17 Chido/Rodgers Blood Group System

17.1 Introduction

Chido and Rodgers antigens are not located on intrinsic red cell structures, but on the fourth component of complement (C4), which becomes bound to the red cells from the plasma. As Chido/Rodgers antigens are readily detected on red cells by conventional blood grouping methods and were considered to be blood group antigens before the association with C4 was disclosed, they have been adopted as the seventeenth blood group system. Currently nine Chido/Rodgers antigens have been defined [1]: Ch1 to Ch6, Rg1, and Rg2 have frequencies greater than 90%; W.H. has an incidence of about 15%. A complex relationship exists between these nine determinants and polymorphic variation of the C4 α-chain.

According to the ISBT terminology, Ch1 to Ch6 are CH/RG1 to CH/RG6, WH is CH/RG7, Rg1 is CH/RG11, and Rg2 is CH/RG12. Numbers CH/RG8 to CH/RG10 are available for additional Ch antigens.

In Section 17.2, anti-Ch and -Rg will be considered as simple, monospecific antibodies, their complexities being discussed in Section 17.5.

17.2 Basic serology

Antibodies to a relatively high frequency antigen, called anti-Chido (anti-Ch) by Harris *et al.* [2], were described as 'nebulous' because antigen strength was variable and difficulty in distinguishing weakly positive from negatively reacting red cells could not be resolved by adsorption experiments. All seven of the original anti-Ch sera were found in multiply transfused patients; in six of them no other atypical antibody was detected [2]. Middleton and Crookston [3] found that the reaction of anti-Ch with Ch+ red cells was inhibited by plasma from Ch+, but not Ch–, individuals. Furthermore, plasma of people with weak Ch expression on their red cells was as effective in inhibition tests as plasma from strongly Ch+ individuals. Inhibition techniques, therefore, are more effective than testing of red cells for phenotype determination. Ch– red cells can be converted to Ch+ by incubation in Ch+ plasma [4]. About 97% of white donors are Ch+ [3,5]. The gene controlling Ch production is inherited as an autosomal dominant character and the locus is strongly linked to *HLA* [6].

When Longster and Giles [7] described anti-Rg (Rodgers), the resemblance to anti-Ch was patent: the antibody reacted with red cells of about 97% of white people, strength of red cell Rg expression was variable, and the reaction with Rg+ red cells was specifically inhibited by Rg+ plasma. The high frequencies of both Ch and Rg precluded an allelic relationship, yet the excessive rarity of the Ch– Rg– phenotype suggested a genetic association. The locus controlling Rg expression is also very tightly linked to *HLA*, with strong association between Rg– and HLA-B8 [8,9]. The gene

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producing Rg antigen is inherited in an autosomal dominant fashion [7,8].

About 2.5% of Rg+ plasmas were partially effective in inhibiting anti-Rg and initially appeared to represent a quantitative variant [7]. Partial inhibition of anti-Ch and -Rg was later shown to be due to some Ch/Rg antisera containing antibodies to more than one determinant [5,10–13]. This polyspecificity became the basis of much of the complexity described in Section 17.5.

Reliable results can be obtained by testing red cells with anti-Ch or -Rg if a suitable technique is used [14,15]. Ch and Rg antigens are expressed less strongly on cord cells than on red cells of adults, although cord and adult plasma are equally effective at inhibiting Ch/Rg antibodies [3,16]. The effect of enzymes on Ch and Rg antigens will be described in Section 17.3.

17.3 Ch and Rg antigens are located on C4

C4 is the fourth component of complement, involved in the classical pathway of complement activation. Activation of C1 by binding to IgG or IgM molecules, usually on a cell surface, results in the cleavage of C4 into a small fragment, C4a, and a large fragment, C4b. C4b immediately becomes covalently bonded to the cell surface as the result of the breaking of an intramolecular thioester bond, a rare type of bond involving the side chains of cysteine and glutamine. When broken, the thioester bond generates a very reactive carbonyl group, which can couple instantaneously to a membrane-bound macromolecule. C4b can then bind C2. The activated C4b,2a complex cleaves C3 and brings about a cascade reaction involving C5–C9 culminating in the puncturing of the cell membrane.

