The prozone effect exerted by the complement-binding anti-Le^a on anti-D

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Abstract:
BACKGROUND: Prozone phenomenon is seen with very high-titer antibodies in an immune serum.
AIM: The prozone effect on anti-D by a low-titer anti-Le^a was investigated associated with neonatal jaundice. MATERIALS AND METHODS: Standard methods were used in investigations.
RESULTS: The child was born at full-term developed mild jaundice. With weak direct antiglobulin test+, her indirect serum bilirubin was progressed to 27.5 mg/dL in 48 h. Anti-D and anti-Le^a were detected in the mother. Both these antibodies were detected in the child’s serum though the eluate from red blood cells (RBCs) contained only anti-D. Mother’s anti-D was masked by anti-Le^a if the RBCs possessed both the antigens together. Anti-D was revealed only with D-positive RBCs lacking Le^a or if the serum was modified by mixing with Le^a+ saliva or was heated at 56°C or fortified with citrate solution.
CONCLUSION: An anti-D showed prozone effect exerted by the complement-fixing anti-Le^a in the test.

Key words:
anti-D, anti-Lewis, complement-mediated inhibition, prozone

Introduction

The prozone phenomenon, described as masking of serological reaction in undiluted serum but increased strength of reaction upon its dilution, is characteristically associated with certain hyperimmune anti-Rh and antiglobulin sera. It is due to an excess of antibody molecules in the serum blocks the most antigenic sites in such a way that prevents cross-linking of the red blood cells (RBCs). However, the prozone effect occasionally observed with respect to a high-titer ABO antibodies was thought to be due to a steric hindrance created by the complement molecules fixed on the cell surface following antigen–antibody complex formation. A similar observation was reported involving complement-fixing anti-Jk^a that had suppressed the reactivity of certain antibodies of the Rh blood groups. We document a case, in which the prozone effect exerted upon anti-D through complement-fixing anti-Le^a. As per the best of our knowledge, this is the first case of its kind where the reactivity of a clinically significant anti-D was markedly suppressed by a low titer clinically less significant complement-binding anti-Lewis antibody.

Materials and Methods

Standard serological methods by saline tube/indirect antiglobulin techniques were used in testing throughout. The ABO and RhD blood grouping and antiglobulin reagents were obtained commercially (Span Diagnostic Ltd). The Rh subtyping antisera were from Ortho Clinical Diagnostics. The Lewis typing was carried out using antibodies identified locally using appropriate controls being run in parallel...
to the test. Heat inactivation of complement in serum was carried out at 56°C water bath. Immunoglobulin nature of the antibodies was determined using the dithiothreitol (DTT).[4] In brief, equal volume of serum was mixed with 20 mmol DTT in saline and left at room temperature for 15 m. Hemagglutination inhibition test on anti-Lea was performed by mixing antiserum with an equal volume of Lea saliva (diluted 1:2 in saline) followed by 20 m incubation and further incubating the test for 60 m after adding group O, Lea+ RBCs. To block the complement activation, ionized calcium was chelated from serum by mixing 1 part of 3.8% trisodium citrate solution to 8 part of serum. Antibody elution from the sensitized RBCs was carried out by heat elution method.

Results

Case detail

The mother, a 26-year-old multipara (4th gravida, 3rd para), with neither having history of blood transfusion nor having hemolytic disease of the newborn (HDNB) to her previous children, delivered her 4th female child at full-term pregnancy. The child weighed 2.3 kg with hemoglobin values of 15.4 g/dL at birth. The child showed a sign of jaundice after 24 h of birth that had progressed to a serum bilirubin level of 27.5 mg/dL (indirect being 24 mg/dL) within the next 48 h. The child was treated with an exchange blood transfusion using 535 ml of homologous blood compatible with her mother’s serum by saline, albumin, and antiglobulin methods. The child was grouped as A+, RhD+, CDe/cde (R1R1); direct antiglobulin test (DAT) was weakly positive. Her serum contained anti-D and anti-Lea, reacting at 37°C by saline, albumin, and antiglobulin methods, was considered as being passively transferred from her mother during intrauterine life. However, eluate from the child’s RBCs showed only anti-D reactive by albumin/enzyme/indirect antiglobulin testing (IAT) at 37°C so was considered as the cause of her jaundice. The mother was grouped as A+, RhD-negative (cde/cde, rr), Lea (a−b−), and the antibody screen test (AST) on her serum was positive due to the presence of the alloantibody (ies) reacting weakly at 37°C by the albumin/enzyme/IAT methods.

Her husband was grouped as A+, RhD positive (CDe/ CDe, RrRr), Lea (a+b−) and reacted with her serum by the methods employed.

Further investigations showed that the serum reacted with the RBCs in variable strength and at different phases indicating toward multiple specificities. Her serum caused complement-mediated hemolysis of the enzyme premodified red cells having Lea antigens indicating to the anti-Lea being one of the specificities. The presence of anti-D was revealed when the serum reacted with all the ten examples of the enzyme premodified RBCs typed as RhD+, Lea (a−) but failed to do so with 11 enzyme-treated RhD−, Lea (a−) red cells. Anti-D was also clearly shown upon neutralization of anti-Lea by saliva from individual with Lea (a+b−) phenotype. To obviate complement-mediated hemolysis that interfered in expression of anti-D, her serum decomplemented by heating at 56°C or the complement activation pathway was blocked by adding sodium citrate solution to serum to arrest the ionized Ca++. The results are summarized in Table 1.

