# 20 Knops Blood Group System and the Cost Antigens



## 20.1 Introduction

The Knops system consists of nine antigens, including three pairs of antithetical antigens (Table 20.1). These antigens are defined by clinically insignificant antibodies that are notoriously difficult to identify. They are all located on complement receptor 1 (CR1, CD35), a member of the complement control protein superfamily, and associated with nucleotide changes in *CR1*. The Helgeson phenotype appears, by conventional serological methods, to be a Knops-null phenotype, though very low levels of CR1 are present on the red cells.

*CR1* is part of the regulators of complement activity gene cluster on chromosome 1q32.

$Cs^a$, an antigen of moderately high frequency, is related serologically to $Yk^a$, but does not appear to be on CR1. $Cs^a$ and $Cs^b$ comprise the Cost collection (Collection 205, Section 20.8).

## 20.2 Complement receptor 1 (CR1) and the Knops system

### 20.2.1 CR1 (CD35)

CR1 is a glycoprotein of about 200 kDa present on red cells, granulocytes, monocytes, B lymphocytes, a subset of T cells, glomerular podocytes, and follicular-dendritic cells in lymph nodes (reviews on CR1 in [1–4]). A soluble form of CR1 (sCR1) is present in plasma. The primary structure of the CR1 polypeptide has been elucidated from the cDNA sequence [5–7]. The most common allotype of CR1 (CR1*1) consists of 2039 amino acids, including a 41 amino acid N-terminal signal peptide (cleaved from the mature protein), a 1930 amino acid extracellular domain, a 25 amino acid transmembrane region, and a 43 amino acid cytoplasmic domain. Like some other complement regulatory proteins, including decay-accelerating factor (Chapter 19), the extracellular domain is organised into regions of amino acid sequence homology, each comprising about 60 residues, called complement control protein repeats (CCPs) or short consensus repeats (SCRs). The extracellular domain of the CR1*1 allotype consists of 30 CCPs (Figure 20.1). Each CCP domain contains four cysteine residues and is maintained in a folded conformation by two disulphide bonds (Figure 20.2). Further homology divides the N-terminal 28 CCPs into four regions called long homologous repeats (LHRs), each comprising seven CCPs.

Four allotypes of CR1 of different molecular weight have been identified: the common CR1*1 allotype (190 kDa under non-reducing conditions), the less common CR1*2 allotype (220 kDa), and the rare CR1*3 (160 kDa) and CR1*4 (250 kDa) allotypes [1–4,8]. These allotypes differ by the numbers of LHRs making up the extracellular domain and may have arisen as a result of intragenic unequal crossing-over [9]. The number of CR1 molecules per red cell differs considerably from person to person,


*Human Blood Groups*, Third Edition. Geoff Daniels.

**Table 20.1** Antigens of the Knops system.

| Antigen | | | | Molecular basis* | | |
|---|---|---|---|---|---|---|
| No. | Name | Frequency | Antithetical antigen | Nucleotides | Exon | Amino acids |
| KN1 | $Kn^a$ | High | KN2 | 4681G (A) | 29 | Val1561 (Met) |
| KN2 | $Kn^b$ | Low | KN1 | 4681G>A | 29 | Val1561Met |
| KN3 | $McC^a$ | High | KN6 | 4768A (G) | 29 | Lys1590 (Glu) |
| KN4 | Sl1 | High | KN7 | 4801A (G) | 29 | Arg1601 (Gly) |
| KN5 | $Yk^a$ | High | | 4223C (T) | 26 | Thr1408 (Met) |
| KN6 | $McC^b$ | Low | KN3 | 4768A>G | 29 | Lys1590Glu |
| KN7 | Sl2 | Low | KN4 | 4801A>G | 29 | Arg1601Gly |
| KN8 | Sl3 | High | | 4828T (A) | 29 | Ser1610 (Thr)† |
| KN9 | KCAM | § | | 4843A (G) | 29 | Ile1615 (Val) |

*Molecular basis of antigen-negative phenotype in parentheses.
†Arg1601 also required for KN8 (Sl3) expression.
§High in Caucasians, relatively low in Africans.