The product of the C4 gene is a pro-C4 molecule, a single polypeptide chain of MW 200 kDa, which is subsequently cleaved into α (95 kDa), β (75 kDa), and γ (30 kDa) chains. These three polypeptides are glycosylated and linked together by disulphide bonds (Figure 17.1). The α-chain occupies most of the molecular surface and is responsible for the majority of the molecule's biological activities. C4b represents the whole molecule minus a short N-terminal fragment, C4a. Degradation of membrane-bound C4b, primarily by factor I, releases most of the molecule and leaves C4d covalently bound to the membrane. A similar effect is produced by trypsin treatment of membrane-bound C4b. For reviews on complement and C4 see [17,18].

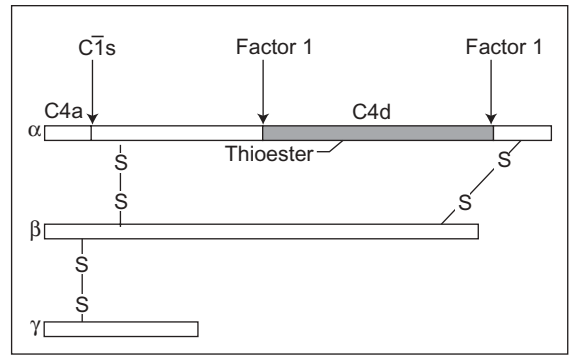

Figure 17.1 Diagram of C4 molecule, showing the three polypeptide chains linked by disulphide bonds, the C4a fragment, which is cleaved by the action of C1s, the site of the thioester bond, which causes the molecule to become covalently bonded to a cell surface, and the C4d region, which carries the Ch and Rg determinants and remains bound to the cell after cleavage of the remainder of the molecule by factor I. After [17], reproduced with permission from Elsevier.

Immunofixation electrophoresis reveals structural polymorphism of C4. C4 of most people falls into one of three patterns:

1 four rapidly migrating bands (C4A);
2 four slowly migrating bands (C4B); or
3 both sets of bands together [19,20] (Figure 17.2).

C4A and C4B represent genes at two very closely linked loci, *C4A* and *C4B*, with common silent alleles at each locus [20,21]. People who only have the faster migrating bands are homozygous for the silent allele at the *C4B* locus (*C4B*Q0*, quantity zero) and those who only have the slower bands are homozygous for a silent gene at the *C4A* locus (*C4A*Q0*). People with both sets of bands have at least one active allele at each locus. The excessive rarity of C4 deficiency arising from homozygosity for silent alleles at both loci is explained by a high level of linkage disequilibrium between the two loci, so that the haplotype *C4A*Q0 B*Q0* very seldom occurs. (For those wishing to read the original papers, it should be pointed out that O'Neill *et al.* [20,21] used the notation C4F (fast) and C4S (slow) for C4A (acidic) and C4B (basic), respectively.)

O'Neill *et al.* [21] showed that the Ch and Rg antigens are associated with C4. Ch+ Rg+ plasma has both C4A and C4B isotypes, Ch+ Rg– plasma has only C4B, and Ch– Rg+ plasma has only C4A (Figure 17.2). Ch and Rg antigens, therefore, appeared to be located on the products of the *C4B* and *C4A* loci, respectively. C4-deficient

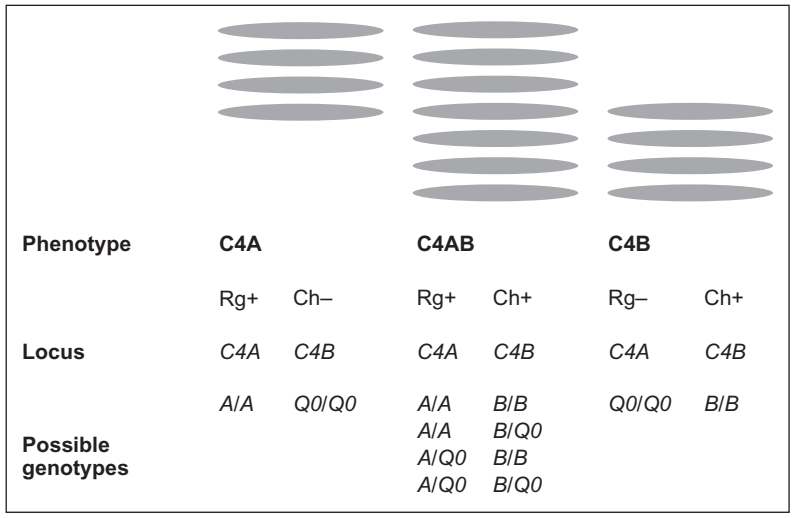

Figure 17.2 Diagram of common C4 electrophoretic patterns showing C4 and Ch/Rg phenotypes, and suggested C4 genotypes [21]. The extremely rare *C4A*Q0 B*Q0* haplotype is not included.

