6 Lutheran Blood Group System

6.1 Introduction

The Lutheran system consists of 20 antigens: LU1 to LU22 in the numerical notation, with two declared obsolete (Table 6.1). Four pairs of these antigens have allelic relationships and represent SNPs in the Lutheran gene, *LU*: Lu^a (LU1) and Lu^b (LU2); Lu6 and Lu9; Lu8 and Lu14; and Au^a (LU18) and Au^b (LU19). The null phenotype, Lu_{null} or Lu(a–b–), in which the red cells lack all Lutheran system antigens, results from homozygosity for inactivating in the Lutheran gene. Individuals with the Lu_{null} phenotype may make an antibody to the Lutheran glycoproteins (Lu-gps), anti-Lu3. Nucleotide changes in *LU* are associated with loss of eight other antigens of high frequency, which are also absent from Lu_{null} cells. The molecular bases for two other antigens of high frequency absent for Lu_{null} cells are unknown.

Red cells of the dominantly inherited In(Lu) and X-linked XS2 phenotypes have very low levels of Lutheran antigens, which are not usually detectable by agglutination methods. Both of these mod phenotypes are governed by genes encoding erythroid transcription factors: In(Lu) results from heterozygosity for inactivating mutations in *KLF1*, the gene for EKLF; XS2 from hemizygosity for a mutation in *GATA1*.

Lutheran antigens are located on two red cell membrane glycoproteins (CD239) of apparent MW 78 and 85 kDa, which belong to the immunoglobulin superfamily of receptors and adhesion molecules. The Lu-gps are ligands for the extracellular matrix glycoprotein, laminin. *LU* or *BCAM* is situated on chromosome 19q12-q13 and consists of 15 exons, with alternative splicing accounting for the two isoforms of the Lu-gps.

6.2 The Lutheran glycoproteins and the gene that encodes them

6.2.1 The Lutheran glycoproteins (Lu-gps)

Components of apparent MW 85 and 78 kDa were revealed by immunoblotting of red cell membranes with monoclonal anti-Lu^b or with alloanti-Lu^a, -Lu^b, -Lu3, -Lu4, -Lu6, -Lu8, -Lu12, -Au^a, or -Au^b [1–3]. The two components were not apparent when Lu_{null} or Lu_{mod} red cells were used.

Parsons *et al.* [4] purified the Lu-gps by immunoaffinity chromatography with a monoclonal antibody, BRIC 221. From the amino acid sequence obtained, they designed redundant oligonucleotide primers and used a PCR product to isolate a cDNA clone of 2417 bp from a human placental cDNA library. The predicted mature protein consists of 597 amino acids: 518 comprising an extracellular domain, 19 a single transmembrane domain, and 59 a cytoplasmic domain. This structure represents the 85 kDa isoform. Immunoprecipitation experiments with a rabbit antiserum prepared to an amino acid sequence of the cytoplasmic domain showed that the 78 kDa structure lacks part of the cytoplasmic domain [4]. The 78 kDa isoform had previously been identified as an epithelial cancer antigen and is often referred to by

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Table 6.1 Antigens of the Lutheran system.

Antigen				Molecular basis*		
No	Name	Frequency	Antithetical	Nucleotides	Exon	Amino acids
LU1	Lu^a	Polymorphic	Lu^b	230G>A	3	Arg77His
LU2	Lu^b	High	Lu^a	230G (A)	3	Arg77 (His)
LU3	Lu3	High		Various		
LU4	Lu4	High		1. 524G (A)	5	Arg175 (Gln)
				2. 524G (T)	5	Arg175 (Leu)
LU5	Lu5	High		326G (A)	3	Arg109 (His)
LU6	Lu6	High	Lu9	824C (T)	7	Ser275 (Phe)
LU7	Lu7	High		Not known		
LU8	Lu8	High	Lu14	611T (A)	6	Met204 (Lys)
LU9	Lu9	Low	Lu6	824C>T	7	Ser275Phe
LU11	Lu11	High		Not known		
LU12	Lu12	High		1. (99-104del)	2	1. (delArg34,Leu35)
				2. 419G (A)	3	2. Arg140 (Gln)
LU13	Lu13	High		1340C (T), 1742A (T)	11, 13	Ser447 (Leu), Gln581 (Leu)
LU14	Lu14	Low	Lu8	611T>A	6	Met204Lys
LU16	Lu16	High		679C (T)	6	Arg227 (Cys)
LU17	Lu17	High		340G (A)	3	Glu114 (Lys)
LU18	Au^a	Polymorphic	Au^b	1615A (G)	12	Thr539 (Ala)
LU19	Au^b	Polymorphic	Au^a	1615A>G	12	Thr539Ala
LU20	Lu20	High		905C (T)	7	Thr302 (Met)
LU21	Lu21	High		282C (G)	3	Asp94 (Glu)
LU22	LURC	High		223C (T)	3	Arg75 (Cys)

Obsolete: LU10, previously Singleton; LU15, AnWj (now 901009).
*Molecular basis of antigen-negative phenotype in parentheses.

its earlier name BCAM [5], or as Lu(v13) [6]. The 85 kDa isoform is 5–10-fold more abundant on red cells than the 78 kDa isoform [7].

6.2.2 The Lu-glycoproteins belong to the immunoglobulin superfamily (IgSF)

The immunoglobulin superfamily (IgSF) is a large collection of glycoproteins, abundant on leucocytes, but also present on other cells, which contain repeating extracellular domains with sequence homology to immunoglobulin variable (V), constant (C1 or C2), or intermediate (I) domains. Each IgSF domain consists of approximately 100 amino acids and is structured into two β-sheets stabilised by a conserved disulphide bond (Figure 6.1). IgSF glycoproteins mostly function as receptors and adhesion molecules, and may be involved in signal transduction [8].

The extracellular domain of the Lu-gps is organised into five IgSF domains, V-C1-I-I-I, with a distinctive bend and flexible junction between domains 2 and 3 [4,5,9,10] (Figure 6.2). There are five potential *N*-glycosylation sites, one in the third domain and the other four in the fourth domain. At least five other IgSF glycoproteins are present in the red cell surface membrane: the Scianna, LW, and Ok blood group glycoproteins (Chapters 13, 16, and 22), CD47 (Chapter 5), and CD58 (LFA-3).

Many of the Lutheran antigens were mapped to an IgSF domain through the expression of *LU* cDNA deletion mutants in K562 erythroleukaemia cells, each construct encoding Lu-gp lacking one, two, three, or four of the IgSF domains [11]. Subsequently the location of most of the Lutheran antigens has been identified by DNA sequencing [11–15] (Table 6.1, Figure 6.2).

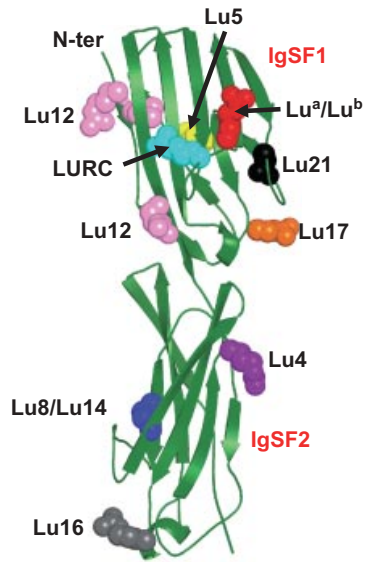

Figure 6.1 Structure of the two N-terminal domains of the Lu-gps (IgSF1 and IgSF2), showing the location of the amino acids associated with some Lutheran antigens [9]. (Thanks to Nicholas Burton for providing the structure.)