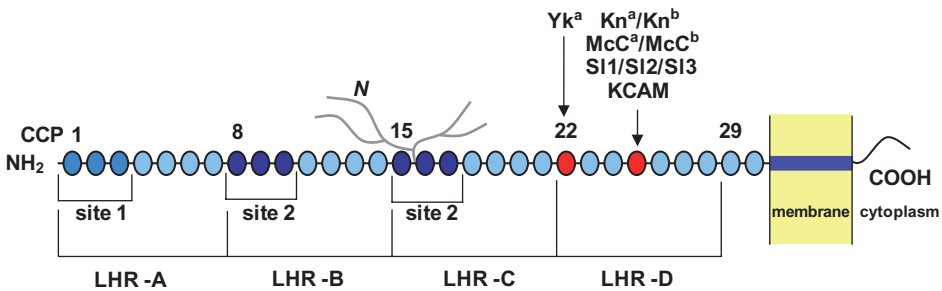


**Figure 20.1** Diagrammatic representation of the most common allotype of CR1 (*CR1*1*), showing the 30 complement control protein repeats (CCP), the four long homologous repeats (LHR), one of the 6–8 *N*-linked oligosaccharides (*N*), the transmembrane region, and the cytoplasmic domain, the active sites (site 1 and the duplicated site 2), the position of the Knops polymorphisms in CCP-22 and CCP-25.

varying from 20 to over 800 [10]. This quantitative polymorphism is independent of the size polymorphism and is associated with several SNPs in *CR1*, including one responsible for a *Hind*III RFLP in white people, but not in African Americans (for reviews on CR1 polymorphisms see [4,11]).

There are 25 potential sites for *N*-glycosylation [6], but only 6–8 *N*-glycans per molecule [1]. CR1 is not *O*-glycosylated [12].

The 133–160 kb *CR1* gene is located on chromosome 1q32, within the regulator of complement activity (RCA) cluster (see Section 19.2.1). *CR1* is organised into 39 exons (*CR1*1* allele) or 47 exons (*CR1*2*). Each LHR is represented by 8 exons. In each LHR, CCPs 1, 5, and 7 are encoded by one exon each; CCPs 2 and 6 by two exons each; and CCPs 3 and 4 by a single exon [9,13] (Table 20.2). The major transcription start site is 111 bp upstream of the translation-initiating ATG codon [9].

## 20.2.2 Knops system antigens are located on CR1

In 1991, Rao *et al.* [14] and Moulds *et al.* [15] independently demonstrated that Knops system antigens are situated on CR1. Immunoprecipitation of radiolabelled red cell membrane proteins with anti-$Kn^a$, -$McC^a$, -Sl1 ($Sl^a$ then), and -$Yk^a$ produced bands on SDS polyacrylamide

gels identical to those produced by precipitation with monoclonal anti-CR1. Furthermore, after immunoprecipitation with monoclonal anti-CR1, affinity purified CR1 could be detected on immunoblots with human anti-Kn/McC serum [14]. When both CR1*1 and CR1*2 allotypes were present, two bands were detected with the monoclonal and human antibodies [14,15]. The location of Kn$^a$, McC$^a$, Sl1, and Yk$^a$ on CR1 was confirmed by neutralisation of the corresponding antibodies with soluble, recombinant CR1 [16] and by the MAIEA assay with monoclonal anti-CR1 [17]. All Knops polymorphisms have now been shown to be associated with single nucleotide polymorphisms in *CR1* (Section 20.4 and Table 20.1). In contrast, the immunochemical methods described above gave negative results with anti-Cs$^a$, suggesting that Cs$^a$ is not on CR1 [15–17].

