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Introduction

An individual's immunologic "self" is encoded in the major histocompatibility complex (MHC). After transplantation, the MHC and minor (non-MHC) antigens of the donor are recognized as "non-self" by the recipient's immune system and a rejection response can occur. However, when immunologically competent cells are transplanted into a recipient, a "reverse-rejection" response can occur in which the immune cells of the donor recognize the MHC and non-MHC antigens of the recipient as foreign and precipitate a graft-versus-host response. Graft-versus-host disease (GVHD) is commonly seen in bone marrow transplantation in which the recipient's own immune system has been ablated to allow engraftment of new, donor bone marrow. GVHD after bone marrow transplantation is manifested by the clinical sequelae of epithelial cell damage of the recipient's skin, gastrointestinal tract, liver and immune system. GVHD is also an unusual, but often lethal, complication after solid organ transplantation. GVHD after solid organ transplantation may be the result of immune cells transplanted with the allograft or immune cells transfused with blood products in the peritransplant period.

GVHD after solid organ transplantation can occur in one of three types. "Antibody-mediated" GVHD can occur after ABO-incompatible liver transplantation in which antibodies of donor origin cause hemolysis in the recipient. "Transfusion-associated" GVHD demonstrates a clinical picture very similar to GVHD after bone marrow transplantation and likely is the result of leukocytes transfused into the recipient along with non-irradiated blood products during or after solid organ transplantation. "Cell-mediated" GVHD is a syndrome in which immunologically mature cells transplanted with the organ gradually reject the recipient's tissues while protecting the allograft. We describe two recent cases of GVHD after liver transplantation at the University of Wisconsin and discuss the types of GVHD along with our current knowledge on this disorder.

Case Reports

Case #1. A 48-year old male with a history of cirrhosis secondary to alcohol abuse and hepatitis C underwent ABO-identical orthotopic liver transplantation. This patient was blood type A and HLA type A1, A11, B41, DR11, DR15. The donor was a 19-year old female blood type A with HLA type A28, B70, DR17, DR18. Eight units of packed red blood cells and 20 units of fresh frozen plasma were transfused during the procedure. Blood products were not irradiated. Postoperative immunosuppression consisted of prednisone (500 mg/d tapering to 30mg/d by postoperative day 9) and tacrolimus. The remainder of the patient's immediate postoperative course was uneventful and he was discharged home on postoperative day 12. This patient was readmitted on postoperative day 18 with complaints of chills and high fever to 39.5°C. Blood cultures, CT scan, MRI, transesophageal echocardiogram, lumbar puncture and CMV DNA capture were all negative or normal. Bone marrow biopsy on postoperative day 28 showed hypocellular marrow. The patient was begun on empiric ganciclovir for possible posttransplant lymphoproliferative disease (PTLD). Liver function tests all remained within normal range with the exception of an elevated LDH. The patient's white blood cell count progressively declined over the next several days reaching a low absolute neutrophil count of 100. Skin biopsy of progressive rash revealed changes consistent with GVHD. Diagnosis of GVHD was confirmed by demonstration of leukocytes of donor HLA type in the patient's peripheral blood. Cytogenetic studies on bone marrow biopsy performed on postoperative day 41 showed 74% cells with a female karyotype. The patient was initially treated with methylprednisolone 1 mg/kg I.V. every 12 hours after holding Prograf for progressive renal failure. Cyclosporine was instituted without improvement in leukopenia. Thymoglobulin (3 gm I.V.) was given for 3 days along with broad spectrum antibiotics including liposomal amphotericin B. The patient developed sepsis from vancomycin-resistant enterococcus and died of multisystem organ failure on postoperative day 51.

Case #2. A 58-year old male with a history of cirrhosis from alcohol abuse underwent orthotopic liver transplantation with an ABO-identical liver graft. The recipient's HLA type was A1, A2, B8, B62, DR4, DR8. The donor was a 22-year-old male with HLA type A1, B8, DR4. Blood products administered around the time of transplantation were not irradiated. The patient's postoperative course was unremarkable and he was discharged home on the tenth postoperative day on tacrolimus and a tapering dose of prednisone. The patient was readmitted on postoperative day 26 with complaints of abdominal pain and headache. Head and sinus CT scan, esophagogastroduodenoscopy (EGD), abdominal CT, liver biopsy and CMV DNA capture were negative. This patient was again admitted on postoperative day 47 with complaints of abdominal pain, anorexia and weight loss. CMV DNA capture returned at 2.8 and patient was discharged home on I.V. ganciclovir for systemic CMV infection. This patient was admitted again on day 61 with elevated liver function tests and a transient rash over the chest, face and back. Skin biopsy was consistent with drug eruption. Gastric,

