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*Review*

# A structural model for the location of the Rodgers and the Chido antigenic determinants and their correlation with the human complement component C4A/C4B isotypes

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## Introduction

Chido (Ch) and Rodgers (Rg) are human plasma and red blood cell antigens which were defined by the alloantibodies anti-Ch (Harris et al. 1967, Middleton and Crookston 1972) and anti-Rg (Longster and Giles 1976). Anti-Rg and anti-Ch are IgG antibodies produced in patients lacking either Rg or Ch antigen, respectively, who had undergone blood transfusion. The genes coding for the Rg and Ch antigens were both mapped to the *h*uman *l*eukocyte *a*ntigen (*HLA*) region by serological studies (Middleton et al. 1974, Giles et al. 1976). The structural genes coding for the fourth component of complement (C4) (Rittner et al. 1975, Tiesberg et al. 1976), and the complement components C2 (Fu et al. 1974) and factor B (Allen 1974), were also mapped to the *HLA* region. Biochemical, genetic, and serological studies revealed that Rg and Ch are part of the antigenic components of the two isotypes of C4, C4A and C4B (previously called C4F and C4S), respectively (O'Neill et al. 1978a, b). Subsequently it was shown that the Rg and Ch antigens were both located in the *C4d* region of C4A or C4B (Tilley et al. 1978). C4d is the factor I-mediated proteolytic degradation fragment of C4 that may be covalently linked to various cell surfaces (see Reid and Porter 1981 for a review). C4A and C4B are highly homologous proteins (Belt et al. 1984, 1985, Yu et al. 1986) having markedly different electrophoretic mobilities, hemolytic activities (Mauff et al. 1983, Sim and Cross 1986), and chemical reactivities (Isenman and Young 1984, 1986, Law et al. 1984, Dodds et al. 1986, Shifferli et al. 1987) (Table 1). The latter is due to the differential reactivities of the thiolester carbonyl group in the two classes of C4: C4A exhibits relatively higher covalent binding affinity to amino groups or peptide antigens than C4B, while C4B binds preferentially to hydroxyl groups or carbohydrate antigens. This phenomenon may also explain the higher hemolytic activity of C4B in the conventional hemolytic assay that is performed using sensitized sheep erythrocytes whose surface is rich in carbohydrate antigens. The identification of C4A and C4B is based functionally on the hemolytic activity and covalent binding activity, or structurally on the apparent relative mass of the alpha chains in modified sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE; $C4A_\alpha$ – 96000; $C4B_\alpha$ – 94000; Roos et al. 1982), or serologically using anti-Rg and anti-Ch sera.

**Table 1.** Comparison of human C4A and C4B proteins

| | C4A | C4B |
|---|---|---|
| 1. Electrophoretic mobility | | |
| (a) Agarose gel | Fast (acidic) | Slow (basic) |
| (b) SDS-PAGE ($\alpha$ chain) | $M_r$ 96000 | $M_r$ 94000 |
| 2. Thioester reactivity | | |
| (a) Hemolytic activity | Lower | Higher |
| (b) Relative covalent binding affinities | | |
| (i) Amino group | Higher | Lower |
| (ii) Hydroxyl group | Lower | Higher |
| 3. Antigenic determinants* (Blood group antigen) | Rodgers (Rg: 1, 2) | Chido (Ch: 1, 2, 3, 4, 5, 6) |

* With exceptions such as C4A1 and C4B5

## Polyspecificity of the alloantisera and multiple determinants for Rg and Ch

The hemagglutination inhibition test that was initially developed to study markers or allotypes of immunoglobulin Gm (Grubb 1956, reviewed in Nisonoff et al. 1975) has been widely applied in the serological typing for the Rg and Ch antigens (Middleton and Crookston 1972, Giles

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1980). To test the antigenicity of C4, the plasma is examined for its ability to inhibit agglutination in an antiglobulin test: (1) the plasma (antigen) is mixed with Rg-specific or Ch-specific antibody to allow Ab-Ag interaction and (2) subsequently added to human erythrocytes coated with standard C4 (free anti-Rg/anti-Ch would bind to the coated C4); the C4-coated red cells are then spun down, washed, and tested for the ability to be agglutinated by anti-IgG. If the test plasma contains the corresponding antigens for anti-Rg or anti-Ch, its interaction with the antisera will eliminate the subsequent reaction of the alloantibody with the standard C4 on red cells; therefore, no antiglobulin agglutination follows. Thus the C4 molecules in the test plasma are termed "complete inhibitors." On the contrary, if the antigens in the test plasma do not match with the test anti-Rg or anti-Ch, the antibodies will bind to standard C4 coated on red cells. Visible agglutination among red cells will result subsequent to the addition of anti-IgG to the reaction mixture. Thus the C4 molecules in the test plasma are called "negative inhibitors" (Giles 1980). On the other hand, the specificities of the antisera can be examined by using plasma of known Rg/Ch type in the test. As an alternative to this approach, the test C4 can be coated on red cells and then examined for its ability to be agglutinated by anti-Rg or anti-Ch.

