A new form of polyagglutination related to Cad

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Four phenotypes of Cad (Cad 1–4) have been characterized by a continuum of polyagglutinability and reactivity with lectins, with the strongest Cad+ red blood cells (RBCs) being polyagglutinable because of the presence of anti-Cad (anti-Sd³) in most normal sera. Over a period of 7 years, a French male blood donor’s RBCs demonstrated polyagglutinability with 50 percent to 70 percent of normal adult sera. The reactivity was characteristic of anti-Sd³ (refractile agglutination at 4°C, 20°C, 37°C, and anti-human globulin test), and was inhibitable by two examples of Sd(a+) urine, but not by Sd(a−) urine or dialysate from Sd(a+) urine. The donor’s RBCs reacted 1+ with Glycine max, but did not react with Dolichos biflorus, Leonurus cardiaca, Salvia borminum, or Arachis hypogaea. The first four of these lectins were reactive with five of five Cad+ RBCs, including one example of Cad 4 RBCs. Polybrene® aggregated the donor RBCs. Dilutions of nine samples of anti-Sd reacted more strongly with the donor RBCs than with normal RBCs. Even though lectin studies failed to classify this donor’s RBCs as Cad, the persistent polyagglutinability and serologic characteristics are consistent with Cad and demonstrate the heterogeneity of this antigen. Immunohematology 1996;12:69–71.

Cad is a rare, inherited form of polyagglutination. Four phenotypes of Cad (Cad 1–4), described by Cazal et al., represent a continuum of polyagglutinability with normal adult sera and reactivity with Dolichos biflorus. Polyagglutination occurs with the stronger Cad RBCs because of the presence of anti-Cad (anti-Sd³) in most sera. Dolichos biflorus, at a dilution specific for A₁, agglutinates group O and B Cad 1, Cad 2, and Sd(a++) RBCs, and does not agglutinate Cad 3 or Cad 4 RBCs. Cad RBCs are also agglutinated by Salvia borminum, Helix pomatia, and Leonurus cardiaca. Glycine max will react with the stronger examples of Cad.²

Cad RBCs have been called “super” Sid (Sd[a+] RBCs), yet the antigenic relationship of Cad and Sd on the RBC membrane has not been fully established. Cad and Sd share a common immunodominant sugar, N-acetylgalactosamine (GalNAc); the pentasaccharide that includes the GalNAc determinant of Cad is a potent inhibitor of anti-Sd³.

Case Report

Over the course of 7 years, RBCs from a French male blood donor from Bermuda demonstrated polyagglutinability with 50 percent to 70 percent of normal adult sera. The reactivity of random sera with the donor RBCs was characteristic of anti-Sd³: mixed-field (mf) microscopic refractile agglutination at 4°C, 20°C, 37°C (prewarmed), and by the anti-human globulin test; reactivity was enhanced (1+–2+mf) at 37°C with ficin-treated RBCs. Cad polyagglutinability was suspected.

Materials and Methods

A complete antigen profile for the common blood group systems was determined with a variety of blood grouping reagents according to the manufacturers’ directions (Immucor, Norcross, GA; Ortho Diagnostic Systems, Inc., Raritan, NJ; American Red Cross, Washington, D.C.). Serologic tests were performed as described previously.³ Normal adult sera were obtained from blood donors. Cad RBCs were obtained from the Serum, Cell, and Rare Fluid (SCARF) Exchange Program and stored in liquid nitrogen.

Urine neutralization studies were done with urine from two Sd(a+) individuals and one Sd(a−) individual; all three samples had been dialyzed at 4°C for 24–48 hours against phosphate-buffered saline (PBS), pH 7.4. Sera found previously to be reactive with the donor’s RBCs were incubated for 30 minutes at room temperature with dialyzed urine, then retested with the donor’s RBCs. The sera were also incubated in parallel with dialysate from Sd(a+) urine (to control nonspecific inhibition) and/or with PBS.

