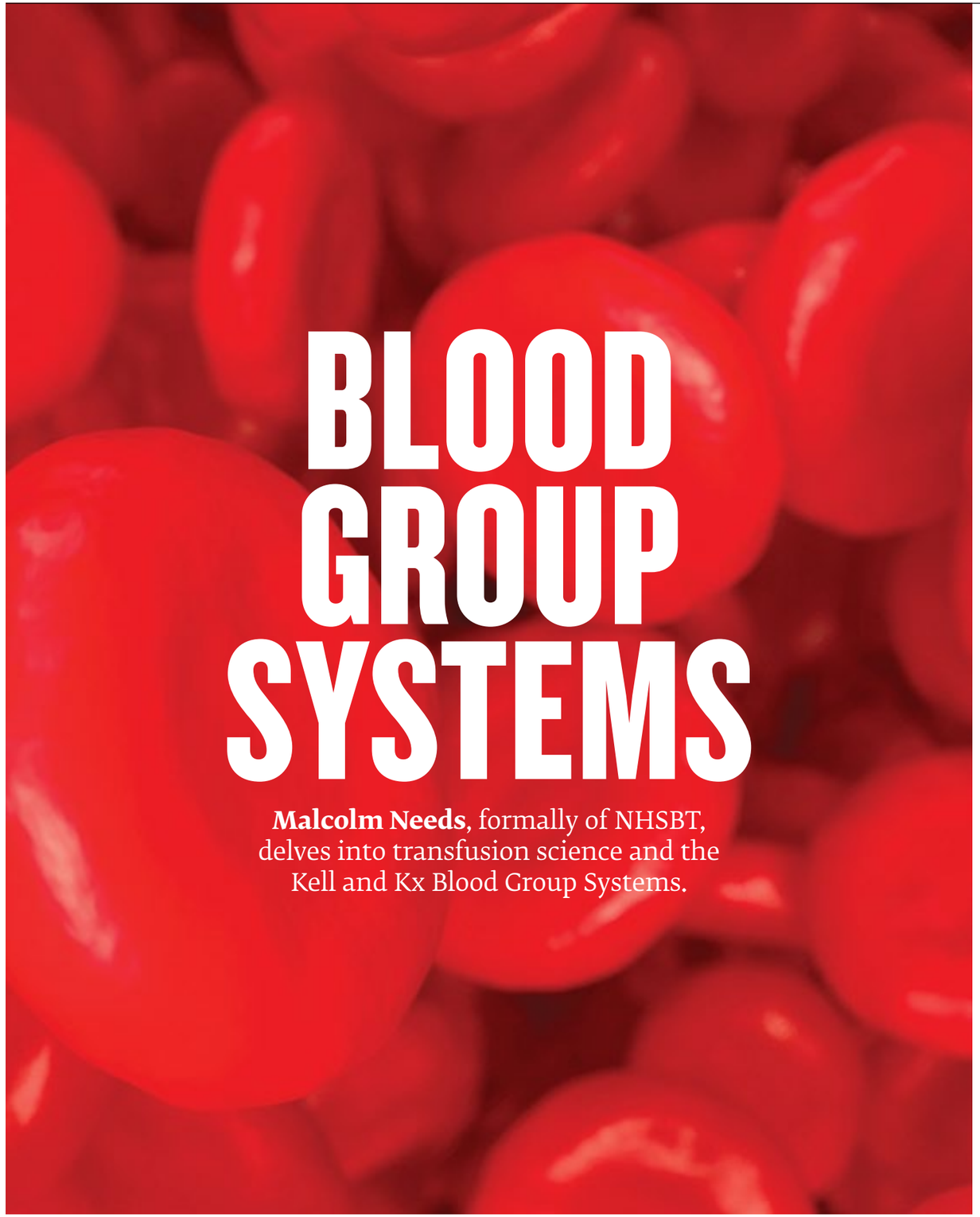


BLOOD GROUP SYSTEMS

Malcolm Needs, formally of NHSBT, delves into transfusion science and the Kell and Kx Blood Group Systems.