plasma lacked both Ch and Rg activity, whether the C4-deficient plasma was obtained from rare C4-deficient individuals [21–23] or was produced by removal of C4 from normal plasma by goat anti-C4 on an affinity column [21]. Red cells coated with C4 *in vitro* are directly agglutinated by anti-Ch and -Rg; antibodies that would normally require anti-human globulin to agglutinate the same red cells when uncoated [24]. C4-coated red cells acquire the Ch/Rg phenotype of the serum donor. Ch and Rg determinants on C4-coated red cells are relatively resistant to trypsin cleavage and must reside on the C4d fragment remaining bound to the red cell after trypsin cleavage of C4b [24]. Ch/Rg activity of uncoated red cells probably results from low level adsorption of C4 *in vivo*, either *via* the classical pathway or by spontaneous cleavage of the thioester [25].

The usual technique for coating red cells with C4 for serological purposes involves dropping freshly drawn citrated blood into a low ionic strength 10% sucrose solution [24]. These red cells are coated with C4 and C3 and are useful either for Ch/Rg typing by direct agglutination with appropriate antibodies or as indicator cells in Ch/Rg plasma inhibition tests. If red cells coated with C4 from another person are required, washed cells are mixed with the required fresh plasma (ABO compatible) and added to the sucrose solution. Trypsin treatment of cells coated with C4 and C3 cleaves the C3 and C4b leaving C4d coated cells. Red cells can also be coated with C4 *in vitro* or *in vivo* by complement fixing IgM antibodies [24].

Serological tests suggest that Ch and Rg antigens on native, 'uncoated' red cells, but not on cells coated with C4 by the 10% sucrose method, are denatured by the proteases trypsin, chymotrypsin, papain, ficin, and pronase [3,7,16,24]. Trypsin treatment reduces the number of detectable C4d molecules by about 50%, which reduces the number of C4d molecules on native cells, but not on coated cells, to a level below the threshold required for detection by agglutination tests with anti-Ch and -Rg [26].

There are four basic serological methods for determining Ch/Rg phenotypes:

1 testing of the subject's red cells with anti-Ch and -Rg;
2 inhibition of anti-Ch and -Rg with the subject's plasma;
3 testing of the subject's red cells coated with C4 from their own plasma;
4 testing of homologous red cells coated with the subject's C4.

17.4 Further complexities of C4

17.4.1 The complex polymorphisms of C4

Following the detection of two structural loci for C4 [20,21], a host of C4 variants were recognised and a variety of different notations used. In 1983 a single

nomenclature for C4 was agreed [27]. Although this has subsequently been modified [28,29], the 1983 nomenclature is used in most publications on the complexities of Ch/Rg, so that nomenclature will be used in this chapter. The protein products of *C4A* generally have the most acidic (anodal) migration on agarose gel electrophoresis and the products of *C4B* generally have the more basic (cathodal) migration. The C4 variants or allotypes are numbered, the most common being C4A 3 and C4B 1. Their genes are designated *C4A*3* and *C4B*1*, respectively. C4A Q0 and C4B Q0 represent unexpressed allotypes. There are at least 24 alleles at the *C4A* locus and 27 at the *C4B* locus, including a silent allele, *C4A*Q0* and *C4B*Q0*, at each [29]. In white people there are four *C4A* and three *C4B* alleles occurring with frequencies greater than 1% and producing variants with different electrophoretic mobilities: *C4A*2*, *C4A*3*, *C4A*4*, *C4A*6*, *C4B*1*, *C4B*2*, and *C4B*3*.

In most cases, C4A expresses Rg and C4B expresses Ch. Exceptions to this rule represent 'reversed antigenicity' [30,31]. For example: C4A 1 reacts with anti-Ch but not anti-Rg; C4B 5 (now called C4B 45) reacts with anti-Rg and with only some anti-Ch. The complexities of Ch and Rg are described in Section 17.5.

