Case Report

Anti-A_1Le^b: a mind boggler

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The Lewis blood group system is unique because antigens are neither alleles of the same gene nor are they synthesized by red blood cells (RBCs); rather, they are adsorbed onto the RBC membrane from plasma as glycolipids. Antibodies against Lewis antigens are predominantly naturally occurring immunoglobulin (Ig)M type that sometimes react at 37°C and the antihuman globulin phase. Lewis compound antigens, ALe^b and BLe^b, have been described that were confirmed because of the presence of antibodies against them. These compound antigens are the result of an interaction between *ABO, H, SE,* and *LE* genes. ***Immunohematology* 2021;37:69–71. DOI: 10.21307/immunohematology-2021-010.**

Key Words: Lewis blood group system, compound antigen, ALe^b, immunohematology

The Lewis blood group system (Le) is unique because it is the only system in which the antigens are not synthesized by red blood cells (RBCs); rather, the antigens are passively adsorbed onto the RBC membrane.[1] Le antigens are soluble carbohydrate moieties formed by tissue cells and secreted by body secretions like saliva, where they appear as glycoproteins; in plasma, however, they appear as glycolipids. The Le phenotype depends on ABH secretor status of an individual, although *FUT2* and *FUT3,* the secretor (*SE*) gene and *LE* gene on locus 19q and 19p, respectively, are inherited independently.[2] Genetic interaction also exists between the *LE* and *ABO* genes because the amount of Le antigen detectable on the RBC is influenced by the *ABO* genes inherited, and the products of *ABO* and *LE* share the same precursor substrate. Individuals who are Le(a+b–) are nonsecretors, Le(a–b+) are secretors, and Le(a–b–) can be secretors or nonsecretors. Although individuals who are Le(a+b–) are nonsecretors, Le^a substance is still secreted regardless of secretor status. An individual can be a nonsecretor (*sese*) of ABH and still secrete Le^a into body fluids, producing the phenotype Le(a+) on the RBCs. Frequency of the Le phenotypes in the Indian population are as follows: Le(a+b–) 20.82 percent, Le(a–b+) 60.57 percent, and Le(a–b–) 18.61 percent.[3] Compound antigens in the Lewis blood group system include ALe^b and BLe^b, which are the result of interaction between *ABO, H, SE,* and *LE* genes.[4] Antibody against these compound antigens (e.g., anti-ALe^b) has been shown to react only when RBCs possess both A and Le^b together.[1]

Here, we describe the immunohematology workup of a rare and interesting case of incompatible crossmatches with negative indirect antiglobulin test and the antibody identified as anti-A_1Le^b.

Case Report

A 61-year-old male patient was admitted to a local hospital with complaints of hematemesis, abdominal distension, decreased appetite, weight loss, weakness, and itchy and yellow discoloration of the skin. He was a known chronic alcoholic and was diagnosed with hepatitis C in 2010. Baseline investigations at the time of admission revealed low hemoglobin (Hgb) of 5 g/dL (normal range 13.5–17.5 g/dL for men), deranged liver function tests with markedly raised bilirubin and enzymes, abnormal coagulation profile with raised international normalized ratio, low total protein, low serum albumin, and negative α-fetoprotein. Because of the low Hgb (5 g/dL), a request was sent for packed red blood cell (PRBC) transfusion. The hospital blood bank performed routine antibody screening and reported a positive result with incompatible PRBC crossmatch. Because of limited resources, the patient's samples were referred to the Department of Transfusion Medicine in our hospital for further antibody identification workup, and 2 units of antigen-negative antihuman globulin (AHG) crossmatch-compatible PRBCs were requested. Before his referral, the patient had been transfused with 1 unit of PRBCs 3 years prior and with 1 unit 2 weeks prior.