IMAGE: SCIENCEPHOTOLIBRARY/SHUTTERSTOCK

The Kell Blood Group System (after ABO and Rh) is commonly regarded as the third most important of the 36 blood group systems recognised by the International Society of Blood Transfusion (ISBT). Coincidentally, there are now 36 antigens recognised by the ISBT in the Kell Blood Group System, third only behind the 55 recognised in Rh and the 49 recognised in MNS. There is just one within Kx.

There are now seven antithetical pairs among these 36 antigens, and one antithetical triplicate, with the latest of these antigens (KEAL) and antithetical pairs (KHUL and KEAL) being recognised by ISBT in June this year.

First described

The antigen K and its cognate antibody were first described in 1946, in the same paper as the description of the direct antiglobulin test. Then, in 1949, the antithetical antigen k was described by Levine *et al*. Here again, it was necessary to emphasise the correct nomenclature of the antigen, which is k, and not Cellano – an all too common mistake.

Kp^a was described by Allen and Lewis in 1957 and Kp^b was described by Allen *et al* in 1958, but Kp^c (originally named Levay) was described by Callender *et al* in 1945, a year before the K antigen was described. So, perhaps the Kell Blood Group System should really be the Levay Blood Group System – but it is a bit late now!

Js^a was described by Giblett in 1958, and Js^b by Walker *et al* in 1963. For some time, these two antigens were thought to be the only two antigens within the Sutter Blood Group System. However, in 1965, Stroup *et al* were able to show that these antigens actually belonged to the Kell Blood Group System.

Phenotypes

When it comes to frequencies of the phenotypes, trends only need to be noted for day-to-day use, rather than absolute percentages. So coming across a patient with either anti-k or anti-Kp^b in their plasma, it would be sensible to look for a compatible donor among the white donor pool, rather than the black donor pool. However, the exact opposite is true for a patient with anti-Js^b in their plasma. In all populations, the K+k- type, the Kp(a+b-) type and the Js(a+b-) type are sufficiently rare that red cells from such donors are offered for cryopreservation.

The Kell glycoprotein is a single-pass red cell membrane glycoprotein, type II. This means that the amino terminus is predicted to be in the cytosol, and the carboxyl terminus to be extracellular. The Kell glycoprotein has structural homology with a family of enzymes termed zinc neutral endopeptidases, and is probably highly folded via approximately 15 cysteine residues external to the red cell membrane.

The K antigen is usually characterised by a methionine residue at position 193, however, a weak expression of the K antigen is seen when an argenine or serine residue replace the methionine residue at position 193. It is important to remember that there is a single amino acid substitution between K and k at position 193. The difference between Kp^a, Kp^b and Kp^c is a single amino acid substitution (tryptophan, argenine and glutamine, respectively) at position 281. The difference between Js^a and Js^b is also a single amino acid substitution (proline and leucine, respectively), this time at position 597.

Immunogenic

The K antigen is the most immunogenic of the antigens within the Kell Blood Group System, because the Thr193Met mutation voids an N-glycosylation site (asparagine 191), exposing a highly immunogenic area devoid of an N-linked sugar.

The Kell glycoprotein carrying the Kp^a antigen has tryptophan (a non-polar, "bulky" amino acid residue) at position 281, which has replaced argenine, and it is possible that the conformation of the protein is affected, so that it is no longer stable. *In vitro* expression studies have revealed that most of the Kp^a form of Kell glycoprotein is retained in the intracellular compartment, consistent with a model in which the Kp^a isoform is not folded correctly, so that less is present on the red cell membrane. Expression of cDNA constructs in human embryonic kidney cells showed the *KPA* mutation causes retention of most of the Kell glycoprotein in a pre-Golgi compartment, due to differential processing, suggesting aberrant transport of Kell glycoprotein to the red cell surface.

