7 Kell and Kx Blood Group Systems

7.1 Introduction

Kell was the first of many blood group systems disclosed by the antiglobulin test [1]. When Allen *et al.* [2] described the fourth Kell system antigen, Kp^b, they concluded prophetically, 'There is, probably, much still to be learned about the Kell blood group system'. There are now 35 antigens in the Kell system (Table 7.1) and Kell is closely related to the Kx system.

There are seven sets of antigens in the Kell system with allelic relationships: K and k; Kp^a, Kp^b, and Kp^c; Js^a and Js^b; K11 and K17 (Wk^a); KEL14 and KEL24; KEL25 and KEL28; KEL31 and KEL38. There are an additional 17 high frequency antigens and three low frequency antigens. All have been shown to be associated with nucleotide changes in *KEL*, except KEL13, which has been shown to be on the Kell-glycoprotein, and Km, which requires the presence of Xk (see below). Recombination as a result of crossing-over has never been observed within *KEL*.

None of the Kell antigens are expressed on cells of the Kell-null phenotype, K_o, which arises from homozygosity for *KEL* inactivating mutations. Ku antigen is present on all cells save those of the K_o phenotype. In K_{mod}, which also arises from *KEL* mutations, all antigens of the Kell system are expressed weakly (Section 7.7).

Several rare phenotypes occur in which all or most of the high frequency Kell antigens are expressed only weakly. Some are due to epistasis, such as the McLeod phenotype and depressed Kell associated with some Gerbich negative phenotypes, and some arise from interactions within the *KEL* gene. In patients with Kell-related autoantibodies, the depressed Kell phenotype may be acquired and transient (Section 7.9).

The Kell antigens are located on CD238, a red cell transmembrane glycoprotein of apparent MW 93 kDa, a metalloendopeptidase that processes endothelin-3 (Section 7.2).

The *KEL* gene is situated on chromosome 7q33 and consists of 19 exons.

McLeod syndrome is a form of neuroacanthocytosis, which includes an abnormal Kell red cell phenotype. McLeod phenotype red cells have depressed Kell antigens and lack the high frequency antigen Kx. The inheritance of Kx is controlled by an X-borne gene, *XK*, and represents a blood group system (the Kx system) independent of Kell. The Xk protein and Kell glycoprotein are linked by a disulphide bond. The Kx system is described in this chapter (Section 7.13) because of its phenotypic and biochemical associations with Kell.

The numerical antigen notation is generally used in this chapter, except for those antigens more commonly known by their traditional symbols: K, k, Kp^a, Kp^b, Kp^c, Ku, Js^a, Js^b, Ul^a, and Km.

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Table 7.1 Antigens of the Kell system.

Antigen				Molecular basis*		
No.	Name	Frequency	Antithetical	Nucleotides†	Exon	Amino acids
KEL1	K	Polymorphic	k	578C>T	6	Thr193Met
KEL2	k	High	K	578C (T)	6	Thr193 (Met)
KEL3	Kp^a	Polymorphic	Kp^b (Kp^c)	841C>T	8	Arg281Trp
KEL4	Kp^b	High	Kp^a	1. 841C (T)	8	Arg281 (Trp)
			Kp^c	2. 842G (A)	8	Arg281 (Gln)
KEL5	Ku	High		Various		Various
KEL6	Js^a	Polymorphic	Js^b	1790T>C	17	Leu597Pro
KEL7	Js^b	High	Js^a	1790T(C)	17	Leu597 (Pro)
KEL10	Ul^a	Low		1481A>T	13	Glu494Val
KEL11	K11 (Côté)	High	KEL17	905T (C)	8	Val302 (Ala)
KEL12	K12 (Boc)	High		1523A (G)	15	His548 (Arg)
KEL13	K13	High		986T (C)	9	Leu329 (Pro)
KEL14	K14 (San)	High	KEL24	1. 539G (C)	6	Arg180 (Pro)
				2. 538C (T)	6	Arg180 (Cys)
				3. 539G (A)	6	Arg180 (His)
KEL16	'k-like'	High		Not known		
KEL17	K17 (Wk^a)	Low	KEL11	905T>C	8	Val302Ala
KEL18	K18	High		1. 388C (T)	4	Arg130 (Trp)
				2. 389G (A)		Arg130 (Gln)
KEL19	K19 (Sub)	High		1475G (A)	13	Arg492 (Gln)
KEL20	Km	High				Absence of Xk
KEL21	Kp^c	Low	Kp^b (Kp^a)	842G>A	8	Arg281Gln
KEL22	K22	High		965C (T)	9	Ala322 (Val)
KEL23	K23	Low		1145A>G	10	Gln382Arg
KEL24	K24 (Cls)	Low	KEL14	539G>C	6	Arg180Pro
KEL25	VLAN	Low	KEL28	743G>A	8	Arg248Gln
KEL26	TOU	High		1217G (A)	11	Arg406 (Gln)
KEL27	RAZ	High		745G (A)	8	Glu249 (Lys)
KEL28	VONG	Low	KEL25	742C>T	8	Arg248Trp
KEL29	KALT	High		1868G (A)	17	Arg623 (Lys)
KEL30	KTIM	High		913G (A)	8	Asp305 (Asn)
KEL31	KYO	Low	KEL38	875G>A	8	Arg292Gln
KEL32	KUCI	High		1271C (T)	11	Ala424 (Val)
KEL33	KANT	High		1283G (T)	11	Arg428 (Leu)
KEL34	KASH	High		758A (G)	8	Tyr253 (Cys)
KEL35	KELP	High		708G (T), 2024G (A)	8,18	Leu260 (Phe), Arg675 (Gln)
KEL36	KETI	High		1391C (T)	12	Thr464 (Ile)
KEL37	KUHL	High		877C>T	8	Arg293 (Trp)
KEL38	KYOR	High	KEL31	875G (A)	8	Arg292 (Gln)

*Molecular basis of antigen-negative phenotype in parentheses.
†1 is the first nucleotide of the translation-initiating codon, which is 120 bp downstream of the traditional position for the first nucleotide in early reports.
Obsolete: KEL8, previously Kw; KEL9, previously KL; KEL15, previously Kx (now XK1).

7.2 The Kell glycoprotein and the gene that encodes it

7.2.1 The Kell glycoprotein

A glycoprotein of apparent MW 93 kDa was isolated from detergent-solubilised red cell membranes in the 1980s by immunoprecipitation with antibodies to Kell-system antigens: anti-K, -k, -Js^b, or -KEL22 [3–5]. Treatment of the Kell glycoprotein with an *N*-glycanase reduced the apparent MW by about 15 kDa, whereas *O*-glycanase had little effect. The Kell glycoprotein is phosphorylated, but not palmitoylated [6,7]. Kell antibodies do not generally react with isolated Kell glycoprotein by immunoblotting, though mouse monoclonal and rabbit antibodies, produced by immunising animals with purified Kell glycoprotein, detect the 93 kDa Kell glycoprotein on immunoblots [3,8]. No Kell glycoprotein was detected on blots of K_o cells or isolated from K_o cells by immunoprecipitation with a variety of polyclonal and monoclonal Kell antibodies.

Based on the amino acid sequence of a tryptic peptide, primers were synthesised and a specific oligonucleotide probe prepared by the polymerase chain reaction. Lee *et al.* [9] used this probe to screen a human bone marrow cDNA library and a clone was isolated with an open reading frame encoding a 732 amino acid polypeptide. Rabbit antibody prepared to a synthetic 30 amino acid peptide derived from the cDNA sequence bound to Kell glycoprotein on an immunoblot.

Hydropathy analysis indicated a type II membrane protein with a single hydrophobic membrane-spanning region, a highly hydrophilic N-terminal cytoplasmic domain of 47 amino acids (or 28 amino acids if the codon for Met 20 is used for translation initiation), and a large, 665-amino acid, C-terminal extracellular domain (Figure 7.1). The N-terminal methionine residue is probably cleaved from the mature protein. The extracellular domain has six Asn-Xxx-Ser/Thr putative *N*-glycosylation sites (positions 94, 115, 191, 345, 627, and 724), though Asn724 is unlikely to be glycosylated as residue 725 is proline, which usually inhibits glycosylation. There are15 extracellular cysteine residues, suggesting the presence of seven intramolecular disulphide bonds, resulting in extensive folding of the molecule. The Kell protein has structural and sequence homology with a family of zinc-binding endopeptidases (for functional aspects see Section 7.12) and has been modelled, based on the crystal structure of the external domains of neutral endopeptidase 24.11 (NEP) and endothelin-converting enzyme 1 (ECE-1) [10,11]. The Kell-glycoprotein has two globular extracellular domains, consisting mostly of α-helical segments. The domain closest to the membrane contains the N- and C-terminal sequences and the enzyme-active site; the outer domain contains almost all of the amino acid sites responsible for Kell-system alloantigenicity [10].

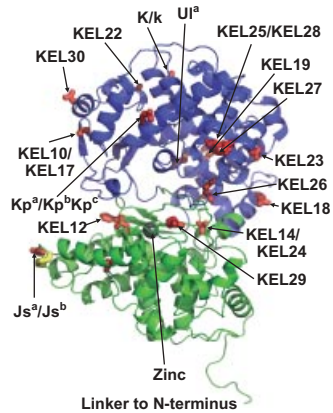

Figure 7.1 Homology model for Kell ectodomain, with the highly conserved peptidase domain in green and the variable distal membrane domain in blue. The linker region is the site of attachment to the transmembrane domain. Positions associated with Kell antigens are labelled. Modified from [11].

Kell glycoprotein is closely associated in the membrane with the Xk protein and a 120 kDa heterodimer can be isolated by immunoprecipitation under non-reducing conditions [12]. The two proteins are linked by disulphide bonding between Cys72 of Kell and Cys347 of Xk [13] (Section 7.13.2). The Kell-Xk heterodimer is part of the 'junctional' or 4.1R red cell membrane complex that contains band 3, Rh proteins, and glycophorin C, and is linked to the spectrin-actin junction of the cytoskeleton through protein 4.1R and p55 [14] (see Section 10.7 and Figure 10.2).