Table 6.2 Exon/intron organisation of *LU* [11,12].

Exon	Domain encoded	Exon size (bp)	Intron size (kb)
1	5′ UT + leader	105	2.0
2	1 IgSF (V)	122	0.7
3	1 IgSF (V)	229	0.09
4	2 IgSF (V)	71	0.5
5	2 IgSF (V)	95	0.09
6	2 IgSF (V)	185	0.53
7	3 IgSF (C2)	137	0.31
8	3 IgSF (C2)	157	3.5
9	4 IgSF (C2)	116	0.1
10	4 IgSF (C2)	142	0.17
11	5 IgSF (C2)	137	0.15
12	5 IgSF (C2)	145	0.09
13	TM + 19 residues cyt	145	0.97
14	cyt (85 kDa isoform)	118	0.09
15	1 residue cyt (85 kDa isoform)	498	

TM, transmembrane; cyt, cytoplasmic.

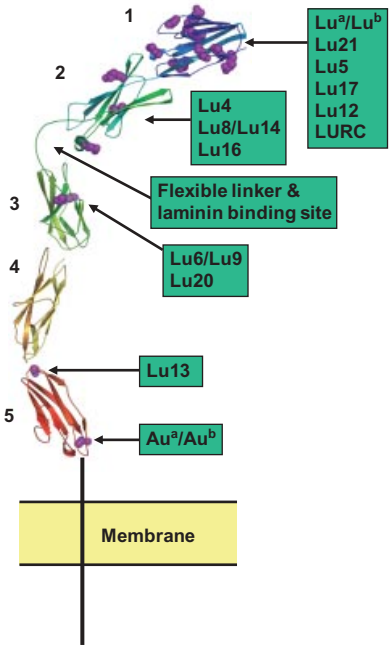

Figure 6.2 Structure of the Lu-gps, showing five IgSF domains and the location of the Lutheran antigens on those domains. (Thanks to Nicholas Burton for providing the structure.)

6.2.3 Organisation of the *LU* gene

The *LU* (*BCAM*) gene is 12.5 kb organised into 15 exons (Table 6.2). Exon 1 encodes the signal peptide; exons 2–12 the five IgSF domains (two exons per domain except domain 2, which is encoded by exons 4–6); exon 13 the transmembrane domain and the cytoplasmic domain common to both isoforms; exons 14 and 15 the C-terminal 40 amino acids of the larger isoform [11,12]. Two *LU* transcripts have been isolated, one of 2.5 kb encoding the larger Lu-gp isoform (85 kDa) and one of 4.0 kb encoding the smaller (78 kDa) isoform [6]. The two transcripts differ as a result of alternative splicing of intron 13. In the 2.5 kb transcript intron 13 has been removed by splicing and exons 14 and 15 encode the C-terminal 40 amino acids of the larger isoform. In the 4.0 kb transcript intron 13 remains, explaining the larger size of the intron. The 5′ end of the intron contains a UGA translation stop codon, so the unspliced intron 13 and exons 14 and 15 are not translated and the protein product has a cytoplasmic domain consisting only of the 19 amino acids encoded by exon 13 [12].

The 5′ flanking region of *LU* does not contain TATA or CAAT boxes, but showed an organisation typical of ubiquitous genes with several potential binding sites for the Sp1 transcription factor. The region between –673 and

–764 upstream of the coding region contains binding sequences for GATA and CACCC or Sp1 transcription factors [12].

6.2.4 Linkage and chromosome location

In 1951, when the Lutheran locus was shown to be genetically linked to the locus controlling ABH secretion (*FUT2*, believed at that time to be the Lewis blood group locus), blood groups were involved in the first recognised human autosomal linkage and, consequently, the first demonstration of recombination due to crossing-over in humans [16,17]. Linkage analysis revealed that *LU–FUT2* was linked to the gene for the third component of complement (*C3*) and indirectly linked to the Lewis gene (*FUT3*), so when *C3* was assigned to chromosome 19 by somatic cell hybridisation *LU* was also assigned to chromosome 19 [18,19]. *LU* (*BCAM*) is located at 19q12-q13 as part of a linkage group that also includes the H gene (*FUT1*) and the LW blood group gene (*ICAM4*).

6.3 Lu^a and Lu^b (LU1 and LU2)

The first Lutheran antibody, anti-Lu^a, was described in 1945 by Callender *et al.* [20] and in more detail the following year by Callender and Race [21]. Ten years later the antithetical antibody, anti-Lu^b, was described by Cutbush and Chanarin [22].

6.3.1 Molecular basis for Lu^a and Lu^b

The Lu^a/Lu^b polymorphism results from a single base change in exon 3 of *LU*, encoding an amino acid substitution in the first IgSF domain of the Lu-gps: *LU*A* His77; *LU*B* Arg 77 [11,12] (Table 6.1). The importance of this amino acid substitution in Lu^a or Lu^b expression was confirmed by *in vitro* site-directed mutation [12]. The nucleotide change is associated with an *Aci*I restriction-site polymorphism.

6.3.2 Frequencies and inheritance of Lu^a and Lu^b

Lu^a is widely distributed amongst Europeans, Africans, and North Americans with a frequency of around 8%, but is very rare or absent from all other indigenous populations studied [23]. Typical frequencies were obtained from tests with anti-Lu^a and -Lu^b on about 1500 white Canadians: Lu^a 6.9%; Lu^b 99.9%; Lu(a–b+) 93.1%; Lu(a+b+) 6.8%; Lu(a+b–) 0.1%; *LU*A* 3.5%; *LU*B* 96.5% [24,25]. Of 922 Chinese in Taiwan tested with anti-Lu^a and -Lu^b, all were Lu(a–b+) [26].

Table 6.3 Lutheran genotype frequencies on four populations of American blood donors, obtained by testing on the BeadChip array [29].

		Genotypes		
Ethnic group	No. tested	*LU*A/A*	*LU*A/B*	*LU*B/B*
Caucasians	1243	0	0.05	0.95
African Americans	690	0	0.06	0.94
Hispanic	119	0.01	0.02	0.97
Asian	51	0	0.02	0.98

In two large surveys, in which donor red cells were screened with anti-Lu^b and all negatives tested with anti-Lu^a, the following results were obtained: South of England, approximately 250 000 tested, 230 Lu(a+b–), 72 Lu(a–b–) [27]; South Wales, 75 614 tested, 39 Lu(a+b–), 15 Lu(a–b–) [28]. In a predominantly European population, therefore, roughly one in 1000 is Lu(b–); approximately two thirds of these being Lu(a+b–) and one third Lu(a–b–) (see Section 6.8.2.9).

Table 6.3 shows *LU*A/B* genotype frequencies in four ethnic groups of Americans determined by SNP testing [29]. All of 1102 Chinese were genotyped as homozygous for *LU*B* [30].