duodenal, and colon biopsies on postoperative day 71 showed mild inflammation without evidence of CMV inclusions or GVHD. Bone marrow biopsy, performed because of progressive pancytopenia, showed only mild hypocellularity but no clear evidence of GVHD. Repeat gastric, duodenal and colon biopsies on postoperative day 86 were suggestive of GVHD. CMV DNA capture on these gastrointestinal tract biopsies were elevated, indicating the presence of CMV and the patient was started on I.V. ganciclovir. Short-tandem repeat (STR) analysis of peripheral blood leukocytes demonstrated that approximately 14% of DNA present was of donor origin. Immunosuppression was decreased and this patient was continued only on prednisone (15-30 mg/d) while stimulating bone marrow with granulocyte colony-stimulating factor (GCSF) and erythropoietin. Over the next week, the patient's clinical course deteriorated from pancytopenia, sepsis and gastrointestinal bleeding. The patient was begun on Omtak (anti-CD25 monoclonal antibody conjugated to diphtheria toxin) in hopes of depleting the activated donor T lymphocytes. After a mild improvement in leukopenia and a decrease in level of chimerism to 10% by STR analysis, the patient began to develop a whole body rash which became exfoliative on postoperative day 96. The patient was continued on supportive measures but deteriorated clinically from sepsis, GI bleeding, progressive respiratory and renal failure, and expired on postoperative day 112.

Discussion

Antibody-mediated GVHD, also known as graft-versus-host hemolysis (GVHH) syndrome is characterized by hemolysis after ABO-compatible but nonidentical liver transplantation. GVHH can happen after a type O liver has been transplanted into a non-O recipient or after a type A or B liver has been transplanted into an AB recipient. Approximately one-half of recipients of ABO-unmatched liver transplants will show evidence of hemolysis 5 to 10 days after transplantation.[1,2] The clinical presentation of GVHH is one of mild fever, hemolytic anemia, hyperbilirubinemia and elevated liver function tests. This condition can be interpreted as early liver allograft rejection and may prompt liver transplant biopsy. Liver biopsy usually reveals mild portal inflammation and erythrophagocytosis and may be confused with early rejection. The degree of erythrophagocytosis on biopsy may correlate with the severity of hemolysis in GVHH.[3] The hemolysis in GVHH is mediated by IgG (produced by B cells transplanted with the liver allograft) directed against unmatched recipient erythrocytes. GVHH is usually mild and self-limiting and can be temporized by transfusing the patient with red blood cells of the donor rather than the recipient blood type.

Transfusion-associated GVHD occurs when lymphocytes transfused around the time of transplantation are responsible for an immune response against both the recipient and the transplanted organ. Transfusion-associated GVHD has been reported in liver, kidney and heart transplantation.[4-6] Along with the clinical manifestations of fever, rash, leukopenia and diarrhea, transfusion-associated GVHD may also show allograft dysfunction. Transfusion-associated GVHD may exhibit two types of immune response: graft (transfused lymphocytes) versus graft (transplanted allograft) and graft- (transfused lymphocytes) versus-host (recipient). Definitive diagnosis of transfusion-associated GVHD is difficult because HLA typing of all donors of transfused blood has often not been performed. Two cases have been reported in which transfused lymphocytes seem to be the origin of GVHD:

1. a male recipient of a renal allograft from a male cadaveric donor developed GVHD with skin biopsy showing 80% lymphocytes of female origin[5] and
2. a liver transplant recipient developed GVHD with affected skin and peripheral blood containing DNA not of donor or recipient origin.[4] We hypothesize that the cases of GVHD detailed above were not transfusion-induced because cells of organ donor HLA type were demonstrated in recipient peripheral blood. Additionally, these patients did not exhibit liver dysfunction during the period when clinical GVHD was present. Transfusion induced GVHD cannot be definitively excluded because HLA typing was not performed on all donors of blood products transfused during the peritransplant period. Because cases of transfusion induced GVHD are difficult to diagnose (because of the necessity for HLA typing of all blood product donors) and rare in the liver transplant literature, irradiated blood products are not routinely used. However, since treatment of transfusion-associated GVHD is often ineffective and risk of patient mortality is high, preventative measures such as irradiation of blood products should be considered.