Table 2 shows the titer values of the two antibodies present in the mother’s serum by three different serological techniques. Anti-Lea reacted by saline, antiglobulin, and enzyme techniques with the titer values of 4, 16, and 32, respectively. On the other hand, anti-D reacted by antiglobulin and enzyme techniques with the titer values of 128 and 64, respectively. A prozone effect on anti-D was apparent when the test was carried out by antiglobulin method and involving red cells possessing both D and Lea antigens, whereas it was not seen if the D+ red cells had lacked Lea.

Discussion

Prozone phenomenon, characterized by a weak or no agglutination of the RBCs in undiluted serum having antibody but agglutinated upon dilution of the serum, has long been recognized and considered to be due to an excess of antibody molecules that block and so deprive the antigen sites for agglutinating antibodies. The prozone effect is usually observed with the hyperimmune anti-D or antiglobulin serum.[1]

Table 1: The reaction pattern obtained on native and modified with the RBCs of different phenotypes carrying D and Lea antigens

| RBCs | n | Native | Neutralized by Saliva | Heated at 56°C | Mixed with Citrate |
|------|----|--------|-----------------------|----------------|-------------------|
|      |    | Saline | Enzyme | Saline | Enzyme | Saline | Enzyme | Saline | Enzyme |
| D+, Lea+ | 6  | 1+ | H | 0 | 4+ | 3+ | 4+ | 2+ | 4+ |
| D+, Leam | 10 | 0 | 4+ | 0 | 4+ | 0 | 4+ | 0 | 4+ |
| D−, Lea+ | 4  | 1+ | H | 0 | 0 | 3+ | 4+ | 2+ | 3+ |
| D−, Leam | 11 | 0 | 0 | 0 | 0 | 0 | 0 | 0 | 0 |
The prozone effect was reported in the hyperimmune ABO blood group antibodies and was thought to be due to the fixation of the C1 complex resulting through the complement activation.[2,3] As upon activation of the complement pathway through antigen–antibody interaction on the red cell surface, an accumulation of the C1q, C1r, and C1s forms a large macromolecular complex, with a molecular weight of 700,000, and its deposition on the sensitized red cells may cause a nonspecific physical hindrance at the second stage of hemagglutination by maintaining an intracellular distance greater than that can be bridged by the antibodies.[5] Complement-mediated suppression of hemagglutination was also observed on the reactivity of anti-Rh other than anti-D by the complement-fixing anti-Jk+a present in the serum.[9] In the present case, the depressed reactivity of anti-D by coexisting complement-binding anti-Lea deserves the merit to be the first of its kind.

Recently, the role of complement activation as the cause of the prozone phenomenon with respect to the human leukocyte antigen antibody has also been reported.[6,7] This phenomenon of the complement-mediated hindrance on serological reaction was thought not same as the prozone phenomenon because while the prozone effect can be obviated by diluting the serum, it is not prevented by adding ethylenediaminetetraacetic acid [EDTA] to the sample or inactivating the serum by heating at 56°C.[8]

A positive DAT on the newborn child and the concurrent presence of a high-titer antibody in the mother point toward the diagnosis of the HDNB. In the present case, these two features were present though, to our surprise, the AST on the mother’s serum was not that strong as to produce the HDNB. As a matter of fact, the mother’s serum showed a variable reaction pattern with different red cells in the panel indicating to a mixture of antibodies to be present. Serum gave gross hemolysis with the papain enzyme-treated red cells carrying Lea antigen indicating to anti-Lea specificity. The anti-Lea sera that fail to lyse untreated test cells almost always lyse enzyme-treated cells.[9] The presence of anti-D was revealed when,

- The D-positive red cells lacking Lea were used in the test, or
- The patient’s serum mixed with saliva containing Lea substance to neutralize anti-Lea, or
- If the serum was heat inactivated for complement at 56°C, or
- The serum was fortified with trisodium citrate, the Ca++ chelating agent that is essential for complement activation.

As eluate prepared from the child’s RBCs showed the presence of the anti-D, hence was incriminated for the disease. However, a possibility of the IgG anti-Lea as contributing factor would still remain open as the anti-Lea coated red cells might have been hemolyzed and might no longer be present in the child’s circulation. The cord red cells were found to be efficiently hemolyzed by complement-binding anti-Lea.[10] Spitalnik et al.[11] detected IgG anti-Lea in 12 of the 13 cord sera tested by kinetic enzyme-linked immunosorbent assay indicating that the IgG anti-Lea antibodies do cross the placenta though they do not cause HDNB because of the low levels of Lewis antigens on fetal red cells. However, the mild HDNB due to anti-Lea[12] and anti-Le[6,13,14] has been documented in literature.

The clinical relevance of the complement-mediated prozone phenomenon needs to be kept in mind while using clinical laboratory evidence in evaluating the HDNB, as anti-Lewis is largely complement binding and often found among the pregnant women.[11,13]

**Conclusion**

*In vitro* masking on expression of anti-D in serum of the mother, who delivered a child with HDNB, was evidently exerted by the complement-fixing anti-Lea.

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**Conflicts of interest**

There are no conflicts of interest.
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