In a study on the anti-Rg, Longster and Giles (1976) observed that some plasma samples exhibit a partial inhibition (p. i.) phenomenon. In other words, the Rg/Ch antigens from some individuals show weaker interaction with certain anti-Rg or anti-Ch, although there is no indication that the related plasma C4 is expressed at a lower level. p. i. was demonstrated for Ch in the C4B2 allotype that is generally expressed in parallel with C4A4 (Nordhagen et al. 1980). The p. i. phenomenon was also detected on the C4B1 allotype that is generally expressed in parallel with C4A6 or with a proportion of C4A3. However, it was found that the anti-Ch attributed to the p. i. in C4B2 did not result in the p. i. phenomenon observed in the C4B1, and vice versa (Giles 1984). This was taken to suggest that the anti-Ch and anti-Rg are polyspecific and that there may be more than one antigenic determinant for Rg or for Ch. Different sources of antisera vary in antibody specificities for Rg/Ch determinants (Giles 1985a). A single partial inhibitor pattern has been detected in Rg so far and is generally detected in the isoexpressed haplotype *C4A3A2*. Thus Giles (1985b) proposed a model with two Rg and three Ch antigenic determinants, and they are designated Rg1, 2 and Ch1, 2, 3, respectively. In both cases, Rg1 and Ch1 were suggested to be the commonest epitopes.

Standardized polyspecific anti-Rg and anti-Ch alloantisera allowed serological characterization of many rare C4 allotypes. This led to not only the subdivision of many common allotypes, but also the invalidation of the C4A-Rg and C4B-Ch dogma. Rare variants of both isotypes such as C4A1 and C4B5, which both possess their isotypic properties (i. e., hemolytic activity, covalent binding affinity to small molecules, and the apparent relative mass of the alpha chain), were found essentially expressing the reversed antigenic determinants (Rittner et al. 1984, Roos et al. 1984). Detailed analysis of the Rg-positive C4B5 protein (i) in the plasma for hemagglutination inhibition test and (ii) coated on standard erythrocytes for direct agglutination experiments uncovered the presence of

**Table 2.** Interrelationship among *C4A/C4B* and *Rg/Ch* haplotypes

| Donors | *C4* haplotypes | *Rg/Ch* haplotypes | | References |
|---|---|---|---|---|
| General cases | | | | |
| F.P. | *A3B1* | *Rg: 1, 2;* | *Ch: 1, 2, 3, 4, 5, 6* | Giles 1987 |
| AW* | *A3B3* | *Rg: 1, 2;* | *Ch: 1, 2, 3, 4, 5, 6* | Yu et al. 1986, Dodds et al. 1986 |
| S. L. | *AQ0B1* | *Rg: −1, −2;* | *Ch: 1, 2, 3, 4, 5, 6* | Giles 1987 |
| J. Mah | *A3BQ0* | *Rg: 1, 2;* | *Ch: −1, −2, −3, −4, −5, −6* | Giles 1987 |
| C. G. | *A4B2* | *Rg: 1, 2;* | *Ch: 1, −2, 3, 4, −5, 6* | Giles 1987 |
| Str. | *A4B5* | *Rg: 1, 2;* | *Ch: 1, −2, 3, 4, −5, 6* | Giles 1987, Hing et al. 1986 |
| Rare cases | | | | |
| M. C. | *A3B1* | *Rg: 1, 2;* | *Ch: 1, 2, −3, 4, 5, −6* | Giles 1987 |
| D. W. | *A3A2BQ0* | *Rg: 1, −2;* | *Ch: −1, −2, −3, −4, 5, 6* | Giles 1987 |
| D. C. | *A3BQ0* | *Rg: 1, −2;* | *Ch: −1, −2, −3, −4, −5, 6* | Giles 1987 |
| MS74 | *A2B3* | *Rg: ND;* | *Ch: −1, 2, −3, 4, 5, −6* | Giles 1987, Skanes et al. 1985 |
| J. M.* | *A4B5* | *Rg: 1, 2;* | *Ch: 1, −2, 3, −4, 5, 6* | Yu et al. 1986, Hing et al. 1986 |
| A. D.* | *A1BQ0* | *Rg: −1, −2;* | *Ch: 1, −2, 3, −4, 5, 6* | Yu et al. 1986 |

ND, not determined

* Genomic DNA libraries have been made for these individuals

another three specificities of the anti-Ch and their corresponding antigenic determinants on C4 were designated Ch4, 5, and 6 (Giles 1987). A correlation of these determinants with the Ch1, 2, and 3 and with the Rg1 and 2 determinants was made among 131 donors (Giles 1987). Assignment of the presence of Rg/Ch determinants on each C4 allotype is usually complicated by the presence of more than one *C4A* or *C4B* gene in each individual, and therefore family studies are always necessary. The expression of some informative *Rg/Ch* haplotypes from the *C4A* and *C4B* haplotypes is listed in Table 2. Cumulative serological observations (Giles et al. 1984, Giles 1987) enabled the following conclusions to be drawn.

