MANAGEMENT OF PATIENTS WITH PREFORMED REACTIVE ANTIBODIES WHO ARE AWAITING CARDIAC TRANSPLANTATION

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Patients with elevated levels of preformed reactive antibodies to HLA antigens have higher rates of organ rejection than do patients without such antibodies. Consequently, before proceeding with transplantation, many medical centers do prospective cross-matching, that is, mix lymphocytes from the organ donor with sera from the prospective organ recipient, to determine whether a higher rejection rate or an immediate episode of rejection will occur. The problem has been compounded by the increased frequency of preformed reactive antibodies in patients with ventricular assist devices who are awaiting cardiac transplantation. Performing a prospective cross-match can be time-consuming and often is impossible because of the unstable condition of the organ donor or travel logistics, leading to increased costs for transplantation and longer waiting times for recipients. A variety of treatments are used in cardiac transplantation programs in attempts to reduce the concentration of preformed reactive antibodies. Each of these treatments has accompanying complications and considerations for the transplant team. Each treatment must also be assessed for therapeutic response. Options for managing patients with preformed antibodies and a case report are presented. (American Journal of Critical Care. 2005;14:46-51)

The HLA complex is vital in distinguishing self-from nonself-proteins (antigens). Antibodies to HLA do not occur naturally; their development requires exposure to foreign (nonself) HLA antigens. In the past, it was understood that persons who have received blood products or a transplanted organ or who have multiple pregnancies can acquire antibodies to HLA antigens (alloantigens) and become sensitized to the antigens (allosensitized). Recipients of ventricular assist devices (VADs) who do not receive cellular blood products may become allo-sensitized because of an immunological reaction at the blood-VAD interface.1-3 Because of the increased use of VADs as a bridge to transplantation, healthcare teams must manage more patients who are allo-sensitized.4

PATIENTS WHO RECEIVE A VENTRICULAR ASSIST DEVICE (VAD) MAY DEVELOP ANTIBODIES TO HUMAN LEUKOCYTE ANTIGENS (IE, BE ALLOSENSITIZED) THROUGH AN IMMUNOLOGICAL REACTION AT THE BLOOD:VAD INTERFACE.

To determine if a patient is allo-sensitized, a lymphocytotoxicity assay is done to measure the serum level of preformed reactive antibodies. In this assay, a sample of the patient's serum is mixed with lymphocytes from persons representing the most common HLA antigens. Each reaction is measured and quantified as a percentage of total samples tested. In other words, the sum of reactions determines the probability of having antibodies that will react to the HLA antigens of a random donor. If a patient has an elevated level of preformed reactive antibodies, usually defined as a reaction to greater than 10% to 15% of the usual HLA antigens, the patient is considered allo-sensitized. Although some

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debate exists about the significance of elevated levels of preformed reactive antibodies in patients awaiting transplantation, evidence suggests that patients who are allosensitive have higher rejection rates and poorer outcomes than do patients who are not allosensitive.4-8

The test for allosensitivity measures preformed reactive antibodies. A reaction to greater than 10% to 15% of antigens used is considered positive.

When a B lymphocyte is exposed to an HLA antigen, the lymphocyte becomes sensitized to the antigen. Upon reexposure to the antigen, the lymphocyte divides into a memory B lymphocyte and eventually differentiates into a plasma cell. The plasma cell synthesizes alloantibodies, antibodies specific for the sensitizing HLA antigen (Figure 1), that can kill cells bearing the antigen. Upon reexposure to the sensitizing HLA antigen, the memory cell remains in circulation, ready to respond to reintroduction of the antigen. Upon reexposure to the sensitizing HLA antigen, the memory cell quickly goes through this sequence again. Preformed cytotoxic alloantibodies, or antibodies against HLA, are left in circulation. The goal for managing a patient who has such antibodies is to markedly reduce the concentration of circulating cytotoxic alloantibodies and antibody-producing cells or to eliminate the antibodies and the cells.9

In many transplantation programs, the healthcare team chooses to wait for a prospective negative donor-specific cross-match before performing transplantation surgery on a patient who has a level of preformed antibodies greater than 10% to 15%. A tissue laboratory can perform a cross-match before (prospectively) or after (retrospectively) transplantation. Although use of prospective cross-matching is routine in kidney transplantation, in heart transplantation, it is usually reserved for patients who have elevated levels of preformed reactive antibodies.

The results of cross-matching assays are usually available in about 4 to 6 hours. An assay is positive if the lymphocytes from a potential organ donor are killed by antibodies in the potential organ recipient’s serum. If a cross-match is positive, no transplantation is done; if the cross-match is negative, then transplantation can proceed.

