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Immunological Basis for Treatment of Graft versus Host Disease after Liver Transplant

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Summary

Graft versus host disease (GVHD) after liver transplant, although a rare disease, has a very high mortality rate. GVHD occurs due to immunoreactions caused by donor T lymphocytes and host cell surface antigens resulting in proliferation and clonal expansion of T lymphocyte. Migration of effector cells, including macrophages, NK cells and cytotoxic T lymphocyte, to the target organs such as skin, intestine and bone marrow results in skin rashes, diarrhea and bone marrow depression. GVHD is diagnosed by clinical symptoms, histopathological findings and by the presence of chimerism. The delayed diagnosis, opportunistic infections and lack of definitive treatment of post orthotopic liver transplant (OLT)-GVHD results in sepsis and multi-organ failure leading to very low survival rates. In this review, we have focused on early diagnosis and critically discuss novel treatment modalities to decrease the incidence of GVHD.

Keywords

Graft versus host disease; liver transplant; Immunoreactivity; T lymphocytes; Surface antigens; Chimerism; Opportunistic infections; Sepsis; Multi organ failure

Introduction

According to American Liver Foundation, there are approximately 17,000 people waiting for liver transplant and this list is growing further and approximately 1,500 people die per year while waiting for liver transplant. Dr. Thomas Starzl and colleagues performed the first successful human orthotopic liver transplant (OLT) in 1967 in Denver, Colorado. Presently more than 6,000 liver transplants are being performed each year in United States with a one-year survival rate of 80–85% [1]. GVHD after orthotopic liver transplantation is a rare and serious complication after OLT with a mortality rate of 85–90% and was first described by Burdick *et al.* in 1988 [2–3]. The incidence after OLT is 0.1 – 2% [2, 4–5] compared to >50% after hematopoietic stem cell transplantation. A study in 2007 reported about 80 cases

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of GVHD after OLT [4] and few more cases have been reported since then [6–11]. Graft Versus Host Disease after liver transplant occurs due to immunoreactions mediated by donor T lymphocytes and recipient cell surface antigens such as human leukocyte antigen (HLA) and major histocompatibility complex (MHC) [12]. Acute GVHD occurs within the first few weeks after transplant. There are also few reported cases of chronic GVHD. Cellular GVHD after liver transplant occurs due to MHC-mismatch resulting in immunoreactions by donor T lymphocyte, while humoral GVHD occurring in an ABO-mismatched liver transplant is mediated by the production of antibodies against the red cell antigen of recipient, by donor T cell lymphocyte [13]. The increased number of cases of liver transplants and more advanced immunosuppressive techniques result in a greater decrease in host immune defense, thus increasing the incidence of GVHD. The decreased immune response in the host after GVHD results in increased severity of infections and mortality rate [12]. Post-transplant GVHD patients die due to invasive viral, bacterial and fungal infection, multi-organ failure, septicemia and stroke. In this critical review, we have focused on the methods of early diagnosis and developing treatment modalities.

Clinical Sign and Symptoms

Acute GVHD mainly displays the inflammatory component while chronic GVHD displays autoimmune features. Humoral GVHD results in minor symptoms of mild self-limiting hemolytic anemia, while cellular GVHD results in multisystem involvement involving skin, mucous membrane, gastrointestinal tract (liver, intestine), and bone marrow with patients having the clinical sign and symptoms related to these organs [12]. Common features of GVHD after liver transplantation are skin rashes, diarrhea, fever and pancytopenia. Acute Post-OLT GVHD presents with fever, skin rash, diarrhea, and pancytopenia, typically 2 to 8 weeks after transplantation [3, 8, 13–14]. In cases of GVHD after hematopoietic stem cell transplantation skin rashes appear on the palm and soles, while in case of GVHD after orthotopic liver transplant, rashes usually appear on the chest and spread to the trunk, neck, and arms, sparing the palms and soles. This makes the diagnosis difficult as it mimics viral infections like cytomegalovirus, and drug reactions. The rash is initially maculopapular but may progress to bullae formation and desquamation. Skin rashes/eruption may consist of erythematous to violaceous macules coalescing into patches [13, 15–16], and are highly suggestive but not specific for post-OLT GVHD. Such eruptions may also occur in drug reactions as toxic epidermal necrolysis and viral infection particularly cytomegalovirus [17–18]. Skin biopsy in such patients will show dermo-epidermal interface lymphocytic infiltration and apoptotic cells. Additionally skin biopsy may show vacuolar degeneration of the basal layer of the epithelium, lymphocytic infiltration of epidermis and necrotic eosinophilic keratinocytes. Spongiosis, basal cell hydropic changes, apoptotic keratinocytes, and lymphocytic exocytosis in the epidermis along with subepidermal cleft formation may also be documented [6]. Chronic post-transplant GVHD is characterized by fibrosis in skin and subcutaneous tissue resulting in contracture and alopecia. Patients may also have involvement of salivary and lacrimal glands in chronic GVHD [13].

