The second example of Lu:–7 phenotype: serology and immunochemical studies

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We describe the second example of red blood cells (RBCs) with the Lu:–7 phenotype in a 37-year-old Latino female (SA). Her RBCs were nonreactive with anti-Lu7 (Mrs. GA) but were reactive with all other antibodies to high-prevalence antigens tested, including those in the Lutheran blood group system. No Lu:–7 RBCs were available for testing. SA's serum was nonreactive by the indirect antiglobulin test against (1) recessive and dominant Lu(a–b–) RBCs and (2) trypsin-treated or α-chymotrypsin-treated RBCs of common phenotype. By immunoblotting, eluates containing anti-Lu7 from both Mrs. GA and SA reacted with apparently the same bands in RBC membranes of common phenotype as did human anti-Lu^b, reacted weakly with Lu(a–b–) RBCs of the dominant type, and were nonreactive with SA's RBC membranes. These findings raise the Lu7 antigen from its Lutheran-related (para-Lutheran) status to a bona fide member of the Lutheran blood group system. ***Immunohematology*** 1996;12:66–68.

The Lutheran blood group system comprises 18 distinct antigens as recognized by the International Society of Blood Transfusion.[1] The system has four sets of antithetical antigens (Lu^a/Lu^b, Lu6/Lu9, Lu8/Lu14, and Lu18/Lu19) and a number of antigens of very high prevalence. The Lutheran-null phenotype has three genetic backgrounds: homozygosity for the recessive gene *LU,* and at the *LU* locus, heterozygosity for the dominant gene *In(Lu)* at a locus not linked to *LU*, and hemizygosity for the X-linked gene *XS2*. Lu(a–b–) red blood cells (RBCs) of the recessive type do not express Lutheran antigens, while Lu(a–b–) RBCs of both the dominant and X-linked types express inherited Lutheran antigens so weakly that they may require adsorption and elution for their detection. For reviews of the Lutheran blood group system, see Crawford[2] and Daniels.[3]

The high-prevalence antigens Lu4, Lu5, Lu7, Lu11, Lu12, Lu13, Lu16, Lu17, and Lu20 are associated with the Lutheran system by virtue of their weak expression on RBCs with dominant and X-linked types of Lu(a–b–) RBCs and their absence from Lu(a–b–) RBCs of the recessive type. Daniels and Khalid[4] showed by immunoblotting that Lu4, Lu12, and Lu17 are probably located on the Lutheran glycoprotein.[5]

We report in this article the second example of anti-Lu7 and the only available source of RBCs with the Lu:–7 phenotype. The proposita (Mrs. GA), reported in 1972, had a compatible brother, thereby showing that Lu7 is an inherited characteristic.[6] Our immunoblotting experiments indicate that the Lu7 antigen is located on the Lutheran glycoprotein.

Case Report

SA is a 37-year-old gravida 1, para 1, untransfused Latino female who was admitted to the hospital in 1993 for laminectomy. The antibody screening test was positive. The antibody was shown to be directed against a high-prevalence antigen in the Lutheran blood group system. In testing RBC samples known to lack high-prevalence Lutheran antigens that were recovered from liquid nitrogen storage, it became apparent that the Lu:–7 sample (tested and found to be compatible) was from SA when she had been investigated 10 years earlier under her maiden name. At that time, the patient had delivered a baby girl with a negative direct antiglobulin test, even though the cord RBCs were agglutinated by the maternal serum. Therefore, the antibody appeared not to have crossed the placenta. SA does not have family members available for testing, but she donated autologous blood, giving us an opportunity to send samples of the plasma to other laboratories through the Serum, Cell, and Rare Fluid (SCARF) Exchange Program.

Materials and Methods

Standard tube hemagglutination procedures were used throughout. RBCs were treated with papain, α-chymotrypsin, and trypsin as reviewed by Daniels[7], and RBCs were treated with DTT or AET as reviewed by Judd.[8] Acid eluates were prepared (Elu-Kit II, Gamma Biologicals, Inc., Houston, TX) after incubating serum containing antibody with antigen-positive RBCs. RBCs

and sera containing antibodies were from our frozen library, and numerous commercial panels, and through the SCARF Exchange Program.

Immunoblotting was performed as previously described.[9] Briefly, RBC membrane proteins separated by SDS-polyacrylamide gel electrophoresis were transferred onto nitrocellulose paper (NCP) (BioRad, Richmond, CA). After blocking in 5 percent milk in phosphate-buffered saline at pH 7.3 (w/v), the NCP was incubated in a supernate containing monoclonal antibody (BRIC 108, David Anstee, IBGRL, England[5]) or in eluates containing human polyclonal antibody. The NCP was washed and then incubated in peroxidase-conjugated anti-mouse or anti-human Ig (BioRad) prior to the visualization step.

