Letter to the Editors

A reminder that ZZAP reagent removes complement in addition to IgG from coated RBCs

A case was submitted to our laboratory for serologic classification of an autoimmune hemolytic anemia (AIHA). The patient's RBCs were spontaneously agglutinated (i.e., the 6% albumin control was reactive) after washing with room temperature and 37°C saline in preparation for the DAT. Thus, the RBCs needed to be treated with a sulfhydryl reagent (DTT or 2-ME) to disperse the IgM-mediated autoagglutination.[1] The patient's DTT-treated RBCs reacted strongly with anti-C3 and did not react with anti-IgG and a 6% albumin control; they reacted microscopically with anti-IgM. The patient's plasma strongly agglutinated (4+, titer = 4) adult group O RBCs at 37°C (prewarmed), but did not agglutinate cord or DTT-treated autologous RBCs. At 30°C, 18°C, and 4°C, the titer was 16, 128, and 128 with adult RBCs and 4, 32, and 64 with cord RBCs, respectively. The titer with autologous RBCs was consistently one tube less than that obtained with cord RBCs. In our AIHA serum screen,[2] the DTT-treated autologous RBCs incubated at 37°C with the patient's serum did not react while autologous RBCs incubated at 18°C or DTT-treated autologous RBCs that were subsequently enzyme-treated were strongly agglutinated. Additional testing confirmed that the 37°C reactive agglutinin was IgM autoanti-I, not an alloantibody to an antigen not expressed or weakly expressed on cord RBCs. In this patient with hemolytic anemia, the serology was consistent with cold agglutinin syndrome (CAS) associated with moderate titer, high thermal amplitude antibodies.

As Dacie noted in 1962, cold agglutinin titers in patients with CAS are often lower with autologous RBCs than with allogeneic RBCs.[3] This appears to be due to steric hindrance by complement (mainly C3dg) accumulated on the RBCs following exposure to anti-I.[4] Typically, for our serologic classification of CAS, autologous RBCs after washing with 37°C saline give an accurate DAT result; i.e., the cold agglutinin is eluted from the RBCs and the 6% albumin control does not react. On occasion, as was noted in this case, the albumin control is still reactive washing with 37°C saline and sulfhydryl treatment is used to disperse the spontaneous agglutination. When 37°C washing or sulfhydryl treatment is required for the DAT, that same preparation of the patient's RBCs is used for other tests requiring autologous RBCs, e.g., titration studies.

Removing immunoglobulin from coated RBCs with ZZAP (DTT plus enzyme) is an alternative method to sulfhydryl reagents alone for obtaining unagglutinated RBCs suitable for testing other than the DAT. ZZAP was originally reported as an effective reagent for dissociating IgG from RBCs of patients with warm AIHA[5] but immunohematologists often forget that the reagent also removes IgM and complement from coated RBCs.[6] Sulfhydryl reagents when used alone dissociate pentameric IgM through cleavage of disulfide bonds connecting the monomeric subunits but do not remove immunoglobulin or complement.

To check our suspicions that the large amount of complement coating the autologous RBCs was blocking agglutination by the anti-I at 37°C, we treated the autologous RBCs with ZZAP reagent and retested with the patient's plasma at 37°C. The ZZAP-treated autologous RBCs were strongly agglutinated (3+) whereas the DTT-treated RBCs were not agglutinated. When tested with anti-C3, the ZZAP-treated autologous RBCs demonstrated a decrease of reactivity to w+ compared with 3+ reactivity with the DTT-treated autologous RBCs tested in parallel. The results of testing of RBCs from four other patients with either warm or cold serum autoantibodies or both confirmed abolished or decreased reactivity with anti-C3 and anti-IgG after the RBCs were treated with ZZAP.

In this case, the pattern of reactivity of the agglutinin in the initial titer and thermal amplitude studies had the appearance of an alloantibody at 37°C. DTT treatment alone appeared to have disassociated the IgM molecules that caused the spontaneous agglutination detected in the DAT but left C3 and at least some IgM monomers on the autologous RBCs. ZZAP treatment of the RBCs confirmed the autoantibody reactivity at 37°C, presumably because of the removal of C3 from the RBCs and enzyme enhancement of the anti-I reactivity. Enzyme treatment

alone would not have enabled testing of the autologous RBCs as enzymes do not abolish spontaneous agglutination.

References

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