Protein modelling from the X-ray crystal structure of the C4d fragment showed that the residues associated with the major Ch/Rg epitopes are proximally located and accessible on the concave surface [32].

17.4.2 Molecular genetics of C4

C4A and *C4B* each consist of 41 exons [33] and are either 20.6 kb or 14.2 kb in length, the longer form having the endogenous retrovirus HERV-K(C4) integrated into exon 9 [34]. Both coding and non-coding regions of *C4A* and *C4B* are highly conserved. There is over 99% identity between the DNA nucleotide sequences of the two genes and between the amino acid sequences of the two proteins [35]. Eight amino acid changes within a region of the C4d fragment of the α-chain, close to the thioester bond, account for differences between isotypes (C4A and C4B) and allotypes (variants of these proteins) [35–38]. Four amino acid residues encoded by exon 26 determine isotype: the sequence for residues 1101–1106 of the pro-C4 molecule is *Pro-Cys*-Pro-Val-*Leu-Asp* in C4A and *Leu-Ser*-Pro-Val-*Ile-His* in C4B. *C4A* and *C4B* are distinguished by an *N1a*IV restriction fragment-length polymorphism (RFLP) [39]. Amino acid substitutions at positions 1054, 1157, 1188, and 1191, encoded by exons 25 and 28, account for the C4 allotypes and the various Ch and Rg determinants (Table 17.1). A detailed structural model to explain the location of Ch/Rg antigenic determinants and their correlation with the C4A and C4B isotypes [40] is described in Section 17.5.

Other complications occur that affect the C4 haplotype. Duplicated C4 genes appear to be quite common [11,42–45]: *C4A*3 A*2* and *C4B*2 B*1* both have frequencies close to 1% in Caucasians. There is also a high incidence of null alleles, about half of which result from a 28 kb DNA deletion [46,47]. Gene duplications and deletions probably arose by gene misalignment and unequal crossing-over [46]. *C4A*Q0* also results from a 2 bp insertion in exon 29 generating a stop codon in exon 30 [48]. Alternatively a *C4B* gene may appear to be absent because it has been changed to an identical copy of its neighbouring *C4A* homologue by a process of gene

Table 17.1 Sequence analysis of different C4 allotypes of known antigenic status (from [40,41]).

	Amino acid residues from N-terminus								Ch/Rg expression								
Allotype	1054	1101	1102	1105	1106	1157	1188	1191	Rg1	Rg2	Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	WH
C4A 3	Asp	Pro	Cys	Leu	Asp	Asn	Val	Leu	+	+	–	–	–	–	–	–	
C4A 1	Gly	Pro	Cys	Leu	Asp	Ser	Ala	Arg	–	–	+	–	+	–	+	+	
C4B 3	Gly	Leu	Ser	Ile	His	Ser	Ala	Arg	–	–	+	+	+	+	+	+	
C4B 1	Gly	Leu	Ser	Ile	His	Asn	Ala	Arg	[–	–	+	+	–	+	+	–]*	
C4B 2	Asp	Leu	Ser	Ile	His	Ser	Ala	Arg	[–	–	+	–	+	+	–	–]*	
C4B 5†	Asp	Leu	Ser	Ile	His	Ser	Val	Leu	+	–	–	–	–	+	–	+	+

*Assumed phenotype.
†Now called C4B 45 [28].

conversion [49,50]. Intragenic unequal crossing-over, resulting in *C4A/B* hybrid genes, could explain reversed antigenicity – C4A variants that express Ch determinants and C4B variants that express Rg [30,51]. (The mechanisms of unequal crossing-over and gene conversion are discussed in Section 3.9.)

The C4 genes are located within the class III region of the major histocompatibility complex on chromosome 6 [52]. They form part of a discrete genetic unit or module known as RCCX comprising four consecutive genes or fragments and inactive mutants of those genes [53]. RCCX stands for serine/threonine nuclear protein kinase **R**P, **C**4, steroid 21-hydroxylase **C**YP21, and extracellular matrix protein tenascin TN**X**. Haplotypes containing 1, 2, and 3 RCCX modules, and therefore 1, 2, and 3 C4 genes, have frequencies of 17%, 69%, and 14%, respectively. Quadruplication of the module is also known, so the diploid contribution of C4 genes can vary between zero and eight.

