The Knops blood group system: a review

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The Knops blood group system finds its roots in the group of antibodies that were previously called high-titer, low-avidity, or HTLA, antibodies. This term was used to describe a group of alloantibodies that appeared to have many of the same characteristics, including very weak reactions at the anti-human globulin (AHG) phase of testing. However, as their specificities were more clearly defined, each was placed into a blood group system, and Knops became the 22nd system recognized by the ISBT Committee on Terminology for Red Cell Surface Antigens. Since that time, the protein bearing the Knops antigens has been identified, the gene has been cloned, and all of the known Knops antigens have been characterized at the molecular level. This has allowed investigators to more precisely study the role of the Knops blood group system in disease.

History

In 1965, the sera from three unrelated patients (Copeland, Stirling, and Wainright) were reported as having an antibody with similar specificity. The antibody was named after the first two antibody producers, Co and St (Cost), and given the designation Csα. Ten years later a new antigen, York (Ykα) was reported. The York serum was initially believed to be an example of anti-Csα until the RBCs of the donor were found to be Cs(a+). This same study found that 12 of 1,246 Caucasian donors were both Cs(a−) and Yk(a−), suggesting that these antigens were at least phenotypically related. Neither, however, was given blood group status, and they became part of the HTLA group of antibodies. Even though Ykα was thought to be associated with Csα at this time, it would later be assigned to the Knops system.

Another similar antibody being investigated in the 1960s was anti-Knα. Anti-Knα was described in a transfused Caucasian woman who had a saline-reactive anti-K plus an unidentified antiglobulin-reactive antibody to a high-frequency antigen. The antithetical Knβ was later reported in the Hall serum, which contained a potent anti-Kpβ and was being used to screen Australian blood donors.

The Knops blood group system began to expand when Molthan and Moulds described a new antigen, McCα, which seemed to be related to Knα. They reported that 53 percent of McC(a−) samples were also Kn(a−). Interestingly, a majority of McCα antibody producers were Black whereas most of those making anti-Knα were Caucasian, thus suggesting that ethnic differences might exist in their respective gene frequencies.

The next pair of alleles, Slα and Vil, was reported in separate abstracts with one author using the term McC for Slα and McCd for Vil. The Sl terminology came from the names of the first two antibody producers, i.e., Swain and Langley. It was preferred by these authors because they believed that this antigen was independent from McCoy. After identification of the protein bearing the Knops antigens, Slα was renamed S11 and Vil became S12.

The last high-incidence antigen identified in the Knops system was KAM, later renamed KCAM by the ISBT Committee on Terminology for Red Cell Surface Antigens. The antibody producer was a Caucasian man who exhibited the Helgeson phenotype, i.e., serologic Kn null. Like Slα, this antigen showed a widely diverse frequency in Blacks vs. Caucasians (Table 1). Although KCAM is a high-frequency antigen in Caucasians, only 20 percent of African Blacks were KCAM+.

<table>
<thead>
<tr>
<th>Population</th>
<th>Knα (%)</th>
<th>Knβ (%)</th>
<th>McCα (%)</th>
<th>McCβ (%)</th>
<th>S11 (%)</th>
<th>S12 (%)</th>
<th>Ykα (%)</th>
<th>KCAM (%)</th>
<th>Refs.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caucasians</td>
<td>98</td>
<td>4</td>
<td>98</td>
<td>1</td>
<td>99</td>
<td>&lt;1</td>
<td>90</td>
<td>98</td>
<td>3, 5, 8, 12, 13</td>
</tr>
<tr>
<td>West Africans</td>
<td>100</td>
<td>NT</td>
<td>89–92</td>
<td>49–54</td>
<td>30–38</td>
<td>95</td>
<td>NT</td>
<td>20</td>
<td>10, 14</td>
</tr>
<tr>
<td>African Americans</td>
<td>99</td>
<td>&lt;1*</td>
<td>90</td>
<td>44</td>
<td>51–61</td>
<td>80%*</td>
<td>98</td>
<td>NT</td>
<td>8, 13, 14</td>
</tr>
<tr>
<td>African Brazilians</td>
<td>98</td>
<td>2</td>
<td>93</td>
<td>42</td>
<td>70</td>
<td>86</td>
<td>NT</td>
<td>53</td>
<td>12</td>
</tr>
<tr>
<td>Asian Brazilians</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>100</td>
<td>0</td>
<td>NT</td>
<td>95</td>
<td>12</td>
</tr>
</tbody>
</table>

*Marilyn Moulds, personal communication.

Note: The publication by Covas et al. incorrectly lists 4870A (isoleucine) as being KCAM negative.

