Introduction

Graft versus host disease (GVHD) following solid organ transplantation is a rare event and the incidence after liver transplant is thought to be 0.1-2% [1, 2]. GVHD results from the transplantation of donor passenger lymphocytes into the recipient. The three classic requirements for GVHD are the transfer of immunocompetent lymphocytes into a host, the incompetence of the host to reject these cells, and an antigenic disparity between host and donor tissue [3,4]. GVHD typically presents 1-8 weeks after liver transplantation with fever or skin rash early in the clinical course often followed by pancytopenia, sepsis and death [1, 5-7]. The diagnosis of GVHD can be difficult because the clinical manifestations and histopathologic features in affected organs are not specific and similar findings can be seen in drug reactions and infection.

This case report describes GVHD manifested by skin rash and progressive cytopenias following second orthotopic liver transplant from a sex-mismatched donor for autoimmune hepatitis. Clinical course was complicated by the development of toxic epidermal necrolysis (TEN)-like skin disease, aplastic anemia and terminal sepsis. Initial skin biopsy revealed vacuolar interface change, keratinocyte necrosis and a mild mononuclear superficial perivascular infiltrate. The bone marrow was markedly hypocellular with scattered CD8 positive T lymphocytes. FISH analysis revealed chimerism with the presence of male donor cells in the skin and bone marrow biopsies. This case illustrates the diagnostic utility of FISH in detecting the presence of donor-derived cells in tissues affected by GVHD.

Keywords: graft versus host disease (GVHD), fluorescence in situ hybridization (FISH), aplastic anemia, liver transplantation
was markedly hypocellular with scattered CD8 positive T lymphocytes. FISH analysis to ascertain the source of the lymphocytes showed a small percentage of XY lymphocytes in both specimens. Analysis of short tandem repeat (STR) polymorphisms failed to demonstrate donor DNA in these tissue samples; however, the number of donor-derived cells may have been below the level of detection for this assay. This report illustrates the usefulness of FISH to demonstrate the presence of donor cells in the skin and bone marrow combined with clinical findings and histopathologic evaluation of affected tissues in the diagnosis of GVHD.

Case Report

A 61-year-old Caucasian female status-post second orthotopic liver transplant, both from HLA-mismatched male donors, was admitted to the hospital on post-operative day 22 with fever, as high as 104°F, and chills for one week. She denied nausea, vomiting, abdominal pain, diarrhea, and cough. Her past medical history was significant for an orthotopic liver transplant for end stage liver disease due to autoimmune hepatitis four months prior to admission. The first liver transplant was complicated by hepatic artery thrombosis and parenchymal abscess. Medications at admission included tacrolimus, prednisone, valganciclovir, voriconazole, and trimethoprim/sulfamethoxazole. Labs showed a mildly increased WBC (18 K/μL; normal range: 3-12 K/μL), mild anemia (Hgb = 12.9 g/dL; normal range: 13-17 g/dL), normal platelet count (221 K/μL; normal range: 150-500 K/μL), mildly elevated liver enzymes: alkaline phosphatase-104 IU/L (32-91 IU/L), AST-164 IU/L (15-41 IU/L), ALT-211 IU/L (5-45 IU/L), total bilirubin-1.5 mg/dL (0.2-1.2 g/dL), and normal albumin of 3.9 g/dL.