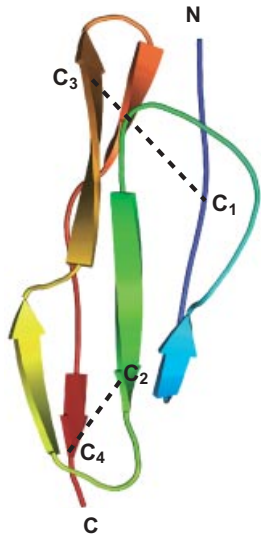


**Figure 20.2** Model of a CCP domain, showing the five β sheets and four conserved cysteine residues (C) forming two disulphide bonds (dashed lines) (Provided by N. Burton.)

## 20.3 Helgeson, a mod phenotype in the Knops system

The major serological characteristic that led to the Knops, McCoy, and Sl antigens being ranked together has been their apparent absence from the red cells of one of the discoverers of Kn$^a$, Margaret Helgeson (M.H.), and from other red cells of the same phenotype [18–22]. Apparent absence of Yk$^a$ from these cells was demonstrated later [10]. In fact, Helgeson phenotype cells do not represent a true Knops-null phenotype because they express very low levels of Knops antigens [10,14,15], and may even be agglutinated in antiglobulin tests by the most potent examples of Knops antibodies [10]. Consequently, Helgeson phenotype is not associated with an antibody to a generic Knops system antigen. The Helgeson phenotype has an incidence of about 1% in Caucasians and African Americans [21,22].

Red cells of the Helgeson phenotype have a very low number of CR1 molecules per red cell, approximating

**Table 20.2** Domains of CR1 encoded by the 39 exons of *CR1*1*.

| LHR-A | | LHR-B | | LHR-C | | LHR-D | | | |
|---|---|---|---|---|---|---|---|---|---|
| Exon | CCP | Exon | CCP | Exon | CCP | Exon | CCP | Exon | Domain |
| 2 | 1 | 10 | 8 | 18 | 15 | 26 | 22 | 34 | 29 |
| 3 | 2a | 11 | 9a | 19 | 16a | 27 | 23a | 35 | 30 |
| 4 | 2b | 12 | 9b | 20 | 16b | 28 | 23b | 36 | TMa |
| 5 | 3,4 | 13 | 10,11 | 21 | 17,18 | 29 | 24,25 | 37 | TMb |
| 6 | 5 | 14 | 12 | 22 | 19 | 30 | 26 | 38 | cyto |
| 7 | 6a | 15 | 13a | 23 | 20a | 31 | 27a | 39 | 3′ UT |
| 8 | 6b | 16 | 13b | 24 | 20b | 32 | 27b | | |
| 9 | 7 | 17 | 14 | 25 | 21 | 33 | 28 | | |

Exon 1 encodes the leader peptide.
LHR, long homologous repeat; CCP, complement control protein domain; TM, transmembrane domain; cyto, cytoplasmic domain; UT, untranslated.

**Table 20.3** Frequencies of Knops antigens.

| Antigen | Population | No. tested | No. positive | Antigen frequency | References |
|---|---|---|---|---|---|
| Kn$^a$ | Caucasians | 4553 | 4498 | 98.8% | [18,21] |
| | African Americans | 894 | 883 | 98.8% | [21] |
| Kn$^b$ | Caucasians | 229 | 10 | 4.4% | [24] |
| McC$^a$ | Caucasians | 3860 | 3802 | 98.5% | [19,21] |
| | African Americans | 1539 | 1461 | 94.9% | [19,21] |
| McC$^b$ | African Americans | 371 | 168 | 45.3% | [25] |
| Sl1 | Caucasians | 833 | 815 | 97.8% | [20,22] |
| | African Americans | 480 | 257 | 53.5% | [20,22] |
| Yk$^a$ | Caucasians | 2889 | 2598 | 89.9% | [21] |
| | African Americans | 1117 | 1098 | 98.3% | [21] |