Diarrhea is caused by lymphocytic infiltration and destruction of the intestinal mucosa resulting in loss of absorptive capacity of the intestine and bowel biopsy in such patients will show apoptosis of crypt cells, gland abscesses and partial mucosal denudation. Although

biopsies from colon and small bowel are gold standard and more GVHD specific, bowel biopsy is not recommended as screening or the first-line investigation for the patients on immunosuppressive drugs due to its invasive nature [4, 6, 19] unless the GI symptoms are present. Further the role of subsequent bowel biopsies to assess the treatment response has also not been determined [20]. The endoscopic appearance of GVHD is non-specific and there are discrepancies between endoscopic and histological assessment. Also the upper GI involvement is more common than lower GI, there is need of revised grading system. Due to risk of perforation, oozing at biopsy site due to thrombocytopenia, lack of sufficient biopsy samples and high variability of endoscopic findings, precaution should be taken before considering bowel biopsies and should be considered in patients diagnosed as GVHD with skin biopsy to confirm the diagnosis of GI GVHD [21].

Pancytopenia, which is uncommon in GVHD after hematopoietic stem cell transplantation, is more common in patients with GVHD after OLT [22–25]. Pancytopenia is most commonly caused by lymphocytes transferred with the donor's liver attacking and destroying the recipient's hematopoietic stem cells. Pancytopenia can also be caused by immunosuppressive drugs and viral, bacterial and fungal infection due to decreased host immune response. There will be a rapid drop in white blood cell count and platelet count, but varies from patient to patient (approx. range from various studies WBC count $<0.1\text{--}0.3 \times 10^9/\text{L}$ and platelet count $<29\text{--}50 \times 10^9/\text{L}$ [26–28]. However, variation in these values in the patients also depends on the time of onset of GVHD and differential hematological effects of individual immunosuppressive drug. Severe aplastic anemia, a late complication after liver transplantation can occur even years after the transplant [27–28]. GVHD can also affect other organs systems such as lung and brain [29].

Fever and skin rash are the most frequent early sign followed by leucopenia [23]. Bone marrow involvement, severe sepsis and gastrointestinal bleeding are the major causes of death [5]. Liver functions are not affected in GVHD after OLT contrary to GVHD after hematopoietic stem cell transplantation, because the transplanted liver lacks host antigen and has matched MHC to the graft T cells [30–32]. Also, the grafted liver and the immunocompetent cells that are responsible for GVHD are of same origin [33–35].

Pathophysiology of GVHD after OLT

GVHD in a post-transplant patient occurs due to the activation and clonal expansion of the immunocompetent donor T lymphocytes, and these activated T lymphocytes mount a destructive cellular immune response against the recipient tissue [6]. ABO-incompatibility in liver transplant results in humoral GVHD whereas major histocompatibility complex mismatch results in cellular GVHD [13]. Immune response after OLT involves activation of donor T lymphocytes by antigen-presenting cells in the transplant recipient, causing an alloreactive T-cell response to recipient tissues mediated by cytotoxic T cells and inflammatory cytokines [7].

The interaction between donor T-lymphocytes and recipient antigen presenting cells can lead to 3 outcomes: (1) strong immune response of the host against the donor, host's immune system overcomes and reacts against the donor organ resulting in graft rejection (2) GVHD

occurs if the donor lymphocytes prevail and react against host tissue, and (3) normal allograft function without GVHD or rejection if the host's and donor's immune systems are in balance [36]. So GVHD will occur in a condition when there is immunocompromised recipient and an immunocompetent donor, but it has also been reported that immunocompromised status of recipient is also not a prerequisite for the development of GVHD [3]. It has also been reported that GVHD can develop even after onset of acute cellular rejection [4].

