Report on anti-Di\textsuperscript{b} encountered in two Hong Kong Chinese


Two cases of anti-Di\textsuperscript{b}, a rarely encountered antibody, were identified in serum samples referred by hospital blood banks during the past 13 months. Case 1 is a 41-year-old female who required blood for elective surgery. Case 2 is a premature infant suffering from mild neonatal jaundice on day 2 after birth. The anti-Di\textsuperscript{b} in both cases exhibited marked dosage effect. The titer/score against Di(a+b+) and Di(a-b+) red blood cells (RBCs) in case 1 was 8/10 and 32/32, respectively, and in case 2, 4/18 and 32/46. The monocyte monolayer assay (MMA) also gave a similar pattern of results, being 15 percent and 100 percent reactive when tested with Di(a+b+) and Di(a+b+) RBCs in case 1, and 0.4 percent (within normal range) and 14.4 percent in case 2. The patient in case 1 underwent her operation without blood transfusion. The infant in case 2 was treated by phototherapy and subsequently recovered without the need for exchange transfusion. Immunohematology 1997;13:17–19.

The Diego blood group system consists of two pairs of antithetical antigens, Di\textsuperscript{a} and Di\textsuperscript{b}, and Wr\textsuperscript{a} and Wr\textsuperscript{b}, located on the red cell anion exchange glycoprotein (band 3).\textsuperscript{1} Anti-Di\textsuperscript{a}, which defines the blood group antigen Di\textsuperscript{a}, was discovered in 1956,\textsuperscript{2} whereas anti-Di\textsuperscript{b} was detected in 1967.\textsuperscript{3} Di\textsuperscript{a} is a low-incidence antigen among Caucasians, occurring in less than 1 in 1,000 individuals\textsuperscript{4} but has an incidence of 5 percent to 15 percent in Japanese,\textsuperscript{4} 3.2 percent in Chinese in Taiwan,\textsuperscript{5} and 5 percent in Chinese in Canton.\textsuperscript{6} A survey conducted by the Hong Kong Red Cross Blood Transfusion Service (HKRCBTS) showed that the incidence of the Di(a+) phenotype was 2.99 percent\textsuperscript{7} among local Chinese blood donors, who are predominantly descendants from the southern province of Guangdong, Canton. The Di(a–b–) phenotype has not been found,\textsuperscript{1} therefore, the occurrence of anti-Di\textsuperscript{b} is confined to individuals who are Di(a+b–).

To date, the incidence of Di(a+b–) has not been determined among Hong Kong Chinese. This paper reports findings in two cases of anti-Di\textsuperscript{b} encountered in our laboratory. The ready availability of Di(a+b+) RBCs enabled us to assess the dosage effect and to perform monocyte monolayer assay (MMA) studies. The ability of the MMA for the prediction of severity of hemolytic disease of the newborn (HDN) and its use in assessing the safety of transfusing incompatible blood in these two cases are also discussed.

Case Reports

Case 1

A 41-year-old female was admitted for elective surgery for treatment of a fractured femur. Ten units of blood were found incompatible by a saline indirect antiglobulin technique (SIAT). Previous transfusion history was unknown, though the patient had a history of postpartum hemorrhages.

Case 2

A premature infant of 30 weeks gestation, with a birth weight of 1.365 kg, suffered from mild jaundice on day 2 after birth. His 41-year-old mother had two terminated pregnancies, followed by two full-term infants, with no complications. The infant suffered from progressive jaundice and anemia, and exchange transfusion was contemplated. Using maternal serum for crossmatching, incompatibility by SIAT was encountered with more than ten units of blood tested. The infant was treated by phototherapy and ultimately recovered without the need for exchange transfusion.

Materials and Methods

Red cell antibody identification panels from Ortho Diagnostics Systems, Inc., (Raritan, NJ), Organon Teknika Corp., (Durham, NC), and from the Blood Bank Victoria, South Melbourne, Australia, were used. Polyspecific anti-human globulin (AHG), anti-IgG, and anti-C3b,C3d reagents were purchased from Ortho Diagnostics Systems, Inc. Anti-Di\textsuperscript{a} was obtained from Biotest AG (Dreieich, Germany), while anti-Di\textsuperscript{b} sera were obtained through the Serum, Cells and Rare Fluids (SCARF) Exchange Program, Houston, TX.

Standard techniques, as described in the Technical Manual of the American Association of Blood Banks, were employed.\textsuperscript{8} Investigation of unexpected antibodies was carried out using the SIAT, polyethylene glycol technique (PEG), and 2-stage enzyme indirect antiglobulin test (IAT). A heat elution method was used to dissociate anti-
bodies from sensitized infant’s RBCs in order to perform Di\textsuperscript{a} and Di\textsuperscript{b} typing.\textsuperscript{8} An eluate from the infant’s RBCs was obtained using xylene.\textsuperscript{8} Titration results were scored according to the method of Marsh.\textsuperscript{9} Differentiation between IgG and IgM antibody was determined by dithiothreitol (DTT) treatment of serum.\textsuperscript{8}

The MMA was carried out according to methods of Nance and Schanfield.\textsuperscript{10,11} Briefly, mononuclear cells, freshly isolated from blood donors, were layered onto microscope slides. Di(a+b+) and Di(a–b+) RBCs, sensitized with fresh serum from the patient, were added. The assay was performed in duplicate. Anti-D, (Gamma Biologicals, Inc., Houston, TX), with an IAT titer of 1,600 versus R\textsubscript{2}R\textsubscript{2} RBCs, was diluted 1:10, incubated with D+ RBCs, and the result used as a positive control. A minimum of 200 monocytes were examined microscopically for phagocytosis and/or adherence of RBCs. The results were expressed as a percentage of reactive monocytes with adherent and/or phagocytosed RBCs (percentage of reactivity). Results above 3 percent reactivity are regarded as indicative of a clinically significant antibody.