10% of normal [10,14,15]. Knops antigens could be detected on CR1 of Helgeson phenotype cells by immunoprecipitation, flow cytometry, or MAIEA [14,15,17]. Levels of CR1 were often normal on red cells of individuals who lacked only one of the Knops system antigens and who had made the corresponding antibody [15]. Expression of Knops antigens, as detected by an antiglobulin test, correlates strongly with the number of CR1 molecules per red cell. Cells with between 20 and 100 CR1 molecules are negative with Knops antibodies by the antiglobulin test (Helgeson phenotype), cells with 100–150 molecules are weak or negative depending on the antibody used, and cells with more than 200 molecules are generally positive with all antibodies tested [10]. Helgeson phenotype appears to result from inherited low copy number of CR1, whereas absence of single antigens in individuals who may make the corresponding antibody results from mutations within the *CR1* gene. There is some, but not complete, correlation between the Helgeson phenotype and the polymorphic genotypes associated with low CR1 copy number, including that responsible for the *Hind*III restriction site [23]. Helgeson phenotype red cells have significantly lower CR1 copy number than the more common 'low copy number' phenotypes.

## 20.4 Antigens of the Knops system

Knops antigens and their molecular backgrounds are listed in Table 20.1 and some antigen and allele frequencies in Tables 20.3 and 20.4. All the Knops polymorphisms are associated with SNPs in exon 29 of *CR1*, encoding amino acid changes in CCP-25, except Yk$^a$, with a SNP in exon 26, encoding an amino acid change in CCP-22.

### 20.4.1 Kn$^a$ and Kn$^b$ (KN1 and KN2)

Kn$^a$, the original Knops antigen first reported in 1970 [18], has an incidence of about 98–99%, although any antigen frequency study in the Knops system will be compromised by the presence of Helgeson alleles. An antibody in a serum containing anti-Kp$^a$, which reacted with red cells of 4.2% of Kp(a–) Australian blood donors, was considered to be anti-Kn$^b$ because it reacted with virtually all Kn(a–) McC(a+) red cell samples, but with no Kn(a–) McC(a–) samples [24]. No other examples of anti-Kn$^b$ are reported.

The Kn$^a$/Kn$^b$ (*KN*A/KN*B*) substitution represents Val1561Met in CR1 [26].

### 20.4.2 McC$^a$ and McC$^b$ (KN3 and KN6)

McC$^a$ (McCoy) was identified and shown to be associated with Kn$^a$ by Molthan and Moulds [19]. Although Kn$^a$ and McC$^a$ have frequencies well in excess of 90%, 53% of McC(a–) individuals were also Kn(a–). The frequency of Mc(a–) is 1–2% in white Americans, but varies between 90 and 97% in different surveys of African Americans and West Africans [19,21,32]. Anti-McC$^b$ is antithetical to anti-McC$^a$ in black people [25,29]: 45.3% of black donors are McC(b+), which includes all Kn(a+) McC(a–), but no Kn(a–) McC(a–) individuals.

The McC$^a$/McC$^b$ polymorphism is associated with Lys1590Glu [29]. Discrepancies between phenotype and genotype in about 6% of samples were accounted for mainly by genes encoding low CR1 copy number.

**Table 20.4** Frequencies of some Knops alleles.

| Population | Alleles | | | | | | | | | | Refs |
|---|---|---|---|---|---|---|---|---|---|---|---|
| | *Kn*$^a$ | *Kn*$^b$ | *McC*$^a$ | *McC*$^b$ | *Sl1* | *Sl2* | *Yk*$^a$ | *Yk*$^{b*}$ | *KCAM*$^+$ | *KCAM*$^-$ | |
| Caucasians | 0.99 | 0.01 | 0.99 | <0.01 | 0.99 | <0.01 | 0.71 | 0.29 | | | [26,27] |
| European Brazilians | 0.97 | 0.03 | 0.98 | 0.02 | 0.97 | 0.03 | | | 0.76 | 0.24 | [28] |
| Malians | 0.90 | 0.10 | 0.69 | 0.31 | 0.31 | 0.69 | | | | | [26] |
| West Africans | | | 0.69 | 0.31 | 0.21 | 0.79 | | | | | [29] |
| Africans | | | | | | | | | 0.1 | 0.9 | [30] |
| African Americans | 0.98 | 0.02 | 0.78 | 0.22 | 0.48 | 0.52 | | | | | [26] |
| African Brazilians | 0.99 | 0.01 | 0.76 | 0.24 | 0.42 | 0.58 | | | 0.32 | 0.68 | [28] |
| Chinese Han | 1.00 | 0 | 1.00 | 0 | 1.00 | 0 | | | 0.82 | 0.18 | [31] |
| Asian Brazilians | 1.00 | 0 | 1.00 | 0 | 1.00 | 0 | | | 0.79 | 0.21 | [28] |