6.3.3 Variation in antigenic strength

The Lutheran antigens are very variable in strength. Lu^a on red cells from different families may vary quantitatively, but the antigenic strength remains roughly constant within the family. Occasionally adsorption and elution tests are required to detect weak Lu^b on Lu(a+b+) cells. There is also heterogeneity of Lutheran antigen strength between individual red cells within a person, which accounts for the characteristic mixed-field agglutination patterns often seen with Lutheran antisera, especially anti-Lu^a [21,22], and the wide range of survival times of Lu(b+) cells introduced into an Lu(a+b–) person with anti-Lu^b [31]. The abundance of Lu^b antigens on red cells, as determined by Scatchard analysis with purified monoclonal anti-Lu^b, is relatively low and shows wide variation. The number of Lu^b sites was estimated at 1640–4070 on Lu(a–b+) cells and 850–1820 on Lu(a+b+) cells [32].

Red cells from cord samples and from infants in the first year of life have markedly weakened expression of

Lu^a and Lu^b compared with those from adults. Ten of 155 cord blood samples had the phenotype Lu(a–b–), which is very rare in adults [33]. Adult levels of Lu^a and Lu^b antigenic expression are reached by the age of 15 [34].

6.3.4 Anti-Lu^a and -Lu^b

6.3.4.1 Anti-Lu^a

The first example of anti-Lu^a was found in a multiply transfused patient, together with anti-c, anti-C^w, anti-Kp^c, and anti-N [21]. The antibody had been stimulated by transfusion of blood from a donor named Lutheran. Anti-Lu^a has been reported after pregnancy and/or transfusion, and often appears together with other antibodies, especially red cell reactive HLA antibodies (anti-Bg) [35]. Anti-Lu^a may also be 'naturally occurring' [36,37], and in some cases a 'naturally occurring' antibody may be augmented by transfusion [38,39]. Anti-Lu^a suitable for grouping reagents is uncommon.

Lu^a antibodies are usually IgM, but, like other Lutheran-system antibodies, often have IgG and IgA components [40]. Anti-Lu^a often agglutinate Lu(a+) red cells directly, with a thermal optimum well below 37°C. Some also react in an antiglobulin test, and a few, predominantly IgG examples, are reactive only by an antiglobulin test.

A single-chain variable-fragment (scFv) with Lu^a specificity has been produced by phage display and recombinant DNA technology, and a monoclonal anti-Lu^a constructed [41].

6.3.4.2 Anti-Lu^b

Anti-Lu^b is relatively rare, often found as a single antibody. It has been stimulated by transfusion and by pregnancy; 'naturally occurring' examples have not been found.

Anti-Lu^b are often optimally active in the antiglobulin test, but directly agglutinating anti-Lu^b have been described, many with a temperature optimum of about 20°C. Most anti-Lu^b are mixtures of IgG and IgM, although IgA may also be present [40]. IgG anti-Lu^b may be predominantly IgG1, although IgG2 and IgG4 may be present [42].

Two monoclonal anti-Lu^b (BRIC 108 and LM342/767.31) have been produced from mice immunised with Lu(b+) red cells [1,43], although adsorption and elution tests demonstrated some binding of BRIC 108 to Lu(a+b–) cells [44].

6.3.4.3 Clinical significance of anti-Lu^a and -Lu^b

On the rare occasions that Lutheran antibodies are implicated in HTRs, they are almost always mild and delayed [36,40,45], although there could be exceptions [46] (see Section 6.4.2). Radiolabelled Lu(a+) red cells injected into a patient with anti-Lu^a survived normally [47]. Similar survival tests in patients with anti-Lu^b showed that at least a proportion of injected Lu(b+) cells could be removed fairly rapidly [31,48]. Least incompatible red cells are usually suitable for transfusion, but, if possible, antigen-negative red cells should be selected for strong examples of the antibody.

No case of HDFN caused by anti-Lu^a or -Lu^b and requiring any treatment other than phototherapy is reported, although raised bilirubin or a positive DAT may be detected [40]. One explanation for this could be poor development of Lutheran antigens on neonatal red cells (Section 6.3.3), but there is another possible explanation. Babies of mothers with high-titre IgG1 anti-Lu^b or -Lu6 had no sign of HDFN, their red cells gave negative DATs, and Lutheran antibody could not be detected in their sera [49]. Maternal IgG1 usually becomes concentrated in the fetal circulation by active placental transfer. As Lu-gp is present on placental tissue [4], it is possible that Lutheran antibodies are adsorbed by placental cells, preventing their transfer to the fetus.

6.4 Other Lutheran antigens and antibodies

In addition to Lu^a and Lu^b, the Lutheran system contains 17 other antigens: three pairs of antithetical antigens – Lu6 and Lu9, Lu8 and Lu14, and Au^a and Au^b – plus 11 antigens of high frequency (Table 6.1). Recombination as a result of crossing-over has never been observed within the Lutheran system. All Lutheran antigens are absent from Lu_{null} cells and absent from or expressed very weakly on In(Lu) cells. Like Lu^a, Lu^b, and Lu3, Lu4, Lu5, Lu6, Lu7, Lu8, Lu12, Lu13, Lu14, Au^a, Au^b, and Lu20 have been shown, by immunoblotting and/or by flow cytometry with K562 cells transfected with *LU* cDNA, to be located on the Lu-gps [2,3,11,50,51]. Nucleotide changes in the *LU* gene encoding amino acid changes in the Lu-gps have been shown to be associated with absence of all of the high frequency antigens or presence of the low frequency antigens, with the exception of Lu7 and Lu11 (Table 6.1). Lu11 has not been shown to be inherited and has not been shown to be located on the Lu-gps or encoded by the *LU* gene, and so should be referred to as a para-Lutheran antigen. Lu3 and anti-Lu3 will be discussed in Section 6.7.

With the exception of anti-Lu8, antibodies to none of the antigens described in this section have been incriminated in a serious HTR or in HDFN. All of the antibodies have been produced in Lu(a–b+) individuals, with the exception of anti-Lu16.

6.4.1 Lu6 (LU6) and Lu9 (LU9)

Lu6 and Lu9, Lutheran antigens of high and low frequency, respectively, have an antithetical relationship, and represent a SNP and *Cfo*I restriction polymorphism in *LU* encoding an amino acid substitution in the third IgSF domain [13] (Table 6.1).

The original anti-Lu9 was found, together with anti-Lu^a, in the serum of a white woman (Mrs Mull) [52]. The anti-Lu9 was responsible for a weak DAT with the red cells of her three babies. Red cells of the husband of Mrs Mull were Lu(a+b+) and Lu:9. Study of his family showed that Lu9 expression was controlled by the *LU* locus, although it did not represent an allele of *LU*A* and *LU*B*. The only other example of anti-Lu9 was found in a multitransfused woman and tests on 200 red cell samples unearthed another Lu:9 sample (0.5%) [53]. Tests with the original anti-Lu9 suggested a higher frequency of 1.7% [52], but that figure may be inaccurate as the serum also contained anti-HLA-B7 (-Bg^a) [35].

Red cells of the original Lu:–6 propositus, and those of her two Lu:–6 siblings, were strongly Lu:9, suggesting homozygosity [54]. All other Lu:–6 individuals (who are not Lu_{null}) have also been Lu:9.

In vivo red cell survival studies, macrophage binding assays, and transfusion of Lu:6 cells to patients with anti-Lu6 have suggested that anti-Lu6 is not usually of any clinical significance, but similar assays in an elderly woman suggested that her IgG1 anti-Lu6 was clinically significant; she was transfused with Lu_{null} cells, which had normal or near normal survival [55]. An Iranian Jewish woman with anti-Lu6 was transfused with two units of Lu:6 red cells with no apparent haemolysis, but after the transfusion a monocyte-based functional assay became positive, although a ^{51}Cr survival test was negative [56]. Red cells of the baby of a woman with high-titre IgG1 anti-Lu6 gave a negative DAT and no anti-Lu6 could be detected in the baby's serum, suggesting that the antibody was unable to cross the placenta [49] (see Section 6.3.4.3).

