FEATURED CASE #19-10; ANSWERS
(Case study by Jim Perkins, 2019)

Indian Immunohematology Initiative

1. What do you think might be going on here? Based on your hypothesis(es) how might you proceed?

   This patient had a negative antibody detection test on three separate samples by a column agglutination test ("gel"), but when 3 allogeneic RBCs were crossmatched by "tube/immediate spin" one donor RBC reacted.

   Explanations that immediately come to mind include:
   (1) one of the 3 initial units labeled as group B was in fact group A or AB and mislabeled as group B (this is why the crossmatches were performed in the first place);
   (2) the patient has a cold-reactive alloantibody that was not detected by the gel method used for the 3 antibody screens;
   (3) the patient has an antibody against a low frequency antigen present on the one donor's RBCs but not on the RBCs used for the screens or on the other two donor's cells;
   (4) the patient has a cold-reactive autoantibody that happened to react more strongly with one donor's RBCs than it did with two others';
   (5) the reactive donor's cells have a positive DAT our other abnormality that is causing them to agglutinate nonspecifically when mixed with the patient's plasma.

2. Now what do you think might be going on? Based on your new hypothesis how might you proceed? Is there anything else you would like the technologist to have done as part of the initial testing?

   The results rule out mislabeling or a positive DAT on the incompatible unit, and the lack of agglutination wit the anti-A and other reagents provide a negative control for spontaneous agglutination due to a high protein medium. They are consistent with a "complete" alloantibody (one that directly agglutinates RBCs without an AHG test) directed against a low frequency antigen on the incompatible donor's cells, the reactions of which are weakened by prewarmed testing. Although somewhat less likely at this point, the patient could also have a cold reactive autoantibody that reacts particularly strongly with the reactive donor's cells for some reason.

   It would have been useful to see an autocontrol with the AHG crossmatches in order to help us distinguish the possibility of an autoantibody from an alloantibody.

3. Do the new findings suggest a hypothesis that can be tested? What tests would you like to do base on your impression from the available results? Is there any testing you think should have been done above?

   Like the positive LISS/tube crossmatch above, these findings suggest the presence of a cold-reactive antibody, although the positive crossmatch reactions are considerably stronger. Again, unfortunately an autocontrol was not done. The technologist typed the patient for Le^a and Le^b, M, N, and P1, presumably suspecting a cold alloantibody of one of the corresponding specificities. The negative P1 phenotype and the fact that 5 out of 6 reactive cells are P1 positive suggest a weak anti-P, but anti-P1 can't explain the weak reaction on room temperature incubation with cell #11. Although the reactions of a cold autoantibody can vary somewhat with different cells, they would be unlikely to vary to this degree.

4. What is your impression now? Is there any data which you would discard from consideration at this point? What would you do now based on your impression?

   The results to this point are conflicting. The initial LISS/tube panel suggested a cold-reactive auto- or alloantibody, with reactions most consistent with anti-P1, but there is no support for this hypothesis from the P1 neutralization results. The cold panel indicates the presence of a cold autoantibody, but it is only detected at 15°C and below whereas the initial panel reacted at RT. When the same panel's RBCs are tested by the gel method the plasma is non-reactive, as are multiple other gel panels. In contrast the patient's antibody reacts strongly by gel with the index donor.

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Taken together the results suggest that the initial LISS panel was over read and was misleading, causing extra work to be done and delaying recognition of what was actually going on. Perhaps the technologist was seeing reactivity from the weak cold autoantibody, the presence of which is within normal limits, but this cold autoantibody doesn't appear to have any relation to the robust reactivity in tubes and gel of the patient's plasma with the index donor! This suggests that we should pursue the possibility of an antibody directed against a low prevalence antigen. Regarding the latter possibility we have some hints; in particular we should look for antibody specificities expected to react at lower temperature as well as in the AHG test, and the antigen appears to be sensitive to ficin.

5. What is your impression now? Are the final data conclusive?

Results of the selected cell panel suggest that the patient has anti-M^g, which identifies a rare allele of M/N (see below). This is consistent with the presentation of this case as a positive abbreviated crossmatch in a patient with a negative antibody detection test.

When an antigen is rare, it is not present on the vast majority screening or panel cells, so the corresponding antibody is only detected by a positive crossmatch with a rare donor or when a child with the antigen is born with a positive DAT or jaundice. Multiple studies have shown that only about 1 in 25,000 antiglobulin crossmatches will be positive due to a potentially significant alloantibody after a negative antibody screen. This is the reason that it is safe to abbreviate or "computerize" the crossmatch when the antibody screen is negative. Usually such unexpected incompatibilities are only detected by a "Coombs" crossmatch, so this case is doubly rare. The abbreviated crossmatch is only needed to detect clerical errors in selection of compatible RBCs. Positive AHG crossmatches are in fact much more common than 1 in 25,000, but the great majority are due to clinically insignificant causes such as a positive donor DAT or recipient cold agglutinin.

Since M^g is an allele of M and N we might consider the patient's M/N typing result to be anomalous. However, anti-N may react with the 26 amino acid sequence at the extracellular terminus of glycophorin B (GYB; the S/s-bearing protein) that is homologous to GYA.N.

Anti-M^g is more common than one would expect given the rarity of the corresponding antigen, occurring in about 1 to 3% of individuals in the U.S. and U.K. (Daniels, Human Blood Groups, pg114.) In this way it is analogous to anti-Wr^a. The antibody is even more common (17%) in a Liberian population, possibly due to a higher incidence of parasitic infection.

6. How would you select RBCs that are safe to transfuse?

The clinical significance of anti-M^g is unknown, but since the patient's antibody reacts strongly with M^g+ RBCs by both tube (with one exception) and gel methods the conservative course would be to crossmatch group B or O RBCs with either one of these methods.

7. What do we know about the antigen the patient's antibody is directed against?

M^g results from a single base pair/amino acid substitution in the N-terminal (extracellular terminal) glycophorin A amino acid sequence of the N-antigen sequence as follows:

GPA.N	*Leu-Ser-Thr-Thr-Glu-*
GPA.M^g	*Leu-Ser-Thr-Asn-Glu-*
GPA.M	*Ser-Ser-Thr-Thr-Gly-*

This amino acid sequence results in decrease of 3 sialoglycans attached to this terminal peptide.

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Take home points:

A rare case presenting as a positive abbreviated crossmatch after a negative antibody screen.

A case in which over reading of saline/tube panel was misleading causing unnecessary testing.

Anti-M^g and the M^g antigen.

The utility of performing an autocontrol even if the DAT is negative.