The patient's RBCs were typed as group A, D+ with a positive autocontrol (2+; AHG phase). Direct antiglobulin tests (DATs) with polyspecific and monospecific AHG reagents were positive (poly = 2+; immunoglobulin [Ig]G = 2+; C3d = 2+). Indirect antiglobulin test (IAT) with a three-cell screening panel was negative. Two randomly selected PRBC units crossmatched by column-agglutination technology (CAT) were found to be incompatible (2+). A repeat crossmatch was performed using a fresh sample with group A, D+; group O, D+; and group A_2, D+ PRBC donor units. Tables 1–6 briefly

Table 1. Basic immunohematologic workup

Testing	Result
Blood group/D type	A_1, D+
DAT	IgG: 2+, C3d: 2+
IAT	Negative
Autocontrol (AHG)*	2+
Anti-A_1 lectin	Positive (4+)
DAT eluate† against A, D+ RBCs	3+
DAT eluate† against O, D+ RBCs	Negative
DAT eluate† against O, D– RBCs	Negative

*Cell separation using microhematocrit tubes.
†Heat elution.
DAT = direct antiglobulin test; Ig = immunoglobulin; IAT = indirect antiglobulin test; AHG = antihuman globulin; RBCs = red blood cells.

Table 2. Crossmatch performed with random PRBC units

Blood group/D type of PRBC unit	Number of units tested	CAT crossmatch	Conventional tube crossmatch	
			RT	37°C
A_1, D+	6	2+	1+	1+
O, D+	6	Compatible	Compatible	Compatible
A_2, D+	2	Compatible	Compatible	Compatible

PRBC = packed red blood cell; CAT = column-agglutination technology; RT = room temperature.

summarize the immunohematology workup performed. (Reagent RBC panel, antisera for phenotyping, CAT gel cards, DAT cards, DTT, enzymes, and 22% bovine albumin were from Bio-Rad, Hercules, CA; Anti-A_1 from J. Mitra, New Delhi, India.) After detailed workup, a suspicion of a compound antibody directed against antigens in two blood group systems (i.e., ABO and Lewis blood group systems) was raised. The offending antibody was identified as anti-A_1Le^b, and 2 units of group A_1, Le(b–) AHG crossmatch-compatible PRBCs were found and issued to the patient (Table 6). No adverse events were reported during or after transfusion.

Discussion

Le antigens are not alleles of the same gene. Their synthesis depends on the interaction of the enzymes (transferases) produced by the *LE* and *SE* genes. The Lewis and secretor transferases fucosylate (add fucose) Type 1 chains, which are predominant types in secretions and plasma and refer to the beta linkage of the first carbon of galactose to the third carbon of *N*-acetylglucosamine (1→3 linkage) residue of the precursor structure.[4] The *SE* gene codes for the enzyme (α1,2-L-fucosyltransferase), which adds terminal fucose to the Type 1 chain to form Type 1H. The *Le* allele codes for α1,4-L-fucosyltransferase, which transfers L-fucose to the Type 1H chain on glycoprotein or glycolipid structures to form Le^b. Small amounts of Le^a are made before the secretor enzyme can add the terminal fucose. If these individuals also have *A* or *B* genes, Type 1H structures will be converted to A or B structures and the Le fucosyltransferase will then produce ALe^b or BLe^b.[1,2]

Le(a–b–) phenotype is not the result of the absence of Lewis antigens on the RBCs caused by the absence of the Lewis gene (*FUT3*), but rather by explicit point mutations in the *FUT3* and other genes.[5,6] Although serologically

Table 4. Inhibition studies performed using saliva

Patient serum antibody	Saliva	Reactivity with A_1, Le(a–b+) RBCs
Anti-A_1Le^b	None	Present
Anti-A_1Le^b	A_1, Le(a+b–)	Present
Anti-A_1Le^b	O, Le(a–b+)	Present
Anti-A_1Le^b	A_1, Le(a–b+)	Inhibited
Anti-A_1Le^b	A_2, Le(a–b+)	Present

RBCs = red blood cells.

Table 5. Testing with 0.1 M DTT-treated serum

Test sample	Reciprocal dilution					Interpretation
	2	4	8	16	32	
Serum + DTT	2+	1+	0	0	0	IgM + IgG
Serum + PBS	2+	1+	1+	0	0	

DTT = dithiothreitol; PBS = phosphate-buffered saline; Ig = immunoglobulin.