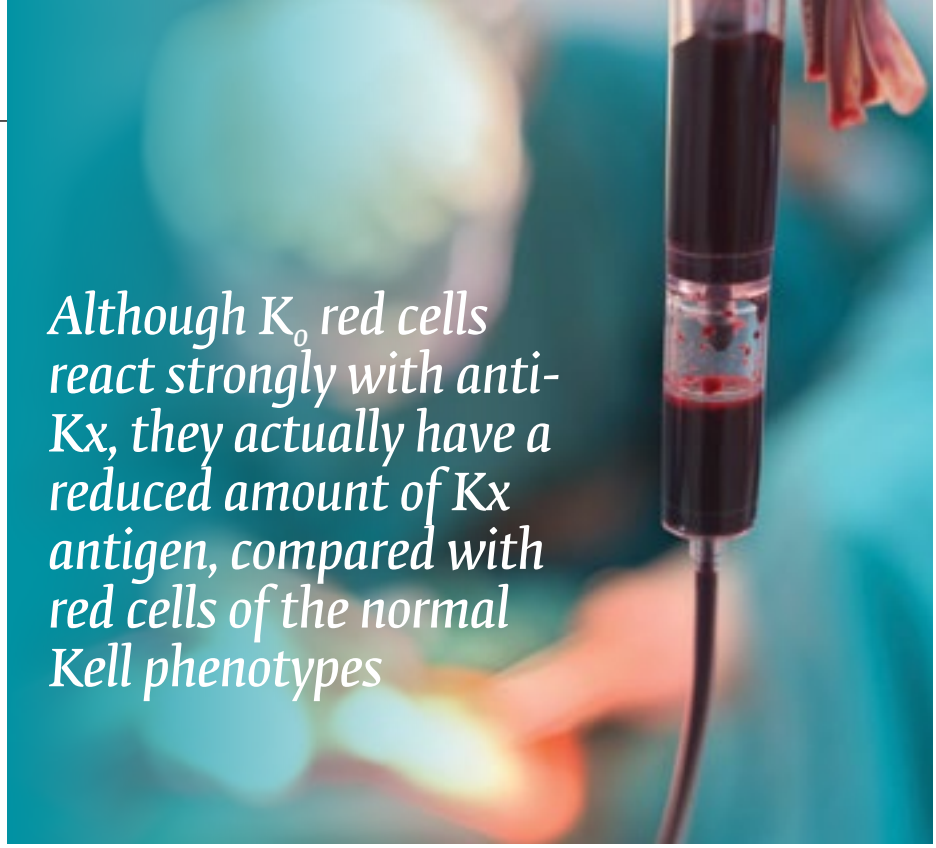
Glycoprotein

The Kell glycoprotein has 15 extracellular cysteine residues that may, through their sulphydryl side chains, form disulphide bonds. The single amino acid substitution (Leu597Pro) associated with the Js^a and Js^b antigens is situated between 2 cysteine residues (at positions 596 and 599), probably explaining why they are more sensitive to reagents that disrupt disulphide bonds than other Kell antigens.

The function of the Kell glycoprotein is probably to transport Zn++ across the red cell membrane, as Kell is a member of the M13 (neprilysin) family of zinc-dependent endopeptidases. These enzymes process a variety of biologically active peptides. Kell activates big endothelin 3 – a potent vasoconstrictor – regulation of vascular tone.

Genes

KEL is mapped to chromosome 7 (7q34) and is organised into 19 exons of coding sequence. For many years, it was thought that no two low prevalence *KEL* genes could be found on the same chromosome. But in 2009, Körmöczi *et al* reported on two individuals (one from eastern Austria, and the other from Germany) the *K* and *KPA* genes were



Although K_o red cells react strongly with anti-Kx, they actually have a reduced amount of Kx antigen, compared with red cells of the normal Kell phenotypes

found in *cis*, rather than *trans*.