Each positive cross-match prolongs a patient’s time waiting for a transplant. In one center in 1999, the mean waiting time for an allosensitized patient to be matched with an acceptable donor (ie, a negative cross-match) was 7.1 months, whereas the waiting time for a potential recipient who was not allosensitized was 3.1 months.10 Because of the time involved, each cross-match can jeopardize the stability of the donor’s condition, add time to the wait of all the other recipients awaiting the organ transplants (the lungs, kidneys, and so on), increase costs, and limit the geographic region from which a potential donor can come.

A variety of strategies are available to reduce the level of cytotoxic alloantibodies in the circulation in preparation for transplantation of an organ. Reducing the level of antibodies cytotoxic to cells bearing HLA antigens increases the likelihood that the transplanted organ will remain a functioning graft, reduces rejection, and decreases the number of positive cross-matches.

Methods to Reduce the Formation of Circulating Cytotoxic Alloantibodies

Several methods are available to prevent the formation of preformed reactive antibodies to HLA antigens. Transfusions of blood products are a frequent cause of the formation of these antibodies. Unfortunately, placement of a VAD is associated with significant blood loss, especially in patients who have had previous sternotomies and thrombocytopenia. The alloantibodies form when a recipient’s T cells detect foreign antigens on nucleated cells. Platelets and red blood cells are not nucleated, but these blood products often contain small numbers of white blood cells,
which are nucleated. Whenever possible, transfusions of all blood products should be limited. Transfusions may not be avoidable, and in these instances, nucleated white blood cells can be removed by using special leukocyte reduction filters.2 Although filters add cost to the transfusion and can theoretically slow the infusion rate, they are 99.9% effective in eliminating nucleated cells (and have the added bonus of eliminating cells infected with cytomegalovirus) and have now been manufactured to allow the infusion of a unit of packed cells in less than 5 minutes.11 Otherwise, to prevent the interaction between T cells and white blood cells, azathioprine or mycophenolate mofetil can be given in conjunction with the red blood cell transfusion. Both of these medications prevent lymphocyte proliferation and therefore may limit the formation of antibodies to HLA antigens.12

**Methods to Reduce the Concentration of Circulating Cytotoxic Alloantibodies**

Intravenous immune globulin (IVIg) is a preparation used in the treatment of allosensitized patients.13,14 Immunoglobulin is collected from pooled plasma from random donors. IVIg most likely blocks the antibody receptors of the target cells (ie, the cells with HLA antigens), thus inhibiting complement-mediated cytotoxicity by alloantibodies.

Apheresis decreases the concentration of cytotoxic alloantibodies. After cardiac transplantation, apheresis is a known treatment for humoral rejection, that is, rejection mediated by B lymphocytes and evidenced by the perivascular presence of immunoglobulin and complement.6 The concentration of cytotoxic alloantibodies is decreased by dividing blood into its components by centrifugation, removing plasma cells, and replacing the cells with fresh-frozen plasma or albumin. This therapy is often used in combination with cyclophosphamide to inhibit proliferation of B lymphocytes.10,12 Cyclophosphamide is commonly used in diseases in which depletion of rapidly dividing cells is desired.3 In allosensitized patients, cyclophosphamide is administered to selectively inhibit B-lymphocyte proliferation and inhibit the immune cascade, which leads to cytotoxic effects.12,15 Mycophenolate mofetil and cyclophosphamide have similar properties. However, compared with cyclophosphamide, mycophenolate mofetil is easier to use, can be given orally twice a day, and has less severe side effects.

**Adverse Events and Management Considerations**

Therapies directed at decreasing the concentration of cytotoxic alloantibodies are associated with a variety of adverse events, which are sometimes serious.

**Infection**

By reducing the number of circulating B cells and the serum concentration of immunoglobulin, therapy to reduce the concentration of cytotoxic alloantibodies can predispose patients to infectious complications. Not unexpectedly, both cyclophosphamide and apheresis can predispose patients to infection. Patients who receive cyclophosphamide can also become neutropenic because of generalized myelosuppression. Patients with an absolute neutrophil count less than 0.50 x 10^9/L are at a particularly increased risk. The degree of neutropenia can be managed by adjusting the dose of cyclophosphamide.15 However, the nadir of neutropenia caused by cyclophosphamide generally occurs 7 to 10 days after treatment, thus predisposing patients to potentially prolonged periods of marked neutropenia. Apheresis can predispose a patient to infection because each exchange procedure during apheresis reduces the concentration of circulating IgG by 70%. This reduction as well as a 30% reduction in complement can put a patient at increased risk for bacterial infections.16,17