The donor’s RBCs and five examples of Cad RBCs were tested according to manufacturers’ directions with lectin panel (Gamma Lectin System, Gamma Biologicals, Houston, TX). Dolichos biflorus (F.W. Schumacher Co., Inc., Sandwich, MA) and Leonurus cardiaca (W.J. Judd, Ann Arbor, MI) lectins were prepared according to the method of Judd, and diluted in 11% wt/vol bovine serum albumin (American Red Cross) with 0.1% vol/vol Tween 20 (Fisher Scientific, Pittsburgh, PA).³

Polybrene® (Sigma Chemical Co., St. Louis, MO) aggregation testing to detect decreased N-acetylneuraminic acid (NeuNAc) content was done by the modified method of Steane.⁵

Dolichos biflorus (F.W. Schumacher Co., Inc., Sandwich, MA) and Leonurus cardiaca (W.J. Judd, Ann Arbor, MI) lectins were prepared according to the method of Judd, and diluted in 11% wt/vol bovine serum albumin (American Red Cross) with 0.1% vol/vol Tween 20 (Fisher Scientific, Pittsburgh, PA).⁴
Dilutions of nine examples of anti-Sd from our frozen inventory were incubated for 60 minutes at 37°C with the donor's RBCs, Cad RBCs, Sd(a–) RBCs, and normal group O RBCs (Gamma Biologicals) and tested with a polyspecific anti-human globulin reagent (Gamma Biologicals).

Results

The donor's RBCs typed as group O, C–Cw–D–E –c+e+V–, P1+, M+N–S+s+, K–k+Kp(a–)Js(a–), Fy(a+b+), Jk(a+b–), and Wr(a+).

Seven of seven normal adult sera, previously reactive with the Bermuda donor's RBCs, were tested with and neutralized by one example of Sd(a+) urine. An additional six of six reactive sera were neutralized by both examples of Sd(a+) urine, but not neutralized by the dialsate from the second Sd(a+) urine nor by PBS. Serum reactivity with one example of control Cad 2 RBCs was neutralized by Sd(a+) urine.

The lectin results are presented in Table 1. Polybrene® aggregated the donor's RBCs and Cad 2 control RBCs.

Of the nine examples of anti-Sd tested, one demonstrated prozone with the donor's RBCs but not with Cad 2 or Cad 4 RBCs, or random group O RBCs; one additional anti-Sd was reactive with the donor's RBCs but not with Cad 2 RBCs or random group O RBCs; and the rest demonstrated stronger reactivity with the donor's RBCs than with normal RBCs but not as strong as the 2–3+mf reactivity seen with Cad 2 RBCs.

Discussion

This donor's RBCs express some of the serologic characteristics of Cad but do not fit into the previously described continuum of Cad reactivity (Cad 1>Cad 2>Cad 3>Cad 4). The polyagglutinability of this novel Cad phenotype would classify this donor's RBCs as Cad 1; however, Dolichos biflorus, Leonurus cardiaca, and Salvia borminum lectins were nonreactive. The reactivity with Glycine max indicates some alteration in the RBC membrane structure. The results of the Polybrene® aggregation test indicate that these RBCs do not have reduced NeuNaC. Stronger forms of Cad RBCs react with Glycine max but do not have a markedly reduced NeuNaC content. The ability of Sd(a+) urine to neutralize reactivity with this donor's RBCs is evidence of the antigenic site recognition by anti-Cad present in normal sera. Additionally, Cad RBCs react more strongly with anti-Sd. This donor's RBCs consistently demonstrated stronger reactivity with nine anti-Sd than did normal RBCs. Watkins postulates that the increased reactivity of Cad+ RBCs with anti-Sd may be due to the presence, on Cad+ RBCs, of increased numbers of the oligosaccharide chains that carry the Sd active structure.

This donor's RBCs demonstrate that there may be even greater heterogeneity of Cad than previously appreciated. Since this donor's RBCs do not appear to fit the described continuum, we have not called this Cad 5 reactivity; perhaps CadBer (Ber=Bermuda) would be a better designation for this donor's RBCs.

An additional interesting finding is this donor's RBCs are Wr(a+). In 1973, Lewis et al. described a family whose members also had red cells positive for Wr and “super” Sd.

References

FIBMS, Senior of Blood Transfusion, and Keith Cunningham, MB, BS, FRCPath, Director of Laboratories and Blood Bank, King Edward VII Memorial Hospital, Paget, Bermuda; George Garratty, PhD, FRCPath, Scientific Director, American Red Cross Blood Services, Southern California Region, Los Angeles, California.

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