FEATURED CASE #19-02; ANSWERS
(Case study by Jim Perkins, © 2018)

Indian Immunohematology Initiative

1. What Might be causing these serologic reactions? Based on your hypothesis(es) how might you proceed?

   The type-and-screen reveals the patient to be O-pos with a positive antibody screen. The initial panel showed reactivity with all RBCs, and the autocontrol and DAT are negative. An Rh phenotype, done per routine, showed the patient to be R2r.

   When a patient's plasma reacts with all panel donor RBCs there are classically three scenarios to be considered, namely an autoantibody, an alloantibody directed against a high frequency antigen, and multiple alloantibodies with or without concomitant autoantibodies. Today however, one might add two more scenarios, namely method-dependent pan-agglutination when initial testing is by column agglutination ("gel") or solid phase technique and treatment of the patient with a drug such as daratumumab which is an antibody which binds to all RBCs. In this case the reaction strength is the same with all panel cells, and the autocontrol and DAT are negative suggesting that she has an alloantibody against a high frequency antigen or a method-dependent antibody.

   In working up a possible alloantibody against a high frequency antigen one of the first considerations is the patient's ethnicity. Since the patient is Caucasian anti-k is one of the most common antibodies she might make that would react with all cells on many panels, as about 2 in 1,000 Caucasians are k-negative, and Kell system antigens are relatively immunogenic. Although most panels prepared for routine or initial antibody identification would not have a K+k- cell, secondary panels from most manufacturer's will have at least one, so such a cell could be tested. Demonstration that the patient lacks a high frequency antigen is strong evidence that the corresponding antibody is present, and commercial typing sera allow phenotyping for RBCs lacking a number of high frequency antigens including k, Fy3 (Fy $^{a-b-}$), Jk3 (Jk^{a-b-}), U (S-s-), and En^{a} (M-N-). Other relatively common antibodies against high frequency antigens show a "High-Titer, Low-Avidity" (HTLA) type of reactivity, demonstrated by testing serial dilutions of the patient's plasma. Another approach many reference laboratories pursue relatively early in their workup when confronted with an apparent antibody against a high-frequency antigen is to test enzyme- or DTT-treated cells. Finally, method-dependent antibodies can be ruled out by showing that the plasma fails to react in a different test system such as PEG/tube.

   In this case the technologists' first hypothesis was that they might be dealing with method-dependent reactivity, so they ran a selected cell panel by the LISS/tube method.

2. What hypothesis is ruled out by the new information? Given your answer what might you do next?

   All RBCs tested still react by a tube IAT with LISS enhancement. Many gel-dependent antibodies can also be eliminated by replacing the medium the commercial panel cells are provided in with a buffer that does not have the same enhancement agents (in this case the "MTS buffer^{TM}"). Such enhancement agents may be mediating some cases of gel-dependent agglutination.

   Having discarded the possibility of a gel-dependent antibody the technologist decided to determine the patient's extended phenotype.

3. Given this new information what new hypothesis might you make? How would you test it?

   The fact that the patient lacks the high frequency k antigen, makes it very likely that the patient in fact is making anti-k.

   The next step would be to test multiple k-negative cells from current and out-of-date panels.

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4. Is the technologist's hypothesis proven? Is any more work needed? If so, what might we do next?

   The presence of anti-k is essentially proven (3 reactive k-positive cells, 3 nonreactive k-negative cells, patient is k-negative), but the workup is incomplete as anti-S is not ruled out. Presumably the technologist tested all of the k-negative cells they had access to, so an anti-S rule-out cell is not available. In general, the problem of ruling out other, underlying antibodies is shared by all forms of panagglutination.

   Of note, had testing with chemically-treated RBCs (typically ficin/papain and DTT/AET) been pursued as a first step to identify the pan-agglutinin, RBCs treated with the sulfhydryl reducing agent DTT would have been expected to be non-reactive with anti-k because the Kell protein depends on disulfide bonds for its overall configuration. This would have been a clue that the patient's antibody was directed against one of the high-frequency antigens in the Kell system.

   We could also use this principle to rule-out anti-S, since S is NOT destroyed by DTT.

5. Is anti-S ruled out?

   Yes! Treatment of screening cell I with DTT renders it k antigen negative as discussed above. The S antigen is NOT destroyed by DTT, so SCI becomes a k-negative, S+s- rule-out cell for anti-S. So the final criterion for proof that the patient has anti-k is met.

Take home points

The immunohematologic differential diagnosis for a patient plasma reacting with all cells on the initial panel(s).
An approach to ruling out gel-dependent antibodies.
Approaches to identifying an antibody directed against a high frequency antigen.
The need to rule out antibodies underlying an antibody reacting with most RBCs by use of multiple techniques including chemical inactivation of antigens.