Billingham [29] described the essential requirement for the development of GVHD. These include: (1) the graft must contain immunologically competent cells; (2) the recipient must be recognized as foreign by the graft; and (3) the recipient must be unable to reject the graft before it mounts an effective immune response [37]. Taylor and colleagues [13] proposed a three-phase model for the pathogenesis of post-OLT GVHD (Table 1). The first phase is characterized by the relatively immunocompromised state of the recipient due to pre-transplant liver disease, the physiologic stress of surgery, and the use of post-transplant immunosuppressants. Activation of donor lymphocytes upon interaction with host antigen-presenting cells, triggering interleukin-2 dependent proliferation with predominantly T helper type 1 (Th1) differentiation is the second. Cell death and tissue dysfunction mediated by the cytotoxic donor T lymphocytes targeting antigens expressed by host tissue, characterizes the third phase. Further activation of the donor lymphocytes is mediated by the cytokines released by targeted host cells. Cytokine-injury-cytokine cycle will result in development of inflammation and finally give rise to GVHD [38]. In addition to it, the destruction of the host's skin, bone marrow, and mucosal epithelium further increases the immunocompromised state [13] (Figure 1).

Hematopoietic Cell Transplant versus Orthotopic Liver Transplant GVHD

10^7 donor lymphocytes per kilogram of recipient body weight are necessary to cause transfusion-associated GVHD in experimental animals, and OLT resembles to hematopoietic cell transplant (HCT) due to the transplantation of about 10^9 – 10^{10} donor-derived immunocompetent leukocytes including monocytes, T-cells and natural killer cells during OLT, and the use of immunosuppressive drugs. The lymphocyte population in the donor liver is distinct from the peripheral blood with a reversed ratio of CD4:CD8 (1:3.5 instead of 2:1) among hepatic lymphocytes and a higher proportion of CD8+ cells and B lymphocytes [39–40]. Also the percentage of CD3+ cells expressing NK cell markers or $\gamma\delta$ receptors is higher in hepatic lymphocytes (7–54% in liver, <6% in peripheral blood). These phenotypic differences may impact GVHD development, but to what extent is not clear. $\gamma\delta$ T lymphocyte predominantly produce Th2 cytokines, which in turn down regulates Th1 response, protecting liver transplant patients from GVHD. It may be a reason for a lower occurrence of GVHD in OLT [9, 13, 41].

Risk factors for post-OLT GVHD

Complete HLA matching between donor and recipient has been documented as an important risk factor for post-orthotopic liver transplant GVHD [42–43]. Donor recipient compatibility for HLA-B but not for HLA-A or DR was identified as a significant risk factor

for the development of GVHD [9, 32]. Relative risk of GVHD after OLT has been estimated to be 1% with an HLA-A and HLA-B mismatch of 3–4; the rate increases to 7% in the presence of 0–1 HLA-A and HLA-B mismatches, and with the additional presence of 0–1 HLA-DR mismatch it increases to 12.5% [13]. In another study it was suggested that the risk of fatal GVHD following live donor liver transplant (LDLT) may depend on the number of loci with donor-dominant one-way HLA matching, and was highest with mismatching in the GVHD direction at all 3 loci (HLA-A, -B and -DR) [44]. Calmus et al. [34] reported a case of fatal GVHD after live donor liver transplant (LDLT) and suggested that in LDLT use of a HLA homozygous donor may result in a complete 1-way donor recipient HLA match (use of an allograft from a homozygous donor to haploidentical recipient) and carries an extremely high risk of developing GVHD. Such donors should be ruled out and pretransplant HLA-type work-up should be done for both donor and recipient [45]. Higher incidences of acute rejection after LDLT are associated with HLA class II matching for DRB1 and DQB1 loci while not with mismatch for HLA class I antigen [46]. It was also argued that since all cases of LDLT using a HLA-homozygous donor do not suffer from GVHD, there is probably a role for other factors. Temporary discontinuation of immunosuppressive therapy and the number of lymphocytes carried by the graft can favor development of GVHD. It has also been reported that the most common Caucasian HLA haplotype A1 B8 DR17 DQ2 is a risk factor for development of GVHD [14, 47–49].

Age of the recipient is also a risk factor and recipient age >65 years carries a nine fold increased risk of developing post-OLT GVHD. Age difference between recipient and donor is also a risk factor and age difference >40 years carries a 10-fold increased risk. With increasing age there may be a loss of immune response against donor lymphocyte, making it a risk factor [3, 6, 14, 33]. Recently a multivariate analysis showed that a difference of more than 20 years between donor and recipient is a major risk factor for post transplant GVHD [50].