6.4.2 Lu8 (LU8) and Lu14 (LU14)

Lu8 and Lu14, Lutheran antigens of high and low frequency, respectively, have an antithetical relationship, and represent a SNP and *Fat*I and *Nla*III restriction polymorphisms in *LU*, encoding an amino acid substitution in the second IgSF domain [13] (Table 6.1).

The original anti-Lu8 was reported in 1972 as an antibody to a high frequency antigen absent from Lu_{null} cells [57]. Two Lu8 antibodies have been implicated in acute HTRs [46,58]; one gave positive results in monocyte monolayer assays [58].

An antibody in the serum of a multiply transfused dialysis patient, reported in 1977, reacted with red cells of 14 of 580 (2.4%) random white donors and also reacted strongly with three examples of Lu:–8 red cells and was numbered anti-Lu14 [59]. The apparent antithetical relationship between Lu8 and Lu14 was supported by family studies. Lu14 also appeared to have a higher frequency in Lu(a–b+) than in Lu(a+b+) samples, suggestive of allelic association [59]. Many other examples of anti-Lu14 have been found since [35,60], which included an IgG anti-Lu14 that was apparently 'naturally occurring'. Monoclonal anti-Lu^b gave consistently higher titration scores with Lu:14 cells than with Lu:–14 cells [61]. The frequency of Lu14 in 610 Danish donors and 600 English donors was 1.5% and 1.8%, respectively. Genomic testing for Lu8 on 11 418 Austrian donors revealed six (0.05%) negatives and 488 (4.3%) heterozygous positives [62].

6.4.3 Au^a (LU18) and Au^b (LU19), the Auberger antigens

Au^a and Au^b represent a SNP in exon 12 of *LU* encoding an amino acid substitution in the fifth IgSF domain (Table 6.1) [11].

For many years the Auberger antigens were considered to represent a system independent of Lutheran, mainly because of results on one family, which showed recombination between *LU*A* and the gene for Au^a [63]. When the family was retested for Au^a and tested for Au^b, errors in the original testing were discovered and the family now supported linkage between Auberger and Lutheran [64]. Family studies confirmed that Auberger and Lutheran antigens are controlled by a single gene [65].

The first anti-Au^a was identified in 1961 in the serum of a multitransfused woman, which also contained anti-E, -K, -Fy^b, and -Bg (-HLA) [66]. Only two other examples of anti-Au^a are reported, also in sera containing multiple antibodies to red cell antigens [67]. Anti-Au^b was not found until 1989 [68] and three further examples were soon found [69]. All four sera containing anti-Au^b also contained anti-Lu^a.

Au^a has an incidence of between 80 and 90% in European populations [66,67]. Au^b has an incidence of about

50% in a European population and 68% in an African American population [68]. Genotyping in Chinese predicted antigen frequencies for Au^a and Au^b of 98% and 24%, respectively [30].

6.4.4 Lu4 (LU4)

The first Lu:–4 propositus, a white woman who had made anti-Lu4, had Lu:–4 siblings [70]. All of about 2700 predominantly white donors were Lu:4. The second example of anti-Lu4 was identified in a prenatal patient with no known history of transfusion or previous pregnancy [71].

Homozygosity for two different mutations within the codon for Arg175, encoding either Gln or Leu, are responsible for the Lu:–4 phenotype in the two propositi [13,71] (Table 6.1). A monoclonal antibody produced in a mouse immunised with Lu-gp bound an epitope on IgSF2, but did not bind if Arg175 was substituted by either Asn or Ala [72].

6.4.5 Lu5 (LU5)

Anti-Lu5, -Lu6, and -Lu7, were initially found at an AABB 'wet' workshop [54]. At least 10 examples of anti-Lu5 have been identified and have been found in both black and white people [35,54,73,74]. Two of the Lu:–5 propositi had an Lu:–5 sibling [73,74]. None of 423 mostly white donors was Lu:–5 [54]. Two Lu:–5 individuals were homozygous for the same missense mutation in *LU* [13] (Table 6.1).

Results of a chemiluminescent functional assay suggested that one anti-Lu5 might cause increased clearance of transfused Lu:5 red cells [74].

6.4.6 Lu7 (LU7)

The original anti-Lu7 was found in an Lu:–7 woman with an Lu:–7 brother [54]. An IgG3 antibody in an Lu:–7 Latino woman was assumed to be another example of anti-Lu7, but Lu:–7 cells were not available for confirmation [51]. Her baby's red cells did not give a positive DAT. None of 285 mostly Caucasian donors was Lu:–7 [54].

Lu7 is located on the fourth IgSF domain of the Lu-gps [11].

6.4.7 Lu11 (LU11), a para-Lutheran antigen

The first example of anti-Lu11, an IgM antibody, was present in the serum of a white woman [75]. At least two other examples have been identified since [35]. There is no evidence that Lu11 is inherited. All of 500 predominantly white donors were Lu:11 [75].

6.4.8 Lu12 (LU12)

The first example of anti-Lu12 was produced by women of Polish and Ukrainian extraction, whose red cells were Lu(a–), but had only weak expression of Lu^b [76]. Red cells of her father, which had a weak Lu12 antigen, and of her Lu:–12 sister, were also Lu(a–b+^{w}) and, like the cells of the propositus, had only weak expression of other high frequency Lutheran antigens. Red cells from all except one of 1050 Canadian donors reacted strongly with anti-Lu12; those from the exceptional donor reacted weakly and were Lu(a–b+^{w}). The second example of anti-Lu12 was found in an Lu:–12 white woman with two Lu:–12 siblings [77]. An *in vivo* red cell survival test suggested that this antibody had the potential to cause accelerated destruction of transfused Lu:12 cells.

Lu:–12 had two different molecular backgrounds in two unrelated individuals: a six-nucleotide deletion in exon 2 encoding and a missense mutation in exon 3 (Table 6.1). Although the mutations are in different exons, when mapped to a three-dimensional schematic presentation of the Lu-gp the amino acid changes appeared to be located in close spatial proximity because of the protein folding [13]. Lu12 and Lu^b are both located on the first IgSF domain, so the weakening of Lu^b could result from conformational changes in the first domain, or could result from reduced expression of the Lu-gps associated with loss of Lu12.

6.4.9 Lu13 (LU13)

The original anti-Lu13 was unpublished. A second anti-Lu13 was found in a Finnish woman, but anti-Lu13 was not available for testing her red cells [78]. A family in which three of five siblings were Lu:–13 has been mentioned briefly [79].

Two unrelated Lu:–13 individuals have the same two missense mutations encoding amino acid substitutions: one in the region of the interface between IgSF domains 4 and 5; the other in the cytoplasmic domain [13] (Table 6.1). Each also had a silent mutation in the codon for Ser557. One of the individuals was homozygous for all three changes, but the other was apparently heterozygous, suggesting the presence of a null allele *in trans*.

