Case Report

A LU:–16 individual with antibodies

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Antibodies against Lutheran blood group antigens have been observed during first-time pregnancy. Samples from a woman of African descent were tested in our immunohematology laboratory on several occasions since 2001. Her samples were phenotyped as Lu(a+b–), and anti-Lu^b was suspected but not identified. She was asked to make autologous donations in preparation for her delivery, which she did. In 2010, two antibodies were identified: anti-Le^a and -Lu^b. Six years later, a third investigation was requested. This time, an antibody directed at a high-prevalence Lutheran antigen was found in addition to the anti-Le^a and -Lu^b previously observed. Her serum was compatible with three out of five Lu(a–b–) reagent red blood cells (RBCs). One of the incompatible Lu(a–b–) reagent RBCs was known to be In(Lu) (*KLF1* mutation). The genetic background of the other reagent RBC was unknown. The *LU* cDNA sequence analysis revealed the presence of the c.230G>A (Lu^a), c.679C>T (LU:–16), and a silent polymorphism c.1227G>T. Anti-Lu16 was highly suspected. This would be the fifth case of LU:–16 with antibodies reported, all within women of African heritage with the Lu(a+b–) phenotype. Hemolytic disease of the fetus and newborn was not noted in these cases. ***Immunohematology* 2017;33:110–113.**

Key Words: Lutheran, high-prevalence antigen, African, pregnancy

Lutheran antigens (blood group system ISBT 006) are carried on type I integral membrane glycoproteins, members of the immunoglobulin super family. According to the International Society of Blood Transfusion (ISBT) Web site, this blood group system comprises 24 antigens.[1] Two additional antigens were presented at the 2016 ISBT meeting in Dubai (LUAC and LUBI).[2] Most Lutheran antigens are of high prevalence. Only four allelic and polymorphic pairs have been found so far: Lu^a/Lu^b, Au^a/Au^b, Lu6/Lu9, and Lu8/Lu14.[3]

The gene encoding the Lutheran antigens, *LU,* spans 12.5 kb, contains 15 exons (14 of which are involved in the coding part) for the shortest transcript, and is located on chromosome 19q13.32 (Unigene NP_005572.2). The molecular basis involved in expression or absence of the Lutheran antigens has been elucidated over the years.[4–10]

In 1980, Sabo et al.[11] presented three cases of Lu(a+b–) women of African descent in whom anti-Lu^b was found along with another antibody against a high-prevalence antigen believed to be in the Lutheran system. This antibody was similar in all three women as well as when tested with Lu(a–b–) cells of the dominant and recessive types. However, the antibody produced by these three women was different from those previously identified. Anti-Lu16 was proposed.

A fourth example was published more recently by Denomme et al.[12] in 2010. It was the case of a young woman of African heritage in her first pregnancy. Her RBCs phenotyped as Lu(a+b–). Anti-Lu^b was found as well as a second antibody against a high-prevalence Lutheran antigen. This patient was found to be homozygous for the c.679C>T (p.Arg227Cys) polymorphism in exon 6 encoding the LU:–16 phenotype. The neonatal outcome of this pregnancy was not associated with any incident.

We here report a fifth LU:–16 case similar to the first four already described.

Case Report

Samples from a woman of African descent born in 1978 were referred for antibody identification during three pregnancies starting in 2001. A fourth sample followed in 2013 and a fifth in 2016. The patient had a total of six pregnancies in 1999, 2001, 2003, 2010, 2013, and 2016. Her phenotype was the following: group A_2B; D+ C– E+ c+ e+ C^w–; M+ N+ S– s+; Le(a–b–); K– Kp(a–); Fy(a–b–); Jk(a+b–); Lu(a+b–). Reagents were from Ortho Clinical Diagnostics (Raritan, NJ), Dominion Biologicals (Dartmouth, Nova Scotia, Canada), Bio-Rad (Montreal, Quebec, Canada), and Lorne (Lower Early, UK).