On hospital day 11 (post-op day 32), a dermatology consultation was obtained to evaluate a rash of one week duration that appeared to be worsening according to the primary team. On examination, a blanchable, erythematous, morbilliform rash was present on the bilateral upper extremities, trunk, and bilateral lower extremities. No characteristic GVHD lesions were found on the ears, palms, or soles. Follicular accentuation, or lesions consistent with Stevens-Johnson Syndrome (SJS) or toxic epidermal necrolysis (TEN) were not seen. Medication-induced dermatitis was proposed and the majority of non immunosuppressive agents were withdrawn 1-4 days prior to skin biopsy of the left anterior thigh. Histologic examination of the punch biopsy showed vacuolar interface change with necrotic keratinocytes throughout all layers of the vital epidermis. There was mild spongiosis and a superficial perivascular inflammatory cell infiltrate that consisted chiefly of CD8 positive cytotoxic T lymphocytes, few histiocytes and rare eosinophils (Fig. 1A and 1B). These morphologic findings were consistent with drug reaction or GVHD. To assess for donor male cells in the skin, FISH with directly labeled probes for the X centromere (DXZ1) and the Y heterochromatic region (DYZ1) (Abbott Molecular, Des Plaines, IL) was performed on the paraffin-embedded skin biopsy. Sections of 4 μm thickness were mounted onto SuperFrost Plus positively charged slides (ThermoShandon, Pittsburgh, PA), baked at 65°C overnight and de-paraffinized using xylene. After protease digestion (Proteinase K Enzyme solution, Qiagen, Gaithersburg, MD) at 55°C for 15 minutes, sections were rinsed in 2X standard sodium citrate solution (2X SSC), dehydrated using a graded ethanol series and air-dried. Hybridizations were carried out at 37°C for 16 to 18 hours in an automated codenaturation oven (HYBrite Hybridization System, Abbott Molecular Inc, Des Plaines, IL) according to the manufacturer’s instructions. Sections were washed to remove excess probe (2X SSC/0.3% NP40 at 72°C for 2 minutes; room temperature 2X SSC for 1 minute). Nuclei were counterstained with 4´,6-Diamidino-2-phenylindole dihydrochloride hydrate (DAPI) and the slides were scored for red (X chromosome) and green (Y chromosome) signals using an epi-fluorescence microscope equipped with single band-pass filters (Abbott Molecular Inc). FISH analysis of the skin biopsy showed few cells with an XY signal pattern located at the dermal-
epidermal junction and epidermis (Fig. 1C). By hospital day 23 (post-op day 44), full thickness desquamation was present with exposed dermis on the back and parts of the arms.

Progressive cytopenias developed simultaneously with the onset of the skin lesions. Her CBC on hospital day 15 (post-op day 36) showed a hemoglobin of 8 g/dL, WBC of 0.14 K/μL and a platelet count of 17 K/μL. A bone marrow aspirate and biopsy revealed a hypoplastic marrow with an infiltrate of CD8 positive T-lymphocytes (Fig. 2A and 2B). Bone marrow cells were directly harvested following a 20 minute incubation in a 0.75M potassium chloride solution and three fixations using 3:1 methanol/acetic acid. The bone marrow cells were placed on glass slides and fluorescent in situ hybridization (FISH) was performed using directly labeled probes for the X centromere (DXZ1) and the Y heterochromatic region (DYZ1) (Vysis/Abbott Molecular, Des Plaines, IL) following the manufacturer’s protocol. Following the hybridization, the nuclei were counterstained with 4,6-diamidino-2-phenylindole (DAPI) and visualized using a BX-60 fluorescence microscope (Olympus, Tokyo, Japan) equipped with a triple bandpass filter for DAPI, FITC, and rhodamine. Analysis of the bone marrow revealed 2% of cells with signal for both the X and Y chromosome probes, consistent with donor origin (Fig. 2C). Donor DNA was not detected by STR polymorphism analysis in the bone marrow or skin biopsies. HLA typing of the peripheral blood was not performed. A liver biopsy performed at this time showed no evidence of rejection or GVHD.

Throughout the hospital course, the patient received intravenous immunoglobulin (IVIG) and was maintained on steroids (prednisone, methylprednisolone, or hydrocortisone) for immunosuppression. Tacrolimus was stopped on hospital day 4 (post-op day 25), but restarted on hospital day 18 (post-op day 39) based on the presence of donor cells detected by FISH analysis of the skin biopsy.

The patient received multiple blood products, all of which were irradiated. Cytomegalovirus (CMV) detection by polymerase chain reaction of plasma consistently showed no active disease (< 500 copies/mL). CMV culture of bone marrow and early antigen immunofluorescence for CMV was negative. Human herpes virus 6, parvovirus, adenovirus and herpes simplex virus 1 and 2 were not detected by PCR of plasma. Epstein Barr virus detection by PCR of whole blood showed no active disease (< 200 copies/mL) on two occasions. EBV was detected by PCR (1906 copies/mL) on hospital day 23 (post-op day 44), which was consistent
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with immunosuppression and not necessarily indicative of active disease. The patient died secondary to overwhelming sepsis caused by vancomycin-resistant Enterococcus faecium on hospital day 27 (post-op day 48).