6.4.10 Lu16 (LU16)

Anti-Lu16 was found together with anti-Lu^b in the sera of four Lu(a+b–) black women [80,81]. Two were homozygous for a missense mutation in exon 6 of *LU* (Table 6.1), in addition to the sequence in exon 1 associated with Lu^a expression [13,81]. In one case the anti-Lu16 plus anti-Lu^b was present in an untransfused

woman during her first pregnancy. There were no indications of HDFN at delivery [81].

6.4.11 Lu17 (LU17)

The only example of anti-Lu17 was found in an Italian woman [82], who was homozygous for a missense mutation in exon 3 of *LU* [13] (Table 6.1). *In vivo* studies suggested that anti-Lu17 might be capable of causing a modest reduction in survival of transfused Lu:17 red cells [83].

6.4.12 Lu20 (LU20)

Anti-Lu20 was identified in the serum of an Israeli thalassaemia patient [50], who was homozygous for a missense mutation in exon 7 of *LU* [13] (Table 6.1). The serum also contained anti-C, -K, and -Fy^b.

6.4.13 LU21

Anti-LU21 was identified in the serum of a Caucasian woman of Israeli origin during her second pregnancy [14]. There was no evidence of HDFN in her second, third, or fourth pregnancies. A MAIEA assay suggested that LU21 was located on IgSF1, the same domain as Lu^b, and the woman was subsequently shown to be homozygous for a mutation encoding an amino acid substitution in IgSF1 [14] (Table 6.1).

6.4.14 LURC (LU22)

LURC appears to be a high frequency antigen involving both Lu^b (Arg77) and Arg75 in IgSF1 [15]. Anti-LURC was produced by a woman with Lu(a+b+^{w}) red cells who was heterozygous at codons 75 and 77. It appears that Cys75, Arg77 results in weak Lu^b and no LURC, whereas Arg75, His77, results in Lu^a and no LURC. The common sequence, Arg75, Arg77 produces both Lu^b and LURC. Val96Ile in IgSF2, encoded by 586G>A in exon 5, also present in the LURC– propositus, is very conservative and would probably cause no conformational rearrangements. This change has been associated previously with Lu^a phenotype with no evidence of antibody formation [84].

6.5 Recombinant Lutheran antigens

Lutheran antigens have been used as models for the application of recombinant proteins in antibody identification [11,85–87]. Recombinant proteins containing all or some of the IgSF domains of the Lutheran protein have been expressed in eukaryote or prokaryote cells. The purified protein was then used in agglutination inhibition tests, attached to polystyrene plates for detection by an ELISA procedure, or coupled to superparamagnetic particles for detection in a particle gel immunoassay. Alloanti-Lu^a or -Lu^b were detected with high sensitivity and specificity.

6.6 Effects of enzymes and reducing agents on Lutheran antigens

Lutheran antigens are destroyed by treatment of red cells with trypsin or α-chymotrypsin; papain has little effect. Sulphydryl reducing agents, such as AET and DTT, break inter- and intra-polypeptide chain disulphide bonds resulting in the unfolding of the protein. Red cells treated with 6% AET or 200mM DTT at pH8.0 did not react with most Lutheran antibodies tested, including many examples of anti-Lu^a and -Lu^b [1,88,89]. This is to be expected, considering that the Lutheran antigens are located in the disulphide-bonded IgSF domains of the Lu-gps.

6.7 Lu_{null} and anti-Lu3 (LU3)

Lu_{null} phenotype is extremely rare and has a recessive mode of inheritance. Lu_{null} cells lack all Lutheran system antigens. Individuals with the Lu_{null} phenotype may make an antibody, anti-Lu3, which reacts with all red cells apart from those with the Lu_{null} phenotype. Lu_{null} red cells have normal expression of those antigens, such as AnWj, that are expressed very weakly on In(Lu) red cells.

Lu_{null} phenotype was first found in 1963 in an English woman [90], followed by three Lu_{null} members of a Canadian family [91] and two in a Japanese family [92]. The presence of anti-Lu3 in the serum of an African American woman with Lu(a–b–) red cells suggested Lu_{null}, but no family study was possible [93].

The molecular background of Lu_{null} has been identified in five individuals. All are either homozygous or doubly heterozygous for inactivating mutations in the *LU* gene.

1 An English woman [90]. Heterozygosity for two inactivated alleles: one a nonsense mutation 691C>T in exon 6, encoding Arg231Stop (*LU*02N.01*), the other a deletion of exons 3 and 4 (*LU*02N.02*) [94].

2 A Japanese blood donor. Homozygosity for a nonsense mutation 711C>A in exon 6 encoding Cys237Stop

(*LU*02N.03*) [94]. His parents were heterozygous for the mutation.

3 A German woman of Czech origin. Homozygosity for a nonsense mutation 361C>T in exon 3 encoding Arg-121Stop (*LU*02N.04*) [94].

4 A Japanese blood donor. Homozygosity for a 27 kb deletion encompassing exons 3 to 15 of *LU* [95].

5 A pregnant Dutch Caucasian woman. Homozygosity for a dinucleotide insertion, 123GG, in exon 2, converting 42Glu-Val-Met to 42Gly-Arg-Stop [96].

6.7.1 Anti-Lu3 (-LU3)

All Lu_{null} propositi have been found following the detection of an antibody to a high frequency antigen, anti-Lu3. Anti-Lu3 has a single specificity and reacts equally strongly with Lu(a+b–), Lu(a+b+), and Lu(a–b+) cells. Adsorption with Lu(a+b–) cells will remove the activity for Lu(a–b+) cells and *vice versa* [90,92]. Lu3 is present on all red cells that express any Lutheran antigen.

An antibody resembling anti-Lu3 was detected in an African American woman whose red cells behaved like Lu_{null} and were AnWj+ (excluding In(Lu), Section 6.8.2.4), but reacted very weakly with anti-Lu^b, as revealed by an adsorption/elution test. An emergency transfusion with Lu(b+) red cells resulted in no indications of haemolysis and a DAT remained negative [97].

Two patients with ovarian cancer and DAT-positive red cells had potent Lu3-like autoantibodies, one of which was responsible for an HTR [98,99]. The red cells were Lu(a–b+) in one patient and apparently Lu(a–b–) Lu:–3 in the other, probably the result of blocking of the Lutheran antigen sites by the antibody.

Two murine monoclonal antibodies (BRIC 221 and BRIC 224) behave serologically like anti-Lu3. They react with all red cells except Lu_{null} cells [4].

6.8 Lu_{mod} : the In(Lu) phenotype

In(Lu) was the name given for a rare, dominant suppressor of the Lutheran antigens [100] and has subsequently also been used to describe the phenotype. Red cells of most individuals with an *In(Lu)* gene appear to be Lu(a–b–) and Lu_{null} by agglutination tests, but will bind selected Lutheran antibodies, as determined by adsorption and elution tests. The first family showed that the In(Lu) phenotype was dominantly inherited through three generations [101]. Unlike true Lu_{null} individuals, these propositi have usually been found in searches of random donors. Fifty-two propositi of families with dominant Lu_{mod} had 63 Lu_{mod} and 61 not-Lu_{mod} siblings [2,27,102,103]. An analysis of Lu_{mod} x not-Lu_{mod} matings revealed 64 Lu_{mod} and 61 not-Lu_{mod} children. Both counts are very close to the 1:1 ratio expected for dominant inheritance.