1. All C4B molecules express Ch4.
2. All C4B molecules expressing Ch1, 2, 3 also express Ch4, 5, and 6 but not Rg1, 2.
3. Ch2 and Ch5 are closely related and never split on the C4B molecule.
4. Ch3 appears related to Ch1 and Ch6 on both C4A and C4B.
5. Both Rg1 and Rg2 may not be expressed by some C4A variants. Nonexpression of Rg1 or Rg2 or both always renders the C4A protein possessing certain Ch epitopes, but never Ch2 and Ch4.
6. Rg1 and Ch1 appear to be the alternative antigenic determinants on the C4 molecule.

As a result, it can be speculated that some of the Ch determinants might be overlapping with other determinants, and that Ch2 and Ch4 might be related to the region of the C4 protein that would determine its isotypic properties.

## A structural model for C4 isotypic and antigenic determinants

In parallel with the advance in serological typing of C4, remarkable progress was made in the structural studies of C4 between 1981 and 1986. This was initiated with the determination of the amino acid sequence around the putative thiolester site and most of the *C4d* region (Campbell et al. 1981, Harrison et al. 1981, Chakravarti et al. 1983, Hellman et al. 1984), and subsequently continued by the isolation and DNA sequencing of C4 cDNA and genomic clones (Carroll and Porter 1983, Carroll et al. 1984a, b, Belt et al. 1984, 1985). Meanwhile, disulfide linkages (Janatova 1986, Seya et al. 1986) and sites of glycosylation (Chan and Atkinson 1985) and sulfation (Karp 1983, Hortin et al. 1986) of C4 have been elucidated. No difference has yet been found in these posttranslational modifications between C4A and C4B. The differential isotypic properties as well as the complex antigenic determinants are therefore more likely due to amino acid differences. Full-length and partial cDNA (Belt et al. 1984, 1985) and genomic DNA sequences (C. Y. Yu, manuscript in preparation) and partial protein sequences (Chakravarti et al. 1983) from pooled serum identified ~17 amino acid changes among some common C4A and C4B allotypes. Surprisingly, only a few variations lie on the beta and the gamma chains, but 12 changes clustered at a small region of 140 amino acids which is ~230 amino acid residues C-terminal to the thiolester site at the C4d region. Presumably, these 17 amino acid variations are the basis of the (i) differential isotypic properties, (ii) antigenic determinants, and (iii) some allelic variations. The small number of variations on the beta and gamma chains are probably responsible for allelic variations or for some other undefined features, as these changes are not common to either C4A or C4B isotypes.

Isotypic and antigenic determinants were dissected by molecular cloning and DNA sequencing of many alleles of the C4 molecules (Yu et al. 1986) with defined functional (Dodds et al. 1986) and antigenic (Giles 1987) properties (Table 3). Comparison of derived amino acid sequences at the *C4d* region between the Rg-positive C4A3a and the Ch-positive C4B3 suggested that there are eight amino acid changes which may be correlated with the isotypic and antigenic determinants. These changes are located at four regions as shown in Figure 1. Regions I and III are due to single amino acid substitutions, while

**Table 3.** Correlation of the serological and structural data in six cloned *C4* genes

| *C4* allotype | *Rg/Ch* haplotype | | Characteristic a. a. sequence at C4d (I) | (II) | (III) | (IV) |
|---|---|---|---|---|---|---|
| A3a | *Rg: 1, 2;* | *Ch: −1, −2, −3, −4, −5, −6* | D | PCPVLD | N | VDLL |
| B3 | *Rg: −1, −2;* | *Ch: 1, 2, 3, 4, 5, 6* | G | LSPVIH | S | ADLR |
| A1 | *Rg: −1, −2;* | *Ch: 1, −2, 3, −4, 5, 6* | G | PCPVLD | S | ADLR |
| B5 | *Rg: 1,* ND; | *Ch: −1, −2, −3, 4, −5, 6* | D | LSPVIH | S | VDLR |
| B2 | *Rg:* ND; | *Ch: 1, −2, 3, 4, −5, 6* | D | LSPVIH | S | ADLR |
| B1a | *Rg:* ND; | *Ch: 1, 2, −3, 4, 5, −6** | G | LSPVIH | N | ADLR |