17.5 Further complexities of Chido and Rodgers

Detection by plasma inhibition methods of three Rodgers phenotypes, Rg+, Rg–, and Rg partial inhibitor [7], and of four Chido phenotypes, Ch+, Ch–, and two types of Ch partial inhibitor [5,10], led Giles [5,12,13] to isolate two Rodgers and three Chido antibodies. Anti-Rg1, -Rg2, -Ch1, -Ch2, and -Ch3 define antigens of relatively high incidence. Rg+ plasmas are Rg:1,2; Rg– are Rg:–1,–2; and Rg partial inhibitors are Rg:1,–2. Rg:–1,2 has not been found and if the model of Yu *et al.* [40] is correct (see below), does not exist. Ch+ plasmas are Ch:1,2,3, Ch– are Ch:–1,–2,–3, and the two types of Ch partial inhibitors are Ch:1,–2,3 and Ch:1,2,–3. Ch:–1,2,–3 and Ch:1,–2,–3 are extremely rare variants [45,54]; Ch:–1,2,3 and Ch:–1,–2,3 have not been detected and may not exist [40]. The frequencies of these phenotypes are shown in Table 17.2. All C4 molecules express either Rg1 or Ch1; no C4 molecule expresses both.

Ch and Rg phenotypes do not correlate with specific C4 allotypes in a straightforward manner (Table 17.3), although a few generalisations can be made [57]. Of the partial inhibitor phenotypes, Rg:1,–2 is found predominantly with the haplotype *C4A*3 A*2 B*Q0*, there is a strong association between Ch:1,–2,3 and *C4B*2*, and Ch:1,2,–3 is most frequently associated with *C4A*6 B*1*, but also with *C4A*3 B*1*.

Table 17.2 Ch (1–3) and Rg phenotypes and frequencies in English (309 tested) and Japanese (89 tested) donors [1,5,55].

Phenotype	English (%)	Japanese (%)
Rg:1,2	95	100
Rg:1,–2	3	0
Rg:–1,–2	2	0
Ch:1,2,3	88	75
Ch:1,–2,3	5	24
Ch:1,2,–3	3	0
Ch:–1,–2,–3	4	1
Ch:–1,2,–3	Very rare	
Ch:1,–2,–3	Very rare	

Further complexities arose with the detection of another three high frequency Ch determinants, Ch4, Ch5 and Ch6 [58]. Antibodies to these specificities can only be reliably detected by testing polyspecific anti-Ch reagents with Ch:–1,–2,–3 red cells coated with C4 of various allotypes, especially those of reversed Ch/Rg antigenicity. Ch4 was detected on all C4B allotypes and Ch4 is not produced by any haplotypes with *C4B*Q0*, including *C4A*1 B*Q0*, which produces Ch1 and Ch3 in the absence of Rg1 and Rg2. Ch5 associates with Ch2 on C4B; Ch2 is only present on C4B, but Ch5 is also detected on C4A 1. Ch6 is associated with Ch3 on C4B, but, unlike Ch3, Ch6 is always detected on Rg:1,–2 C4 allotypes.

Another Ch/Rg antibody, W.H., was identified in the serum of a multiply transfused man, which also contained anti-Ch1 and -Ch4 [41]. W.H. expression is associated with allotypes producing Ch6 and Rg1 in the absence of Rg2 [41,59].

All Rg antisera contain anti-Rg1 and anti-Rg2; with very rare exceptions [60] all Ch antisera contain anti-Ch1, which is often the only specificity [12,13]. The approximate frequencies with which each of the other specificities have been found in anti-Ch reagents are as follows: anti-Ch2, 25%; anti-Ch3, 10%; anti-Ch4, 75%; anti-Ch5, 16%; and two examples each of anti-Ch6 and W.H. antibody [1]. Rg antibodies are only found in Rg:–1,–2 individuals. Ch antibodies are usually found in people with the Ch:–1,–2,–3,–4,–5,–6 phenotype, but a few exceptions are reported: anti-Ch2 plus -Ch5 in a Ch:1,–2,3,4,–5,6 person [60]; anti-Ch2 plus -Ch4 in a Ch:1,–2,3,–4,5,6 person [61]; anti-Ch1 in a Ch:–1,–2,–3,

Table 17.3 Nine antigenic determinants and one hypothetical determinant (Rg3) in 16 combinations predicted by Yu *et al.* [40] and Giles and Jones [41], 13 of which have been detected (from Giles [56]). The 1983 allotype nomenclature is used.