*$Yk^b$ represents absence of Yk$^a$ as anti-Yk$^b$ has not been found.

Recombinant soluble CR1 (sCR1) containing Lys1590 inhibited anti-McC$^a$, but not anti-McC$^b$, whereas sCR1 containing Glu1590 inhibited anti-McC$^b$, but not anti-McC$^a$.

### 20.4.3 Sl1, Sl2, and Sl3 (KN4, KN7, and KN8)

Sl1 (previously named Sl$^a$, Swain-Langley, and McC$^c$) is an antigen of high incidence in white people, but of distinctly lower frequency in black people [20,22]. McC(a–) black people are Sl1–, whether they are Kn(a–) or Kn(a+), but 45% of Kn(a+) McC(a+) African Americans are also Sl1–. Kn(a–) McC(a–) white people are also Sl1–, but only 1% of Kn(a+) McC(a+) white people were Sl1– and all four Kn(a+) McC(a–) individuals found in testing 722 white donors were Sl1+. Sl1– has a frequency of around 70% in West Africans [32].

Anti-Sl2 (originally called anti-Vil) appeared to be antithetical to anti-Sl1 when testing red cells from black people, but no Sl2+ white person was found [20].

The Sl1/Sl2 polymorphism is associated with Arg1601Gly in CR1 [29]. Discrepancies between phenotype and genotype in about 12% of samples were mainly accounted for by genes encoding low CR1 copy number. Soluble CR1 containing Arg1601 inhibited anti-Sl1, except when it also contained Glu1590 (McC$^b$), but not anti-Sl2. The change from a positively charged lysine to a negatively charged glutamic acid at position 1590 probably affects the conformation of the Sl1 epitope around position 1601 [29]. McC(a–) Sl(a+) phenotype is not found in black people [22,25,29]. Soluble CR1 containing Gly1601 inhibited anti-Sl2, but not anti-Sl1.

The specificity previously named anti-Sl$^a$ was subdivided when an Sl(a+) donor was found to have an apparent anti-Sl$^a$ [33]. Her red cells reacted with six anti-Sl$^a$, renamed anti-Sl1, but not with two anti-Sl$^a$, renamed anti-Sl3. She was homozygous for 4810A (Arg1601), typical of Sl1+, but also homozygous for 4828T>A, encoding Ser1610Thr. Consequently, it appears that Sl3 is a conformational epitope requiring both Arg1601 and Ser1610. No homozygotes for 4828A, encoding Sl3– phenotype, were found in 89 Caucasians, 41 black Africans, or 86 Asian Americans, but allele frequencies for 4828A of 0.04, 0, and 0.01 were found in those three populations, respectively [33].

### 20.4.4 Yk$^a$ (KN5)

Yk$^a$ was described as a new antigen related to Cs$^a$ because the original anti-Yk$^a$ (York) failed to react with two Cs(a–) samples, yet the red cells of Mrs York were Cs(a+) [34]. The frequency of the *Yk*$^a$ allele, calculated from the data shown in Table 20.3, is 0.6826 in white Americans and 0.8696 in African Americans [21], and by molecular testing is 0.71 in Caucasians [27].