NT = not tested.
Knops Protein

In 1991, two groups identified complement receptor one (CR1) as the protein carrying the Kn\(^n\), McC\(^a\), and Sl\(^a\) blood group antigens.\(^{15,16}\) In addition, Moulds et al.\(^{17}\) also located Yk\(^a\) to CR1 and suggested that the Helgeson phenotype was attributable to low CR1 copy numbers on the erythrocytes (E-CR1). Several years later, Petty et al.\(^{18,19}\) confirmed these reports using monoclonal antibody-specific immobilization of erythrocyte antigens (MAIEA) analyses and also confirmed that Cs\(^a\) was not on CR1. Thus, Cs\(^a\) and Cs\(^b\) have remained a collection as defined by ISBT.

CR1 is a membrane-bound glycoprotein and, with the exception of platelets, is found on most human peripheral blood cells. Depending on the methods used, erythrocytes display approximately 300 to 800 CR1 molecules per cell whereas leukocytes display approximately 10,000 to 30,000 molecules per cell. Because erythrocytes are present in the peripheral circulation at concentrations 100-fold higher than the peripheral blood mononuclear cells (PBMCs), they account for greater than 85 percent of CR1 in the blood. E-CR1 binds immune complexes (ICs) that are shuttled to the liver or spleen for transfer to and ingestion by macrophages, leading to their elimination. IC-free erythrocytes return to the circulation, where they may continue participating in IC clearance. We have noted that individuals with high CR1 copy numbers may exhibit a weak false-positive DAT in the gel technique, most likely as a result of increased binding of ICs.

Knops Antigen Characteristics

The Knops antigens can vary greatly in strength (Table 2), and weakly reactive cells can be falsely phenotyped as negative if the cells are stored for some duration or have low E-CR1 numbers. Chemicals that can disrupt disulfide bonds, i.e., DTT and AET, can destroy Knops, McCoy, Swain-Langley, and York antigens because they destroy the conformational structure of the short consensus repeats (SCRs). There are no examples of these antibodies that are enhanced by enzyme treatment of RBCs, and most are still reactive (sometimes weaker) with either ficin- or papain-treated cells.\(^{18,19}\) However, all Knops system antibodies currently identified are nonreactive with trypsin-treated RBCs. It is known that a trypsin cleavage site exists in SCR 28 of the CR1 protein. Because the blood group antigens identified to date have been found in SCR 25, they are lost on trypsin treatment of the cells. This fact can be a useful tool not only in antibody identification but also for absorption to remove other antibodies from a sample.

Although the variability in antigen strength can be inherited, it may also be acquired. Diseases that have high levels of ICs being processed, e.g., systemic lupus erythematosus or HIV infection, can cause an increased loss of E-CR1, resulting in weakened Knops antigen strength.\(^{20}\) In addition, when RBCs are stored, vesicles are budded from the membrane, and these vesicles contain CR1.\(^{20}\) Therefore, the longer RBCs are stored, the weaker the Knops antigens become. This may explain why In(Lu) RBCs were initially reported to have weakened Knops antigens.\(^{22}\)

<table>
<thead>
<tr>
<th>Table 2. Characteristics of the Knops antigens</th>
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</thead>
<tbody>
<tr>
<td>Inherited as Mendelian-dominant traits</td>
</tr>
<tr>
<td>High-frequency RBC antigens (except Kn(^n) and McC(^a))</td>
</tr>
<tr>
<td>Developed on cord blood cells but may be weaker</td>
</tr>
<tr>
<td>Weaker on stored RBCs</td>
</tr>
<tr>
<td>Not destroyed by ficin or papain</td>
</tr>
<tr>
<td>Destroyed by trypsin treatment</td>
</tr>
<tr>
<td>Not found on platelets</td>
</tr>
<tr>
<td>Found in low levels in plasma but not in urine or saliva</td>
</tr>
</tbody>
</table>

Knops Antibodies

The Knops antibody characteristics are shown in Table 3. Two characteristics that were often attributed to Knops antibodies were (1) they were not neutralized with plasma, saliva, or urine, and (2) they were difficult to adsorb and elute. The latter most likely reflects the low density of the CR1 protein on the RBC membrane. However, Race and Sanger\(^{23}\) reported that adsorption performed with buffy coats (WBCs) were able to remove anti-Kn\(^n\) from serum. This led to the speculation that anti-Kn\(^n\) and related specificities were WBC antibodies. The ability of antigen-positive WBCs to adsorb Knops antibodies most likely relates to the fact that the CR1 copy number on WBCs is in the tens of thousands compared with a few hundred on RBCs.