First Investigation, 2001 (Second Pregnancy)

Two samples were received in 2001: one in March and one in April. The patient was 22 weeks pregnant at the time the first sample was received. The hospital results showed a weak reaction with Lu(a+b+) reagent RBCs tested in gel at 37°C. The results obtained by an immunohematology reference laboratory (IRL) showed no reactivity with three different Lu(a+b–) reagent RBCs in a polyethylene glycol indirect antiglobulin test (PEG-IAT; Immucor, Norcross, GA) and in gel at 37°C (MTS, Ortho Clinical Diagnostics) and showed

positive reactivity with all Lu(a–b+) panel cells in PEG-IAT. All reactions were negative at 4°C; auto control was negative. Titers performed in PEG with Lu(a+b+) reagent and with fresh RBCs were 1 and 128, respectively.

The report sent to the hospital mentioned high-titer, low-avidity (HTLA) reactivity, and anti-Lu^b could not be ruled out. Because the patient had a rare blood phenotype, she was encouraged to make autologous donations in preparation for her delivery. These donations were made in July.

Second Investigation, 2010 (Fourth Pregnancy)

Hospital results on samples submitted in 2010 during her fourth pregnancy showed weak to 1+ panagglutination with two commercial panels (Immucor). The auto control was negative. The IRL tested cells in different media: gel–low-ionic-strength saline (LISS) (Diagast, Loos, France), gel-ficin (Sigma-Aldrich, Oakville, Ontario, Canada), gel-dithiothreitol (DTT) (Sigma-Aldrich), and gel-trypsin, (Sigma-Aldrich). Reactions varied from weak to 2½+, except in gel-trypsin where reactions were weaker. Auto controls were negative. No reactions were seen with nine Lu(a+b–) reagent RBCs, and a 2½+ reaction was observed with three Le(a+b–) Lu(a+b–) reagent RBCs.

In-house polymerase chain reaction–sequence specific primer (PCR-SSP) analyses were performed to confirm the Duffy phenotype (c.125G>A for *FY*01/FY*02* and c.–67T>C for *FY*01N.01*). A polymorphism was found at the GATA-1 binding site at position –67 within the *FY* gene causing the Fy(a–b–) phenotype.

The report sent to the hospital mentioned two antibodies: anti-Le^a and -Lu^b. The "HTLA" reactivity could not be found.

Third Investigation, 2016 (Sixth Pregnancy)

Two samples were sent to the IRL in 2016 during the patient's sixth pregnancy: one in March and one in June (Table 1). The March sample showed 1½+ to 2+ reactivity with five Lu(a+b–) reagent RBCs in gel-LISS and gel-papain (Immucor), 2+ to 2½+ reactivity with two Lu(a+b–) reagent RBCs in gel-DTT, and no reactivity with two Lu(a+b–) reagent RBCs in gel-trypsin. The auto control was negative in all of the media tested. No reaction was observed after alloadsorption onto Lu(a–b+) RBCs.

Testing with cord blood samples showed no reactivity with one of the cells; adult cells were reactive 1+ in saline at 22°C. One cord blood sample was negative in gel-LISS, and one showed very weak reactivity.

Testing with two Lu(a–b–) cell samples showed no reactivity with one sample in gel-LISS and gel-papain. The second cell sample was very weakly reactive in gel-LISS and 2+ in gel-papain (Table 1).

Serum adsorbed with rabbit erythrocyte stroma (RESt, Immucor) was tested with two group A, Lu(a+b–) cell samples in gel-papain with negative reactions and with two group O Lu(a+b–) cell samples in gel-papain in which one of the two

