ABID CASE #27, ANSWERS

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1. What can you say about this problem so far? What possibilities should we consider? What additional testing might you do at this point?

   This patient had a positive antibody detection test (antibody screen), so per routine a DAT and an antibody identification panel were tested. This laboratory routinely performs an extended Rh phenotype on patients with a positive antibody screen, but additional phenotyping was done in this case.

   The plasma reacted at varying strengths with all but two cells in the panel. "Crossing out" on the non-reactive panel cells failed to rule out anti-c, anti-E, anti-K, anti-S, and anti-M. The DAT was non-reactive suggesting that the reactivity was alloantibody(ies). Anti-c plus anti-K plus anti-S or anti-M could have explained all of the positive reactions with the panel cells, but none of these combinations was suggested by the pattern of reaction strengths.

   The phenotype results are suspect due to the transfusion 45 days ago, and the mixed field result in the C type suggests that donor RBCs are indeed present. Nonetheless, the other Rh typing results appear to be much stronger, consistent with the patient being c and E positive. If this is true anti-c and anti-E are ruled out as alloantibodies. But anti-K, anti-S and anti-M cannot explain the reactions with panel cells #6 and 9. Taken together with the pattern of reaction strengths as discussed above, this suggests that the plasma reactivity is not due to common alloantibody specificities.

   One might test the plasma against cells expressing antigens corresponding to the possible specificities from the first panel in isolation, and if possible with double dose expression, so that they could be both "rule-in" and "rule-out " cells depending on their reactivity. It would also be useful to see how the plasma reacts by a tube method to rule out non-specific, gel-dependent reactivity.

2. Did the additional testing help? Can you form a new hypothesis that will lead to further investigation?

   The gel panel cells were selected as discussed in the last paragraph above, and all reacted. Thus they did not help narrow down the possibilities. The saline panel however demonstrated weak (all but one reaction microscopic only) reactivity with most of the cells tested. The non-reactive cells could be regarded as ruling out anti-c, anti-K. anti-M and anti-S, but it could also be that the antibody is directed against a high-prevalence antigen but is just too weak to agglutinate all cells bearing the antigen. Regardless, the reactivity does not appear to be restricted to the gel method.

   Confronted with an antibody directed against a high-prevalence antibody many reference laboratories would try testing the plasma against reactive cells that have been chemically treated with enzymes and a sulfhydryl reducing agent such as AET or DTT. These agents have different effects on antigens of different blood group systems which may give a hint as to the antibody specificity.

   Some antibodies directed against high prevalence antigens show "high-titer, low-avidity" ("HTLA") behavior, the presence of which may also suggest certain specificities. HTLA phenomenon is demonstrated by testing serial dilutions of the plasma, again, against a cell shown to be reactive in previous tests.

3. What do the new test results show? How was the AET control performed and what did it demonstrate? How might we proceed now?

   LISS and PEG additive reagents did not enhance the reactions of this plasma; with both reagents the plasma reactions are still only detected under the microscope ("vw+") with screening cell II. Ficin treatment appears to have eliminated the reaction with SCII at AHG phase, but new reactivity has appeared after 37oC/30' incubation with both screening cells, as well as in the autocontrol, presumably representing reactions of a weak cold autoantibody. This is a common result with ficin-treated cells since ficin removes negative-charge-bearing glycophorins, allowing cold autoantibodies, possessed by most individuals, to agglutinate most RBCs.

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AET treatment of screening cell II did not eliminate the RBCs' reactivity with the patient plasma. Screening cell I ("SCI") expressed the k antigen. Since Kell antigens are destroyed by AET, lack of reactivity with anti-k with SCI after AET treatment demonstrated that the AET worked as expected.

The patient's undiluted ("neet") plasma reacts only weakly in the indirect antiglobulin test, and yet microscopic examination of reactions with her plasma diluted by a factor of 32 still reveal agglutinates. This is classic for an "HTLA".

High prevalence antigens that are destroyed by ficin and resistant to AET include, Ch/Rg (Chido/Rogers), En^a and Gerbich (GE2 and GE4). Anti-Ch/RG is classically an HTLA, so we might hypothesize that anti-Ch/Rg is present. Since Ch/Rg antigen is present in plasma, anti-Ch/Rg is neutralized by incubation with pooled normal plasma and can be demonstrated by a plasma neutralization test.

4. What does the plasma neutralization test demonstrate? What is the identity of this antibody? Why must a dilution control be done?

Incubation of the patient's plasma 1:1 with pooled normal plasma eliminated the reactivity, demonstrating that although the antigen is expressed on RBCs, it is also present in plasma. This is characteristic of antibodies directed against the Chido and Rogers polymorphisms, which are carried on C4, the fourth component of complement. Although the fine specificities of Ch/Rg antibodies are quite complex, use of pooled normal plasma ensures that all will be neutralized. The dilution control demonstrates that loss of reactivity was not simply due to dilution of the antibody.

5. Would donor RBCs reactive with this antibody cause hemolytic transfusion reactions? Has any other type of reaction been associated with this antibody specificity? What do we know about the Ch/Rg antigen(s)?

Anti-Chido/Rogers antibodies do not destroy RBCs bearing the corresponding antigens, corresponding to the fact that they are mostly composed of IgG2 and IgG4. However, some individuals with this antibody (including one from this hospital) have been reported to have severe allergic reactions when receiving plasma-containing components.

Ch and Rg are carried by two homologous and closely linked proteins, C4B and C4A respectively, differing by 4 amino acids in the C4d portion of the C4 protein, which is left covalently linked to the RBC after C4b has been bound by in the classical pathway of complement activation and subsequently inactivated by complement factor I. Presumably, this residual C4d represents slow, non-specific "ticking over" of the classical cascade on the surface of RBCs. Expression of Ch/Rg on RBCs is highly variable.

Other amino acid sequence differences result in additional allotypes of Ch and Rg, and multiple null alleles and hybrid genes exist. These differences are largely academic from the point of transfusion practice, but complete and partial deficiencies of C4 and C4A are associated with systemic lupus erythematosis (SLE) and other autoimmune disorders.