<table>
<thead>
<tr>
<th>Table 3. Characteristics of Knops system antibodies</th>
</tr>
</thead>
<tbody>
<tr>
<td>Titer is dependent on E-CR1 levels</td>
</tr>
<tr>
<td>Can demonstrate variable reactions</td>
</tr>
<tr>
<td>Not neutralized by pooled serum or other body fluids</td>
</tr>
<tr>
<td>Difficult to adsorb and elute from RBCs</td>
</tr>
<tr>
<td>IgG reacting by AHG technique</td>
</tr>
<tr>
<td>Do not bind complement</td>
</tr>
<tr>
<td>Usually not clinically significant</td>
</tr>
</tbody>
</table>

Although CR1 has not been found in the saliva, low levels have been found in both urine\(^{24}\) and plasma.\(^{25}\) These are believed to be the result of proteolytic cleavage of CR1 from leukocytes.\(^{26}\) Serum CR1 is present only in nanogram amounts,\(^{27}\) and therefore the levels are insufficient to neutralize Knops antibodies using routine serologic techniques. Hence, Moulds and Rowe\(^{28}\) developed an inhibition technique using recombinant, soluble CR1 (sCR1). Because their source of sCR1 was genetically positive for Kn\(^a\), McCoy, Sl\(^a\), and Yk\(^a\), it would only inhibit these antibodies and not inhibit anti-Kn\(^n\) or McCoy.\(^{28}\) It must be remembered that the Knops phenotype of the sCR1 will be dependent on the gene chosen for its production. More recently, these investigators

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have used mutated CR1 constructs to produce peptides capable of inhibiting anti-McC and S12 (Vil).29

**Clinical Importance**

Because the Knops antibodies are found in the serum of multiply transfused individuals, these sera often contain additional alloantibodies directed at (for example) K, E, and Duffy antigens. Most of the Knops antibodies are not considered clinically significant because they do not cause overt hemolytic transfusion reactions or hemolytic disease of the fetus and newborn. Some examples of Knops system antibodies have been studied using in vitro tests such as the monocyte monolayer assay (MMA). Arndt and Garratty30 studied three anti-Kn, three anti-Yk, and five anti-Kn/McC and found that only one had a macrophage index of greater than 20, suggesting increased RBC destruction. Other MMA studies of two anti-Kna, two anti-Sla, one anti-K/Mc and found that only one had a macrophage index of greater than 20, suggesting increased RBC destruction. Other MMA studies of two anti-Kn, two anti-Sla, one anti-K/McC, and one anti-Yk/Cs all gave indexes of less than 0, indicating no enhanced RBC destruction (J.J. Moulds, unpublished data).

**The CR1 Gene**

The CR1 gene resides on chromosome 1 (1q32) and comprises 39 exons spread out over approximately 133 kilobase (kb) pairs of DNA.31 These exons encode SCRs of approximately 60 amino acids in the functional CR1 protein. Seven SCRs are organized into larger units called long homologous repeats (LHRs). The most common size protein product, CR1-1, is made up of four LHRs (A, B, C, D), a transmembrane region (TM), and a cytoplasmic tail (CYT), as shown in Figure 1. The binding sites for C3b and C4b have been localized to SCRs 8-9 and 15-16 (LHRs B and C) and to SCRs 1-2 (LHR-A), respectively.

**Molecular Basis of Knops Antigens**

Using CR1 deletion constructs, Moulds et al.29 first localized the McCoy and S11 (Sl′) antigens to LHR-D of CR1. By direct DNA sequencing they were then able to identify two separate mutations in SCR 25 that correlated with these two blood group antigens. The McC/CMcC polymorphism is at base pair 4795, where an A encodes proline (McCα) and a G encodes aspartic acid (McCβ). The S11/S12 mutation is only 11 amino acids away; at base pair 4828, an A encodes arginine and a G encodes glycine. Accordingly, the ISBT has now assigned these antigens to the Knops system with the numbers shown in Table 4.38

<table>
<thead>
<tr>
<th>ISBT number</th>
<th>Antigen</th>
<th>Nucleotide</th>
<th>Nucleotide†</th>
<th>Amino acid</th>
</tr>
</thead>
<tbody>
<tr>
<td>KN1</td>
<td>Knα</td>
<td>4708G</td>
<td>4681G</td>
<td>Val11561</td>
</tr>
<tr>
<td>KN2</td>
<td>Knβ</td>
<td>4708A</td>
<td>4681A</td>
<td>Met11561</td>
</tr>
<tr>
<td>KN3</td>
<td>McCα</td>
<td>4795A</td>
<td>4768A</td>
<td>Lys1590</td>
</tr>
<tr>
<td>KN4</td>
<td>S11</td>
<td>4828A</td>
<td>4801A</td>
<td>Arg1601</td>
</tr>
<tr>
<td>KN5</td>
<td>Ykα</td>
<td>Unknown</td>
<td>Unknown</td>
<td>Unknown</td>
</tr>
<tr>
<td>KN6</td>
<td>McCβ</td>
<td>4795G</td>
<td>4768G</td>
<td>Glu1590</td>
</tr>
<tr>
<td>KN7</td>
<td>S12</td>
<td>4828G</td>
<td>4801G</td>
<td>Gly1601</td>
</tr>
<tr>
<td>KN8</td>
<td>S13 (provisional)</td>
<td>4828A, 4855T*</td>
<td>4801A, 4828T*</td>
<td>Arg1601, Ser1610</td>
</tr>
<tr>
<td>KN9</td>
<td>KCAM</td>
<td>4870A</td>
<td>4843A</td>
<td>Ile1615</td>
</tr>
</tbody>
</table>