With Fab fragments of three monoclonal antibodies directed at epitopes on the Kell glycoprotein, figures of 4000–8000 sites per red cell were obtained, but Fab fragments from a fourth antibody gave a figure of 18 000 sites per cell [15]. The number of K antigen sites per red cell has been estimated as 4000–6200 on K+ k– cells and 2500–3500 on K+ k+ cells by use of radioiodinated polyclonal and monoclonal anti-K [16,17].

7.2.2 Organisation of the *KEL gene*

KEL spans about 21.5 kb organised into 19 exons of coding sequence [18] (Table 7.2). Exon 1 encodes a possible translation initiating methionine residue and Sp1 and GATA-1 binding sites. The exon 1 region is involved

Table 7.2 Exon/intron organisation of *KEL*.

Exon	Codons	3′ intron size kb	Comments
1	5′UT Met1	0.34	
2	2–27	0.29	Cytoplasmic
3	28–74	0.26	Transmembrane
4	75–133	~2.6	KEL18
5	134–175	0.33	
6	176–224	~3.2	KEL14/KEL24, K/k
7	225–245	0.093	
8	246–308	0.23	KEL25/KEL28, KEL27, KEL34, KEL35, $Kp^a/Kp^b/Kp^c$, KEL31, KUHL, KEL11/KEL17, KEL30
9	309–358	~1.3	KEL22, KEL13
10	359–401	~6	KEL23
11	402–438	~1.6	KEL26, KEL32, KEL33
12	439–471	0.24	KEL36
13	472–497	0.44	KEL19, Ul^a
14	498–531	0.19	
15	532–568	0.15	KEL12
16	569–590	0.23	HELLH
17	591–647	0.35	Js^a/Js^b, KEL29
18	648–679	~1.3	KEL35
19	680–732 3′UT		

UT, untranslated; HELLH, consensus sequence for zinc neutral endopeptidases.

in negative regulation of the promoter in non-erythroid tissue [19]. Exon 2 encodes the cytoplasmic domain and a second possible translation initiation site at Met20, exon 3 the membrane-spanning domain, and exons 4–19 the large extracellular domain. The 5′ flanking region to nucleotide −176 contains two GATA-1 binding sites and a CACCC box [18].

7.2.3 Linkage and chromosome location

KEL was located on 7q33 through linkage to *PIP*, the gene for prolactin-inducible protein [20], indirect linkage to the cystic fibrosis gene (*CFTR*) [21], and *in situ* hybridisation with cDNA encoding the Kell protein [22,23]. An analysis of 31 families informative for segregation of Yt blood group gene and *KEL* revealed loose linkage between these loci, with maximum likelihood of a recombination fraction of 0.26 [24].

7.3 K and k (KEL1 and KEL2)

In 1946, in the first report on the applications of the direct antiglobulin test, Coombs, Mourant, and Race [1] described an antibody of new specificity. This antibody, originally called anti-Kell and subsequently anti-K or anti-KEL1, reacted with the red cells of the husband and two children of the antibody producer and with about 7% of random blood samples [25].

Three years later, Levine *et al.* [26] described anti-Cellano, an antibody antithetical to anti-K. As *k* had already been used to represent the common allele of *K*, the symbol k was subsequently adopted for the product of that gene, despite K and k being products of co-dominant alleles.

Kell antigens are well developed at birth. K was found in fetuses of 10–11 weeks gestation and k at 6–7 weeks [27].

7.3.1 The molecular basis of the K/k polymorphism

The k/K polymorphism results from a C578T transition within exon 6 of the *KEL* gene, which gives rise to an amino acid substitution in the Kell glycoprotein: Met193 in K and Thr193 in k [28,29]. A *Bsm*I (*Bsa*MI) restriction site is present in *KEL*01* (*K*), but not *KEL*02* (*k*) [28] (Table 7.3). In the *KEL*02* product, Asn-Arg-Thr193 is a

Table 7.3 Sequences from nucleotides 571–582 of *KEL*, encoding amino acids 191–194, in four alleles encoding k, K, and two variants.

Allele						Antigen
				578		
k	*KEL*02*	AAC	CGA	A**C**G	CTG	
		Asn[N]	Arg	**Thr**	Leu	k
K	*KEL*02*	AAC	C<u>GA</u>	<u>ATG</u>	<u>C</u>TG	
		Asn	Arg	**Met**	Leu	K
	*KEL*01.2* [30]	AAC	CGA	**TC**G	CTG	
		Asn[N*]	Arg	**Ser**	Leu	weak K, possibly k
	*KEL*01M.01* [31]	AAC	CGA	A**G**G	CTG	
		Asn*	Arg	**Arg**	Leu	very weak K
				193		

N, *N*-glycosylation. **N***, probable *N*-glycosylation. *, probable no *N*-glycosylation. Underlining in *K* allele sequence shows *Bsm*I restriction site.

consensus sequence for *N*-glycosylation of Asn191, whereas Asn-Arg-Met in the product of *KEL*01* is not. Immunoblotting revealed that the K and k proteins are of apparent MW 110 and 115 kDa, respectively, supporting the suggestion that Asn191 is glycosylated in the latter, but not in the former [32,33]. Enzymatic deglycosylation of the Kell glycoprotein on red cells did not affect binding of either anti-K or -k to cells of the appropriate phenotype, demonstrating that k expression is not dependent on *N*-glycosylation of Asn191 [33].

7.3.2 Frequencies of K and k

In tests on nearly 10 000 English blood donors (mostly white), 9.02% were K+ [34]. From this figure the following gene and genotype frequencies have been calculated: *K* 0.0462; *k* 0.9538; *K/K* 0.0021; *K/k* 0.0881; *k/k* 0.9097 (assuming *k* is the only allele of *K*). K is much less common in Africans and extremely rare in eastern Asia and in Native Americans [35] (Table 7.4). K achieves its highest level among people of the Arabian and Sinai peninsulas, where up to 25% may be K+.

The k antigen has a high incidence in all populations. From the gene frequencies given above it can be estimated that the incidence of K+ k– would be 1 in 476. The incidence of k– was found to be one in 549 London blood donors [50].

Table 7.5 shows genotype frequencies in Austrian blood donors obtained by genotyping with allele-specific primers [51].

7.3.3 K/k genotyping

A variety of methods, involving SNP testing in exon 6 of *KEL*, are available for predicting K/k phenotypes for DNA. All these tests may give a false prediction if a Kell null or mod allele or the *K* variant allele *KEL*01.2* is present (Sections 7.3.4 and 7.7).

Anti-K is a relatively common cause of severe HDFN (Section 7.3.5.2), so in pregnant women with anti-K it is advantageous to predict fetal K phenotype. This can be done from fetal DNA in maternal plasma, thus avoiding invasive procedures such as amniocentesis or chorionic villus sampling (see Section 5.7.1). Finning *et al.* [52] achieved 98.6% accuracy by real-time quantitative PCR, involving the application of locked nucleic acids to prevent mispriming of the *KEL*01*-specific primer on the *KEL*02* allele. This technology is now employed in England to provide a routine service to pregnant women with anti-K, with a level of accuracy of around 99.6% [53]. A method for fetal *K* detection incorporating matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF MS) gave 94% accuracy [54].

K genotyping has also been applied to preimplantation genetic diagnosis (PGD). In K– women with a K+ k+ partner, half of the embryos derived from *in vitro* fertilisation (IVF) will have a paternal *KEL*01* allele and half will not. K genotyping of DNA from individual blastomeres obtained by IVF was used for PGD to ensure implantation of a K– embryo in two women with anti-K,

Table 7.4 Frequency of some Kell system antigens and deduced gene frequencies.

Antigen	Population	No. tested	Positive (%)	Gene frequency	References
K	English	9875	9.02	0.0462	[34]
	Parisians	81 962	8.55	0.0437	[36]
	Finns	5000	4.10	0.0207	[37]
	African Americans	4079	1.50	0.0075	[38]
	Japanese	14 541	0.02	0.0001	[39]
Kp^a	White people	18 934	2.28	0.0114	[34,40–42]
Kp^c	Japanese (Osaka)	4442	0.32	0.0016	[*]
	Japanese (Miyagi)	5974	0.18	0.0009	[43]
Js^a	African Americans	1298	15.87	0.0828	[38,44,45]
	Black Africans	593	15.68	0.0818	[46]
Ul^a	Finns	2620	2.6	0.0131	[37]
	English	5000	0	0.0000	[37]
	Swedes	501	0.2	0.0011	[37]
	Chinese	12	1 pos.		[37]
	Japanese	8000	0.46	0.0023	[47]
KEL17	English	11 044	0.29	0.0015	[48]
KEL31	Japanese	400	1.5	0.0075	[49]

*H. Yamaguchi, Y. Okubo, T. Seno, unpublished observations.

Table 7.5 Results of genotyping tests on about 11 000 Austrian blood donors [51].

	*KEL*02* *k*	*KEL*04* *Kp^b*	*KEL*07* *Js^b*	*KEL*11* *K^{11}*
Homozygous positive (%)	91.98	98.31	99.99	99.61
Heterozygous positive (%)	7.94	1.67	0.01	0.39
Negative (%)	0.08	0.02	0	0

both of whom had previously lost babies as a result of fatal HDFN [55].

7.3.4 Unusual K and k expression

Red cells of two unrelated Swiss-German blood donors, and the mother of one of them, reacted with most polyclonal and monoclonal anti-K, albeit slightly less strongly than with normal K+ cells, but gave significantly weak or negative reactions with some anti-K reagents [30]. Like *KEL*02* homozygotes, they were homozygous for 578C, but were also heterozygous for 577A>T, encoding Ser193 (*KEL*01.02*) (Table 7.3). Ser193 would be expected to support *N*-glycosylation of Asn191. It is somewhat surprising that Ser193 would be responsible for K expression, particularly as flow cytometric analyses suggested a homozygous dose of k. Consequently the *KEL*01.02* allele appears to produce both variant K and k antigens. Poole *et al.* [30] suggest that anti-k detect an epitope that is not substantially altered by a Thr193Ser substitution, whereas anti-K recognise a distinct conformational epitope created by any substitution of Thr193. A third unrelated example of this variant was found in an American Caucasian [56]. Despite encoding K, *KEL*01.02* would be recognised as a *KEL*02* allele by most genotyping systems.

