Case Report

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An autoantibody to Rh-Ce protein causing positive direct antiglobulin test in a healthy blood donor

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Abstract:

A positive direct antiglobulin test (DAT) is of diagnostic feature for the patient with autoimmune hemolytic anemia (AIHA). However, on rare occasions, for obscure reasons, it is found among healthy blood donors. The present report is aimed to elucidate serological and immunological characteristics of such autoantibody in a healthy donor aged 62 years found with positive DAT. There was no history of Leishmaniasis, nor having a significant illness. His red blood cells (RBCs) showed incompatible cross-match results with every recipient tested in the antiglobulin phase. He was found to be DAT+. As his plasma had very little presence of autoantibody, hence was augmented by elution from his in vivo sensitized RBCs for the study. Autoantibody with immunoglobulin IgG showed predominant specificity of anti-Ce. It is certainly a rare case of autoantibody to RhCe compound antigen yet being innocuous in a healthy blood donor with a positive DAT.

Keywords:

Autoanti-Ce antibody, healthy blood donor, positive direct antiglobulin test

Introduction

A positive direct antiglobulin test (DAT) has diagnostic significance for autoimmune hemolytic anemia (AIHA). However, on rare occasions, it is found among healthy blood donors with no sign of clinical disease. While occurring in a frequency of 1:13,000 among healthy individuals,[1] its cause remains obscure with speculations as to being associated with certain infections such as malaria[2] or kala-azar[3,4] or due to premalignant hematological conditions.[5] A presence of complement fraction on the RBC membrane may indicate the complement-fixing immunoglobulin M cold agglutinins or if the immunoglobulin G (IgG) molecule is detected it suggests the warm temperature reacting autoantibodies. In the latter case, serological specificities are found against the antigens of the Rh blood groups.[6] Sometimes autoantibodies with a high affinity to the antigen are found only on the red blood cells (RBCs) membrane with little presence of free antibodies in circulating plasma. In such a situation, one needs to elute the IgG autoantibody molecules from the RBCs for determining the serological specificity. The aim of the present study was to understand the serological and immunoglobulin nature of the autoantibody found in a healthy donor showing a strong DAT+.

Materials and Methods

A donor's blood samples and the eluate prepared from his red cells were used to carry out various serological tests. Rh subtyping was carried out using commercial antisera (Tulip Diagnostics, India) reacting in the saline tube phase. Reagents such as papain and phosphate-buffered saline (PBS) were prepared in house. Standard protocol was

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followed for antibody identification using a commercial cell panel (Immucor, USA). Ether elution method was used to augment antibodies from the donor's RBCs already sensitized *in vivo*. A slight modification in testing the eluate was employed to avoid interference in reading the results due to the dense hemoglobin (Hb) present in the eluate prepared as described earlier.[7] In brief, the reagent RBCs, the eluate, and the low-ionic-strength solution were mixed and incubated in a tube at 37°C for 15 min. The sensitized RBCs washed using PBS were superimposed onto the gel card and centrifuged, and read the result.

Results

The case

A 62-year-old male healthy blood donor never transfused nor had any significant medical history except surgery for a kidney stone. There was no history of Malaria or kala-azar, although he hailed from the region known for kala-azar prevalence. He used to take alprazolam whenever required. Three months before blood donation, he had received the prophylactic dose of the COVID-19 vaccine. He passed through all the criteria for blood donation with parameters such as Hb, 13.0 g/dl; blood pressure, 142/80 mmHg; pulse, 90/min; body temperature, 98.2°F. His peripheral reticulocyte count was found to be 2%. His health status remained uneventful throughout 1 year of the follow-up.

Grouped as B, RhD+ (Rh-subtype Dce/Dce, R_1R_1), his RBCs were found incompatible in the antiglobulin phase during crossmatch test with several homologous recipients. The DAT on his RBCs was positive with the broad-spectrum antiglobulin reagent explaining the incompatibility with the serum/plasma of every recipient tested. His serum/plasma showed very little antibody and the DAT was positive by monospecific anti-IgG but not by anti-C3d, the antibody was eluted from his sensitized RBCs for study purposes. The antibody tested with the RBC panel showed anti-E-like specificity as it reacted with the cells having E antigen but not with those lacking the antigen E [Figure 1]. Interestingly, the eluate tested by the conventional tube method reacted stronger (Grade 4+) with the RBC typed R_1R_1 (DCe/DCe) as compared to that typed rr (dce/dce) in Grade 3–2+ that lead us to perform a titration of the antibody using selected RBCs. The RBCs typed R_1R_1 (DCe/DCe) showed a titer value of 1:64, whereas those with rr (dcd/dce) type showed the titer of 1:4, thereby indicating the preferential specificity as anti-Ce (anti-Rh_1). However, reactivity against the RBC typed rr (dcd/dce) was not separable from that against the RBCs typed R_1R_1 (DCe/DCe) by the selective adsorption method used.