Cell-mediated GVHD after solid organ transplantation has become more common as the volume of organ transplantation has increased over the past decade. Cell-mediated GVHD can arise when immunocompetent lymphocytes are transported with the new liver and perihepatic lymphoid tissue into the recipient.[7] HLA class I and minor histocompatibility antigens present on the recipient's enterocytes and keratinocytes are recognized as foreign by the "passenger" lymphocytes and a rejection response against the recipient ensues.[8] If the recipient's immune system is unable to suppress this "reverse rejection" process, clinical GVHD appears. The host tissues most prominently affected in GVHD include the skin, GI tract and bone marrow. The attack on these organ systems generates the most common clinical signs of GVHD—skin rash, diarrhea and pancytopenia. The organ system not adversely affected in cell-mediated GVHD is that of the allograft—the only tissue the offending activated lymphocytes do not recognize as foreign. Cell-mediated GVHD after liver transplantation can be differentiated from transfusion-mediated or antibody-mediated GVHD because liver allograft function improves as clinical GVHD progresses.

The clinical manifestations of cellular GVHD include fever, skin rash, diarrhea and pancytopenia and most often present within 2 to 12 weeks after transplantation. The

differential diagnosis of skin rash after transplantation includes GVHD, toxic epidermal necrolysis, erythema multiforme and drug eruption. However, all of these entities can histologically demonstrate epithelial cell necrosis and basal cell vacuolization. The demonstration of the presence of donor lymphocytes on the skin biopsy or correlation of skin biopsy findings with other clinical signs can support the diagnosis of GVHD. While skin rash is often seen in patients with cellular GVHD, it may not present until late in the course of the disease (as seen in patient #2), making the clinical diagnosis difficult.[9] Severe diarrhea associated with cellular GVHD may reach volumes of 1 to 3 liters per day and can present concurrently with an exfoliative skin rash. The pathogenetic mechanism of diarrhea in patients with GVHD (presumably similar to the cause of skin rash) is the attack of recipient enterocytes by activated donor lymphocytes. Colon and rectal mucosa show diffuse erythema and edema and biopsies reveal individual epithelial cell necrosis (apoptosis) and invasion of the lamina propria by lymphocytes, PMNs and eosinophils.[9,10] Pancytopenia in cellular GVHD arises from the destruction of recipient bone marrow elements by donor lymphocytes. Leukopenia is the most devastating complication of GVHD because of the likelihood of death resulting from septic complications. Leukopenia and treatment consisting of increased immunosuppression predispose patients with GVHD to invasive opportunistic infections such as CMV, yeast and antibiotic-resistant bacteria. Lethal bone marrow hypoplasia and neutropenia may persist after treatment despite resolution of the skin rash and diarrhea.[9]

Diagnosis of cellular GVHD is based on the presence of clinical manifestations, demonstration of chimerism and histologic evidence. Any or all of the clinical signs of fever, diarrhea, skin rash and pancytopenia may be seen during the initial presentation of cellular GVHD. Many of the clinical signs of GVHD may also be seen with cytomegalovirus infection. The presence of CMV in a patient with GVHD may confuse the appropriate diagnosis and delay treatment (patient #2). A significant association between acute GVHD in bone marrow transplantation and CMV is documented[11] and may be related to the pancytopenia resulting from bone marrow depletion by attacking donor lymphocytes. Since the clinical presentation of cellular GVHD is inconsistent, a high degree of suspicion is necessary to pursue a diagnosis and institute timely therapy. Methods including HLA typing of peripheral blood,[10,12-18] restriction length polymorphism,[19] and fluorescent in situ hybridization of peripheral blood[20] have been used to demonstrate chimerism in recipients with suspected GVHD after liver or pancreas transplantation. Chimerism at the tissue level has been shown by PCR, tandem N-terminal repeat analysis and in situ hybridization techniques in the skin[17,20-22] and bone marrow[17,21,23] in patients with GVHD after solid organ transplantation. Peripheral blood chimerism has been reported after routine liver transplantation, but levels of donor lymphocytes in recipient serum peak within the first 7 days post-transplant and decline rapidly over the following weeks.[24,25] The presence of fever, diarrhea, skin rash or pancytopenia and significant peripheral blood chimerism weeks after transplantation should prompt the search for further evidence of GVHD at the tissue level. Demonstration of donor cells in recipient skin, bone marrow, or the GI tract along with histologic evidence of selective target organ destruction support the clinical diagnosis of cellular GVHD.