a. a., amino acid; ND, not determined
* Assumed *Ch* haplotype

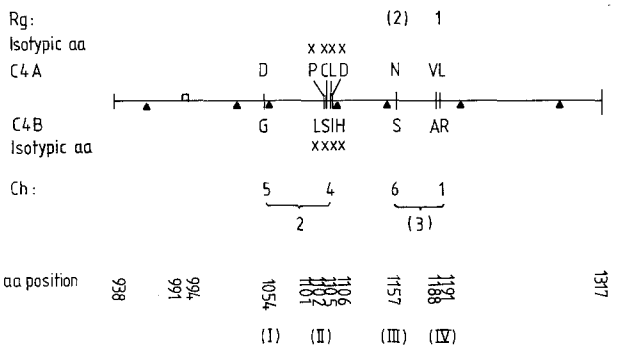


**Fig. 1.** A structural model for the location of the Rg and the Ch antigenic determinants and the correlation with the human complement C4A/C4B isotypes. The C4d fragment is from position 938 to 1317. The isotypic residues of C4A and C4B are marked by *crosses*. The eight amino acid changes at regions I, II, III, and IV constitute Ch5, Ch4, Ch6, and Ch1, which are probably continuous epitopes. In addition, they may form two discontinuous epitopes, Ch2 and Ch3. The locations of the Rg determinants are derived from the serological observation that on a C4 molecule Rg1 and Ch1 appear to mutually exclude each other, while Rg2 may be an alternative to Ch6 or Ch3, and from the structural and serological data of C4B5 and C4A1. Deduced/assumed locations are enclosed in *parentheses* (i. e., Ch3 and Rg2; Rg2 can also be a conformational epitope). A *solid triangle* shows the position of intron. An *open square* shows the location of the thiolester residues

region II involves four clustered changes and region IV involves two changes. Thus C4A3a has the sequence D-PCPVLD-N-VDLL and C4B3 has the sequence G-LSPVIH-S-ADLR at these regions. The fact that the sequences of the Rg-negative C4A1 and C4B3 are identical at the *C4d* region except for the four changes at region II suggests that PCPVLD/LSPVIH at position 1101–1106 are the isotypic residues for C4A/C4B. Further data from the C4B5 allotype support this finding, as it maintains the C4B-specific sequence LSPVIH 1101–1106, but acquires the C4A-related residues, D 1054 and VDLL 1188–1191.

Since the Rg and Ch determinants are located in the *C4d* region (Tilley et al. 1978) and the eight substitutions at regions I–IV are the only changes detected among the Rg-positive C4A3a, Chido-positive C4B3, Rg-negative C4A1, and Rg-positive C4B5, these substitutions should also be related to the expression of the Rg/Ch antigenic determinants on the C4 molecules. Based on the structural data and the *Rg* and *Ch* haplotypes of these four cloned *C4* genes, and also on the cDNA and genomic sequences of *C4A4, C4A3b, C4B1a, C4B1b,* and *C4B2,* whose *Rg/Ch* haplotype can only be derived indirectly (Table 3), it is possible to correlate some of the antigenic determinants with the amino acid residue changes at these four regions. For the Chido determinants, it appears that there is not a one-to-one direct correlation between an epitope and the amino acid change(s) at each region. For example, C4B2 differs from C4B3 only at region I (i. e., D 1054 cf. G 1054) and almost all C4B2 is Ch −2 and Ch −5 (Giles et al. 1987); C4A1 differs from C4B3 at region II and is Ch −2 and Ch −4. Thus the amino acid change(s) at each region may be involved in the formation of more than one epitope. Some of the epitopes are discontinuous or conformational, which involves more than one region (probably two); this is in accord with serological results as already mentioned. Two *C4B* genes were partially sequenced at the polymorphic *C4d* region (Belt et al. 1985). The sequence of C4B1b is identical with that of C4B3, while that of C4B1a is unusual in having the C4A-related region IV (i. e., N 1157). It is possible that this C4B1a corresponds to the "partial inhibitor", which is Ch −3 and Ch −6. Based on these assumptions, the probable

**Table 4.** Comparison of *Ch* haplotypes and amino acid sequences of the C4d fragment of some C4 allotypes with a Ch-positive C4B allotype (e. g., C4B3)

| Allotypes | Polymorphic sequences at C4d | | | | *Ch* haplotypes* |
|---|---|---|---|---|---|
| | (I) G 1054 | (II) LSPVIH 1101-6 | (III) S 1157 | (IV) ADLR 1188-91 | |
| C4A1 | | − | | | *−2, −4* |
| C4B2 | − | | | | *−2, −5* |
| C4B1a | | | − | | *−3, −6*† |
| C4B5 | − | | | − | *−1, −2, −3, −5* |
| C4A3 | − | − | − | − | *−1, −2, −3, −4, −5, −6* |
| Deduced location of the Ch epitope | 5 ⌣ 4 | 2 | 6 ⌣ 1 | 3 | |

−, Absence
* Only negative haplotypes are shown
† Assumed

locations of the six Chido antigenic determinants have been deduced and are listed in Table 4. The C4B-related sequences at regions I, II, III, and IV, respectively, constitute the Ch5, Ch4, Ch6, and Ch1 determinants. These four determinants may be continuous (or sequential) epitopes. On the other hand, Ch2 and Ch3 may be discontinuous (or conformational) epitopes: the former involves the amino acids from regions I and II, while the latter involves the amino acids from regions III and IV. As the C4B isotypic residues are related to the expression of Ch2 and Ch4, this explains the fact that these two epitopes are never expressed on the C4A molecules.