Weak K is produced by the very rare *KEL*01* alleles that also produce Kp^a [57] (Section 7.4). Whereas this K could be missed by some serological methods, it would be predicted by genotyping.

The term K_{mod} was used for four individuals with very weak K that could only be detected by adsorption and elution, no k, and weak expression of high frequency Kell-system antigens [31]. All four were homozygous for a *KEL* 578C>G encoding Thr193Arg (*KEL*01M.01*) (Table 7.3). Weakness of other Kell antigens was due to reduced quantity of Kell glycoprotein. Heterozygosity for

other K_{mod} alleles on a *KEL*01* background has also been responsible for weakened expression of K.

A K+ woman had an antibody resembling anti-K in the serum. Her red cells and those of her daughter expressed a weak K antigen, which did not react with her K-like antibody [58]. Weak expression of K has also occurred in the McLeod [59] and Gerbich-negative [60] phenotypes (Sections 7.10 and 7.13).

During a terminal episode of sepsis, red cells of a patient previously known to be K– k+ became K+, as did K– transfused cells [61]. Post-mortem blood samples contained a Gram-positive organism, *Streptococcus faecium*. K– red cells incubated with a culture containing disrupted *S. faecium* were converted to K+.

Weakness of k in K+ k+ individuals, associated with a missense mutation in the *KEL* gene, is likely to result from heterozygosity for a K_{mod} allele and *KEL*01*, the weakness of the other high frequency Kell antigens being masked by normal strength antigens being produced by the *KEL*01* allele (Section 7.7.2).

7.3.5 Anti-K

7.3.5.1 Alloanti-K

Anti-K is the most common immune red cell antibody outside of the ABO and Rh systems; about two-thirds of non-Rh red cell immune antibodies are anti-K [62]. Giblett [63] estimated the relative potency of antigens in stimulating the formation of antibodies and, excluding ABO and D, K attained the highest score with a relative potency of twice that for c, about 20 times that for Fy^a, and over 100 times that for S. Anti-K is often found in sera containing antibodies to high incidence Kell system antigens. Seventy-five percent of people with IgG autoantibodies related to the Kell system also have alloanti-K in their serum [64]. Conflicting results were obtained in two studies comparing HLA Class II genotypes with anti-K production. In one, anti-K alloimmunisation was not restricted by HLA-DRB1 genotype [65], whereas in the other study frequencies of *HLA-DRB1*11* and *HLA-DRB1*13* were significantly higher in patients with anti-K, than in those without [66].

Anti-K, like other Kell system antibodies, are generally IgG, and predominantly IgG1 [67]. Although IgG anti-K may occasionally agglutinate K+ red cells directly, the antiglobulin test is usually the method of choice. Anti-K often react poorly in low ionic strength solutions (LISS) [68,69] and fewer molecules of anti-K bind to red cells in LISS than in normal strength saline [70]. Problems in detecting anti-K have also been encountered in automated systems [71].

A few examples of apparent non-red cell immune anti-K have been described in untransfused, healthy, male blood donors [72,73]. Microbial infection has been associated with the presence of IgM or IgA anti-K [74–78]. *Escherichia coli*, *Enterococcus faecalis*, *Morganella morganii*, and mycobacterium, responsible for pulmonary tuberculosis, have been implicated. In some cases cell-free preparations from these stool cultures inhibited IgM anti-K and K antigens were detected on the bacterial cells [74,79].

Human monoclonal anti-K are generally used as grouping reagents. Murine monoclonal anti-K have been produced by immunising mice with plasmids encoding K, followed by a boost injection of plasmid-transfected cells [80].

7.3.5.2 Clinical significance of anti-K

All Kell-system antibodies must be considered potentially clinically significant and, where possible, antigen-negative red cells should be selected for transfusion. Anti-K can be responsible for severe and fatal HTRs.

Anti-K can cause severe HDFN [62,81]. In one series of tests [82], maternal anti-K was detected in 127 of 127 076 pregnancies (0.1%); 13 of the pregnancies with maternal anti-K produced a K+ baby, five (38%) of whom were severely affected with HDFN. K immunisation usually results from transfusion. In a Dutch survey, 83% of women with anti-K had a history of red cell transfusion [83]. It is common practice, therefore, for girls and premenopausal women to be transfused only with K– red cells. In addition, first trimester screening for red cell antibodies in the Netherlands resulted in an improvement from 61 to 100% survival of severely affected fetuses in K alloimmunised pregnancies [84].

Unlike RhD (Section 5.18.1.4), no prophylaxis is available for the prevention of K alloimmunisation during pregnancy and at delivery. A 15-residue peptide representing the K protein, with Met179 at the C-terminus, was identified as the major helper T-cell epitope in the alloresponse to K [85]. Administration of this peptide via a suppressive route, such as the nasal mucosa, may have the potential to reduce or prevent K alloantibody production in susceptible women.

The pathogenesis of anti-K HDFN differs from that caused by anti-D. Severity of the anti-K disease is harder to predict than the anti-D disease. There is very little correlation between anti-K titre and severity of disease [62], though severe HDFN due to anti-K of titre less than 32 is extremely rare [86]. Anti-K HDFN is associated with

lower concentrations of amniotic fluid bilirubin than in anti-D HDFN of equivalent severity and post-natal hyperbilirubinaemia is not prominent in babies with anaemia caused by anti-K [82,87,88]. There is also reduced reticulocytosis and erythroblastosis in the anti-K disease, compared with anti-D HDFN. These symptoms suggest that anti-K HDFN is associated with a lower degree of haemolysis and the fetal anaemia appears to result predominantly from a suppression of erythropoiesis [87,88]. Kell glycoprotein appears on erythroid progenitors very early in erythropoiesis, whereas the Rh proteins are late to appear [89,90]. Vaughan *et al.* [91] found that *in vitro* growth of K+ erythroid blast-forming units (BFU-E) and colony-forming units (CFU-E) was specifically inhibited by monoclonal and polyclonal anti-K. They speculated that the Kell glycoprotein, an endopeptidase (Section 7.13), might be involved in regulating the growth and differentiation of erythroid progenitors, possibly by modulating peptide growth factors on the cell surface. Consequently, binding of anti-K to the Kell glycoprotein might impede its enzymatic activity and suppress erythropoiesis. Unfortunately, this theory does not take into account the K_o phenotype, in which no Kell glycoprotein is present on the surface of erythroid cells, yet erythropoiesis is apparently normal. It is more likely, therefore, that anti-K suppresses erythropoiesis through the immune destruction of early erythroid progenitors. Early erythroid progenitors cultured from $CD34^+$ cells derived from K+ neonates expressed K and elicited a strong response from monocytes in a functional assay in the presence of anti-K; no response was obtained with anti-D because Rh antigens do not appear on erythroid cells until much later, when they have become haemoglobinised erythroblasts [92]. Anti-k and anti-Kp^b also inhibit BFU-E growth *in vitro* [93].

In addition to inhibiting erythropoiesis, Kell antibodies also inhibit *in vitro* proliferation of granulocyte-monocyte and megakaryocyte progenitors (CFU-GM and CFU-MK) [75,76]. Pronounced thrombocytopenia, leukopenia, and neutropenia have been detected in cases of HDFN caused by anti-K [94,95].

7.3.5.3 'Mimicking' autoanti-K

Autoantibodies that appear to have K specificity, but which can be adsorbed and eluted from K– cells, have been detected in the serum and red cell eluates of K– patients [96–98]. These antibodies caused strong DATs and were not associated with any weakening of high frequency Kell-system antigens.

7.3.6 Anti-k

Less than 2 in 1000 people are k– and capable of making anti-k, yet many examples of this rare antibody have been described [99]. Most anti-k are IgG (often IgG1 [67]) and work best by the antiglobulin test, but cold agglutinating IgM anti-k are known [100,101]. Anti-k has been responsible for HTRs [99,102] and HDFN [99,102–104]. The characteristics of fetal anaemia (reticulocytopenia and normal bilirubin levels) caused by anti-k are similar to those due to anti-K, suggestive of suppression of erythropoiesis [104] (Section 7.3.5.2)

IgG1 and IgG2a monoclonal anti-k, which could not be adsorbed and eluted from k– red cells, have been raised in mice [105–107]. One antibody which behaved like anti-k, but did not react with k+ KEL:–22 red cells [108] (k and KEL22 are probably spatially related [109], Figure 7.1). Some murine monoclonal antibodies react with red cells of all Kell phenotypes except K_o, but react more strongly with K– k+ and K+ k+ cells than with K+ k– cells and may behave as anti-k at an appropriate dilution [80,107,110].

7.4 Kp^a, Kp^b, and Kp^c (KEL3, KEL4, and KEL21)

In 1957, Allen and Lewis [40] described anti-Kp^a and its probable antithetical antibody anti-Kp^b. Kell became a complex blood group system in the following year when Kp^a and Kp^b alleles were shown to be linked to *K* and *k* [2]. Family evidence confirmed this very close linkage; K+ Kp(a+) people never receive both *K* and Kp^a from the same parent and never pass them on to the same child. Despite numerous studies of families with K+ Kp(a+) propositi, the KKp^a allele was never been found. However, in 2009 gene sequencing demonstrated that two K+ k+ Kp(a+b+) unrelated Caucasians with very weak K antigens were heterozygous for KKp^a (*KEL*01.03*) and kKp^b (*KEL*02.04*) [57]. The suppressive effect of Kp^a on other Kell antigens expressed on the same molecule is described in Section 7.7.3.