The incidence of the Cs(a–) Yk(a–) phenotype in white Americans is 1.63% [21]. If there were no association between Cs$^a$ and Yk$^a$, the expected phenotype frequency would be 0.48%, about three times less than that observed. Cs(a–) Yk(a–) has an incidence of 0.35% in African Americans [21], about 17 times that expected

assuming no association. Of McC(a–) white people, 37% were Yk(a–) and 29% Cs(a–); 2.2% of McC(a–) black people were Yk(a–) and 17% were Cs(a–) [19]. These figures differ substantially from those expected if no relationship existed between McC$^a$, Yk$^a$, and Cs$^a$; 100 times more McC(a–) donors were Yk(a–) Cs(a–) Kn(a–) than would be predicted from the frequencies of these antigens. Although the original Helgeson phenotype cells (M.H.) are Yk(a–) Cs(a–), three other examples were Yk(a–) Cs(a+) [10].

Yk(a–) results from homozygosity for 4223C>T in exon 26 of *CR1*, encoding Thr1408Met in CCP-22 [27].

### 20.4.5 KCAM (KN9)

The original anti-KCAM was initially considered to be anti-McC$^a$, but recognises the product of a SNP encoding Ile1615 in KCAM+ and Val1615 in KCAM– [30]. Four of nine anti-McC$^a$ and six of 19 anti-'Kn/McC' also appeared to have KCAM specificity. Serological tests do not appear to show reliable concordance between phenotype and genotype [35]. Frequencies of KCAM predicted from molecular genotyping are as follows (Table 20.4): Chinese 97%; Caucasians 94%; African Brazilians 54%; Africans 19% [28,30,31].

### 20.4.6 Some other serological characteristics of Knops antigens

Knops system antigens are generally resistant to treatment of the red cells with ficin and papain, although this may depend on the antibody and method of enzyme treatment used. Kn$^a$, McC$^a$, and Yk$^a$ are destroyed by trypsin and chymotrypsin treatment of the cells, which helps to distinguish them from Cs$^a$, and also destroyed, or at least weakened, by the disulphide bond reducing agents AET and DTT, also distinguishing them from Cs$^a$ [36].

Knops antigens show a variation in strength between individuals that correlates with red cell CR1 levels [10]. A reduction in expression of Knops antigens has often been observed to occur with red cell storage, but this could not be demonstrated conclusively in controlled tests with Knops antibodies against freshly bled red cells and cells stored for 35 days [37]. CR1 density per red cell decreases with cell aging, possibly due to protease cleavage of the protein near its stalk [2].

Strength of the Knops antigens is also affected by presence of an *In(Lu)* gene (Section 6.8.2) [38]. In(Lu) $Lu_{null}$ cells gave lower mean titration scores with anti-Kn$^a$, -McC$^a$, -Yk$^a$, and -Sl1, and with anti-Cs$^a$, than did Lu(a–b+) or Lu(a+b+) cells from members of the same family. This effect of *In(Lu)* was not confirmed in a later study, by comparing In(Lu) cells with unrelated donors of common Lutheran phenotype [39].

Knops antigens are generally well expressed on red cells from cord samples. Two babies of McC(a–) mothers with high titre anti-McC$^a$ were also McC(a–) at birth, but became McC(a+) within their first year of life [40]. Maternal anti-McC$^a$ may have been responsible for impaired McC$^a$ antigen expression *in utero*.

## 20.5 Knops system antibodies

### 20.5.1 Antibody characteristics

The term 'high-titre low-avidity' (HTLA) was used for many years to describe antibodies to a variety of antigens, including those of the Knops system. Although most of these antibodies react at high dilution despite their low avidity, some examples do not share these characteristics and the HTLA label is of little value.

Knops antibodies are generally troublesome to work with. This partly results from the variation in antigen strength, but also because it is difficult to adsorb the antibodies to completion or to obtain active eluates from weak antigen-positive cells. Consequently, it is almost impossible to distinguish antigen-negative cells from weakly positive cells, especially when stored or 'travelled' red cells are used.

Knops antibodies are generally IgG; they react by an antiglobulin test and do not bind complement [21,41]. There is little information regarding IgG subclass: one Knops system antibody was IgG4 [42]; another contained IgG1, IgG3, and IgG4, as well as IgA [43].