The situation for the location of Rg determinants is less obvious. As C4A1 does not express Rg1 and Rg2, the C4A isotypic residues may not be related to the Rg determinants, unless they are involved in the formation of conformational epitopes. As C4B5 expresses Rg1 and as Rg1 and Ch1 seem to be alternative in a C4 molecule, Rg1 is probably determined by the C4A-related sequence VDLL (1188–1191). It remains to be determined whether Rg2 is a conformational or a sequential epitope. Accumulated serological data infer that Rg2 could be the alternative to Ch6 (Giles 1987) or to Ch3, if it is a conformational epitope. Therefore, the Rg2 determinant may be related to the C4A-related region IV sequence (i. e., N 1157) and possibly to the region III (i. e., VDLL 1188–1191). Thus a molecular model for the location of the C4A/C4B isotypic residues and the Rg/Ch antigenic determinants has been formulated and is presented in Figure 1.

This model can be partly tested by determining the DNA sequences at the *C4d* regions for the *C4B1* gene in linkage to *C4A6*, and for the isoexpressed haplotype *C4A3 C4A2 (C4BQ0)*. For the first case, N 1157 is expected, as the C4B1 protein expressed from the *C4A6 C4B1* haplotype is Ch −3 and Ch −6. The C4 proteins from the *C4A3 C4A2 (C4BQ0)* haplotype have been found to be Rg1, −2; Ch5, 6 (Giles 1987). If Rg2 is alternative to Ch6 or to Ch3 and because the negative traits of the Rg and Ch determinants are expressed in a recessive manner, S 1157 (Ch6/Rg −2) in region III are expected to be present on both the C4A2 and C4A3 molecules, and G 1054 (Ch5) in region I may be present on either or both of these isoexpressed C4A molecules. Alternatively, the model can be tested by using synthetic peptides with amino acid sequences corresponding to the proposed antigenic sites for competition binding to the alloantibodies. On the other hand, the model is in accord with all of the serological data and no exception has been found so far (C. M. Giles, personal communication).

## Prospective and implications

The elucidation of the C4A/C4B isotypic residues significantly advanced our understanding of the structure and function between two highly homologous proteins which exhibit diversified reactivities. It suggests the presence of a region in the C4 molecule that may modulate the covalent binding reaction of the thiolester carbonyl group (Yu et al. 1986). The determination of the molecular basis of Rg/Ch antigenic determinants would provide insights into the alloantigenicity of human proteins. It is interesting to observe that only a single amino acid difference on a 1745-residue human serum protein can stimulate an immune response after blood transfusion. For instance, a blood transfusion patient with the *C4A4 C4B2* haplotype (Rg1, 2; Ch1, −2, 3, 4, −5, 6) produced antiserum specific for Ch2 and Ch5 (Giles et al. 1987). In this aspect, the Rg/Ch antigenic determinants of C4 are remarkably similar to the genetic markers in the constant regions of the immunoglobulin molecules, Gm. The presence of multiple antigenic determinants on the same molecule, and some identical determinants on different isotypes, and the polyspecificities of the corresponding alloantisera are all common features shared by the two groups of serum proteins. In comparison, study of the antigenicities of C4A and C4B has the advantage of a manageable complexity.

The high immunogenicity of a protein region would suggest that this region is exposed to or readily accessible for antibody-antigen interaction (Novotny et al. 1987). This infers that the *C4d* region may be located on the surface of the C4 molecule. A characteristic antigenic site of a protein probably spans about six amino acid residues (Kabat 1970, Schechter 1971), and they can be continuous (sequential) or discontinuous (conformational) in nature (Benjamin et al. 1984, Van Regenmortel 1987). Therefore, the proposed locations for Rg/Ch determinants stand for stretch(es) of amino acids at which the polymorphic residue(s) are located. The proposed conformational epitopes Ch2 and Ch3 would infer that G 1054 and LSPVIH 1101–1106 are reasonably proximal to each other, and so is S 1157 and ADLR 1188–1191. To this end, it is interesting to note that a novel epitope that requires Ch6 (i. e., S 1157) and Rg1 (i. e., VDLL 1188–1191) in *cis* configuration has been described (Giles and Jones 1987). As already noted, the C4A isotypic residues appear nonimmunogenic. This might be the result of the unique, and probably very rigid structure formed by a cysteine and two proline residues. It was suggested that the C4A isotypic residues cause the retardation of the alpha chain mobility (therefore, larger apparent relative mass) in modified SDS-PAGE (Roos et al. 1982, Chan and Atkinson 1985).