Discussion

A positive DAT on RBCs with broad-spectrum antiglobulin reagent is associated with acquired hemolytic anemia with free autoantibody present in circulating plasma.[8] The use of monospecific antiglobulin reagents is of diagnostic significance as the cold agglutinin disease usually shows a presence of complement fraction on to the RBCs, whereas the other autoimmune conditions involved with the warm temperature reacting autoantibody may show the presence of IgG molecules on the cell surface, thus providing a differential diagnosis and thereby a line of treatment to be administered to the patients. Some rare healthy individuals with a positive DAT were found in a frequency of around 1:13,000,[1] although a higher incidence of 1:1000 donors was also shown when the cases showing weakly positive DAT were included.[9] Antibody in the majority of such donors, including that in the present study, showed the immunoglobulin subclass as IgG1 which is otherwise known to cause clinical hemolysis, yet remained innocuous for unknown reasons. Many times, such healthy donors with positive DAT are noticed during crossmatch tests in the antiglobulin phase[1,10] as was the case with our donor.

Serological specificity of the autoantibody involved was often related to anti-Rh along with anti-Wr^b.[6] Autoantibody specificity in the present case was also found against the RH-Ce protein. While gel column technology showed an anti-E specificity of the autoantibody and the manual tube test, followed by titration of antibody clearly showed differential strength of agglutination with the RBCs typed R_1R_1 (DCe/DCe) and rr (dce/dce), thereby indicating to the dual specificities as anti-Ce (-Rh_1) and anti-E, although both the entities were not separable by selective absorption. This observation reflects on the limitation of the advanced gel card technology against the age-old tube method in distinguishing different specificities in antibody identification exercises. In one study, involving a large number of cases, numbering 150, with positive DAT among the patients with AIHA and α-methyldopa in-takers along as well as the healthy individuals, the authors had observed only one case with the specificity of anti-Ce alongside other specificities that too in the patients having AIHA.[6] Anti-Ce as autoantibody found in normal healthy donors, therefore, merits as the first case of its kind ever to be documented in the literature.

There are scanty reports in the literature on normal individuals with a positive DAT due to the presence of complement or IgG on the RBCs.[1,6,9] A positive DAT associated with malaria was presumed to be a process in the development of immunity to malaria[2] or as nonspecific adsorption of IgG/complement on RBCs in conditions like kala-azar.[3,4] However, the present case did not give a recent history of malaria,

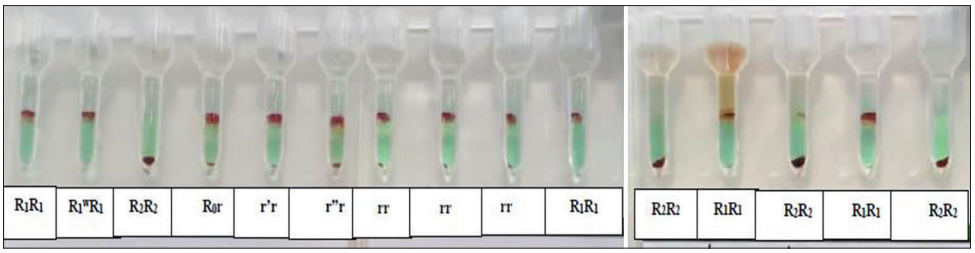

Figure 1: Results on the eluate tested with red cell panel

although a possibility of the asymptomatic subclinical infection of leishmaniasis still remains open as he hails from the region where the endemic kala-azar has been documented.[11] While a positive DAT without any evidence of a hemolytic process is found in patients taking α-methyldopa or a variety of other drugs,[6] the present case had no history of taking α-methyldopa, although he used to take anti-depressant medication whenever required, this drug is not known to cause a positive DAT. The DAT+ healthy individuals have a risk of developing AIHA[1] or hematologic malignancy.[5] However, our donor remained in excellent health even after 1 year of the follow-up period.

Conclusion

A rare case of a healthy blood donor with positive DAT by IgG1 anti-Ce is described. While the case of anti-Ce autoantibody associated with AIHA has been documented, anti-Ce as an autoantibody in healthy individual merits the first of its kind in the available literature.

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Conflicts of interest
There are no conflicts of interest.

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