Tests with anti-Kp^a on just under 19000 white people from Europe and North America [34,40–42], showed 2.28% to be Kp(a+), a gene frequency of 0.0114 for Kp^a (Table 7.4). A Kp^a gene frequency of 0.0086 in Austrian blood donors was obtained by molecular genotyping [51] (Table 7.5). Only 1.21% of K+ people are Kp(a+) [34]. Although about 9% of white people are K+, only 2.7% of Kp(a+) mostly white people from Boston were K+ [40].

Kp^a appears to be extremely rare in other ethnic groups. Kp^b is a public antigen in all populations.

In 1979, Yamaguchi *et al.* [111] found that the red cells of a Japanese blood donor were Kp(a–b–) with otherwise unremarkable Kell antigens. Her red cells reacted with the serum containing anti-Levay [112], an antibody to the first inherited private red cell antigen, originally reported in 1945 [113,114]. Study of the informative family of the Japanese propositus proved that Levay is the product of *Kp^c*, a third Kp allele. In one Japanese family, two Kp(a–b–c+) members appeared to be heterozygous for *Kp^c* and a Kell-null gene *K^o* [43]. The incidence of Kp^c in Japan is shown in Table 7.4. Other than the original Levay-positive propositus and her family, only one Kp(c+) individual has been found outside Japan: a Kp(a+b–c+) Spanish American with anti-Kp^b.

Kp^a and *Kp^c* differ from the common allele, *Kp^b*, by single nucleotide changes at adjacent sites within the same codon in exon 8 [115]. *Kp^b* has CGG encoding Arg281; *Kp^a* has TGG, Trp281 and *Kp^c* has CAG, Gln281. The *Kp^a* and *Kp^c* mutations introduce *Nla*III and *Pvu*II restriction sites, respectively. Site-directed mutagenesis experiments confirmed that the single base change is responsible for the Kp^a/Kp^b polymorphism [29].

7.4.1 Anti-Kp^a

The first (Penney) appeared to be 'naturally occurring' but, as with most anti-Kp^a, reacted best by the antiglobulin test [34,40]. Anti-Kp^a can cause delayed HTRs; only one case is reported as severe [116].Anti-Kp^a very rarely causes severe HDFN, but there are reports of a requirement for neonatal transfusion [117,118], one case of hydrops fetalis [119], and one neonate who presented with purpura, respiratory failure, severe liver dysfunction, hyperbilirubinaemia, and anaemia [120]. In one case, symptoms are described as consistent with suppression of erythropoiesis in addition to immune red cell destruction [118]. Owing to the relative rarity of Kp^a, anti-Kp^a, and serious clinical sequelae of incompatible transfusion, Kp(a+) red cells are not required in antibody screening panels [121].

Murine monoclonal anti-Kp^a have been produced by immunising mice with plasmid DNA followed by a boost injection of plasmid-transfected cells [80].

7.4.2 Anti-Kp^b

The first anti-Kp^b (Rautenberg) was found during routine crossmatching [2]; the serum also contained anti-K, as do some other examples [34]. Although anti-Kp^b is usually IgG (IgG1 + IgG4 [67]), two anti-Kp^b that appear to be 'naturally occurring' and did not react by the antiglobulin test have been mentioned [34].

Serious HDFN due to anti-Kp^b is very rare, but two cases are reported where obstetric intervention and, in one case, transfusion, were required [122,123]. Both mothers had been transfused during childhood. *In vivo* red cell survival studies and monocyte monolayer assays predict that anti-Kp^b has the potential to cause reduced survival of transfused Kp(b+) cells [124,125]. Anti-Kp^b has been responsible for a delayed HTR [124], although Kp(b+) units of blood have been administered to patients with anti-Kp^b, with no indications of transfusion reaction or reduced red cell survival [126,127].

Autoanti-Kp^b has been responsible for AIHA [128–130], in one case in a 12-week-old infant [129]. One autoanti-Kp^b was pure IgM [131]. Autoantibodies to Kell-system antigens are often associated with weakened expression of Kell (Section 7.9).

A murine monoclonal antibody (BRIC 203) defined an epitope shared by Kp^b and Kp^c, but not Kp^a [15]. A human single-chain Fv (scFv) antibody fragment specific for Kp^b has been isolated from a V gene phage-display library derived from non-immunised donors [132].

7.4.3 Anti-Kp^c

The first anti-Kp^c, which was called anti-Levay for 34 years, was made in response to transfusion in a patient who also made the first examples of anti-Lu^a, -C^w, and human anti-N [113,114]. Several more anti-Kp^c have been found since, all immune and all in Japanese.

7.5 Js^a and Js^b (KEL6 and KEL7)

Js^a, a new antigen present on the red cells of about 20% of African Americans, but in none of 500 white people, was first described in 1958 [44,133]. Anti-Js^b was found in 1963 in the serum of a Js(a+) black woman with four Js(a+) children [134,135]. This antibody failed to react with the red cells of 13 of 1269 black donors. Twelve of the 13 were tested with anti-Js^a and all were positive. The antibody did not react with the Js(a+) red cells of two sisters, believed to be homozygous for *Js^a* because all of their 10 children were Js(a+).

The first hint that Js^a and Js^b might belong to the Kell system came from the observation that cells of the Kell-null phenotype (K$_o$) were Js(a–b–) [38]. A search of 4000 black donors revealed six K+ Js(a+) propositi and the

subsequent family studies suggested control of Js^a and Js^b at the *KEL* locus. This was confirmed by four large Brazilian families with K+ Js(a+) propositi [136].

Js^a is almost completely confined to people of African origin [35]. The incidence of Js^a among African Americans is about 16%, giving a frequency of 8% for the Js^a gene (Table 7.4). Js^a is very rare in white people. Of 11 000 African Americans tested with anti-Js^b, 34 were Js(b–) [137]. The Js(a+b–) phenotype has not been reported in a person of non-African origin. Genetic testing revealed Js^a (*KEL*06*) frequencies of 8.18% and 11.68% in Afro-Caribbean donors and from donors originating from Ngazidja (an island off the east coast of Africa), respectively [138].

An 1790T>C SNP in exon 17 encoding a single amino acid substitution in the Kell glycoprotein is responsible for the Js^b/Js^a polymorphism: Leu597for Js^b; Pro597 for Js^a [139] (Table 7.1). This has been confirmed by site-directed mutagenesis experiments [29]. An *Mnl*I restriction site is eliminated in the Js^a allele. The Leu597Pro substitution is between two cysteine residues and could affect disulphide bonding and, consequently, folding of the molecule. In a A>G1899 synonymous SNP at Leu633 codon, about 80% of Js^b alleles have 1899A and 20% G1899 [140].

7.5.1 Anti-Js^a

Anti-Js^a generally react best by the antiglobulin test and are red cell immune in origin. An apparently 'naturally occurring' IgM anti-Js^a in a Japanese woman directly agglutinated Js(a+) cells [141].

Anti-Js^a has been responsible for HDFN, including hydrops in one case [142–144]. Two anti-Js^a, barely detectable by routine serological tests, caused delayed HTRs [145,146].

7.5.2 Anti-Js^b

All examples of anti-Js^b have been found in black people. They generally work best by the antiglobulin test. Anti-Js^b has caused severe HDFN resulting in fatal hydrops fetalis [147,148]. The poor predictive value of anti-Js^b titres is typical of Kell-system antibodies [149]. The mother of a hydropic baby received a transfusion of 275 ml Js(b+) red cells and suffered no symptoms of an HTR, although the survival of the Js(b+) cells was substantially reduced [147]. Anti-Js^b has been responsible for a delayed HTR [150]; multiple transfusions of Js(b+) red cells to transfusion-dependent patients with anti-Js^b have resulted in no adverse reactions [151].

Autoanti-Js^b, enhanced by polyethylene glycol, was detected in the serum of a Js(a–b+) renal patient whose red cells gave a weakly positive DAT [152].

A potent murine monoclonal IgG anti-Js^b was raised by immunising a mouse with a murine erythroleukaemia (MEL) cell line expressing recombinant human Kell glycoprotein [153]. Human-mouse chimeric IgM antibodies have been engineered from this clone, in order to produce a directly agglutinating reagent [154].

7.6 Other Kell-system antigens

In addition to the Kell polymorphisms – K/k, $Kp^a/Kp^b/Kp^c$, and Js^a/Js^b – a number of other Kell-system antigens are known, all of either high frequency or low frequency (Table 7.1). They are absent from K_o cells and expressed either weakly or not at all on McLeod phenotype cells. All have been allocated to the Kell system through family evidence, location of the antigen on the Kell glycoprotein by immunochemical means, and/or association of antigen presence or absence with a sequence change in the *KEL* gene. Ku and Km are described in Sections 7.7 and 7.13.3, respectively.

7.6.1 Ul^a (KEL10)

An incompatible crossmatch revealed an antibody, named anti-Ul^a, that reacted with the red cells of 2.6% of Helsinki blood donors, but is rare in most other populations [37]. Three families with K+ Ul(a+) members had demonstrated that Ul^a belongs to the Kell system [155]. An antibody antithetical to anti-Ul^a has not been found. Ul^a is often considered a predominantly Finnish characteristic, but 0.46% of Japanese [47] and one of 12 Chinese [37] were Ul(a+) (Table 7.4).

Ul^a results from a mutation encoding a Glu494Val substitution and acquiring an *Acc*I restriction site [115].

Anti-Ul^a is very rare. No anti-Ul^a was detected in the serum of 19 Ul(a–) mothers of Ul(a+) children [37]. One case of HDFN caused by anti-Ul^a is reported [156].

7.6.2 KEL11 and KEL17 (Wk^a)

The original anti-KEL11, found, in the serum of a French Canadian woman (Mrs Côté), reacted with all red cells tested except for her own, those of two siblings, and K_o phenotype cells, and reacted extremely weakly with McLeod phenotype cells [157]. Thus Côté serum appeared to contain an antibody recognising a new high frequency antigen related to the Kell system.