**Renal Dysfunction**

The use of IVIg therapy in patients with heart failure can cause reversible acute renal insufficiency.18,19 Between 1981 and 1998, the Food and Drug Administration received 114 reports describing acute renal dysfunction in patients treated with this product. Furthermore, acute renal dysfunction was associated with 17 deaths.20 Predisposing factors such as diabetes mellitus, preexisting acute renal insufficiency, or hypertension place patients at increased risk for acute renal insufficiency. The mechanism may be related to IVIg products that contain sucrose. Renal histopathological studies of patients with acute renal insufficiency after IVIg therapy reveal an osmotic injury to the proximal...
renal tubules. Injury to the proximal renal tubules is similar to injury due to sucrose therapy. Recommendations for avoiding renal dysfunction in patients awaiting cardiac transplantation who are receiving IVIg therapy include the following:

- Identify patients at risk for acute renal insufficiency. This category includes any patient with preexisting renal insufficiency, diabetes mellitus, age greater than 65 years, volume depletion, sepsis, or paraproteinemia and patients who are receiving known nephrotoxic drugs.
- Ensure that the patient is well hydrated (a requirement that can be difficult in patients in unstable condition with advanced heart failure).
- Dilute the dose of IVIg, do not exceed the recommended dose, and infuse the drug at a slow rate. The goal of therapy is to administer the lowest concentration at the slowest rate practical. The maximum infusion rate should not exceed 3 mg of sucrose per kilogram of body weight per minute.
- Monitor serum levels of urea nitrogen and serum creatinine and reassess periodically. Should these renal indices deteriorate, consider discontinuing IVIg therapy.

Anemia
Cyclophosphamide causes generalized myelosuppression. Renal insufficiency associated with IVIg can result in decreased production of erythropoietin and result in anemia. Apheresis may cause marked hemolysis of red cells. Because infusion of blood products should be avoided, administration of erythropoietin may be a preferable treatment for anemia.

Electrolyte Disturbances
Ionized calcium binds to the anticoagulants used in the extracorporeal circuitry for apheresis and potassium is lost during apheresis. Fifteen percent of patients undergoing apheresis have symptomatic hypocalcemia; signs and symptoms include tingling around the lips and fingertips, muscle spasms or cramps, tetany, heart failure, and cardiac arrhythmias. Hypokalemia results in muscle cramps and spasms, arrhythmias, and death. Electrolyte levels should be monitored, and patients should report any signs or symptoms suggestive of electrolyte imbalance. Often calcium gluconate and potassium chloride are infused during apheresis therapy.

Nausea
Fluid replacement during therapy may cause such a dysequilibrium that patients experience a vasovagal episode with concomitant bradycardia and hypotension. Almost every patient experiences nausea to some degree during therapy. Antiemetics administered before apheresis therapy may reduce the degree of discomfort from nausea. Antihistamines may also be administered to relieve nausea that may be associated with the feeling of motion sickness.

Bleeding Disorders
Cyclophosphamide causes generalized suppression of bone marrow and can result in thrombocytopenia. Thrombocytopenia is also associated with apheresis and is due to deposition of platelets in the extracorporeal circuitry. After a single exchange of plasma, platelet counts can be decreased by as much as 30%. The removal of clotting factors, fibrinogen, and platelets leads to elevated prothrombin, partial thromboplastin, and thrombin times. When hemolyzed red blood cells are reinfused, disseminated intravascular coagulopathy can be triggered. Platelet counts and bleeding times should be monitored. Selecting fresh-frozen plasma, platelets, cryoprecipitate, and specific clotting factors rather than other solutions for volume replacement can minimize some of the aforementioned complications. Also, clinicians should be aware that hypercoagulation may occur during apheresis. Ironically, hypercoagulation may be due to low levels of antithrombin III. The levels may return to normal by 24 hours after apheresis, but the biological activity of antithrombin III can remain compromised for 48 hours. Cases of pulmonary emboli and thrombosis after apheresis have been reported.

Case Study
JB was a 52-year-old man with type 2 diabetes mellitus and a history of congestive heart failure. He had undergone mitral valve replacement with a Starr-Edwards valve in 1982 and a Medtronic valve in 1991. On April 28, 1999, he was admitted to the hospital because of dizziness. He had decreased renal function with a serum creatinine level of 318 µmol/L (3.6 mg/dL) and a hematocrit of 0.21. His condition improved with administration of intravenous fluids, 2 units of packed red blood cells, and dopamine and dobutamine therapy. The serum level of creatinine decreased to 80 µmol/L (0.9 mg/dL), and the hematocrit increased to 0.30.