Infection with cytomegalovirus (CMV) and herpes simplex virus (HSV) can depress immunocompetence and increase the risk for GVHD after OLT [51]. Diabetes mellitus type I and II, various autoimmune diseases, infectious state of liver and hepatocellular carcinoma cause immunodeficient states and increases the risk for GVHD. Also alcoholic liver disease, particularly in combination with hepatocellular carcinoma and glucose intolerance, confers a very high risk for GVHD [52].

Diagnosis of post-OLT GVHD

Post-OLT GVHD poses a diagnostic challenge and may be under-diagnosed due to the lack of specific clinical or histopathologic features. Early diagnosis of GVHD is important to decrease the severity of infections as well as mortality rate. The diagnosis may be delayed, because similar presentations can be seen with drug reactions and bacterial or viral infections. Diagnosis is made on the basis of clinical and histological evidence and the presence of chimerism. Post-OLT GVHD can be classified into four stages based on the clinical sign and symptoms (Table 2). Serologic and molecular techniques such as polymerase chain reaction and flow cytometry for detection of HLA alleles have been used to diagnose post-transplant GVHD. Due to the nonspecific features of rash and other clinical

symptoms, chimerism is an important investigation to confirm OLT related GVHD, in the presence of symptoms [5]. Chimerism is the presence of donor lymphocytes in the recipient's peripheral blood, skin, or bone marrow. Chimerism described as the presence of genetically different cells in the same organ or organism, was first reported in the context of liver transplantation in 1969 and systemic microchimerism, establishment of donor derived cells (especially hematopoietic cells), in recipient in 1992. Chimerism after a liver transplant most often occurs after a period of 3–4 weeks, with a peak in the first two weeks then declining in the third and fourth weeks [10], hence GVHD predominantly occurs in first few weeks (2–8 weeks). It has been suggested that macrochimerism with > 1% of the circulated donor T-cells in the peripheral blood of recipient confirms donor engraftment, and an increased level of >10% donor CD8+ T/NK cells is indicative of GVHD. Though chimerism is an important investigation, presence of chimerism in absence of clinical symptoms and histological findings is non-specific, making macrochimerism only a diagnostic tool [6, 53–54]. Also the severity and duration of chimerism varies with the evolution of patient and it may be a pre-requisite for acceptance of the graft. So presence of donor HLA in recipient blood within first week post-transplant may not be used to diagnose GVHD. Higher incidences of acute rejection of liver after transplant may be associated with low level of chimerism [12]. Presence of donor lymphocytes in recipient is most commonly assayed by HLA typing through serologic or polymerase chain reaction based techniques, or by polymerase chain reaction based assays of highly polymorphic short tandem repeats within genomic DNA. Application of short tandem repeat (STR) assays to detect donor DNA within affected tissue specimen gives an advantage over chimerism [31, 55]. HLA typing also helps in confirming that the engrafted lymphocytes are of liver donor origin and not from blood transfusion. STRs are very useful in initial diagnosis, but results of HLA typing are available more quickly. STRs are also useful in following the course of treatment [3]. Fluorescence in situ hybridization (FISH) studies of skin biopsy specimen directed at the Y-chromosome in sex-mismatched patient with OLT can be used for early diagnosis of chimerism in post-transplant GVHD [34, 56–57].

The skin manifestations of GVHD may provide a clue of ongoing GVHD and may facilitate in changing the course of treatment. Other similar skin manifestations should be ruled out by histological and immune-histological criteria. Histological changes of skin in GVHD include the following: in grade I, lymphocytic infiltrates in the upper dermis without epidermal changes; in grade II, vacuolization of basal cells; in grade III, subepidermal clefts through confluence of basal vacuolization; and, in grade IV, massive necrosis of keratinocytes resembling toxic epidermal necrolysis [58].

It is important to note that though GVHD occur most commonly in the first few weeks post transplant, Pollock et al reported a case of OLT with gastrointestinal symptoms after 8 months [14]. In the literature there are reported cases of recurrent GVHD as well as late onset chronic GVHD occurring months and years after liver transplant [49, 59–61].

Existing Treatment Modalities for post OLT-GVHD

Most of the treatment modalities described in literature depend on the conceptual model of the pathogenesis of GVHD [13] involving epithelial destruction and release of pro-

inflammatory cytokines leading to proliferation and clonal expansion of donor T cells. Treatment modalities include either reduction or amplification of immunosuppressive therapy, but most of the studies have favored immunosuppressive therapy. Support of hematopoiesis with cytokines (G-CSF or GM-CSF) and discontinuation of drugs causing myelosuppression and start of treatment before the onset of severe pancytopenia has also been suggested for better outcomes [3]. There is no US Food and Drug Administration approved treatment for post-OLT GVHD.