Results

SA's RBCs are group O, D-positive, and are direct antiglobulin test–negative. Her RBCs had unremarkable Rh, Kell, Kidd, Duffy, and Lutheran phenotypes, except for being Lu:–7. The Lu7 typing was performed with serum from Mrs. GA.[6] SA's RBCs were agglutinated by anti-Lu3, –Lu4, –Lu5, –Lu6, –Lu8, –Lu13, –Lu17, and –Lu20. Mrs. GA's RBCs had previously been tested additionally with anti-Lu11, –Lu12, –Lu16, and anti-Au^a, thereby demonstrating the independence of Lu7 from other Lutheran antigens.

SA's serum reacted 1+ to 2+ with all panel RBCs by saline, albumin, papain, LISS, and polyethylene glycol (PEG) indirect antiglobulin test (IAT). The anti-Lu7 (a presumed specificity since it could not be tested with Lu:–7 RBCs) had a titer of 64 and a score of 55. It was nonreactive by IAT using trypsin, and α-chymotrypsin-treated RBCs. The antibody was nonreactive with Lu(a–b–) RBCs of recessive (n = 1) and dominant (n = 5) types and was adsorbed by and eluted from RBCs with common Lutheran phenotypes. The serum agglutinated RBCs with the following phenotypes: Lu:–4, Lu:–5, Lu:–6, Lu:–8, Lu:–13, Lu:–18, Lu:–19, and Lu:–20. Additionally, Mrs. GA's serum agglutinated Lu:–11, Lu:–12, and Lu:–17 RBCs. The presumed anti-Lu7 in SA's serum was of the IgG3 subclass.

The results of immunoblotting are shown in Figure 1. Eluates containing anti-Lu^b or anti-Lu7 from the RBCs of Mrs. GA and SA reacted with a membrane component with an apparent molecular mass of 78,000 to 85,000 Daltons in RBCs with the common Lutheran phenotype. No reaction was obtained in this region with an eluate from SA's RBCs against her own RBC membranes, or against membranes prepared from RBCs with the recessive Lu(a–b–) phenotype.

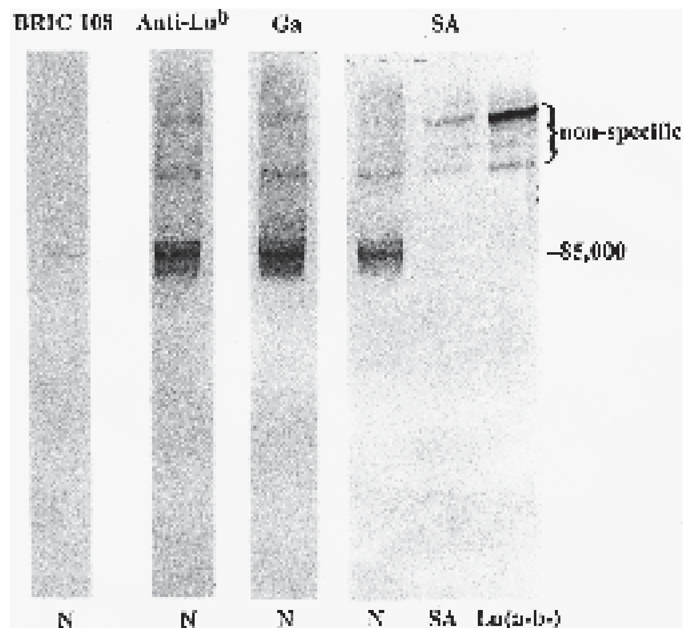

Fig. 1. Immunoblotting with anti-Lu7. Bottom legend: N = membranes prepared from RBCs with the common Lutheran phenotype; SA = membranes prepared from Lu:–7 patient SA; Lu(a–b–) = membranes prepared from RBCs with the recessive Lu(a–b–) phenotype. Top legend: BRIC 108 = murine monoclonal anti-Lu^b; anti-Lu^b = eluate containing human anti-Lu^b; GA = eluate containing anti-Lu7 from the original proposita Mrs. GA, and SA = eluate containing anti-Lu7 from patient SA.

Discussion

The serum from SA is the second example of anti-Lu7 and her RBCs are the only known source of Lu:–7. Immunoblotting located the Lu7 antigen on the Lutheran glycoprotein, thereby raising the Lu7 antigen from its Lutheran-related (para-Lutheran) status to a bona fide member of the Lutheran blood group system.

During the past 2 years, we have made an exhaustive but unsuccessful effort to locate either cryopreserved Lu:–7 RBCs, or to locate Mrs. GA or her compatible brother. If anyone has access to Lu:–7 RBCs, we would welcome the opportunity to test them with SA's serum.

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and her compatible brother. We thank David Anstee from the IBGRL, Bristol, England, for supplying BRIC 108, the anti-Lu^b monoclonal antibody. We also thank Rita Batts for performing the immunoblotting experiments, Robert Ratner for preparing the manuscript, and Telle Huima-Byron for preparing Figure 1.

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