There is only one report of an apparently 'naturally occurring' Knops system antibody; anti-Kn$^a$ in a woman who denied previous pregnancy or transfusion [44]. Of 602 blood donors lacking one or more of the Knops antigens or Cs$^a$, none had made a corresponding antibody [21]. Most people with one or more Knops antibodies have been transfused, but there are a few examples of anti-Kn$^a$, -McC$^a$, and -Yk$^a$ stimulated by pregnancy alone. About 50% of sera with Knops antibodies or anti-Cs$^a$ also contained antibodies to other red cell antigens [21].

### 20.5.2 Clinical significance

Knops antibodies are clinically benign, apart from the hazard of masking the presence of more dangerous antibodies that are commonly present in the same serum. Knops antibodies should be ignored when selecting

blood for transfusion. There are numerous accounts of patients with one of these antibodies being transfused with no ill effects. Radiolabelled incompatible red cells in patients with Knops antibodies show either normal or only slightly reduced survival [42–45], and *in vitro* phagocytosis assays often give very low scores [42,44]. *In vitro* functional assays involving monocytes may, however, give false positive results with Knops antibodies, because these antibodies can bind red cells to monocytes *via* CR1 rather than the Fc receptor, FcγR1 [46].

There is no report of HDFN caused by a Knops antibody, despite numerous opportunities. CR1 expression on red cells is reduced during pregnancy, reaching its nadir in the third trimester and returning to normal within 48 h post-partum [47].

## 20.6 Functional aspects of CR1, a complement-regulating protein

Red cell CR1 binds C3b/C4b-coated immune complexes and transports them to the liver and spleen where they are transferred to macrophages for processing. Ligation of CR1 triggers a complex $Ca^{++}$-dependent signalling cascade that promotes phosphorylation of the cytoskeletal proteins α-adducin and β-spectrin. This correlates with increased membrane deformability, which could play an important role in the immune-adherence clearance process [48].

CR1 has decay-accelerating activity for C3 and C5 convertases of the classical and alternative pathways and acts as a cofactor for the factor I-mediated cleavage of C3b and C4b (reviews in [1,4]). *In vivo* and *in vitro* haemolysis of PNHIII red cells, which have CR1 but are deficient in DAF and CD59 (Chapter 19), suggests that CR1 plays a minor role in protection of red cells from complement-mediated lysis.

CR1 appears to represent a privileged site on red cells for IgG binding, as relatively large quantities of IgG may be bound to CR1 without subsequent lysis or phagocytosis of the red cells [49]. This may explain why Knops system antibodies do not significantly reduce the survival of transfused incompatible red cells (Section 20.5.2).

## 20.7 CR1 associations with malaria and other pathogens

Red cells infected with selected cultures of the malarial parasite *Plasmodium falciparum* form rosettes with infected and uninfected red cells. Rosetting is associated with severe disease by clogging the microvasculature of vital organs including the brain. Rowe *et al.* [50] showed that infected cells do not form rosettes with uninfected red cells that have very low levels of CR1 (Helgeson phenotype) and that there is substantially reduced rosetting with Sl1– red cells, compared with Sl1+ cells. Furthermore, compared with Sl1+ cells, Helgeson phenotype red cells and other cells with the Sl1– phenotype showed reduced levels of binding to COS-7 cells transfected with the *P. falciparum var* gene expressing PfEMP1, the parasite ligand involved in rosetting. In Kenya, children with the genotype for Sl1– were less likely to have cerebral malaria than children with the genotype for Sl1+ and, in particular, those with Sl1– genotype and heterozygous for $McC^a$ and $McC^b$ were less likely to have cerebral malaria [51]. Sl1– is present in about 70% of West Africans, 40–50% of African Americans, but in only about 2% of white Americans (Section 20.4.3), so it is feasible that the Sl1– phenotype has a selective advantage in areas where *P. falciparum* malaria is endemic. The relationship between CR1 and malaria, however, is far from straightforward [52,53]. In Thailand a low level of red cell CR1 appeared to be a risk factor for developing severe malaria [54] and in the Gambia no association between severe malaria and *CR1* alleles encoding Sl2 or $McC^b$ was observed [55]. Studies with soluble CR1 fragments provided no evidence that either the $McC^a$/$McC^b$ or Sl1/2 polymorphisms had any effect on red cell *P. falciparum* invasion or rosette disruption [56].