Immunosuppression with antimetabolites, alkylating agents, anti-T cell antibodies, anti-B cell antibodies, intravenous immunoglobulin, cytokine inhibitors along with steroids, immunostimulation, cellular therapy and HSCT have been tried [9] (Table 3). Increased immunosuppression with high-dose steroids and antibody preparations such as antithymocyte globulin, antilymphocyte globulin and prednisolone comprises standard treatment of GVHD and steroids alone are not effective in GVHD. It has been reported that treatment with anti-lymphocytic agents is not adequate and survival is poor [4]. An early start of therapy for GVHD is no determinant to outcome. As bacterial and fungal complications are more frequent in post transplant period, antibiotics and antifungal play a pivotal role [6, 22].

Corticosteroids as monotherapy have been described in the literature due to their ability to induce apoptosis in lymphocytes and profound anti-inflammatory effect. It was also documented that high doses of corticosteroids may increase the risk of mortality in these patients due to infection and multi-organ failure as a side effect of high dose corticosteroids [4]. Corticosteroid along with immunosuppressive therapy has also been documented with inconsistent results. Chaib et al. [6] reported a case of liver transplantation treated with immunosuppressant (tacrolimus) and corticosteroid pulse therapy. Patient was discharged after recovery but was readmitted with a presumptive diagnosis of GVHD. Specific infectious agents could not be identified. The patient was treated with methylprednisolone and antithymocyte globulin, but the patient died due to multiorgan failure. Though the tacrolimus and corticosteroid were given as immunosuppressive agents, patient did not survive and died due to post-transplant GVHD [6].

Rogulj and co-investigators [9] reported a case of a 53-year-old woman who received a liver transplant from a cadaveric donor and was admitted with a working diagnosis of bone marrow suppression due to GVHD with no other signs or symptoms of GVHD. Immunotherapy was withdrawn and rabbit anti-thymocyte globulin (ATG) 1.5 mg per kg for three consecutive days and alefacept 30 mg per dose, twice weekly for three doses were given to the patient. The patient's bone marrow aplasia persisted and further erythematous, pruritic and painful skin rash, sepsis and post-transplant lymphoproliferative disorder developed. Patient's condition deteriorated more even after hematopoietic stem cell transplant and she died from septicemia, pulmonary aspergillosis and respiratory distress syndrome [9]. Perri et al. [4] reported treatment with immunosuppressive therapy in 5 post-OLT GVHD patients with anti-thymocyte globulin, but none of the patients survived. These reports show the inadequacy of corticosteroids and immunosuppressants for GVHD treatment. However successful treatment of GVHD in second liver transplant patient with

immunosuppression has also been reported [62]. Recently with antithymocyte globulin, significantly lower rate of chronic GVHD after allogenic transplant has been reported [63].

Cytokines released as a result of leukocyte destruction play an important role in development of GVHD. Interleukin-2 plays an important role in the pathogenesis of post transplant GVHD and hence its membrane bound ligand CD25, appears to be an attractive target for intervention in GVHD. Anti-CD25 monoclonal antibodies (daclizumab, basiliximab) have been successfully tried in post transplant patients for the treatment of GVHD. It has also been suggested that small bowel resection, not responding to immunosuppressive therapy aids in treatment of GVHD [30, 64]. Kohler et al. [8] reported a case in which post-mortem biopsy of skin, lung and kidney showed no histological sign of GVHD, only gut biopsy showed the histological sign of GVHD.

Tumor necrosis factor –alpha could also be a therapeutic target in GVHD treatment, but treatment with infliximab, a chimeric anti-tumor necrosis factor-alpha monoclonal antibody, hasn't shown promising results with reports of increased infectious complications [65–66]. First successful treatment of OLT-GVHD with anti-tumor necrosis- α agent, etanercept was reported by Thin et al. It was also suggested that antifungal agents should be used along with etanercept for fungal infections. Etanercept therapy also has less infectious complication as compared to infliximab and these differences may be due to cytolytic effect of infliximab [10].