Anti-Wk^a reacted with red cells of 0.3% of English blood donors (Table 7.4), but with those of only 0.1% of K+ donors [48]. None of 1000 Kp(a+) donors was Wk(a+). The families of five K+ Wk(a+) donors showed that Wk^a was always inherited with k; there was no recombinant and 13 non-recombinants. KEL:–11 red cells were found to be Wk(a+) and the allelic status of *KEL11* and *Wk^a* was confirmed by family studies [48,158]. As KEL11 has never been called Wk^b, the numerical notation for Wk^a, KEL17, will now be used here.

*KEL*11* and *KEL*17* differ by a single nucleotide, creating an *Msc*I restriction site in the *KEL*17* allele. *KEL*11* encodes Val302; *KEL*17* encodes Ala302 [115].

Anti-KEL11 and -KEL17 are rare antibodies. A patient with anti-KEL11 was transfused with 11 units of KEL:11 red cells with no adverse clinical outcome [159]. ^{51}Cr-labelled KEL:11 cells survived normally and there was no increase in reactive monocytes in a monocyte monolayer assay. One example of anti-KEL11 was implicated in severe fetal anaemia, despite results in a chemiluminescent functional assay suggesting that the antibody was not clinically significant [160].

7.6.3 KEL12

Five examples of anti-KEL12 and four KEL:–12 propositi are reported [161–164]. All are white (although one was originally described as black [165]). Two of the propositi each had a KEL:–12 sibling [163,164]. One of the KEL:–12 propositi and her KEL:–12 sister had both been transfused and both had anti-KEL12 [163]. Two of the propositi were transfused with KEL:12 blood with no evidence of *in vivo* destruction [163,164].

Two unrelated KEL:–12 individuals had a mutation encoding His548Arg and abolishing an *Nla*III restriction site [32].

7.6.4 KEL13

The only reported KEL:–13 propositus was a much transfused man of Italian parentage [166]. His red cells and those of his KEL:–13 sister displayed weakened expression of k, Kp^b, Js^b, Ku, and KEL12 and gave an enhanced score with anti-Kx (typical of cells from a K^o heterozygote), suggesting that the KEL:–13 siblings have a K^o allele. This was confirmed by molecular testing, which revealed heterozygosity for one allele encoding Leu329Pro and the other containing a nonsense mutation, Gln532stop [167]. KEL:–13 can be considered a K_{mod} phenotype (*KEL*02M.03*). In transfected human embryonic kidney cells, fewer Leu329Pro mutant Kell proteins were transported to the cell surface, compared with control cells [167].

7.6.5 KEL14 and KEL24

The original anti-KEL14 was found in the serum of a white woman [161,168], and KEL14 was shown to be an inherited character retrospectively when Dp, a previously described public antigen [169], was found to be KEL14 [170]. The KEL:–14 propositus, a white woman with consanguineous parents, had four KEL:14 and two KEL:–14 siblings [169].

IgG and IgM murine and IgG human monoclonal anti-KEL14 have been produced [105,108].

An antibody in the serum of a white woman, which reacted with the red cells of her baby and several of the baby's relatives, appeared to be antithetical to anti-KEL14 and was numbered anti-KEL24 [171]. Anti-KEL24 reacted with all three KEL:–14 samples tested, but with none of 700 other red cell samples, and gave a higher titre with KEL:–14,24 cells than with KEL:14,24 cells.

Two unrelated KEL:–14,24 individuals had a mutation introducing a *Hae*III restriction site [172]: KEL14 represents Arg180; KEL24, Pro180. Two other mutations in two unrelated KEL:–14 Japanese encoded Arg180Cys and Arg180His [173], and the Arg180Cys mutation was also found in a KEL:–14 patient of Middle Eastern descent with anti-KEL14 [174] (Table 7.1).

7.6.6 KEL18

The only three reported KEL:–18 individuals were white and had anti-KEL18 [175–177]. Despite being serologically identical, the first two unrelated propositi had different single base mutations in the same codon, encoding different amino acid substitutions: Arg130Trp and Arg130Gln [32]. The two mutations created *Eco*571 and *Taq*II restriction sites, respectively. No example of KEL:–18 was revealed by tests on 54 450 blood donors [178].

In vivo survival studies and mononuclear phagocyte assays predicted that the original anti-KEL18 would not cause an acute HTR, but that transfusion therapy with KEL:18 red cells would be ineffective in all but an emergency [178]. One anti-KEL18 caused mild HDFN, necessitating phototherapy for hyperbilirubinaemia [177].

7.6.7 KEL19

The first anti-KEL19 was found in a KEL:–19 woman with a KEL:–19 brother and two KEL:19 sisters [179]. The second anti-KEL19, identified in the serum of a

black man, caused a delayed HTR, eliminating four units of incompatible blood [180]. None of 10 757 donors tested with anti-KEL19 was KEL:–19 [180]. Two unrelated KEL:–19 individuals had a mutation encoding Arg492Gln [32].

7.6.8 KEL22

Two examples of anti-KEL22 were found in the sera of unrelated Israeli women of Iranian Jewish origin, with a total of three KEL:–22 siblings [181,182]. Three unrelated KEL:–22 individuals had a mutation encoding Ala322Val [32].

Anti-KEL22 in the second KEL:–22 propositus was responsible for mild HDFN in her fourth and fifth children and severe HDFN in her sixth child, requiring exchange transfusion with the mother's washed red cells [182,183]. The IgG isotype was IgG1 during the fourth and fifth pregnancies and IgG1 plus IgG3 during the sixth.

7.6.9 KEL23

An antibody in the serum of a white woman of Italian ancestry reacted with red cells of her two children, her husband, and his mother, but with none of 2100 reference samples [184]. The antibody precipitated Kell glycoprotein from the husband's red cells and the antigen was designated KEL23. Red cells lacking high frequency Kell antigens were all KEL:–23. Two KEL:23 family members were heterozygous for a mutation encoding Gln382Arg and creating a *Bcn*I restriction site [32].

Anti-KEL23 caused a strongly positive DAT on the red cells of the third baby of the propositus, but did not cause HDFN [184].

7.6.10 KEL25 (VLAN) and KEL28 (VONG)

KEL25 and KEL28 are low frequency antigens representing mutations in the same codon encoding Arg248Gln and Arg248Trp, respectively [185,186]. Both antigens were initially shown to be located on the Kell glycoprotein by MAIEA analyses [186,187]. KEL25 was detected on the red cells of a Dutch blood donor when they were crossmatched with the serum of a patient of unknown transfusion history [187]. Two sisters and a niece of the donor were also KEL:25. Anti-KEL25 consisted of IgG1 and IgG2 isotypes and directly agglutinated KEL:25 red cells. None of 1068 donors was KEL:25. Anti-KEL28 was responsible for fetal anaemia, suggestive of suppressed erythropoiesis, in a family of ethnic Chinese from Timor [186].

7.6.11 KEL26 (TOU)

KEL26 is an antigen of high frequency absent from K_o cells and shown to be located on the Kell glycoprotein by a MAIEA analysis [188]. Two examples of anti-KEL26 have been identified, one in a Native American man and the other in a Latino woman. Neither had been transfused, but the woman had been pregnant twice. A mutation was detected, encoding Arg406Gln in three KEL:–26 samples from two families [32]. A monocyte monolayer assay suggested that the original anti-KEL26 was not clinically significant [188].

7.6.12 KEL27 (RAZ)

KEL27 is a high frequency antigen, not present on K_o cells, expressed weakly on McLeod phenotype cells, and located on the Kell glycoprotein as determined by a MAIEA assay [189]. Anti-KEL27 was found in a Kenyan Indian woman, the only KEL:–27 person known. She is homozygous for a mutation encoding Glu249Lys [185].

7.6.13 KEL29 (KALT)

Anti-KEL29 was found in a Mexican with a history of pregnancies, but no transfusion. She was homozygous for a mutation encoding Arg623Lys and deleting a *Tfi*I restriction site. KEL29 is the most C-terminal of the Kell antigens on the Kell glycoprotein and is unique for a Kell-system antigen because it is destroyed by trypsin treatment of intact red cells [190]. Consequently, the most N-terminal trypsin cleavage site must be between Leu597 (Js^b, trypsin-resistant) and Arg623.

Red cells of the baby of the propositus gave a positive DAT, but no treatment for HDFN was required.

7.6.14 KEL30 (KTIM)

Anti-KEL30 was made by a white American, with a history of pregnancies and transfusion. KEL:–30 resulted from homozygosity for a mutation encoding Asp305Asn eliminating a *Taq*I recognition site [190].

7.6.15 KEL31 (KYO), KEL38 (KYOR), and KEL37 (KUHL)

Anti-KEL31 was found through routine antibody screening of Japanese blood donors, but no further example of anti-KEL31 was found in 100 000 donors [49]. Six of 400 Japanese donors were KEL:31. KEL31 was located on the Kell glycoprotein by a MAIEA analysis and results from an Arg292Gln substitution. Two KEL31 Japanese patients who were homozygous for the mutation encoding Arg-292Gln had antibodies to KEL38, the antigen antithetical to KEL31 [191].

The only two known KEL:–37 individuals are an Asian woman and her sister with apparently normal Kell phenotypes, except that the red cells of the sister were KEL:–31 [192]. Both sisters are homozygous for a mutation encoding Arg293Trp, in the codon adjacent to that responsible for KEL:–31. Despite the propositus being Kp(a–b+), the antibody initially presented as an alloanti-Kp^b, but 4 years later reacted strongly with Kp(b–) red cells, but not with K_o cells.

7.6.16 KEL32 (KUCI) and KEL33 (KANT)

KEL32 and KEL33 are serologically related Kell antigens of high frequency. Anti-KEL32 was produced by a previously transfused African American woman with a KEL:–32 sibling [193]. Anti-KEL33 was produced by an individual of European origin. KEL:–32 red cells are also KEL:–33, but KEL:–33 red cells appear to be KEL:32 [193]. KEL:–32 results from homozygosity for a mutation encoding Ala424Val. The KEL:–33 individual was heterozygous for a mutation encoding Arg428Leu and a Kell-null mutation encoding Arg406stop. The close proximity of the amino acid changes responsible for the KEL:–32 and KEL:–33 phenotypes probably explains the partial serological compatibility.