The *P. falciparum* adhesin PfRh4 binds to CR1 on red cells, but only to CCPs 1–3 [57], remote from the site of the Knops polymorphisms in LHR-D (Figure 20.1). In addition to the role played in rosetting, CR1 is involved in control of complement activation and immune complex formation during malaria infection and is also a receptor for parasite invasion [53]. PfRh4 binding to CR1 affected neither C3b nor C4b binding, but it did inhibit decay-accelerating activity [57].

Some pathogenic bacteria, such as *Mycobacterium tuberculosis*, *M. leprae*, and *Legionella pneumophila*, utilise CR1 for invading phagocytes through adherence and phagocytosis [58]. Presence of $McC^b$ and *Sl2* alleles appears to be associated with resistance to *M. tuberculosis* infections in Mali [59] and homozygosity for $McC^b$ to confer protection from leprosy in Malawi [60]. Complement-dependent binding of gram-negative bacteria (*Escherichia coli* and *Neisseria meningitidis*) to red cell CR1 reduces intravascular phagocytosis and oxidative burst by monocytes and neutrophils, with the

**Table 20.5** $Cs^a$ frequencies.

| Population | No. tested | No. Cs(a+) | $Cs^a$ frequency | References |
|---|---|---|---|---|
| Northern Europeans | 363 | 354 | 97.5% | [62] |
| Black Africans and Americans | 53 | 51 | 96.2% | [62] |
| White Americans | 2028 | 1931 | 95.2% | [21] |
| African Americans | 894 | 883 | 98.8% | [21] |
| Yk(a–) white Americans | 96 | 84 | 87.5% | [34] |
| Yk(a–) African Americans | 13 | 12 | 92.3% | [34] |

possible effect of reducing systemic inflammatory responses [61].

## 20.8 The Cost collection: $Cs^a$ and $Cs^b$ (COST1 and COST2)

When Giles *et al.* [62] described three patients with antibodies reactive with the red cells of 98% of Northern Europeans, they named this antibody anti-$Cs^a$ after two of the original patients, Mrs Co. and Mrs St. Numerous family studies have shown that $Cs^a$ is inherited as a dominant character and that it is not part of the ABO, MNS, Rh, Kell, Duffy, Kidd, Yt, or Scianna systems, is probably not part of P and Lewis, and that there is a possible association between $Cs^a$ and $Do^a$ [62,63]. Table 20.5 shows the results of frequency studies with anti-$Cs^a$.

Anti-$Cs^a$ share many characteristics with Knops system antibodies (Section 20.5). They are difficult to work with, primarily because of the variability in expression of the $Cs^a$ antigen, and anti-$Cs^a$ are of no significance clinically.

An antibody in a multiply transfused woman with a weak $Cs^a$ antigen was named anti-$Cs^b$ [64]. Fifty-six of 59 Cs(a–) samples were Cs(b+); the remaining three were Cs(a–b–), suggesting the presence of a third allele. Fifty-five (31%) of 175 Cs(a+) samples were Cs(b+).

Despite phenotypic associations with the Knops system antigens, especially $Yk^a$ (see Section 20.4.4), $Cs^a$ and $Cs^b$ are not included in the Knops system for the following reasons: $Cs^a$ could not be shown to be on CR1 [15,17]; $Cs^a$ was easily detected on three of four Helgeson phenotype samples [10]; and $Cs^a$ is resistant to treatment of red cells with trypsin, chymotrypsin, and AET [36]. The nature of the association between $Cs^a$ and the Knops system remains obscure.

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