The K_o phenotype is the null type within the Kell Blood Group System, first described by Chown *et al* in 1957. The individual was found to have an enhanced Kx antigen. At the time, this strongly suggested that Kx is a precursor for the Kell antigens, in the same way as the H antigen is for the A and B antigen.

It is now known, however, that, although K_o red cells react strongly with anti-Kx, they actually have a reduced amount of Kx antigen, compared with red cells of the normal Kell phenotypes. The phenomenon of weak Kx in a normal Kell phenotype is caused by steric hindrance.

"Artificial" K_o red cells can be made for use *in vitro* by treatment of red cells with a normal Kell phenotype with either 100–200 mmol dithiothreitol (DTT or ZZAP, a mixture of DTT and papain) or 2-aminoethylisothiouronium bromide (AET), reducing agents that disrupt disulphide bonds. These cells are not suitable for use *in vivo*.

The "McLeod" phenotype results in extremely weakened Kell antigens when human-derived Kell antibodies are used, although not necessarily when monoclonal Kell antibodies are used. This phenotype was first described in a healthy dental student by Allen *et al* in 1961. The phenotype usually results from hemizygosity for a rare gene at the X-borne locus, XK.

The Kx antigen of the Kx Blood Group System was first described by Marsh *et al* in 1975. Kx is described as a multi-pass membrane protein of 444 amino acid residues. Its possible function is of transport across the red cell membrane and the maintenance of normal membrane integrity ("McLeod" red cells are often acanthocytic).

The phenotype is usually found in males and is very often associated with X-linked chronic granulomatous disease (CGD). Symptoms of CGD include muscle wasting, diminished deep tendon reflex, choreiform movements, cardiomyopathy and an increased susceptibility to infection. There is an increased level of serum creatine kinase.

Phenotypes

Individuals with this phenotype can produce two antibodies: anti-Kx and anti-Km. Anti-Kx reacts with all red cells except those from other individuals with the "McLeod" phenotype.

Anti-Km reacts with all red cells, except those from other individuals with the "McLeod" phenotype and from individuals with the K_o phenotype. This mixture of antibodies was originally thought to be a single antibody, known as anti-KL (anti-K9).

In November 2000, however, Russo *et al* reported a case of an anti-Kx in a person with the "McLeod" phenotype, but who did not have CGD, and there has been another example in the US.

Antibodies within the Kell Blood Group System are usually IgG, reacting optimally by the indirect antiglobulin technique. They are all potentially clinically significant in terms of both haemolytic transfusion reactions and haemolytic disease of the foetus and newborn (HDFN), with anti-Js^b being reported as being implicated in a severe case of HDFN in 2014.

The pathogenesis of anti-K HDFN is different, and the severity of anti-K HDFN is harder to predict, as there is little correlation between the anti-K titre and the severity of the resultant HDFN. There are lower concentrations of amniotic fluid bilirubin and postnatal hyperbilirubinaemia, and reduced reticulocytosis and erythroblastosis; in other words, there is foetal anaemia without the biproducts of haemolysis.

It was thought that the binding of anti-K to Kell glycoprotein might impede enzymatic activity and cause foetal anaemia due to the suppression of erythropoiesis, rather than haemolysis.

Individuals, with the rare K_o phenotype have no such pathological conditions.

Daniels *et al* performed exquisite experiments that showed Kell present on early erythroid progenitors (before Rh). They showed that CD34+ stem cells, derived from K+ neonates elicited strong monocyte response in presence of anti-K (by chemiluminescence testing). At the same stage of maturation, there was no response to anti-D – Rh appears later in haemoglobinised erythroblasts. In other words, there is immune destruction of early erythroid progenitors.

It now seems that there is both immune destruction and suppression of erythropoiesis. BMS

Malcolm Needs was the Reference Service Manager at NHSBT –Tooting Centre and is an IBMS Scientific Advisory Panel member.

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