The mainstay of therapy for cellular GVHD is based on augmented immunosuppression to halt the assault of donor lymphocytes on recipients tissues. Steroids, calcineurin inhibitors, antithymocyte globulin, OKT3 and anti-CD25 monoclonal antibodies have been used to treat cellular GVHD with unpredictable results. Increased immunosuppression in an already severely immunocompromised patient risks opportunistic infection, sepsis and posttransplant lymphoproliferative disease. Roberts et al reported two cases of cellular GVHD which responded to treatment consisting of methylprednisolone and Minnesota ALG. The patients each showed some improvement after treatment but ultimately succumbed to PTLD and multisystem organ failure resulting from disseminated aspergillosis.[9] Because of the risks of increased immunosuppression, it has been postulated that deceasing immunosuppression may allow the recipient immune defenses to recover and destroy offending donor lymphocytes. The presence of donor peripheral chimerism and lymphopenia negates the risk of allograft rejection in the face of decreased immunosuppression. This "minimalistic" strategy would eliminate broad-based immunosuppression (prednisone, mycophenolate, calcineurin inhibitors) while possibly employing monoclonal antibody therapies targeting specific subsets of immunocompetent donor cells. Despite newer approaches and therapeutic modalities, the mortality of cellular GVHD after solid organ transplantation remains as high as 80%.[10,21,23]

The ability to identify transplant recipients at risk for cellular GVHD remains poor in spite of increased knowledge of this disease. In 1966, Billingham formulated the donor and host requirements for the induction of GVHD:

1. the graft must contain immunologically competent cells,
2. the host must possess important transplantation isoantigens that are lacking in the graft donor so that the host appears foreign to it, and is therefore capable of stimulating it antigenically,
3. the host must be incapable of mounting an effective immunologic reaction against the graft, at least for sufficient time for the latter to manifest its immunologic capabilities.[26] With the exception of identical twins, all solid organ transplants will fulfill the first two criteria. It would appear that the critical factor in the development of cellular GVHD is the inability of the host immune system to mount a sufficient response to the graft and its immunologically competent cells.

The importance of the number of donor lymphocytes transferred during the transplant procedure has been questioned. Initial

fears of the risk of GVHD after small bowel transplantation were spawned by concerns over transfer of large numbers of donor lymphocytes in the lymphoid tissue of the small bowel under intense immunosuppression. These fears have not been realized as only one case of cellular GVHD after small bowel transplantation has been reported.[20] While the overall number of cases of cellular GVHD is small, it would appear that the inoculum size of passenger donor lymphocytes does not have overriding importance in the development of cellular GVHD. Risk factor analysis among bone marrow allograft recipients shows that stem cell estimates alone do not predict the development or severity of cellular GVHD. Bortin[27] reported that the immunologic characteristics of the patients, as well as the donor, are closely linked to the onset of GVHD after bone marrow transplantation.

There are several potential explanations for how donor lymphocytes are able to prevail over recipient lymphocytes. The donor allograft may contain numerous lymphocytes which have been presensitized through transfusions of erythrocytes or platelets sharing recipient haplotypes or antigens. The recipient's immune system may be deficient or particularly susceptible to immunosuppressive therapy and allow the donor cells to gain an advantage. It is possible that the recipient's HLA phenotype is particularly immunogenic to the donor's immune system. Cellular GVHD may arise when the donor is homozygous for a haplotype that is shared with the donor (see patient #2). In this situation, the recipient is unable to recognize the donor cells as foreign but the donor lymphocytes see the recipient as "non-self" and mount the GVHD response. This scenario is thought to arise in 1 of 500 donor/recipient combinations.[13]

Growth of solid organ transplantation, particularly liver transplantation, has been accompanied by increasing reports of cellular GVHD. While the low incidence of this entity has not allowed intensive study, a review of current knowledge of cellular GVHD prompts a few conclusions. Diagnosis of cellular GVHD after solid organ transplantation must be a clinical diagnosis based on the presence of symptoms (rash, fever, neutropenia or diarrhea) and demonstration of peripheral chimerism. The propensity for developing cellular GVHD hinges on transfer of donor lymphocytes, genetic differences or similarities, pharmacologic immunosuppression and the intrinsic immunologic responsiveness of both donor and recipient. Therapy based on augmented and reduced immunosuppression have not been successful to date. Further studies on mechanism of disease and selective anti-donor therapy are necessary for improved survival of this rare, but often fatal, disorder.

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