7.6.17 KEL34 (KASH)

The antibody defining KEL34 is non-reactive with the red cells of the antibody maker, a woman of Pakistani origin, and with those of her brother, both of whom have a K_{mod} phenotype and are homozygous for a *KEL* allele encoding a Tyr253Cys substitution [194]. The additional cysteine could disrupt disulphide bonding, causing misfolding in the Kell glycoprotein and the K_{mod} phenotype.

7.6.18 KEL35 (KELP)

A pregnant woman with an antibody to a high frequency Kell-system antigen was homozygous for two *KEL* mutations, encoding two amino acid substitutions: Leu260Phe, which is surface exposed and most likely the cause of the KEL:–35 phenotype, and Tyr253Cys, which is buried a short distance from the surface (6Å) and could affect the conformation of a nearby surface-exposed loop [195]. KEL:–35 red cells are also KEL:–12, but no explanation is provided by the protein model.

7.6.19 KEL36 (KETI)

Anti-KEL36 was found in a British patient homozygous for a *KEL* allele encoding Thr464Ile [196]. Anti-KEL36 was non-reactive with two examples of K_o red cells and with K_{mod} KEL:–34 cells.

7.7 The Kell-null and Kell-mod phenotypes and anti-Ku (-KEL5)

7.7.1 K_o, the Kell null phenotype

In the same year as the discovery of Kp^a and Kp^b, Chown *et al.* [197] found a new Kell phenotype, K– k– Kp(a–b–), in two sisters. The consanguineous parents and two other sisters were of the common Kell phenotype K– k+ Kp(a–b+). The propositus had made an antibody that reacted with all but K– k– Kp(a–b–) cells. This antibody was used to search for another example of the new phenotype [198,199]: the 3122nd blood tested did not react and was also K– k– Kp(a–b–), and the rare phenotype was named K_o [2]. Family studies showed that K_o results from apparent homozygosity for an amorph gene at the *KEL* locus [34]. In several families heterozygosity for a silent gene producing no K or k explains abnormal inheritance.

K_o phenotype results from homozygosity or compound heterozygosity for a variety of mutations, including numerous nonsense mutations, single nucleotide insertions or deletions, splice site mutations, and several missense mutations [200–205]. The presence of null alleles has explained unexpected *KEL*01/02* genotyping results in individuals with K+ k– red cells and occasionally individuals with K– k+ cells. Many null and mod alleles are listed in the dbRBC and ISBT databases [206,207]. The Kell-glycoprotein has a large, extracellular C-terminal domain, so many of the mutations that cause early termination of mRNA translation might be expected to produce a truncated protein that could be detected by immunoblotting with Kell antibodies, but there is no evidence for this [3,8,201]. One possible explanation involves nonsense-mediated mRNA decay, which clears eukaryotic cells mRNA molecules containing premature termination codons [203,205]. Ser363Asn and Ser676Asn mutants, expressed in human embryonic kidney cells, were retained in a pre-Golgi compartment and not transported to the cell surface [201]. Arg128stop mutations, homozygous in two African Americans, were present in Js^a (*KEL*06*) alleles [201].

K_o red cells lack expression of all Kell antigens, including, by definition, Ku and Km. The strength of Kx antigen detected on the surface of intact K_o red cells is reported to be enhanced [208], yet the quantity of Kx protein is reduced [209] (Section 7.13.2). K_o red cells demonstrate no morphological abnormality [210] or unusual

expression of antigens belonging to other blood group systems, except Kx.

Only one K_o was found from testing 16 518 white donors with the serum of the original K_o propositus [42]. Several studies provided only one example of K_o from 24 953 white people [34]. These results suggest a frequency of about 0.007 for K^o alleles in white people. One K_o was found among 14 541 Japanese, suggesting a similar frequency [39].

7.7.2 K_{mod} phenotype

Marsh and Redman [211] introduced K_{mod} as an umbrella term to describe phenotypes in which Kell antigens are expressed very weakly, often requiring adsorption/elution tests for detection, and in which Kx antigen expression is elevated. K_{mod} cells have reduced quantity of the Kell glycoprotein. Because some K_{mod} red cells have very weak expression of Kell antigens, the distinction between K_o and K_{mod}, in some cases, may be dependent on the serological reagents and methods used.

K_{mod} usually arises from homozygosity for a missense mutation in the *KEL* gene, or heterozygosity with another such mutation or a null mutation [167,204,206,207]. KEL:–13 (Leu329Pro) [167] and KEL:–34 (Tyr253Cys) [194] (Section 7.6.17) could also be considered K_{mod} phenotypes. A synonymous mutation within the Gly573 codon (*KEL*02M.04*), 16 bp downstream of the 3′ splice site of exon 16, caused exon 16 skipping and introduction of a premature splice site [212]. The weak expression of Kell antigens probably results from some normal splicing events. Homozygosity for a Ser363Asn mutation (*KEL*02M.01)* resulted in a K_{mod} phenotype [167], whereas heterozygosity for the same mutation and for a null allele (Arg192stop or intron 3 splice site mutations) gave rise to K_o phenotype [201]. Based on transfection studies with HEH cells, the majority of the Ser363Asn and Tyr677Cys (*KEL*02M.02)* mutant proteins are degraded intracellularly and not transported to the cell surface, whereas Leu329Pro (*KEL*02M.03)* and Gly703Arg (*KEL*02M.04)* proteins are degraded to a lesser extent, but more so than in controls [167,201].

In Austria, genotyping of 401 apparent K+ k– samples revealed *KEL*01/02* (578C/T) heterozygosity in 14 (3.5%) cases: nine were genuinely k– and six of these had null splice site or nonsense mutations, whereas in the other three no *KEL* mutation was detected; in four of the remaining five, k could be detected by adsorption and elution and all four had mod mutations; and the other one had weak k and was heterozygous for a Kp^a allele (Section 7.3) [204].

7.7.3 The Kp^a effect

In the original description of Kp^a, Allen and Lewis [40] noted some difficulty in k typing some K+ Kp(a+) family members, now known to result from weakening of k and other Kell antigens owing to a reduced quantity of Kell-glycoprotein with the Arg281Trp substitution responsible for Kp^a expression [29]. The Kp^a effect is only recognised under certain conditions:

1 when an alternative allele, such as *K*, is present on the opposite chromosome;

2 when there is a K^o gene *in trans* [213,214]; or

3 with difficulty, when there is homozygosity for Kp^a. Kp^c, a low incidence allele of Kp^a, does not appear to produce a similar effect when *in trans* with K^o [43].

Expression of cDNA constructs in human embryonic kidney cells showed that the Kp^a mutation causes retention of most of the Kell glycoprotein in a pre-Golgi compartment owing to differential processing, suggesting aberrant transport of the Kell glycoprotein to the cell surface [29].

7.7.4 Anti-Ku (-KEL5)

Anti-Ku is the typical antibody of immunized K_o individuals and detects an antigen present on all red cells apart from those of the K_o phenotype. It appears to be a single specificity and cannot be separated into components of other Kell specificity by adsorption and elution [215]. Exceptional K_o individuals with anti-Kp^b or -k have been reported [34,216].

Anti-Ku has been responsible for severe and fatal HTRs [197,217,218] and for HDFN characterised by fetal anaemia [219]. Monocyte monolayer assays on 11 examples of anti-Ku suggested a high potential for causing HTRs and HDFN [220].

Some K_{mod} individuals make an antibody that resembles anti-Ku, but differs from anti-Ku by being nonreactive with the weakly Ku+ K_{mod} cells of the antibody maker. Antibodies made by different K_{mod} individuals are often not mutually compatible, because K_{mod} arises from a variety of different amino acid substitutions [167]. Anti-KEL13, -KEL34, and even -Kp^b could be considered as Ku-like antibodies in individuals with K_{mod} phenotypes.

7.8 Kell depression in Gerbich-negative phenotypes

The phenomenon of Kell depression associated with some Gerbich-negative phenotypes was first recognised

in a K+ woman and her brother with the rare Ge:–2,–3 phenotype [60]. Both showed weakened expression of K, k, and Kp^b, with about half the number of K antigen sites of K+ k+ Ge:2,3 cells [16]. Nine of 11 red cell samples from Ge:–2,–3 people showed at least some degree of weakening of Kell antigens [221], as did red cells of the Ge:–2,–3,–4 Leach phenotype [222,223]. All six Ge:–2,3 samples had normal expression of Kell antigens [221]. Red cells with the K_o, K_{mod}, and McLeod phenotypes have normal expression of Gerbich antigens. The biochemical nature of the phenotypic association between Gerbich and Kell is not understood, but studies on mice suggest that the Kell glycoprotein belongs to the same membrane complex as glycophorin C, which expresses Gerbich antigens [14].

7.9 Acquired and transient depressed Kell phenotypes

In 1972, Seyfried *et al.* [128] described the case of a boy with severe AIHA whose red cells gave a weakly positive DAT and had weak expression of k, Kp^b, Js^b, and Ku. His serum contained a potent anti-Kp^b responsible for an HTR. Within 16 weeks of the start of the investigation, his positive DAT had virtually disappeared, there was no sign of the anti-Kp^b, and his Kell antigens were back to normal strength. Similar examples of Kell-related autoantibodies associated with weak Kell antigens have been described since [224–228].

Anti-Kp^b was responsible for a positive DAT on the cells of a patient who was genetically Kp(a+b–) [226]. Her k and Js^b antigens were weakly expressed, but she had strong Kp^a. Nine months later the DAT was negative, the anti-Kp^b undetectable, her k and Js^b back to the strength expected for Kp(a+b–) cells, and own anti-Kp^b from the initial study no longer reacted with her cells.