Table 6. Crossmatch with phenotype-matched PRBC units

ABO group	Lewis type	CAT crossmatch	CTT crossmatch
A_1	Le(a–b–)	Compatible	Compatible
A_1	Le(a–b+)	Incompatible (2+)	Incompatible (1+)
A_2	Le(a–b+)	Compatible	Compatible
O	Le(a–b+)	Compatible	Compatible

PRBC = packed red blood cell; CAT = column-agglutination technology; CTT = conventional tube test.

Table 3. Extended phenotype (testing on pre-transfusion sample)

D	C	E	c	e	K	Jk^a	Jk^b	Fy^a	Fy^b	M	N	S	s	Le^a	Le^b	Control*
+	+	Neg	Neg	+	Neg	+	Neg	+	+	+	Neg	Neg	+	Neg	Neg	Neg

*22% bovine albumin. Neg = negative.

Lewis antigens are absent on the RBCs, Lewis and ABO substances can be detected in the tissues and secretions of the Le(a–b–) secretors, depending on the secretory status and ABO genotype of the person. The reported prevalence of the Le(a–b–) phenotype in the Indian population is 18 percent.[3]

Our patient had a compound antibody, anti-A_1Le^b, reacting only with RBCs possessing both A_1 and Le^b antigens (i.e., against compound antigen A_1Le^b). Anti-A_1Le^b was first identified by Seaman et al.[7] in 1968 in a patient with blood group A_1B and the Le(a–b–) phenotype. They reported that the antibody reacts with A_1 Le(b+) RBCs but not with RBCs having A_1 or Le^b alone.[7] This antibody of compound specificity was called "Siedler." A literature search on antibodies that react with RBCs containing antigens against two separate blood group systems includes "Magard," which reacts against RBCs with secretor phenotype A_1 Le(a–b–).[7] Tilley et al.[8] in 1975 reported that A, B, and ALe^b substances detected in the lipid fractions are glycosphingolipids that react with anti-A, anti-B, or anti-ALe^b, respectively.

Our patient's sample tested as group A_1, D+ with incompatible crossmatch and negative IAT. Heat elution of DAT+ RBCs reveals an eluate that was reactive with A_1 RBCs (2+; AHG) but was nonreactive against group O RBCs (Table 1). Multiple blood units were crossmatched using CAT and conventional tube testing (Table 2); among them, all group A_1, D+ RBC units were found to be incompatible, whereas all group O, D+ and A_2, D+ blood units were compatible with patient's serum. Ruling out all donor- and patient-related causes of incompatible crossmatch (both at room temperature and 37°C) with a negative IAT, the diagnosis was narrowed down to a compound antibody reacting against two different antigens. Such a compound antibody was identified as anti-A_1Le^b, which reacts only in the presence of both A_1 and Le^b antigens (Table 6).

Lewis antibodies are generally naturally occurring IgM antibodies that sometimes react at 37°C and AHG phase, although weakly, and are enhanced by enzyme treatment. Our patient has mixed IgM + IgG antibody (Table 5), which was clinically significant. Patient Lewis phenotype, performed by cell separation using microhematocrit tubes, was Le(a–b–), as seen in Table 3; a person with this phenotype could produce anti-Le^b. Table 4 shows that reactivity of anti-A_1Le^b with group A_1, Le(a–b+) RBCs can be inhibited with group A_1, Le(a–b+) saliva but not with group O Le(a–b+), A_2 Le(a–b+), A_1 Le(a–b–), or A_1 Le(a+b–) saliva. One group A_1, Le(a+b–) PRBC unit was typed, crossmatched, and transfused to the patient; the post-transfusion period was uneventful.

We find this case interesting, since the immunohematology workup created confusion about the type and nature of the antibody. This case report also highlights the importance of using the traditional crossmatch at the AHG phase because reactivity could be missed with the electronic crossmatch or type-and-screen protocol.

Acknowledgments

We are thankful to our senior residents, Drs. Varuni Yadav, Anupa Pokhrel, Prerna Sachdeva, and Harkiran Arora, for reviewing this manuscript.

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