A monoclonal antibody which can distinguish between Ch1 and Rg1 has been described (Dodds et al. 1986, Giles and Ford 1986). This antibody has proved useful in the separation of many C4A and C4B allotypes (Dodds et al. 1985, 1986).

Using a C4d-specific probe for Southern blot analysis, the presence of DNA sequence determining the expression

of Rg1/Ch1 epitopes and the C4A/C4B isotypic residues can be detected, respectively, by the EcoO 109 and Nla IV restriction fragment length polymorphisms (RFLPs) (Yu and Campbell 1987). The application of these definitive RFLPs enabled the studies of *C4* genetics at the DNA level that has proved useful in the elucidation of the molecular basis of the *C4* null alleles. It is also possible to distinguish between DNA sequences related to the Ch6 (and to the Rg2) epitope by an Alu I RFLP at the corresponding position. The DNA sequence determining the Ch5 epitope cannot be recognized by a known restriction enzyme. However, its presence can be characterized by a specific oligonucleotide probe or by direct nucleotide sequencing. Application of these recombinant DNA techniques would be helpful in the typing of C4 that is important in the studies of *HLA* genetics.

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## References

Allen, F. H., Jr.: Linkage of HL-A and GBG. *Vox Sang. 27:* 382–384, 1974

Belt, K. T., Carroll, M. C., and Porter, R. R.: The structural basis of the multiple forms of human complement component C4. *Cell 36:* 907–914, 1984

Belt, K. T., Yu, C. Y., Carroll, M. C., and Porter, R. R.: Polymorphism of human complement component C4. *Immunogenetics 21:* 173–180, 1985

Benjamin, D. C., Berzofsky, J. A., East, I. J., Gurd, F. R. N., Hannum, C., Leach, S. J., Margoliash, E., Michael, J. G., Miller, A., Prager, E. M., Reichlin, M., Sercarz, E. E., Smith-Gill, S. J., Todd, P. E., and Wilson, A. C.: The antigenic structure of proteins: a reappraisal. *Annu. Rev. Immunol. 2:* 67–101, 1984

Campbell, R. D., Gagnon, J., and Porter, R. R.: Amino acid sequence around the thiol and reactive acyl groups of human complement component C4. *Biochem. J. 199:* 359–370, 1981

Carroll, M. C. and Porter, R. R.: Cloning of a human complement component C4 gene. *Proc. Natl. Acad. Sci. U.S.A. 80:* 264–267, 1983

Carroll, M. C., Campbell, R. D., Bentley, D. R., and Porter, R. R.: A molecular map of the major histocompatibility complex class III region linking complement genes C4, C2 and factor B. *Nature 307:* 237–241, 1984a

Carroll, M. C., Belt, K. T., Palsdottir, A., and Porter, R. R.: Structure and organisation of the C4 genes. *Philos. Trans. R. Soc. Lond. 306:* 379–388, 1984b

Chakravarti, D. N., Campbell, R. D., and Gagnon, J.: Amino acid sequence of a polymorphic segment from C4d of human complement component C4. *FEBS Lett. 154:* 387–390, 1983

Chan, A. C. and Atkinson, J. P.: Oligosaccharide structure of human C4. *J. Immunol. 134:* 1790–1798, 1984

Dodds, A. W., Law, S. K. A., and Porter, R. R.: The origin of the variable haemolytic activities of the human complement component C4 allotypes including C4-A6. *EMBO J. 4:* 2239–2244, 1985

Dodds, A. W., Law, S. K. A., and Porter, R. R.: The purification and properties of some less common allotypes of the fourth component of human complement. *Immunogenetics 24:* 279–285, 1986

Fu, S. M., Kunkel, H. G., Brusman, H. P., Allen, F. H., and Fotino, M.: Evidence for linkage between HL-A histocompatibility genes and those involved in the synthesis of the second component of complement. *J. Exp. Med. 148:* 1044–1051, 1974

Giles, C. M.: Antigens in plasma. *In: A Seminar on Antigens on Blood Cells and Body Fluids*, pp. 33–49, American Association of Blood Banks, Washington, 1980

Giles, C. M.: A new genetic variant for Chido. *Vox Sang. 46:* 149–156, 1984

Giles, C. M.: 'Partial inhibition' of anti-Rg and anti-Ch reagents. I. Assessments for Rg/Ch typing by inhibition. *Vox Sang. 48:* 160–166, 1985a

Giles, C. M.: 'Partial inhibition' of anti-Rg and anti-Ch reagents. II. Demonstration of separable antibodies for different determinants. *Vox Sang. 48:* 167–173, 1985b