Adsorption and elution tests with anti-Lu^a and -Lu^b permitted the determination of the true Lutheran genotype in some In(Lu) members of families, demonstrating recombination between *In(Lu)* and *LU* and, therefore, that *In(Lu)* is not inherited at the *LU* locus [27,28,100].

With two possible exceptions [104], no Lutheran-system antibody has been detected in the serum of any person with an *In(Lu)* gene, because of the weak expression of Lutheran system antigens on the red cells of most In(Lu) individuals. Sera of at least 12 In(Lu) women with not-Lu_{null} children have been tested [27,105].

6.8.1 The molecular genetic background to In(Lu)

Erythroid Krüppel-like factor (EKLF) is an erythroid-specific transcription factor with three zinc fingers homologous to those found in the Krüppel family of transcription factors [106]. EKLF binds the sequence CCACACCCT and *KLF1*, the gene encoding EKLF, is only expressed in bone marrow and spleen [107]. EKLF functions synergistically with the major erythroid transcription factor, GATA-1, and with the ubiquitous Krüppel protein Sp1, to activate transcription of erythroid genes [108]. EKLF is essential for terminal differentiation of erythroid cells and EKLF absence in mice leads to embryonic death from severe anaemia [108,109].

In 2008, Singleton *et al.* [109] demonstrated that In(Lu) resulted from heterozygosity for mutations in *KLF1*, in the presence of a normal *KLF1* allele (Table 6.4). Expression profiles from erythroblasts cultured from In(Lu) individuals revealed a reduced expression of erythroid genes in general, including the genes for the following blood groups: Colton, Lutheran, Ok, Indian (CD44), Duffy, Scianna, MN (glycophorin A), and Diego (band 3). Expression of the Lutheran gene throughout *ex vivo* erythropoiesis was very low in In(Lu) cells, compared with control cells. Singleton *et al.* [109] suggest that this dramatic reduction in Lutheran expression in In(Lu) cells may be related to its transcriptional activation being at a later stage in erythropoiesis than other erythroid-specific genes. No individual homozygous for *In(Lu)* has been found; no surprise considering the embryonic lethality of this condition in mice.

Table 6.4 Mutations in *KLF1* associated with In(Lu).

ISBT symbol	Mutation	Exon	Encoded protein change	References
*KLF1*BGM 01*	–124T>C		Promoter	[109]
*KLF1*BGM 11*	90G>A	2	Trp30stop	[104]
*KLF1*BGM 12*	304T>C	2	Ser102Pro	[104]
*KLF1*BGM 02*	380T>C	2	Leu127stop	[109]
	517–519delC	2	Pro173Pro fs stop64	[110]
	551–556GGACCG>A	2	Gly184Glu fs stop167	[110]
*KLF1*BGM 03*	569delC	2	Pro190Leu fs stop47	[109]
	637C>T	2	Glu213stop	[110]
	802C>T	2	Arg268stop	[110]
	809C>A†	2	Ser270stop	[111,112]
*KLF1*BGM 04*	874A>T	2	Lys292X	[109]
	889T>C	2	Leu300Pro	[110]
*KLF1*BGM 05*	895C>T	2	His299Tyr	[109]
	902inT	2	Arg301Leu fs stop52	[110]
	947G>A	3	Cys316Tyr	[110]
*KLF1*BGM 06*	954dupG	3	Arg319Glu fs stop34	[109,112]
	968C>G	3	Ser323Trp	[110]
	977T>G†	3	Leu326Arg	[112]
*KLF1*BGM 07*	983G>T	3	Arg328Leu	[109]
*KLF1*BGM 08*	983G>A	3	Arg328His	[109]
*KLF1*BGM 09*	991C>G	3	Arg331Gly	[109]
	991C>T	3	Arg331Trp	[110]
	994A>G	3	Lys332Glu	[112]

†Deduced from amino acid changes.

6.8.2 Other effects of *In(Lu)*

Despite a general effect on expression of erythroid genes by *In(Lu)* [109], there are a number of phenotypic effects of *In(Lu)* in addition to suppression of all high frequency Lutheran antigens.

6.8.2.1 P1 antigen

The effect of *In(Lu)* on expression of P1 antigen is less obvious than that on Lutheran antigens. Amongst 236 members of 41 In(Lu) families the distribution of P_1 and P_2 among the In(Lu) members was significantly different from that observed in the not-In(Lu) members and in the general population [27,103,113] (Figure 6.3). The 36 In(Lu) P_1 people may have possessed a strong $P1^+$ allele, or been homozygous for $P1^+$, or both. There is no evidence that P1 antigen is suppressed in recessive Lu_{null} individuals [113]. Three families in which P_2 In(Lu) and P_2 Lu(a–b+) parents have a P_1 Lu(a–b+) child confirm the effect of *In(Lu)* on P1 [27,28,114].

6.8.2.2 i antigen

The monomorphic i antigen is also suppressed by *In(Lu)*, as judged by selected anti-i [27,113,114]. Red cells of neonates have a strong i antigen and this is not dramatically suppressed in red cells of babies with an *In(Lu)* gene [115]. The i antigen was of normal strength in two recessive Lu_{null} people [113]. I antigen is unaffected by *In(Lu)* [113].

6.8.2.3 CD44 and antigens of the Indian system

The CD44 glycoprotein, which is present on a variety of tissues including red cells, carries the antigens of the Indian system (Chapter 21). Expression of CD44, and consequently of the three high frequency Indian antigens, is suppressed by *In(Lu)*, although these determinants are still easily detected on In(Lu) cells [116–118]. *In(Lu)* has little or no effect on leucocyte or serum expression of CD44 [119], reflecting the erythroid-specific influence of

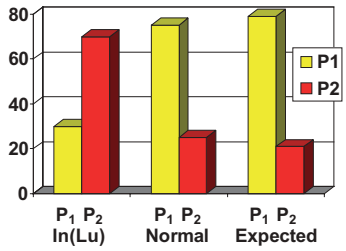

Figure 6.3 Suppression of P1 by *In(Lu)* in propositi and relatives from 41 families [27,103,113], showing the proportion of P_1:P_2 in In(Lu) and normal Lu phenotype family members, and the expected ratio in a normal population.

EKLF. CD44 and In^b are expressed normally on Lu_{null} cells of the recessive type [117,120].

6.8.2.4 AnWj antigen

AnWj is an antigen of very high frequency, which is probably associated with the CD44 glycoprotein (Chapter 21). It is not expressed, or at least is expressed only very weakly, on red cells of individuals with an *In(Lu)* gene. AnWj was initially given the number LU15, but this became obsolete when AnWj was found to be expressed normally on recessive Lu_{null} cells [121]. The one family showing inheritance of AnWj demonstrated recombination between the genes for AnWj and Lu^a [122].

6.8.2.5 Other antigens suppressed by *In(Lu)*

Analysis of a series of families suggested that In(Lu) red cells are more often weak for the Knops system antigens, Kn^a, McC^a, Sl^a, and Yk^a, and for Cs^a antigen, than are red cells from the general population [123]. Antigens of the Knops system reside on the red cell C3/C4 receptor, CR1 (CD35) (Chapter 20).

MER2 is a red cell polymorphism on CD151 (Chapter 23). Strength of expression of MER2 is variable. When MER2 antibodies were titrated with red cells from members of a large three-generation Sardinian family with the *In(Lu)* gene, lower scores were obtained with red cells of nine members with In(Lu) phenotype, compared with those of 12 Lu(a–b+) members [88].

