A case report: cold hemagglutinin disease in a pancreatic and renal transplant patient

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Abstract: A 33-year-old white male, 30 days postpancreatic transplant, with a history of juvenile onset diabetes mellitus and previous renal transplant, appeared to have cold hemagglutinin disease (CHD). He was being treated for acute organ rejection and had received two units of red blood cells (RBCs) on postoperative day 11, at which time no serum antibodies were detectable. On postoperative day 30, serum studies showed an autoanti-I with a titer of 512 in 30 percent albumin at 4°C and a maximum thermal amplitude of 37°C. The patient had a weakly positive direct antiglobulin test (DAT) with only complement detectable on the red cells. The patient recovered spontaneously. The etiology of the CHD is unclear. The use of cyclosporin-A, OKT3 monoclonal antibody, and anti-thymocyte globulin (ATG) to treat acute rejection could have played a part.

Cold hemagglutinin disease (CHD) is a type of cold antibody-induced hemolytic anemia where red cell destruction is not always continuous but may be episodic. It may occur as an acute or chronic condition. The acute form is often secondary to a lymphoproliferative disease or to *Mycoplasma pneumoniae* infection. The chronic form is often seen in elderly patients with lymphoid malignancies.[1]

The causative antibody is generally a monoclonal IgM autoagglutinin that readily binds complement and frequently shows specificity within the I blood group system.[2] Antibody titers greater than 500 at 4°C in albumin and thermal ranges reactive up to 30°C or higher may be seen.[3] When such a patient is exposed to the cold, antibody binds to red cells.

Treatment of CHD varies according to the severity of the disease and may include one or more of the following: avoidance of the cold; the use of immunosuppressive drugs to inhibit autoantibody production; and careful "warm" plasmapheresis to remove the autoantibody.

Most patients with CHD tolerate mild or moderate anemia and survive many years with minimal discomfort. However, other patients with more severe CHD may succumb to progressive anemia, hemosiderosis from blood transfusion,[4] or to an underlying malignant disorder.

We recently had the opportunity to study the development of transient CHD in a patient who had received a pancreatic transplant.

Case History

A 33-year-old Caucasian male received a cadaveric pancreatic transplant and was subsequently treated for acute rejection with cyclosporin A, anti-thymocyte globulin (ATG), and OKT3 monoclonal antibody. He had a history of juvenile onset diabetes mellitus and had undergone successful renal transplantation a year earlier. A drop in hemoglobin to 6.8 g/dL caused the patient's physician to order two units of RBCs for transfusion. Pretransfusion testing revealed a potent cold-reactive autoantibody and a positive DAT due to complement bound to the red cells.

The patient's hemoglobin fell again to 6.5 g/dL with a total bilirubin of 4.6 mg/dL (2.0 mg/dL indirect). The uncorrected reticulocyte count was 4.4 percent. There was no hemoglobinuria or hemoglobinemia. A few spherocytes, some basophilic stippling, and polychromasia were evident on a peripheral blood smear.

CHD persisted for less than 10 days. During that period, the patient received four units of RBCs without incident. The pancreatic transplant failed and was removed after 70 days.

Materials and Methods

Tests were performed using blood samples collected and maintained at 37°C. Standard serologic methods were used for ABO and Rh testing.[1] DATs were performed using polyspecific and anti-IgG antihuman globulin (Ortho; Raritan, NJ) and anti-C3b, -C3d (Gamma; Houston, TX). The tests were read at immedi-

ate spin (IS) and after a five-minute incubation at 25°C. An ether eluate was prepared by the method of Rubin[1] and tested with a panel of reagent red cells (BCA; West Chester, PA). The saline supernate from the last wash was tested in parallel as a control.

The patient's serum was tested using a panel of saline-suspended reagent red cells and read at IS, 25°C, and 37°C. A polyspecific reagent was used at the antiglobulin stage. The method utilizing prewarmed samples for panel studies was performed according to standard technique.[1]

Titration studies were performed using serial doubling dilutions of the patient's serum in 30 percent bovine albumin, group O, I+ adult screening cells (BCA) and group O, I− cord cells. Tests were incubated for 30 minutes at 25°C and 4°C, centrifuged, and read for agglutination. The antibody titer was recorded as the reciprocal of the last dilution giving a 1+ macroscopic reaction.

Thermal amplitude studies were performed by testing the patient's serum with reagent screening cells at 25°C, 30°C, and 37°C. The test serum and reagent red cells were incubated separately for 10 minutes at the respective temperatures before mixing. After a 30-minute incubation, the tubes were centrifuged and read for agglutination, washed x4 with 37°C saline, and tested with anti-IgG and anti-C3 reagents.