Table 1. Third serology study summary done in 2016

Red blood cells			Reactivity	
Phenotype	Source/Donor #	Method	March sample	June sample
	15-cell in-house panel	Gel LISS	1½+ to 2½+	2½+ to 3+
		Gel papain	w+ to 2½+	2½+ to 3+
Group O, Lu(a+b–)	C5147	Gel LISS	1½+ to 2+	NT
		Gel papain	1½+ to 2+	NT
	04 10 486280	Gel LISS	1½+ to 2+	NT
		Gel papain	1½+ to 2+	NT
		DTT	2+ to 2½+	NT
		Gel trypsin	0	NT
	04 10 492148	Gel LISS	1½+ to 2+	NT
		Gel papain	1½+ to 2+	NT
		DTT	2+ to 2½+	NT
		Gel trypsin	0	NT
Group A, Lu(a+b–)	03 11 650893	Gel LISS	1½+	3+
		Gel papain	1½+	3+
	04 13 792604	Gel LISS	1½+	NT
		Gel papain	1½+	NT
	03 11 870278	Gel LISS	NT	3+
		Gel papain	NT	3+
Group O, Lu(a–b–)	03 10 297468*	Gel LISS	vw+	NT
		Gel papain	2+	NT
	09 012795	Gel LISS	0	NT
		Gel papain	0	NT
	06 12 000021	Gel LISS	NT	0
		Gel papain	NT	0
	06 12 000189	Gel LISS	NT	0
		Gel papain	NT	0
	06 12 001623	Gel LISS	NT	1+
		Gel papain	NT	1+
	Alloadsorptions onto three Lu(a–b+) cells (D1, D2, D3)	Gel LISS	0	NT
		Gel papain	0	NT

*03 10 297468: In(Lu) phenotype. This individual was found heterozygous for *KLF1* c.874A>T (*KLF1*BGM04*). Unknown genetic background for the other Lu(a–b–) cells tested.

LISS = low-ionic-strength saline; w = weak; NT = not tested; DDT = dithiothreitol; vw = very weak.

samples gave a negative reaction. RESt is known to adsorb cold-reactive autoagglutinins, anti-B, -P, and some monoclonal IgM alloantibodies.[13] The initial report sent to the hospital mentioned the presence of anti-Lu^b and a cold agglutinin.

In-house PCR–restriction fragment length polymorphism analyses predicted the following Knops phenotype: Kn(a+b–), McC(a+b+), Sl1/Sl2, Yk^a+. *LU* and *KFL1* mRNA were sequenced. The *LU* sequence showed three homozygous polymorphisms: c.230G>A (p.Arg77His), c.679C>T (p.Arg227Cys), and c.1227G>T (p.Leu409Leu), encoding a LU:1,–2,–16 phenotype. The *KLF1* sequence showed two novel heterozygous polymorphisms: c.544T>C (p.Phe182Leu) and c.894G>T (p.Ala298Ala).

The second sample, received a few months later, showed stronger reactions (2½+ to 3+) in gel-LISS and gel-papain even with Lu(a+b–) reagent RBCs. Auto controls were negative. No reactions were seen with two of three Lu(a–b–) reagent RBCs.

A new report was sent to the hospital stating a known anti-Lu^b and a suspected anti-Lu16.

Discussion

The five LU:–16 cases reported until now have been women of African heritage with a Lu(a+b–) phenotype. According to Reid et al.,[3] these individuals should be transfused with Lu(a–b–) of either dominant [*KLF1* heterozygous mutation or In(Lu)] or recessive types (true Lu_{null}). Our results, however, showed an incompatibility with two of the five Lu(a–b–) reagent RBC samples tested. Blood should be carefully selected before transfusion.

The Erythrogene database[14] shows the highest *LU*01* allele frequency in those of African descent at 0.76 percent and a frequency of 0.14 percent in Caucasians, for a mean allele frequency of 0.22 percent. It also indicates the presence of *LU*01.–16* allele in 0.61 percent of individuals of African origin. The *KLF1* polymorphism c.544T>C (p.Phe182Leu) found in this study was listed in the Erythrogene database in association with other polymorphisms different from the one found in this case [c.894G>T (p.Ala298Ala)]. It is difficult to conclude whether this patient's polymorphism would have an effect on the expression of the Lutheran or other blood group antigens, since her Lu^a typing was normal.

Most Lu(a–b–) cells tested were from the Serum, Cells, and Rare Fluids Exchange (SCARF) without genetic background details, except one known to have a *KLF1* c.874A>T heterozygous polymorphism causing a dominant In(Lu) (p.Lys292Ter, *KLF1*BGM04*).[15] In such a phenotype, Lutheran antigens are weakly expressed and could explain the reactions seen. These cells were not tested with other Lutheran antibodies, however.

This case was referred to us for antibody identification during three pregnancies. Several antibodies were found without any adverse effects on the neonates.

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