*Note: The publication by Moulds et al.29 incorrectly lists the 4855 mutation as A>G. The correct substitution is T>A.
†Nucleotide number when nucleotides are counted beginning with the A of the AUG start codon.

Two other mutations have been identified in SCR 25, one of which was found in a Caucasian and is related to S11. KMW was initially believed to have produced anti-Sl′. However, after gene sequencing it was found that she was not only homozygous for S11 but was homozygous for another SNP at position 4855 that substituted threonine for serine at amino acid 1610. After extensive molecular studies, Moulds et al.39 proposed a model in which S13 was
a conformational epitope needing S11 (1601R) and S14 (1610S) to react. S14 and the antithetical antigen S15 are proposed epitopes awaiting the identification of the appropriate antibodies before they can be officially recognized. However, the related SNPs can be identified molecularly. Limited population studies of Caucasians, Blacks, and Asians predict that S14 may be a high-frequency antigen in all populations and that S15 predominantly occurs in Caucasians.\(^{12,39}\)

The final antigen assigned to the Knops system is KCAM (initially reported as KAM). Interestingly, the SNP producing KCAM was already known but had not been associated with a blood group specificity until a McCoy-like antibody was found in a Caucasian blood donor. On DNA sequencing, it was discovered that he was homozygous for an SNP at bp 4870 that substituted valine for isoleucine.\(^{10}\) Hence, it was assigned the number KN9, and the antigen was renamed KCAM (KC for the city where the donor was found and AM for the donor’s initials).

### Disease Associations

In 1997, Rowe et al.\(^{40}\) identified CR1 as a ligand for the rosetting of *Plasmodium falciparum*-infected RBCs with uninfected cells. The ability of erythrocytes infected with *P. falciparum* to form rosettes is a property shown by only some parasite isolates, but is of importance because it has been associated with severe malaria.\(^{41}\) These authors showed that CR1 on uninfected erythrocytes was required for the formation of rosettes by demonstrating that CR1-deficient erythrocytes (Helgeson phenotype) had reduced rosetting and soluble recombinant CR1 could inhibit rosetting. RBCs having the Sl:–1 phenotype showed reduced binding to the parasite rosetting ligand PfEMP1.\(^{14}\) Thus, the authors hypothesized that this polymorphism may have been selected for in malaria-endemic regions by providing protection against severe malaria. The hypothesis was then tested by studies of Africans in Mali and Kenya, where it was found that the combined Knops haplotype Kn(a+)/McC(a−)/Sl:–1 appeared to be protective.

CR1, as well as other complement receptors, has been identified as a receptor facilitating cell entry for a variety of pathogenic organisms. Pathogens using CR1 include *Leishmania major* (monocyte-macrophage),\(^{42}\) *Legionella pneumophila* (monocyte-macrophage),\(^{43}\) *Leishmania panamensis*,\(^{44}\) and *Mycobacterium tuberculosis* (monocyte-macrophage).\(^{45}\) In an AABB abstract, Noumsi et al.\(^{46}\) reported that individuals heterozygous for McC\(^{a}\) and McC\(^{b}\) were less likely to be infected by *M. tuberculosis* (odds ratio, 0.42; 95% confidence interval, 0.22 to 0.81; \(P = 0.007\)). These results suggested that McC\(^{a}\) may have evolved among African populations in the context of *M. tuberculosis* pressure, and conferred a survival advantage in its heterozygous form.

### Conclusions

The molecular identification of the Knops blood group polymorphisms holds the promise for a better means of typing for these antigens, the only exception to this being the yet unidentified Yk\(^{a}\). Because of the inherited or acquired changes in RBC expression of CR1, i.e., Knops antigens, genotyping may become the method of choice for identifying these antigens, as it is for those of the Dombrock system. It is interesting to note that all of the known Knops polymorphisms are in SCR 25 of the CR1 protein. However, additional SNPs have been found in the CR1 gene, which suggests that the identification of new Knops blood group antigens is not yet at an end and will surely provide a challenge to serologists in the future.

### References


37. Cockburn IA, Rowe JA. Erythrocyte complement receptor 1 (CR1) expression level is not associated with polymorphisms in the promoter or 3′ untranslated regions of the CR1 gene. Int J Immunogenet 2006;33:17–20.


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