**Results**

**Case 1**

Reactivity was obtained with the patient’s serum in the SIAT, enzyme IAT, and PEG-IAT with all RBCs in the panel. This observation, together with the negative autocontrol and negative direct antiglobulin test (DAT), indicated the presence of an antibody to a high-incidence antigen. The SIAT was negative when the patient’s serum was tested with three samples of Di(a–b–) RBCs and positive with six samples of in-house Di(a+b+) RBCs by SIAT, confirming the presence of anti-D\textsuperscript{b}. Other antibodies to common RBC antigens were ruled out. The SIAT titer/score against Di(a+b+) and Di(a–b+) RBCs were 8/10 and 32/32, respectively. The patient was typed as Group B, C+c+D+E+e+, Di(a+b–). The anti-D\textsuperscript{b} did not fix complement. Reactivity of the anti-D\textsuperscript{b} in the MMA was found to be 15 percent against Di(a+b+) RBCs, whereas 100 percent phagocytosis/adherence was observed against Di(a–b+) RBCs. In view of the high MMA score, obtained even with the use of Di(a+b+) RBCs, autologous blood transfusion was recommended. The patient, however, underwent surgery without blood transfusion.

**Case 2**

On day 2, the DAT on the baby’s RBCs was moderately strong using anti-IgG. His hemoglobin level was 144 g/L and bilirubin was 118 \(\mu\text{mol/L}.\) The maternal serum reacted with all RBCs in the panel by SIAT and 2-stage enzyme IAT, and the autocontrol was negative. Both the maternal serum and the neonatal eluate strongly reacted with three samples of Di(a–b–) RBCs and were negative with two samples of Di(a+b+) RBCs that ruled out common clinically significant RBC antibodies. Maternal RBCs were typed as Di(a+b–). These serologic findings indicated that the maternal serum contained anti-D\textsuperscript{b}, which had crossed the placenta and coated the infant’s RBCs. The titer/score of the maternal serum against Di(a+b+) RBCs and Di(a–b+) RBCs were 4/18 and 32/46, respectively. The baby’s heat-eluted RBCs were typed as Group A, C+c+D+E+e+, Di(a+b+). Maternal family studies were not possible, but her husband’s blood was available. The antigen typings were as follows: mother, A, C+c+D+E+e+, Di(a+b+); and father, B, C+c+D+E–e+, Di(a–b+). Reactivity of the anti-D\textsuperscript{b} in the MMA was 0.4 percent when tested against Di(a+b+) RBCs and 14.4 percent when tested against Di(a–b+) RBCs (normal = 0–3%). The infant was treated with phototherapy and ultimately recovered without the need for exchange transfusion.

**Discussion**

The majority of reported Di\textsuperscript{b} antibodies appear to be of limited clinical significance,\textsuperscript{1,4,12,13} yet anti-D\textsuperscript{b} occasionally causes moderately severe HDN\textsuperscript{14} and transfusion reactions (TRs).\textsuperscript{4,8} Anti-D\textsuperscript{b} usually shows dosage effect\textsuperscript{5,13,14} and occasionally some Di(b+) RBCs, including those that express a double dose, fail to be agglutinated by a particular anti-D\textsuperscript{b}. Incompatible Di(a+b+) blood has occasionally been used for transfusion to recipients with anti-D\textsuperscript{b} due to the lack of suitable Di(a+b–) or Di(a+b+) units.\textsuperscript{15} There was a case report of an infant with HDN due to anti-D\textsuperscript{b} who recovered after two exchange transfusions with incompatible Di(a–b+) units.\textsuperscript{16}

The MMA often has been found useful in the prediction of the severity of HDN due to anti-D,\textsuperscript{17–20} however, several studies have shown a questionable correlation of MMA results with HDN severity.\textsuperscript{21,22} In view of the rarity of Di(a+b–) and Di(a+b+) blood, we decided that the MMA may have a useful application in our two cases. The anti-D\textsuperscript{b} in both cases reported exhibited a marked dosage effect, as shown by their stronger reaction in the MMA with Di(a+b+) RBCs. It is understood that the application of the MMA for definitive assessment of clin-
ical significance of a particular antibody necessitates careful standardization against examples of that antibody that have caused HDN or TRs. Although such reference standards for anti-D^<b>^> are lacking, the difference in MMA results obtained with RBCs from individuals homozygous and heterozygous for D^<b>^> was so significant in both cases that it was useful in predicting the in vivo lytic potential of both antibodies. MMA reactivity of anti-D^<b>^> in case 1 against D(a+b+) RBCs was 15 percent. This result suggested that even transfusion of D(a+b+) RBCs could be detrimental to that patient, and therefore autologous transfusion was recommended. MMA reactivity of anti-D^<b>^> against D(a+b+) RBCs in case 2 was within normal range at day 2 of birth. The jaundice that the infant developed was mild and he was treated with phototherapy. The mildness of the jaundice correlated with the normal MMA result against D(a+b+) RBCs, the same phenotype as that of the infant’s RBCs. MMA would probably be considered in the determination of HDN in future pregnancies of case 2, bearing in mind that her anti-D^<b>^> might well increase in titer and potency as a result of further stimulation.

Encountering two cases of anti-D^<b>^> within a period of 13 months, with no similar cases reported by the HKRCBTS in the past 10 years, was exciting but could be a mere coincidence. The D(a+) phenotype, in a study done by the HKRCBTS, was found to be 2.99 percent, and an incidence of 5.00 percent was reported in Chinese in Canton. Therefore, with marriage among cousins, individuals who are homozygous for the D^a antigen could develop anti-D^<b>^> as a result of alloimmunization due to transfusions or pregnancies.

References


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