Equine anti-lymphocyte globulin reacts with red cells, but reacts less strongly with In(Lu) cells than cells of common Lutheran type or Lu_{null} cells [124].

Agglutination with concanavalin A lectin of red cells of all five In(Lu) individuals from two families was reduced, compared with cells of the other family members [125]. Band 3, the red cell anion exchanger (Chapter 10), is the major concanavalin A binding protein, so this reduction in binding was interpreted as suggesting an abnormality in glycosylation of band 3 in In(Lu) cells.

6.8.2.6 CD75

CD75 represents a cluster of monoclonal antibodies reacting with lactosaminyl sequences, either sialylated (CD75s) or non-sialylated (CD75) [126]. These antibodies are unique as their reactivity with red cells is enhanced by the presence of an *In(Lu)* gene. Guy and Green [127] showed by haemagglutination tests and radiobinding assays that there was a substantial increase in expression of CD75 on In(Lu) red cells compared with cells of common Lutheran type. Lu_{null} red cells have normal CD75 expression, but Lu_{mod} cells of the X-linked type (Section 6.8.3) are CD75-negative [128]. Protease treatment of red cells did not abolish the CD75 determinant. One CD75 monoclonal antibody reacted with sialidase-treated red cells, but two others did not [128], so both CD75 and CD75s appear to be enhanced by *In(Lu)*. It is not obvious how mutations in *KLF1* could enhance expression of these carbohydrate antigens, apart from removing some masking effect, possibly excessive sialylation, in erythroid cells.

6.8.2.7 Abnormal red cell morphology and electrolyte metabolism associated with *In(Lu)*

Individuals with an *In(Lu)* gene are generally healthy with no obvious anaemia or reticulocytosis, although there may be some association with a degree of acanthocytosis [125,129] and with increased fetal haemoglobin levels [111,112]. Autologous *in vivo* survival of In(Lu) red cells is normal [129].

Osmotic fragility of In(Lu) cells is normal, although incubation of these cells in plasma for 24 hours at 37°C resulted in significant resistance to osmotic lysis compared with cells of common Lutheran type, in which osmotic fragility increases [125]. Before incubation, In(Lu) and control cells had similar concentrations of Na^+ and K^+ ions; during incubation, In(Lu) cells, but not control cells, lost K^+ and, to a lesser extent, gained Na^+ ions. This reduction in total cation content in In(Lu) red cells could explain their relative resistance to osmotic lysis. Significant haemolysis of In(Lu) cells was observed within a few days of storage at 4°C in modified Alsever's solution [129]. This haemolysis could be reduced by the addition of glucose or ATP.

6.8.2.8 Variable effects of *In(Lu)*

The typical phenotype of individuals with an *In(Lu)* gene is Lu(a–b–) with Lutheran system and AnWj antigens only detectable by extremely sensitive methods and P1, i, In^b and some other antigens depressed to a lesser extent. In some families the effect is less extreme, and weakly expressed Lutheran system antigens and AnWj can be detected by direct testing [54,130]. In the person with the *KLF1* promoter sequence mutation, −124TC (Table 6.4), expression of the Lutheran antigens and of In^b was weaker than on normal red cells, but stronger than on most In(Lu) cells, suggesting production of some EKLF [109].

6.8.2.9 Frequency of Lu(a–b–) and In(Lu) phenotypes

In(Lu) is by far the least rare type of Lu(a–b–) phenotype. Analysis of the families of 50 Lu(a–b–) propositi demonstrated that 41 were inherited in a dominant fashion; the genetical background of the other nine could not be determined, but serological tests suggested that most of these were also of In(Lu) type [27,28]. Several large surveys in England and Wales have shown that the incidence of Lu(a–b–), as determined by testing with either anti-Lu3 or with anti-Lu^b and testing the negatives with anti-Lu^a, varies between 0.005 and 0.032% [27,28,90,103] (Table 6.5).

6.8.3 Lu_{mod} of the X-linked type

Five members of a large Australian family had an Lu_{mod} phenotype with serological features characteristic of both In(Lu) and Lu_{null} [134]. The red cells were Lu(a–b–) and lacked the other Lutheran antigens by agglutination tests, yet anti-Lu^b could be adsorbed and eluted from the cells. The cells were AnWj+ and appeared to have slightly enhanced i antigen. They also had weak P1 antigens, although this may have resulted from the presence of a weak $P1^+$ gene in the family. The mode of inheritance of Lu_{mod} in this family showed the features of a recessive X-borne inhibitor gene. All the Lu_{mod} members were males and, although the Lu_{mod} phenotype occurred in successive generations, there was no example of transmission of the phenotype from parent to child. The regulator locus was called *XS*: *XS1* common allele; *XS2* rare inhibitor allele.

The zinc-finger transcription factor GATA-1 plays a central role in erythroid and megakaryocyte development through its interaction with multiple proteins, including EKLF [108]. It is encoded by a gene (*GATA1*) on the short arm of the X-chromosome at Xp11.23. *GATA1* hemizygous male knock-out mice embryos die from severe anaemia, with erythropoiesis arrested at a proerythroblast-like stage [135]. A variety of missense mutations in *GATA1* are associated with macrothrombocytopenia and anaemia and several acquired mutations are associated with malignancies, especially in children with Down syndrome [136].

The X-chromosome location, plus the recognition that the *In(Lu)* gene is the transcription factor EKLF, led Singleton *et al.* [137] to investigate *GATA1* in the Australian family with X-linked suppression of Lutheran. In *GATA1* of the Lu_{mod} propositus they found 1240T>C converting the termination codon (TGA) to an arginine codon (CGA), predicting a GATA-1 protein with an extraneous 41 amino acids at the carboxy terminus. The sister of the propositus, who had normal Lutheran antigens, had the common *GATA1* sequence. The effects of the *GATA1*

Table 6.5 Frequency of Lu_{null} and Lu_{mod} in several populations.

Population	No. tested	No. of $Lu_{null/mod}$	Incidence of $Lu_{null/mod}$	Antibodies used for screening	References
S. London, UK	~250 000	79	~0.0003	Anti-Lu^b (-Lu^a)	[27]
Sheffield, UK	18 069	1	0.0001	Anti-Lu3	[90]
Cambridge, UK	3197	1	0.0003	Not stated	[103]
S. Wales	75 614	15	0.0002	Anti-Lu^b (-Lu^a)	[28]
Houston, USA	42 000	8	0.0002	Anti-CD44*	[125]
Portland, USA	2400	3	0.0012	Anti-AnWj*	[131]
Detroit, USA; African Americans	7314	2	0.0003	Not stated	[132]
Taiwan Chinese	1922	1	0.0005	Anti-Lu^b, -Lu^a	[26,133]

*Only In(Lu) phenotype detected.

mutation in the propositus appear to have gone further than his unusual blood group phenotype: he had a haemoglobin level slightly below normal (122 g/L) and a low platelet count (86×10^9/L) with occasional macrothrombocytes and a history of bruising.

6.9 Acquired Lu(a–b–) phenotypes

A bizarre case is reported of an autoimmune thrombocytopenic purpura (AITP) patient with an antibody resembling anti-Ku, whose red cells had temporarily lost their Kell system antigens [138]. These red cells had normal expression of Lu^a, Lu^b, and LW^a. One year later the Kell antigens had returned to normal and the anti-Ku-like had disappeared, but now the cells lacked Lutheran antigens and the patient had produced an antibody resembling anti-Lu3. Expression of LW^a was also extremely depressed. Lu(a–b+) red cells of another patient with AITP became Lu(a–b–), but retained normal AnWj and LW expression [139]. This patient also had an antibody Lu3-like.