Giles, C. M.: Three Chido determinants detected on the $B5Rg^+$ of human C4: their expression in Ch-typed donors and families. *Hum. Immunol. 8:* 111–122, 1987

Giles, C. M. and Ford, D. S.: A monoclonal anti-C4d that demonstrates a specificity related to anti-Ch. *Transfusion 26:* 370–374, 1986

Giles, C. M. and Jones, J. W.: A new antigenic determinant for C4 of relatively low frequency. *Immunogenetics 26:* 392–394, 1987

Giles, C. M., Gedde-Dahl, T., Robson, E. B., Thorsby, E., Olaisen, B., Aenason, A., Kissmeyer-Neilsen, F., and Schreuder, I.: Rg (Rodgers) and the HL-A region; linkage and associations. *Tissue Antigens 8:* 143–149, 1976

Giles, C. M., Batchelor, J. R., Dodi, I. A., Fielder, A. H. L., Rittner, C., Mauff, G., Bender, K., Levene, C., Schreuder, G. M. T., and Wells, L. J.: C4 and HLA haplotypes associated with partial inhibition of anti-Rg and anti-Ch. *J. Immunogenet. 11:* 305–317, 1984

Giles, C. M., Hoffmann, M., Moulds, M., Harris, M., and Dalmasso, A.: Allo-anti-Chido in a Ch-positive patient. *Vox Sang. 52:* 129–132, 1987

Grubb, R.: Agglutination of erythrocytes coated with 'incomplete' anti-Rh by certain rheumatoid arthritis sera and some other sera. The existence of human serum groups. *Acta Pathol. Microbiol. Scand. 39:* 195–197, 1956

Harris, J. P., Tegoli, J., Swanson, J., Fisher, N., Gavin, J., and Noades, J.: A nebulous antibody responsible for crossmatching difficulties (Chido). *Vox Sang. 12:* 140–142, 1967

Harrison, R. A., Thomas, M. L., and Tack, B. F.: Sequence determination of the thiolester site of the fourth component of human complement. *Proc. Natl. Acad. Sci. U.S.A. 78:* 7388–7392, 1981

Hellman, U., Eggertsen, G., Lundwall, A., Engstrom, I., and Sjoguist, J.: Primary sequence differences between Chido and Rodgers variants of tryptic C4d of the human complement system. *FEBS Lett. 170:* 254–258, 1984

Hing, S. N., Giles, C. M., Fielder, A. H. L., and Batchelor, J. R.: *HLA* haplotypes with C4B5; evidence for further allelic heterogeneity. *Immunogenetics 23:* 151–155, 1986

Hortin, G., Sims, H., and Strauss, A. W.: Identification of the site of sulphation of the fourth component of human complement. *J. Biol. Chem. 261:* 1786–1793, 1986

Isenman, D. E. and Young, J. R.: The molecular basis for the differences in immune haemolysis activity of the Chido and Rodgers isotypes of human complement component C4. *J. Immunol. 132:* 3019–3027, 1984

Isenman, D. E. and Young, J. R.: Covalent binding properties of the C4A and C4B isotypes of the fourth component of human complement of several C1-bearing cell surfaces. *J. Immunol. 136:* 2542–2550, 1986

Janatova, J.: Detection of disulphide bonds and localisation of interchain linkages in the third (C3) and the fourth (C4) components of human complement. *Biochem. J. 233:* 819–825, 1986

Kabat, E.: Heterogeneity and structure of antibody-combining sites. *Ann. N.Y. Acad. Sci. 169:* 43–54, 1970

Karp, D. R.: Post-translational modification of the fourth component of complement. Sulphation of the α-chain. *J. Biol. Chem. 258:* 12745–12748, 1983

Law, S. K. A., Dodds, A. W., and Porter, R. R.: A comparison of the properties of the two classes, C4-A and C4-B, of the human complement component C4. *EMBO J. 3:* 1819–1823, 1984

Longster, G. and Giles, C. M.: A new specificity, anti-Rg$^a$, reacting with a red cell and serum antigen. *Vox Sang. 30:* 175–180, 1976

Mauff, G., Alper, C. A., Awdeh, Z., Batchelor, J. R., Bertrams, T., Bruun-Petersen, G., Dawkins, R. L., Demant, P., Edwards, J., Grosse-Wilde, H., Hauptmann, G., Klouda, P., Lamn, L., Mollenhauer, E., Nerl, C., Olaisen, B., O'Neill, G., Rittner, C., Ross, M. H., Skanes, V., Teisberg, N., and Wells, L.: Statement on the nomenclature of human C4 allotypes. *Immunobiology 164:* 184–191, 1983

Middleton, J. and Crookston, M.: Chido substance in plasma. *Vox Sang. 23:* 256–261, 1972

