Rg	n	Ch	n	Ch	n
A*3A*2,B*QO	5	A*4,B*2	17	A*6,B*1	11
A*3,B*QO	4	A*QO,B*2	4	A*3,B*1	6
A*2,B*QO	1	A*3,B*2	4		
		A*2,B*2	1	A*6,B*3	1
A*3,B*2	1			A*QO,B*3	1
A*5.1,B*1	1	A*1,B*QO	3	A*3,B*2	1
		A*3,B*6	1	A*QO,B*w2	1
		A*4,B*5.1	1		
Totals	12		31		21

The combination of Rg/Ch typing with C4 allotyping yields more precise data and helps with haplotype interpretation.

Aberrant C4/Rg/Ch allotypes

The first finding of a C4B allotype lacking Ch and a C4A allotype with Ch was made in 1982 (Rittner et al. 1984a). The A*1,B*QO haplotype, when paired either with A*QO or B*QO in the study of family Bk (Rittner et al. 1984b) showed that this A*1 allele lacked Rg1 and Rg2 determinants but had Ch1 and Ch3 (Ch:1,-2,3). The A*4,B*5 haplotype when paired with B*QO was of two types, Ch+ and Ch- (now termed Ch1); analysis of the C4B α chain confirmed this but also demonstrated that Rg1 was present on the B5 that lacked Ch1 (Roos et al. 1984).

The aberrant A1 and B5 allotypes could be hybrid proteins, the products of fusion genes of the Lepore (A/B) and anti-Lepore (B/A) types respectively (Giles et al. 1984); this hypothesis predicted the correct gene order for C4A and C4B but as yet there is no scientific evidence to support it.

Monoclonal anti-C4B

O'Neill (1984) reported a monoclonal anti-C4B which he suggested might replace haemolytic overlay in defining the products of all C4B alleles. It was important to study its reactivity in relation to the aberrant C4 allotypes, A1 and B5. In agglutination tests with red cells of different C4 coats and in inhibition tests, the serological specificity was deduced as anti-Ch1 and not anti-C4B. It reacts with the A1 Ch:1,-2,3 allotype

but not with the B5 Ch:-1,-2,-3 allotype (Giles, unpublished observations). The immune response of the animal (mouse) in this instance is closer to that of humans, though no details of the immunisation procedure have been given. The haemolytic overlay remains the only method that defines the A1 and B5 allotypes as products of C4A and C4B genes.

Ch determinants on B5 Ch:-1,-2,-3 allotype

The B5 Ch:-1,-2,-3 allotype was termed B5 Rg^+ by Roos and colleagues (1984) but the C4B α chain reacted with a proportion of anti-Ch which could be translated into serological activity. Ch:-1,-2,-3 red cells coated with C4 from the B5 reacted weakly with many anti-Ch but it was evident with inhibition that more than one determinant involved. Recent work (Giles, in preparation) has defined three more Ch determinants expressed at a much lower level. They subdivide existing Ch phenotypes and are inherited as part of the C4/Ch haplotype. The complexity of Ch has therefore been doubled.

Conclusions

The simplistic interpretation for Rg and Ch as single determinants on C4A and C4B no longer holds true; the terms are not synonymous. Both Rg and Ch are multifactorial and are expressed in an increasing number of detectable phenotypes. The association of the Rg/Ch phenotypes with C4 allotypes is strong but they have no direct relationship. The Rg:1,-2 and Ch:1,2,-3 phenotypes subdivide the common A3 and B1 allotypes which are indistinguishable in electrophoretic analysis. When combined with HLA and C4 haplotypes, Rg/Ch haplotypes define the extended haplotype more accurately.

Rg/Ch typing relies on the supply of antisera from transfused patients and the dependence on inhibition, which detects the lack of a determinant, also limits the information obtained.

The aberrant C4 allotypes indicate that the Rg/Ch epitopes are probably independent of the C4A and C4B class differences. Rg/Ch epitopes do not relate to charge differences in the protein and may reflect amino acid differences since the C4d fragment contains only 6% carbohydrate. The precise location of Rg/Ch epitopes on C4 will have to await amino acid or nucleotide sequencing.

DNA studies of C4 will undoubtedly uncover more variation (Palsdottir et al. 1983, Whitehead et al. 1984), but it is the serological and electrophoretic

variation that provides the guide to most studies of C4 molecular genetics.

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