6.10 Distribution, functions, and disease associations

6.10.1 Distribution of the Lu-glycoproteins

Lu^b was not detected on lymphocytes, granulocytes, monocytes, platelets, or the erythroleukaemic cell lines K562 and HEL [1,140,141]. The Lu-gps are widely distributed. They are under developmental control in the liver, with a high level of expression on fetal hepatic epithelial cells during the first trimester. They are also present in placenta, in arterial walls of a variety of adult tissues, including tongue, trachea, oesophagus, skin, cervix, ileum, colon, stomach, and gall bladder, and in the basement membrane region of superficial epithelia and around mucous glands [4]. Both *LU* transcripts were detected in all tissues analysed, with the 2.5 kb transcript, encoding the larger (85 kDa) isoform, predominant except in a colon carcinoma cell line [6].

6.10.2 Functional aspects

The Lu-gps are members of the immunoglobulin superfamily of adhesion molecules, receptors, and signal transducers with five (V-C1-I-I-I) IgSF domains (Figure 6.2). Laminin is a family of extracellular matrix glycoproteins abundant in basement membranes and also present in vascular endothelia. They are heterotrimers composed of three genetically distinct chains, α, β, and γ. At least 12 laminin isoforms exist, derived from combinations of five different α chains, three β chains, and three γ chains [142,143]. Lu-gps bind specifically and with high affinity to the laminin-type globular domains 1-3 of the α5 chains of the two isoforms of laminin that contain α5 chains (LN-511 and -521) [144,145]. Lu_{null} red cells, which have no Lu-gps but normal expression of the other putative laminin binding protein CD44, bind no laminin [146]. Both isoforms of the Lu-gp have the same laminin binding capacity [147,148]. Transfection of human and murine erythroleukaemia cell lines with *LU* cDNA induced binding of solubilised and immobilised laminin [144,146,147]. The laminin-binding site is formed by Asp312 and a surrounding group of negatively charged residues in the region of the flexible linker between IgSF domains 2 and 3 of the Lu-gps (Figure 6.2) [9,10], although Arg175 in IgSF2, the key residue for Lu4 expression (Section 6.4.4), was crucial for binding of a monoclonal antibody that inhibits laminin binding [72]. *N*-glycosylation is not involved in laminin binding [146].

The cytoplasmic domain of the 85 kDa isoform, but not the 78 kDa isoform, contains an SH3 binding motif and five potential phosphorylation sites [4]. Adhesion events involving laminin appear to be controlled, at least in the 85 kDa isoform, by phosphorylation of serines 596, 598, and 621 [7] (see Section 6.10.3). Both isoforms interact directly with αI-spectrin, the main component of the red cell cytoskeleton, through Arg-Lys at positions 573–574 of their cytoplasmic tails [144,149–151]. This interaction with spectrin appears to modulate the adhesive activity of the Lu-gps as disruption of the interaction resulted in weakened linkage to the cytoskeleton and enhanced adhesion of red cells to laminin [150,151]. Phosphorylation of the cytoplasmic tail might weaken its interaction with spectrin, enabling the freely floating transmembrane molecules to cluster and generate a larger adhesive force. Lu-gps in kidney epithelial cells bind non-erythroid αII-spectrin through Arg573–Lys574, mediating actin reorganisation during cell adhesion and spreading on LN-511/521 [152].

During erythropoiesis *ex vivo*, the Lu-gps appear on the erythroid cells at about the orthochromatic erythroblast stage [153,154]. This late appearance correlates with binding of the cells to laminin [147]. The presence of LN-511/521 on the subendothelium basement membrane of bone marrow sinusoids has led to speculation that the Lu-gps are involved in facilitating movement of maturing erythroid cells from the erythroblastic islands

of the bone marrow (see Section 16.8), across the sinusoidal endothelium, to the peripheral circulation [144,153]. No obvious pathology, however, is associated with Lu_{null} or Lu_{mod} phenotypes, in which erythroid cells lack or have very low expression of the Lu-gps.

A mouse gene encoding a protein with 72% identity to human Lu gp binds LN-511/521 [144,155], but murine red cells do not express Lu-gps [156]. Lutheran-null mice are healthy and develop normally, yet, despite apparently normal renal function, 90% of their glomeruli exhibited abnormalities [156].

6.10.3 Disease associations

The IgSF gps expressing Lutheran and LW (Chapter 16) blood group activity are overexpressed on SS red cells in sickle cell disease: SS red cells express about 67% more Lu-gp than normal cells and bind increased quantities of laminin [146–148]. Enhanced binding of the Lu-gps to LN-511/521 on the endothelia of inflamed or damaged blood vessels could contribute to blockage of the vessels and the painful episodes of vaso-occlusion often suffered by sickle cell patients [157]. Although LN-511 and -521 are usually considered unique ligands of the Lu-gps, the integrin $\alpha4\beta1$ or very late antigen-4 (VLA-4) on SS reticulocytes and peripheral blood mononuclear cells may bind Lu-gps on mature red cells and on endothelial cells, contributing to the vaso-occlusion [158,159].

Only SS red cells bind laminin and resist high shear-stress forces, despite the presence of Lu-gps on normal (AA) red cells [146,147]. Protein kinase A (PKA)- or Rap-1-dependent phosphorylation of Lu-gp Ser621 in SS red cells, stimulated by the physiological stress mediator epinephrine through the $\beta2$-adrenergic receptor, could induce conformational changes to the external domains of these proteins, modulating their attraction to their corresponding ligands on endothelial cells [7,157,160,161]. Hydroxyurea, a drug that reduces the frequency and severity of vaso-occlusive crises in sickle cell disease, increased levels of Lu-gp on SS red cells, but decreased their adhesion to laminin by inhibiting phosphorylation [162].

Red cells of two hereditary spherocytosis patients with 40% spectrin deficiency demonstrated enhanced adhesion to laminin under physiological flow conditions [151]. This adhesion, which was completely abolished by soluble Lu-gp, was brought about by impaired interaction between the Lu-gps and the cytoskeleton, rather than by phosphorylation (see Section 6.10.2).

Polycythemia vera (PV) is a chronic myeloproliferative disease in which clonal proliferation of multipotent haemopoietic cells results in an increase in the red cell mass. It is usually associated with a somatic mutation in the gene for JAK2 tyrosine kinase (Val671Phe) and is often complicated by thrombotic events [163]. The Lu-gps are phosphorylated in PV, but not in normal cells under the same conditions [164]. Expression of recombinant JAK2 containing the PV mutation in an erythroid cell line potentiated Lu-gp phosphorylation. As phosphorylation of the Lu-gps increases red cell adhesion, this increased red cell adhesiveness could be a factor promoting thrombosis in PV [164].

B-CAM, the 78 kDa isoform of the Lu-gps, is upregulated following malignant transformation of a variety of cell types [5,157]. For example, it is strongly induced in basal cell carcinomas and squamous cell carcinomas, two of the most frequent human malignancies [165]. Furthermore, laminin was also upregulated in sites surrounding the tumour nests, suggesting that interaction between the Lu-gp and laminin may be involved in progression of epithelial skin cancers.

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