No.	Rg1	Rg2	(Rg3)*	Ch1	Ch2	Ch3	Ch4	Ch5	Ch6	WH	C4 isotype	Associated C4 allotype(s)
1	+	+†	+	–	+	–	+	+	–	–	B	B3
2	+	+	+	–	–	–	–	+	–	–	A	A3
3	+	+	+	–	–	–	+	–	–	–	B	B5
4	+	+	+	–	–	–	–	–	–	–	A	A1‡ A2 A3 A4 A5 A6
5	+	–†	–	–	+	–	+	+	+	+	(B)	Not found
6	+	–	–	–	–	–	–	+	+	+	A	A3 A(3,2)§
7	+	–†	–	–	–	–	+	–	+	+	B	B5
8	+	–	–	–	–	–	–	–	+	+	A	A3
9	–	–†	+	+	+	–	+	+	–	–	B	B1 B3
10	–	–†	+	+	–	–	–	+	–	–	(A)	Not found
11	–	–†	+	+	–	–	+	–	–	–	(B)	Not found
12	–	–†	+	+	–	–	–	–	–	–	A	A1‡
13	–	–	–	+	+	+	+	+	+	–	B	B1 B3
14	–	–	–	+	–	+	–	+	+	–	A	A1‡
15	–	–	–	+	–	+	+	–	+	–	B	B2 B3 B5 B6
16	–	–†	–	+	–	+	–	–	+	–	A	A1‡

*Hypothetical determinant.
†Predicted Rg phenotype not detected by haemagglutination inhibition.
‡C4 A1 is a heterogeneous electrophoretic group of allotypes.
§Duplicated C4A haplotypes.

–4,5,6 person [62]; and anti-Ch1, -Ch3, and -Ch4 in a Ch:–1,–2,–3,–4,5 person [62]. Serum of a Ch– Rg– C4-deficient patient contained an antibody to C4d not recognisable as any Ch or Rg antibody, although traces of anti-Ch and -Rg may also have been present [23].

From serological data on the two Rg and six Ch determinants, and from derived amino acid sequences of several C4 allotypes, Yu *et al.* [40] devised a model to explain Ch/Rg antigenicity. The model is represented in the diagram in Figure 17.3 and in some of the data presented in Table 17.1. The model involves sequential epitopes, which are dependent on one or more amino acid residues within a single sequence of a few residues, and conformational epitopes, which are more dependent on the shape of the molecule and require the presence of more than one sequential epitope. Ch1 and Ch6 are sequential epitopes. Ch1 requires Ala1188 and Arg1191; Ch6 requires Ser1157. Ch3, a conformational epitope, requires the presence of both Ch1 and Ch6. Ch4 and Ch5 are sequential epitopes. Ch4 requires Leu1101, Ser1102, Ile1105, and His1106 (defining the C4B isotype). Ch5 requires Gly1054. The conformational epitope Ch2 requires the presence of both Ch4 and Ch5. Rg1, a sequential epitope, requires Val1188 and Leu1191. Rg2, a conformational epitope, requires the expression of Rg1 and Asn1157. Asn1157 was assumed to represent a sequential epitope called Rg3, although no anti-Rg3 has been found. W.H. represents a conformational epitope expressed when valine and leucine occupy 1188 and 1191, respectively (Rg1) and when Ser1157 (Ch6) is present [41]. A study of 325 families supported the extended model, without exception [56]. The model allows for the 16 possible combinations shown in Table 17.3, 13 of which have been recognised.

In addition to anti-Rg1 and -Rg2, two of 10 Rg antisera contained an antibody specific for an epitope on the β-chain of C4 [63]. The antibody could not be separated from anti-Rg2 and a strong, but incomplete, association exists between Rg2 and the β-chain epitope.