A patient with autoimmune idiopathic thrombocytopenic purpura (AITP) had a potent antibody to a high frequency Kell antigen. His red cells gave a negative DAT and displayed profound depression of Kell system antigens. Transfused cells also lost their Kell antigens. Five months later the antibody had disappeared and the patient's Kell antigens had returned to normal. An environmental agent, possibly of microbial origin, may have been responsible [229]. Another similar case in an AITP patient is reported [230]; in remission his Kell antibody disappeared and his Kell antigens returned to normal, but his red cells lost their Lutheran antigens during a subsequent relapse (see Section 6.9).

Another patient with AITP and with IgM anti-Kp^b was typed as K– k– Kp(b–), but Js(b+), with a positive DAT with anti-IgM, but not with anti-IgG [131]. When the anti-Kp^b disappeared, his red cells were K– k+ Kp(b+) Js(b+), with no DAT. Boscoe *et al.* [131] suggest that there was no reduction in expression of Kell glycoprotein in this patient, but that his apparent Kp(b–) phenotype resulted from blocking of the Kp^b epitopes by the IgM autoantibody, and that the large IgM molecules also blocked k epitopes by steric hindrance. This is consistent with the relatively close proximity of k and Kp^b as predicted by a three-dimensional model of the Kell glycoprotein [10] and by a competitive MAIEA assay [109].

The possibility that Kell glycoprotein is expressed on megakaryocytes [94] might provide an explanation for the ITP in these patients.

7.10 Effects of enzymes and reducing agents on Kell antigens

Treatment of red cells with the proteases papain, ficin, or trypsin does not reduce expression of Kell antigens (with the exception of KEL29 [190] and KEL38 [191], Sections 7.6.13 and 7.6.15, which are trypsin-sensitive); the effects of α-chymotrypsin and pronase are variable [231]. Treatment of red cells with a mixture of trypsin and chymotrypsin, with trypsin followed by chymotrypsin, or *vice versa*, abolishes activity of Kell antigens [231,232], but some Kell-related monoclonal antibodies continue to agglutinate red cells treated in this way [15].

The Kell glycoprotein has 15 cysteine residues in its extracellular domain and thiol reducing agents, which dissociate disulphide bonds between cysteine residues, destroy Kell antigens on intact red cells. Kell antigens are conformational and are destroyed by 100–200 mM DTT and by 6% AET at pH 8 [233,234]. Js^a and Js^b are inactivated by substantially lower concentrations of DTT (<2 mM) [233]. Two cysteine residues flank the position of the amino acid substitution associated with the Js^a/Js^b polymorphism (residue 597) and probably form part of the Js^a and Js^b epitopes [139]. Like natural K_o cells, artificial K_o cells produced by AET treatment of red cells have enhanced expression of Kx antigen [234]. AET treatment of red cells is not a reliable way of identifying Kell-system antibodies as AET destroys many other red cell antigens,

including the Lutheran, Yt, Dombrock, LW, Knops, and JMH antigens.

7.11 Kell antigens on other cells and in other species

7.11.1 Other cells and other tissues

No Kell-system antigens or Kell glycoprotein has been detected on human lymphocytes, granulocytes, monocytes, or platelets, by flow cytometry with several Kell-related monoclonal antibodies [235] or by immunoblotting with a monoclonal antibody to purified Kell glycoprotein [8]. Cells of the human erythroleukaemic line, K562, do not express Kell antigens, unless induced to synthesise haemoglobin by hemin [235,236]. Kell mRNA transcripts were detected in haemopoietic tissue, bone marrow, and fetal liver, and in peripheral blood leucocytes [237]. There is also indirect evidence that Kell glycoprotein could be present on progenitors of granulocytes, monocytes, and megakaryocytes [93,94] (Section 7.3.5.2).

KEL mRNA transcripts were found to be about equally abundant in erythroid tissues and testis, and were detected in lesser amounts in lymph node, brain, colon, spleen, skeletal muscle, and several other tissues. Immunoblotting and immunohistochemistry revealed Kell glycoprotein in testis, lymphoid tissues, and skeletal muscle [19,237]. Kell glycoprotein was co-isolated with Kx protein in skeletal muscle [237].

7.11.2 Evolutionary aspects

Chimpanzees (*Pan troglodyte*) have the Kell phenotype that is common in humans, except that they are Js(a+b–) [238]. Antigens k, Kp^b, and Js^a are present on the red cells of gorilla and gibbon, K and Js^a on those of old world monkeys, and k on those of new world monkeys [239].

KEL and its mouse homologue, *Kel*, share 80% sequence identity; their glycoprotein shares 74% amino acid sequence identity [240]. The mouse Kell glycoprotein is disulphide-linked to Xk protein.

7.12 Functional aspects

Kell protein shares a pentameric zinc-binding motif, His-Glu-Xxx-Xxx-His (HEXXH, HELLH in Kell, Figure 7.1), with zinc-dependent endopeptidases [9]. Closest homology is with the neprilysin (M13) family, which includes neutral endopeptidases 24.11 (NEP or CD10) and two endothelium-converting enzymes (ECE-1 and ECE-2) [241]. These enzymes process a variety of biologically active peptides. ECE-1 and ECE-2 cleave big endothelin (ET)-1, big ET-2, and big ET-3, inactive peptides of about 40 amino acids, to create 21-amino-acid peptides with vasoconstrictor activity, ET-1, ET-2, and ET-3. A *KEL* cDNA construct lacking the regions encoding the cytoplasmic and membrane-spanning domains was expressed in insect (sf9) cells and a truncated Kell glycoprotein was secreted. This secreted glycoprotein cleaved big ET-3 at Trp21-Ile22 to produce ET-3. It could also process ET-1 and ET-2 from big ET-1 and big ET-2, but to a much lesser extent [242]. Secreted Kell glycoprotein was inactivated by mutating the HELLH motif, essential for catalytic activity, to HGLLH. Red cells of normal Kell phenotype, but not those of the K_o phenotype, were also capable of processing ET-3. Clapéron *et al.* [33] claimed that Kell-glycoprotein expressing K is enzymatically inactive, but this is disputed by Sha *et al.* [243], who found that the proteins expressing either K or Js^a had similar activity to the common form. ET-3 is a biologically active peptide with multiple roles, so the function of the Kell glycoprotein remains unclear. *Kel* knockout mice, like K_o humans, have no obvious abnormalities, but minor effects suggested possible physiological functions of Kell glycoprotein in heart, red cell ion transport, neovascularisation in tumours, and motor function [244].

Kell glycoprotein appears on erythroid progenitor cells at an early stage of erythropoiesis, before glycophorin A or band 3 [89,90]. When K562 erythroleukaemia cell line is stimulated with hemin to develop as an erythroid line it expresses Kell but no NEP, but when stimulated into megakaryocyte development with phorbol ester it expresses NEP but no Kell [245]. Kell may, therefore, play a role in erythropoiesis (but see Section 7.3.5.2).

7.13 The Kx blood group system

The Kx system consists of one antigen, Kx (XK1 or 019001), located on the Xk protein, which is encoded by an X-linked gene, *XK*. Absence of Kx from red cells results in severe reduction in expression of Kell antigens, the McLeod phenotype.

7.13.1 The Xk protein and the gene that encodes it

Xk, a red cell membrane protein of apparent MW 37 kDa, was isolated from red cells by immunoprecipitation with

human alloanti-Kx [246]. Xk protein is not glycosylated [12,247]; it is phosphorylated and palmitoylated [6,7].

Patients with McLeod syndrome (MLS) lack Kx and occasionally have interstitial deletions of chromosome Xp21. Ho *et al.* [247] assembled a cosmid contig of 360 kb covering the region between genes flanking *XK*. They then hybridised the cosmids with genomic DNA from patients with MLS and identified the breakpoints of a deletion of about 50 kb. Genomic fragments that spanned deletion endpoints were used to screen human cDNA libraries and a consensus full-length transcript for a candidate *XK* gene was derived from seven cDNA clones. The open reading frame predicted a 444 amino acid polypeptide with no *N*-glycosylation site and a calculated MW of 50 913 Da. Rabbit antibodies raised to synthetic peptides with sequences corresponding to the *XK* cDNA clone bound to the 37 kDa Xk protein on immunoblots of membrane proteins derived from red cells of common phenotype, but not to blots of proteins from McLeod phenotype red cells [209]. The N-terminal 22 amino acids of the protein were in accordance with the corresponding nucleotide sequence of the cDNA clone [12].

Hydropathy analysis of the amino acid sequence of Xk suggested a protein that spans the membrane 10 times, with internal N- and C-termini [247] (Figure 7.2). The predicted topographical arrangement is similar to that of members of a family of proteins that co-transport a neurotransmitter together with Na^+ and Cl^- ions [248], the amino acid sequence bearing closest resemblance to an Na^+-dependent glutamate transporter [247]. Xk also shares similarity with ced-8, a protein of the nematode *Caenorhabditidis elegans*, which is involved in the regulation of apoptosis [249].

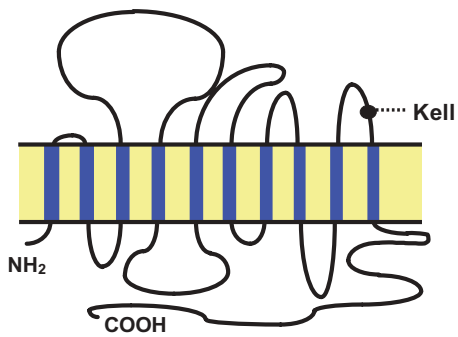

Figure 7.2 Diagrammatic representation of the topology of the Xk protein in the red cell membrane, showing cytoplasmic termini, 10 membrane-spanning domains, and (•) Cys347, which is linked by a disulphide bond with Cys72 of the Kell glycoprotein.

XK is organised into three exons, encoding amino acids 1–82, 83–168, and 169–444, respectively [249]. *XK* mRNA showed widespread distribution, with high levels of expression detected in fetal liver and in adult skeletal muscle, brain, and heart [249]. Immunohistochemical analysis showed Xk in human type II muscle fibres [250] and, in the rat, Xk is widespread in the brain, but its neuronal location is cytoplasmic and not at the cell surface [251]. Kx was not detected on neutrophils [252].

