ABID CASE #28, ANSWERS

Case study by Jim Perkins, M.D. (©2009)

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1. What is the probable identity of this antibody? (Hint: look up the use of the IMMUCOR reagent HPC™.)

   The antibody screen was positive by the gel method as were 3 cells on the initial gel panel without a specific pattern. A selected cell panel tested by the PEG/tube method was completely negative. The screening cell reaction was weakened by adsorption of the plasma with Human Platelet Concentrate (HPC™), and the 3 panel cell reactions were eliminated. Neutralization by HPC is consistent with anti-HLA {human leukocyte antigen(s)} antibodies.

   HPC is a preparation of membranes (stroma) of pooled donor platelets that can be prepared in-house or obtained commercially in lyophilized form (this laboratory uses, but does not endorse, HPC manufactured by IMMUCOR). Since platelets strongly express class I HLA, HPC will adsorb class I HLA antibodies from plasma. Therefore, if otherwise non-specific plasma reactivity is removed by incubation with HPC it can be inferred that the reactivity was due to anti-HLA. In making this inference an important caveat must be kept in mind, namely that other RBC-reactive antibodies may be adsorbed by HPC as well, including anti-A, -B, -H, -I, -Le^a, -Le^b, and -PP1P^k as listed in the manufacturer's product insert (see Featured case #19-05). Because of the concern that HPC might adsorb other antibodies, panel cells that were previously reactive but which are non-reactive with HPC-adsorbed plasma should, if possible, not be used as "rule out" cells, and because it adsorbs anti-A and -B, HPC-adsorbed plasma cannot be used for crossmatching.

   HLA antibodies reactive with RBCs, were previously termed Bennett-Goodspeed antibodies or "anti-Bg".

2. Is any further workup needed to prove it? Are there any other methods that could have been used other than HPC neutralization of the antiserum?

   No. All of the common, clinically significant blood group antibodies that must be ruled out in a routine workup have been, and neutralization of the reactions demonstrates the antibody's specificity.

   Chloroquine diphosphate and glycine EDTA destroy class I HLA antigens, and elimination of a RBC's reactivity with patient plasma by one of these treatments has the same implication as its elimination by HPC adsorption.

3. What is the probable source of the immunizing stimulus in this case?

   The most common source of immunization to HLA is pregnancy. A male patient with these antibodies would presumably have been transfused, and transfusion remains common in patients with chronic renal failure even with the availability of erythropoietin. Plasma with class I HLA antibodies are often muotispecific.

4. Does this antibody cause hemolytic transfusion reactions? How would we select compatible RBCs for this patient? Do these antibodies cause hemolytic disease of the fetus and newborn? What other transfusion reactions are caused by anti-HLA?

   Although generally treated as clinically insignificant for RBC transfusion, rare cases of significant hemolysis are reported (see Daniels, "Human blood Groups", chapter 32), so an antiglobulin crossmatch should be performed.

   Anti-HLA antibodies or "leukoagglutinins" were originally demonstrated by Dausset in multiply transfused thalassemia patients with frequent febrile transfusion reactions("non-hemolytic transfusion reactions" or "FNHTRs"), so patients with anti-HLA in RBC compatibility testing are presumed to have a higher risk of these reactions due to lysis of donor leukocytes with consequent liberation of fever-causing cytokines ("endogenous pyrogens"). Today we understand that cytokines may also be released into the plasma/anticoagulant supernatant of blood components, particularly platelets, during storage.

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5. Are there any other transfusion-related problems for which this patient might be at risk?

Anti-HLA antibodies are also responsible for most cases of hig-grade immune-mediated refractoriness to platelet transfusion, and the author has observed the new appearance of anti-HLA in RBC antibody screening in conjunction with development of new platelet refractoriness. Formation of anti-HLA and development of platelet refractoriness can be prevented or delayed by leukocyte reduction of transfused RBCs and platelets.

6. What is the biochemical nature of the antigen?

HLA class I antigens are carried by complex glycoproteins involved in "presenting" antigens synthesized by the same cell, including viral antigens, to various cells of the immune system. Therefore HLA class I antigens are generally considered to be present on all nucleated cells which have the capacity to synthesize proteins (excludes RBCs but not platelets). HLA on RBCs probably represents residual class I HLA that was synthesized on [nucleated] erythroblasts. Class I HLA(s) are in fact variably expressed on the RBCs of a significant number of different individuals. Their frequency of detection depends on the sensitivity of the technique used to detect them, and certain HLA class I specificities are more commonly present on RBCs. (E.G. HLA B7 or Bg^a is detectable by sensitive techniques on RBCs from all individuals with the antigen on their WBCs).

Class I HLA molecules are present on the RBC (or other) surface as heterodimers of a specific α-chain and a β2-microglobulin chain. The α-chain passes through the membrane bilayer 3 times. Chloroquine and EDTA-glycine result in loss of the β2-microglobulin chain with consequent loss of antigenicity as discussed above.