C4 genes producing Ch1 or Rg1 determinants can be distinguished by an *Eco*O 109 RFLP, regardless of C4A or C4B isotype [39]. PCR-based methods combining

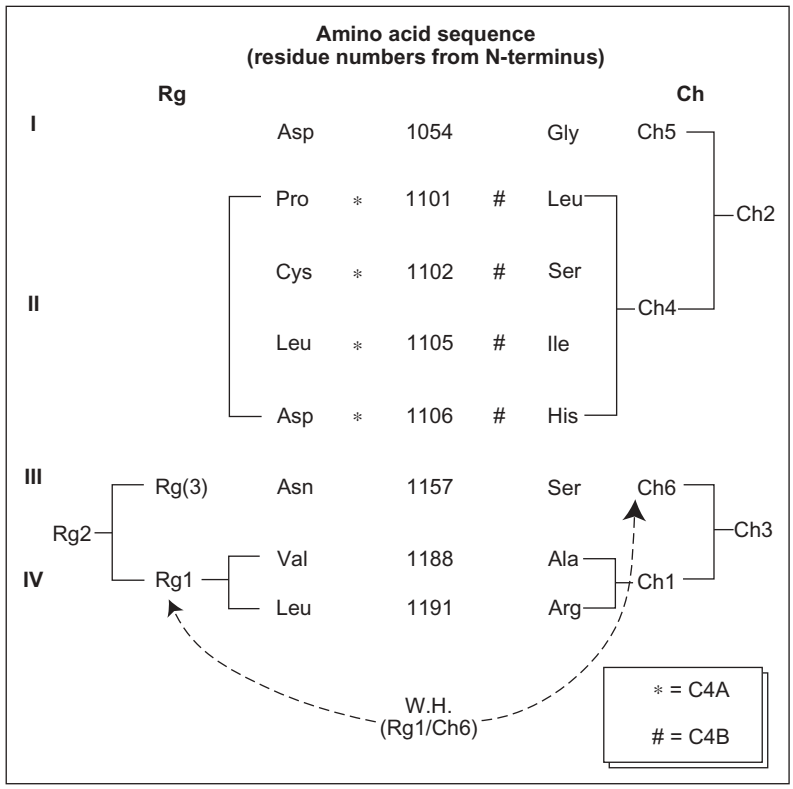

Figure 17.3 A structural model for the location of Ch/Rg determinants on C4 [40,56]. Ch1, Ch4, Ch5, Ch6, and Rg1 represent sequential epitopes; Ch2, Ch3, Rg2, and W.H. represent conformational epitopes involving two sequential epitopes; Rg(3) represents a hypothetical sequential epitope.

isotype- and allele-specific amplification with sequence-specific primers or direct sequencing enable the prediction of all Ch and Rg determinants [64–66].

17.6 Chido/Rodgers antibodies: clinical significance

Ch/Rg antibodies are IgG, mostly IgG2 and IgG4 [67], and they are not considered clinically significant from the red cell transfusion aspect. Ch/Rg antibodies have not caused any obvious signs of an HTR and radiolabelled Ch+ cells transfused to patients with anti-Ch survive normally [2,68–73]. Anti-Ch and -Rg have, however, been implicated in severe anaphylactic reactions following infusion of fresh frozen plasma, plasma fraction, or platelet concentrates containing plasma [74–76], though these events are exceptional.

Ch/Rg autoantibodies, responsible for a DAT, appeared in a woman at the 35th week of pregnancy, and disappeared a few months after delivery [77]. This occurred in two pregnancies and in both cases the red cells of the baby gave a negative DAT.

17.7 Associations with disease

C4 deficiency and its associated Ch/Rg-null phenotype is rare and is usually accompanied by autoimmune or immune complex disorder, typically systemic lupus erythematosus (SLE) [78]. This association may result from ineffective dissolution and removal of immune aggregates in the absence of C4 [17]. There is a significantly greater susceptibility to SLE in individuals with a *C4A* gene deletion (*C4A*Q0*) than in the general population [79–81]. In European Americans, absence or just a single

copy of *C4A* in the diploid genome are risk factors for SLE, whereas three or more copies appear to be protective [82]. It is not surprising, therefore, that symptoms of SLE have been diagnosed in substantially more Rg– individuals, mostly homozygous for *C4A*Q0*, than Rg+ people [83]. *C4A*Q0* has also been associated with several other autoimmune diseases, including Graves' disease and rheumatoid arthritis [84].

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