A mouse homologue of *XK* encodes a protein with 82% identity to human Xk protein with 10 predicted membrane-spanning domains [253].

7.13.2 Association of Xk protein and Kell glycoprotein

When immunoprecipitation experiments were performed under non-reducing conditions with monoclonal anti-K and K+ red cells, a disulphide-bonded heterodimer comprising the 93 kDa Kell glycoprotein and the 37 kDa Xk protein was detected [12]. Rabbit antibody raised to the purified complex reacted with both components on immunoblots and precipitated the Xk protein from K_o red cells, but not from McLeod phenotype cells.

Precipitation with a rabbit antibody raised to a peptide representing the second extracellular loop of Xk (Figure 7.2) isolated the Kell-Xk complex from COS-1 mammalian cells co-transfected with *KEL* and *XK* cDNA. Conversion of cysteine residues to serine by site-directed mutagenesis demonstrated that Cys347 on the fifth extracellular loop of Xk is linked by a single disulphide bond to Cys72 of the Kell glycoprotein [13]. Time-course studies on the *KEL* and *XK* transfected COS-1 cells demonstrated that the Kell-Xk complex is assembled in the endoplasmic reticulum and transported to the cell surface [254].

The relative quantity of Xk protein in K_o is lower than in cells of common Kell type [201,209]. In contrast, Kx antigen, as determined by serological techniques with alloanti-Kx, is expressed more strongly on K_o cells than on cells of common Kell type [208], suggesting that the presence of the Kell glycoprotein may impair access of the anti-Kx to the Xk protein in intact cells. Unlike Kell system antigens, Kx is not denatured by the disulphide-bond reducing agents DTT and AET. On the contrary, red cells treated with the appropriate concentrations of these chemicals resemble K_o cells and have enhanced serological expression of Kx [234,255].

Both *KEL* and *XK* transcripts were detected in early stages of human erythropoiesis, but in mice *Kel*, but not *Xk* transcripts were detected in erythroid progenitors [256].

7.13.3 McLeod phenotype and McLeod syndrome (MLS)

Routine tests on medical students led Allen *et al.* [257] to recognise that one of the students, Mr McLeod, had an unusual Kell phenotype. In the McLeod red cell phenotype all high frequency Kell antigens are expressed weakly, the degree of depression of these antigens varying in different individuals. K is also weakly expressed when present [59]. McLeod phenotype red cells lack the Kx (XK1) and Km (KEL20) antigens (Section 7.13.5).

McLeod phenotype is very rare and no frequency estimate has been published. Two unrelated men with the McLeod phenotype were found as a result of testing, with anti-k, red cells from many thousands of donors from South East England [258].

McLeod phenotype is only one of a number of characteristics that collectively make up a wider phenomenon known as McLeod syndrome (MLS), a form of neuroacanthocytosis [259]. Originally described as a 'benign X-linked myopathy with acanthocytes' [258], MLS is now recognised as a multisystem degenerative disorder sharing many features with Huntington's disease [260]. McLeod cells are acanthocytic [258,259,261] with decreased whole cell deformability [262] and reduced *in vivo* survival [263]. A variety of muscular, neurological, and psychiatric defects have been associated with MLS, with an average age of onset of around 40 years [260, 264,265]. Symptoms include muscle wasting, diminished deep tendon reflex, choreiform movements, and cardiomyopathy, sometimes leading to chronic heart failure and death, personality disorders, and generalised seizures. Elevated serum creatine phosphokinase is almost a constant feature. The reason for the association between Xk deficiency and neuroacanthocytosis and muscular defects is unknown, but the relationship of Xk protein to mammalian neurotransmitter transporters could provide a clue.

MLS red cells appear biochemically and physiologically relatively normal, despite their grossly abnormal morphology and the virtual absence of Kell system antigens. The protein profile as determined by SDS PAGE is normal, suggesting no defect of the membrane skeleton [246,266,267]. Phospholipid content is essentially normal [268,269], but there is evidence for abnormalities in the composition of the membrane lipid bilayers [270] and enhanced transbilayer mobility of phosphatidylcholine [269]. Electrolyte transport in McLeod cells is normal, but osmotic water permeability is reduced [268].

7.13.4 Inheritance and molecular genetics of Kx and McLeod syndrome

Expression of Kx antigen, present on all red cells save those of the McLeod phenotype, is controlled by an X-borne gene. MLS is, therefore, inherited as an X-linked recessive disease. With only rare exceptions [271], McLeod has always been found in males and the rare gene is inherited from the mother.

MLS has resulted from hemizygosity for a large variety of inactivating mutations including deletions of the whole gene, exon deletions, deletion of one or a few nucleotides introducing frameshifts, nonsense mutations, and splice site mutations (listed in [206,207,260, 264,272,273]). Two missense mutations, encoding Arg-222Gly (*XK*N.27*) [272,273] and Glu327Lys (*XK*N.29*) [274], have been found in two pairs of brothers manifesting no or minimal symptoms of MLS, despite all being over 50 years of age. Xk with Arg222Gly did not travel to the surface of transfected COS-1 cells [272]. A deletion of exon 2 of *XK* (*XK*N.04*) was detected in a man with MLS and in his grandson, predicting that the boy will be afflicted with the disease in the future [275]. Studies of sets of brothers with identical *XK* mutations revealed significant variation in clinical presentation, including causes of morbidity and mortality [276]. A nonsense mutation in *XK* exon 3 (*XK*N.21*) of a man with MLS was probably a new mutation as it was not present in his mother or sister [277].

A mutation in the fifth nucleotide of the 5′ donor splice site of intron 2 of *XK* (*XK*N.25*) was found in a man with almost no Kell antigens on his red cells and in his two daughters [278]. He did not have neuroacanthocytosis or muscle defects, possibly because of some degree of normal *XK* RNA splicing. The extreme reduction in Kell antigen was attributed to the combined effects of homozygosity for a Kp^a allele and the Kx deficiency (Section 7.7.3).

Chronic granulomatous disease (CGD) is an inherited immunodeficiency that may be X-linked (about 60% of cases) or autosomal. X-linked CGD result from deletion of *CYBB*, an X-linked gene that encodes the NADPH oxidase NOX2, or from mutations within that gene [279]. A small minority of CGD patients, all of the X-linked type, have MLS. The locus for X-linked CGD and the *XK* locus are discrete and the association of MLS with CGD results from a deletion of part of the X-chromosome that

encompasses both genes. Some large deletions also include genes for Duchenne Muscular Dystrophy (*DMD*), retinitis pigmentosa (*RPGR*), and ornithene transcarbamylase deficiency (*OTC*), causing a condition known as contiguous gene deletion syndrome [280–284].

7.13.4.1 X-chromosome inactivation

XK is subject to the phenomenon of X-chromosome inactivation, in which all somatic cells in female mammals have one active X-chromosome and one inactive X-chromosome (described in Section 12.7.1). Mixed populations of Kx+ and Kx– red cells, or of red cells with strong and weak Kell antigen expression, have been recognised in many female carriers of genes responsible for MLS. The proportion of McLeod phenotype red cells in female McLeod carriers usually varies from 5 to 85% [211]. This dual population of red cells is often difficult to detect serologically, especially if Kell antibodies and not anti-Kx are used, but flow cytometry permits an accurate estimation of the two red cell populations [211,285].

The only female described with MLS was heterozygous for a single basepair deletion in exon 2 of *XK* (*XK*N.08*) [271]. Her severe neurological and muscular defects and weakening of her Kell antigens were attributed to extreme skewing of inactivation of her X-chromosome carrying the normal *XK* gene.

7.13.5 Anti-Kx and -Km (-KEL20)

A five-year-old boy afflicted with recurrent infections (later presumed to be CGD), the second example of the McLeod phenotype, had anti-Kx and -Km (initially called anti-KL) and suffered an HTR. The antibody reacted with all cells tested, except for his own and those of Mr McLeod [286,287]. Anti-Kx reacts strongly with K_o cells, weakly with red cells of common Kell phenotype, and not at all with McLeod phenotype cells [208] (Table 7.6).

Table 7.6 Expression of Kx, Km, and Ku on red cells of common, K_o, McLeod, and K_{mod} phenotypes.

Phenotype	Kx	Km	Ku*
Common	Weak	Strong	Strong
K_o	Strong	Negative	Negative
McLeod	Negative	Negative	Weak
K_{mod}	Strong	Not tested	Weak

* Ku represents all high frequency Kell antigens.

Adsorption of anti-Kx + -Km serum with K_o cells removes anti-Kx and isolates anti-Km. The anti-Kx can be recovered by elution. Unfortunately, the separation of anti-Kx from anti-Km is often difficult to achieve and sera containing these antibodies are in very short supply. Anti-Km reacts with red cells of common Kell phenotype, but not with K_o or McLeod phenotype red cells [208] (Table 7.6). The Kx determinant is located on the Xk protein whereas Km is probably a discontinuous antigen, the product of interaction between Kell glycoprotein and Kx protein. Although Km has been numbered KEL20, it could belong to the Kx system.

Anti-Kx + -Km is the typical immune response of McLeod phenotype patients with CGD following transfusion [208,286,288], and has been responsible for an HTR [263,286]. An untransfused McLeod phenotype CGD patient made anti-Km during septic shock [289]. Two transfused McLeod individuals without CGD made anti-Km, but no anti-Kx [290,291]. When one of these patients required further transfusion, a monocyte monolayer assay was strongly positive and no radiolabelled red cells of common Kell phenotype survived, *in vivo*, 24 hours after injection. Consequently, the patient received four units of K_o and one unit of K_{mod} blood. The transfusion was successful and, despite receiving strongly Kx-positive red cells, the patient did not make anti-Kx [291]. However, some patients with MLS, but no CGD, can, make anti-Kx [292,293] or anti-Kx + -Km [294] in response to transfusion. Whether the molecular background to MLS affects the immune response is not clear.

An IgG autoanti-Kx in a man with common Kell phenotype did not cause haemolysis of autologous or transfused Kx+ cells [295].

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