**Discussion**

The pathophysiology of GVHD involves the introduction of foreign, immunocompetent T-lymphocytes from transplanted donor tissue into an immunocompromised host. The incidence of GVHD following allogeneic hematopoietic stem cell transplantation is approximately 50% [6]. GVHD following solid organ transplantation occurs uncommonly and the frequency is directly proportional to the number of graft lymphocytes. Accordingly, GVHD very rarely occurs following heart or kidney transplants due to the paucity of lymphoid tissue in these organs [1,2]. Although the incidence of GVHD after liver transplant is infrequent (~1%), analysis of 12 cases of GVHD from 1082 liver transplantations identified several risk factors. These include closely matched HLA recipients, patients older than 65 years of age, and recipients with donors more than 40 years younger [8]. Another analysis of 4 cases of GVHD from 205 liver transplantations identified the risk factors of autoimmune hepatitis or the combination of alcoholic liver disease, hepatocellular carcinoma, and glucose intolerance receiving a steatotic donor liver [9]. Although our patient received two livers from HLA-mismatched donors and was less than 65 years of age at the time of transplantation, she had a history of autoimmune hepatitis.

GVHD typically presents 1-8 weeks following liver transplantation with fever, skin rash, diarrhea, pancytopenia or a combination of these features while liver function remains unaffected [1,5-6]. One of the earliest signs of GVHD is skin rash; however, dermatologic complications following liver transplantation are not uncommon and must be differentiated from drug reaction and infection. In a single-center study, 12.5% of patients (14 of 112 patients) presented with a dermatologic problem following liver transplantation, and included one case of GVHD [4]. It has been noted that no distinguishing histological features, including the presence of tissue eosinophils, can differentiate a drug reaction from GVHD [10]. Similar to dermatopathic lesions, there are no morphologic criteria identified in bone marrow specimens that distinguish aplastic anemia secondary to GVHD from other etiologies.

While histopathologic findings of affected tissues may be nonspecific, the diagnosis of GVHD is facilitated by the detection of donor lymphocytes by HLA markers or FISH analysis using sex chromosome probes if the graft is from a sex-mismatched donor and both methods have been used to identify GVHD after liver transplant [7, 11-12]. A recent report revealed the utility of C4d immunoreactivity in colonic biopsies in the diagnosis of chronic GVHD in patients following allogeneic bone marrow transplant [13], suggesting a humoral response to foreign antigens. In our case, CD8 positive cytotoxic T-lymphocytes were predominant in the skin and bone marrow biopsies and a subset of lymphocytes were of donor origin based on sex chromosome FISH analysis. A PCR-based assay to evaluate several polymorphic single tandem repeat (STR) of genomic DNA (applied Biosystems STR kits) failed to demonstrate donor DNA in the skin and bone marrow specimens. It is probable that these tissue samples did not contain sufficient numbers of donor cells for STR analysis as the reported detection limit of this technique is approximately 5% donor lymphocytes [11,14]. Some studies have shown that STR analysis sensitivity is increased using enriched peripheral blood cell populations [15]. Cell enrichment techniques were not employed in the skin or bone marrow specimens. HLA typing has been used to monitor the donor cells in the recipient’s blood [16]; however, the peripheral blood was not evaluated in our patient.

We present the case of a patient who simultaneously developed a morbilliform rash and progressive pancytopenia following second liver transplant that could not be
directly attributed to a drug reaction or viral exanthem through drug history or viral detection. Furthermore, transfused blood products were irradiated, making transfusion-associated GVHD less likely. The development of GVHD after a second liver transplant has been previously reported [17]. An interesting observation in our case is that the rash did not initially present in the characteristic locations typically seen in GVHD including the soles, palms, and ears. However, clinical progression and detection of donor-derived cells in the skin and bone marrow was consistent with GVHD and raise several questions. For example, do solid organ transplant patients often or rarely have donor lymphocytes at these sites? If transplant patients have such cells frequently, does the presence of these lymphocytes predict a strong likelihood of GVHD or are these cells relatively harmless? If such donor cells are present in small numbers, as in our patient, are they sufficient to account for this patient’s rash and bone marrow failure? Could these activated donor T cells induce or recruit the host’s T cells to attack the recipient’s own skin or bone marrow? These questions remained unanswered but are the subject of further study.

References