For the Donath-Landsteiner test, a 1-mL aliquot of freshly drawn blood from the patient was incubated for one hour at 4°C. The sample was then placed in a 37°C incubator for a second hour. A control tube with 1 mL of the patient's blood was maintained at 37°C for two hours. Following the second incubation period, both the test and control tubes were centrifuged for five minutes at 1,000 × g and the sera were examined for hemolysis.

Results

The patient typed as group O, Rh_o(D) negative. Direct antiglobulin tests revealed only complement on his red cells (Table 1). An ether eluate was nonreactive with a panel of reagent red cells.

The patient's serum reacted with all reagent red cells tested at 4°C, 25°C, and 37°C, and at the indirect antiglobulin phase. Using a prewarmed technique, the antibody was nonreactive at the antiglobulin phase using polyspecific antihuman globulin. In titration studies (Table 2), the antibody reacted to a higher titer with adult I+ and autologous red cells than with cord I− red cells, suggesting an autoanti-I specificity. In tests for thermal amplitude, the patient's serum agglutinated and bound complement to reagent red cells at 25°C, 30°C, and 37°C. The Donath-Landsteiner test for detection of a biphasic hemolysin was negative.

Table 1.
Direct antiglobulin tests

Antihuman globulin	vs.	Patient's RBCs
Polyspecific		2+
Anti-IgG		0
Anti-C3		W+

Table 2.
Titration studies in 30 percent bovine albumin

Type of RBC	25°C	4°C
Adult I+	32	512
Cord I−	2	128
Auto	8	512

Discussion

Clinical data and serologic testing of this patient's blood suggested a diagnosis of CHD. Some characteristic serologic findings included a positive DAT due to complement only, an autoanti-I with a titer greater than 500 at 4°C, and maximum thermal range of antibody activity above 30°C.

The pathological autoantibody in CHD differs serologically from the benign form of autoanti-I that can be demonstrated in the serum of many hematologically normal adult individuals. The latter usually have titers of <64 at 4°C and thermal ranges <25°C.[2] A negative Donath-Landsteiner test ruled out an IgG biphasic hemolysin diagnostic of a rare type of antibody-induced hemolytic anemia, paroxysmal cold hemoglobinuria (PCH).

The etiology of CHD in this case is unclear, and the role played by the administration of cyclosporin-A, OKT3 monoclonal antibody, or ATG for organ rejection is unknown. Future reports of CHD in transplant patients may reveal a causal relationship.

References

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BOOK REVIEW

Methods in Immunohematology, W. John Judd. Miami: Montgomery Scientific Publications, 1988. 266 pages, $27.50

Readers familiar with John Judd's *Handbook of Serological Techniques for Use in Investigative Immunohematology* can only be grateful that he has taken the time and trouble to update and improve this useful volume and to shorten its title. Here at last, in a simple format, is a comprehensive list of blood bank procedures useful for problem solving. It includes everything from routine procedures to research methods such as monocyte assays and enzyme-linked antiglobulin tests.

The book is divided into 14 sections that include procedures for antibody identification, the use of enzymes, elutions, the investigation of autoantibodies, and drug-induced antibodies. There are sections on high-titer, low-avidity antibodies, hemolytic disease of the newborn, ABO typing problems, polyagglutination, micromethods, the use of cell separation techniques, reagent preparation and storage, and a miscellaneous section of unusual procedures that would, at the very least, allow the blood banker to determine if and when he or she would need to use these special techniques. There is also a useful directory for equipment, supplies, and reagents.

Each section begins with introductory remarks, and each step-by-step procedure includes its primary application, materials needed, interpretation, and notes. There is also suggested reading and some references, although the references, in most cases, do not refer to any specific area of the text.

Anyone who has labored over a procedure manual cannot help but appreciate the detail and thorough nature of this manual. The methods described in it are, of course, not the only possible ones, but they are methods that are used by workers with extensive experience.

There are a number of useful charts and tables, especially the ones on the serologic characteristics of blood group antibodies, the selection of antigen-negative blood, and a list of drugs associated with drug-induced hemolytic anemia. Some of the procedures refer the user back to earlier procedures, but given the nature of blood bank methods, this is understandable and does save space. The ring binding and cover are appropriate for a book that is designed to be used at the bench. The computer-printed typeface is a little hard to read, but this has allowed a very useful book to be published rapidly and at a reasonable cost.

To an experienced technologist, this book would be a useful how-to manual; however, as the author warns, the book is not a detailed treatise on blood group serology; the novice problem solver would do well to use the listed references at the end of each section as well as to pay particular attention to the "Interpretation" and "Notes" sections. Blood bank educators should not feel that the book is too detailed for their students even though its very comprehensiveness is overwhelming. It should remain an up-to-date resource for blood bankers for many years to come.

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