A blood sample for measurement of preformed reactive antibodies was collected on June 2, and his sera reacted to 99% of the most frequently encountered HLA I and II antigens in the general population.
JB received more packed red blood cells on June 5, 8, and 11. In an attempt to reduce the level of preformed reactive antibodies, therapy with IVIg was started on June 11, and a protocol to reduce the concentration of preformed reactive antibodies was initiated (see Table). IVIg was infused at a dose of 0.5 g/kg per day for 4 consecutive days.

During the course of the week, JB gained weight, and his dosages of diuretic and inotropic agents were increased. The weight gain was thought to be related to the large intravenous volume associated with the IVIg infusion. During this course of therapy, his renal function also deteriorated each day; the serum level of creatinine increased to 203 µmol/L (2.3 mg/dL). Renal function returned to close to baseline value the following week when he began treatment with cyclophosphamide, 0.5 g/m² on treatment day 10. His hematocrit continued to be low (0.25); erythropoietin was administered on day 20. The white blood cell count decreased to 3.5 x 10⁹/L. During the next several days, the neutropenia resolved spontaneously without intervention, consistent with the myelosuppressive effect and subsequent recovery of bone marrow associated with treatment with cyclophosphamide.

On treatment day 21, the level of preformed reactive antibodies decreased to 84%, and IVIg therapy was resumed, but the dose was increased to 0.75 g/kg per day for 4 consecutive days. On day 28, JB went into renal failure, with a serum creatinine level of 354 µmol/L (4.0 mg/dL) and worsening heart failure. He was transferred to the intensive care unit for a week, where his dosage of inotropic agents was increased and hemodialysis was started. Treatment to reduce the concentration of preformed reactive antibodies was postponed during dialysis, and on treatment day 45 he was transferred back to the cardiac care unit. He had renal failure, end-stage heart failure, and a level of preformed reactive antibodies of 47%.

On treatment days 47 and 74, potential heart donors for JB were identified. Prospective cross-matches were performed, and an immediate cross-reaction between JB’s serum and the lymphocytes from both donors was detected; therefore, the donors were deemed unsuitable for him. On treatment day 52, apheresis therapy was instituted on a Monday, Wednesday, and Friday schedule. Cyclophosphamide was administered every third Friday to allow a 3-day apheresis-free period. After the start of apheresis therapy, JB experienced nausea and vomiting during and after each session of apheresis. Dronabinol (Merinol) was administered, but despite the medication and changing the timing of administration of the dronabinol, he remained nauseated. On treatment day 94, ondansetron was added to the regimen; however, this treatment did not alleviate the nausea. Diphenhydramine given before apheresis was effective in suppressing the nausea and vomiting; various antiemetics were less effective. By treatment day 67, the anemia was managed by twice a week administration of erythropoietin, and JB no longer required administration of packed red blood cells.

A third potential donor was identified on day 102, and a prospective cross-match was negative for pre-

(Figure 2).
formed reactive antibodies. JB underwent cardiac transplantation on September 20, 1999. Echocardiograms obtained immediately after surgery and 8 weeks later showed normal biventricular function. Further evaluation via endomyocardial biopsy indicated no evidence of significant rejection.

Conclusion

As the case study illustrates, treatment of patients to reduce concentrations of preformed reactive antibodies is a complex endeavor. The case study also exemplifies the complications associated with treating patients who have elevated levels of preformed reactive antibodies. In many transplantation programs, therapies are instituted to reduce the concentration of cytotoxic alloantibodies. Anticipating adverse events associated with treatments to reduce the concentration of these antibodies allows the healthcare team to minimize complications.

**Protocol for treating patients with preformed reactive antibodies to HLA antigens**

**Cycle 1**

**Week 1:** Infusion of intravenous immune globulin (IVIg). Start with 2 g/kg in 4 divided daily doses.

**Week 2:** Intravenous cyclophosphamide, 0.5-1 g/m². Infuse as a single dose over 4-6 hours.

**Week 3:** No treatment.

**Cycle 2**

**Week 4:** IVIg, 2 g/kg in 4 divided daily doses. If the level of preformed reactive antibodies has not declined, then consider increasing the dose of IVIg to as much as 3 g/kg (maximal dose).

**Week 5:** Intravenous cyclophosphamide, 0.5-1 g/m². Infuse as a single dose over 4-6 hours.

**Week 6:** No treatment.

**Cycle 3**

If the level of preformed reactive antibodies is continuing to decrease, resume the preceding cycles. If, however, the level has not decreased, substitute plasmapheresis for IVIg. Perform apheresis every other day: Monday, Wednesday, Friday or Tuesday, Thursday, Saturday. Cyclophosphamide should still be given every 3 weeks.
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