Middleton, J., Crookston, M. C., Falk, J. A., Robson, E. B., Cook, P. J. L., Batchelor, J. R., Bodmer, J., Ferrara, G. B., Festenstein, H., Harris, R., Kissmeyer-Nielsen, F., Lawler, S. D., Saches, J. A., and Wolf, E.: Linkage of Chido and HL-A. *Tissue Antigens 4:* 366–373, 1974

Nisonoff, A., Hopper, J. E., and Spring, S. B.: *The Antibody Molecule,* pp. 370–388, Academic Press, New York, 1975

Nordhagen, R., Olaisen, B., Teisberg, P., and Gedde-Dahl, T., Jr.: Association between the electrophoretically determined C4M haplotype product and partial inhibition of anti-Ch$^a$. *J. Immunogenet. 7:* 301–306, 1980

Novotny, J., Handschumacher, M., and Bruccoleri, R. E.: Protein antigenicity: a statistic surface property. *Immunol. Today 8:* 26–31, 1987

O'Neill, G. J., Yang, S. Y., Tegoli, J., Berger, R., and Dupont, B.: Chido and Rodgers blood groups are distinct antigenic components of human C4. *Nature 273:* 668–670, 1978a

O'Neill, G. J., Yang, S. Y., and Dupont, B.: Two HLA-linked loci controlling the fourth component of human complement. *Proc. Natl. Acad. Sci. U.S.A. 75:* 5165–5169, 1978b

Reid, K. B. M. and Porter, R. R.: The proteolytic activation systems of complement. *Annu. Rev. Biochem. 50:* 433–464, 1981

Rittner, C., Hauptmann, G., Gross-Wilde, H., Grosshans, E., Tongio, M. M., and Mayer, S.: Linkage between HL-A (major histocompatibility complex) and genes controlling synthesis of the fourth component of complement. *In: Histocompatibility Testing,* pp. 945–953, Munksgaard, Copenhagen, 1975

Rittner, C., Giles, C. M., Roos, M. H., Démant, P., and Mollenhauer, E.: Genetics of human C4 polymorphism: detection and segregation of rare and duplicated haplotypes. *Immunogenetics 19:* 321–333, 1984

Roos, M. H., Mollenhauer, E., Demant, P., and Rittner, C.: A molecular basis for the two locus of human complement component C4. *Nature 298:* 854–855, 1982

Roos, M. H., Giles, C. M., Demant, P., Mollenhauer, E., and Rittner, C.: Rodgers (Rg) and Chido (Ch) determinants on human C4: characterisation of two C4B 5 subtypes, one of which contains Rg and Ch determinants. *J. Immunol. 133:* 2634–2640, 1984

Schechter, I.: Mapping of the combining sites of antibodies specific to polyalanine chains. *Ann. N.Y. Acad. Sci. 190:* 394–419, 1971

Schifferli, J. A., Hauptmann, G., and Paccaud, J.-P.: Complement-mediated adherence of immune complexes to human erythrocytes. Difference in the requirements for C4A and C4B. *FEBS Lett. 213:* 415–418, 1987

Seya, T., Nagasawa, S., and Atkinson, J. P.: Localisation of the interchain disulphide bonds of the fourth component of human complement (C4): evidence based on the liberation of fragments secondary to thiol-sulphide interchange reactions. *J. Immunol. 136:* 4152–4156, 1986

Sim, E. and Cross, S. J.: Phenotyping of human complement component C4, a class-III HLA antigen. *Biochem. J. 239:* 763–767, 1986

Skanes, V. M., Larsen, B., and Giles, C. M.: C4B3 allotype with a novel Ch phenotype. *Immunogenetics 22:* 609–616, 1985

Teisberg, P., Akesson, I., Olaisen, B., Gedde-Dahl, T., Jr., and Thorsby, E.: Genetic polymorphism of C4 in man and localisation of a structural C4 locus to the HLA gene complex of chromosome 6. *Nature 264:* 253–254, 1976

Tilley, C. A., Romans, D. G., and Crookston, M. C.: Localisation of Chido and Rodgers determinants to the C4d fragment of human C4. *Nature 276:* 713–715, 1978

Van Regenmortel, M. V. H.: Antigenic cross-reactivity between proteins and peptides: new insights and applications. *Trends Biochem. Sci. 12:* 237–240, 1987

Yu, C. Y. and Campbell, R. D.: Definitive RFLPs to distinguish between the human complement C4A/C4B isotype and the major Rodgers/Chido determinants; application to the study of *C4* null alleles. *Immunogenetics 25:* 383–390, 1987

Yu, C. Y., Belt, K. T., Giles, C. M., Campbell, R. D., and Porter, R. R.: Structural basis of the polymorphism of human complement C4A and C4B: gene size, reactivity and antigenicity. *EMBO J. 5:* 2873–2881, 1986

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