Treatment of post-OLT GVHD by withdrawing immunosuppressive therapy has given some promising results, but initial worsening of symptoms has been observed with an increased risk of graft rejection. It has been suggested that withdrawal of immunosuppressive therapy will allow the recipient's immune system an opportunity to reject the transplant [32, 47, 53, 67–68]. Calmus et al. [45] suggested that in cases of HLA-homozygous donor in a haploidentical recipient, reduced or withdrawal of immunosuppressive therapy will not be helpful and GVHD should be treated with increased immunosuppression and hematopoietic growth factors.

Review of the literature suggests that increasing the dose of methylprednisolone increases the incidence of fungal infection. Long term sequel of the steroids can be prevented by combine use of infliximab and pentostatin. Mycophenolate by interfering with lymphocytes though plays an important role in treatment of stage I and II of acute GVHD but there is increased risk of pancytopenia. For treatment and preventing the occurrence of stage III and IV GVHD, tacrolimus is more potent than cyclosporin [38]. Worsening of the GVHD can be caused by OKT3 by increased release of pro-inflammatory cytokines due to lymphocyte destruction [8].

Abatacept is a US Food and Drug Administration approved selective T-cell costimulation modulator and an antibody that is directed against cytotoxic T lymphocytes. Abatacept has been used in treatment of rheumatoid arthritis. Elfeki et al. [7] reported the treatment of acute GVHD after OLT using abatacept in a 60-year-old patient, who underwent OLT owing to familial amyloidosis. Abatacept infusion was given after withdrawal of

immunosuppressive therapy and it was reported that patient showed clinical and laboratory improvement [7].

Another therapeutic strategy for the prevention and treatment of GVHD is to increase the immunocompetent status of the recipient [32, 69]. Restoration of the recipient immune system can be achieved by either inducing a host-versus-graft alloimmune response or by withdrawal of immune-suppression; so that the recipient's immune system may reconstitute itself. Restoration of the recipient's immune system will reduce the risk of infection [69]. Autologous bone marrow transplant after GVHD and re-infusion of ex-vivo enriched lymphocytes has shown promising results with the resolution of symptoms of GVHD [68, 70–71]. It has also been proposed that in patients with a peripheral chimerism of more than 10% after OLT, immunosuppressants can be reduced or even withdrawn without increased risk of rejection [72]. It has been proposed that depletion of graft T cells by perfusion of anti-T cells, irradiation of graft, removal of obvious nodal tissue from graft, and elimination of host antigen presenting cells plays a role in preventing GVHD after HCT [73–74]. Treating GVHD by removing the aggressive graft and re-transplanting has not been supported by the research data [6, 8].

Treatment of acute graft versus host reaction after orthotopic liver transplant with anti-metabolites (azathioprine), alkylating agents (cyclophosphamide), anti-T cell antibodies (anti-thymocyte globulin, OKT3, Campath, Alefacept), anti-B cell antibodies (rituximab), immunoglobulins, cytokine inhibitors (TNF- α inhibitors-infliximab, etanercept, interleukin-2 inhibitors-daclizumab, basiliximab), immunostimulants (thymosin- α) and cellular therapy with hematopoietic cell transplantation and ex vivo T-cell expansion have been tried. But to date there is no single successful treatment strategy that has been identified and treatment of post transplant GVHD continues to be a challenge. There is a need for further study and research for early diagnosis and novel modalities for treatment of GVHD (Figure 2).

Novel Treatment Modalities for post OLT-GVHD

Anti-T cell antibody therapy has shown success in treating steroid resistant GVHD, but there are risks of viral reactivation and development of post-transplant lymphoproliferative disease. Cytokine inhibitors therapy has also shown some benefit but the need for antifungal prophylaxis has been suggested. Allogenic HCT may be a treatment of GVHD but many cases reported death of patient even after HCT. Decreasing the lymphocyte burden in donor liver and minimizing the immunosuppression of recipient increases the chance of graft rejection. Immunosuppression of the recipient can also lead to increased rates of graft rejection if immunosuppression of recipient is more than that of donor [9]. It is also important to know that the rationale of treatment in HCT-GVHD and OLT-GVHD is different. HCT associated GVHD treatment goal is to manage symptoms and to allow for tolerance of the recipient tissue to develop in the donor immune cells, while the goal in case of OLT associated immunotherapy is to prevent rejection [4]. So the approach to treatment should proceed accordingly. Mesenchymal Stem Cell therapy has been successfully used in treatment of HCT-GVHD and can be considered for OLT-GVHD [75–77]. Mesenchymal stem cell therapy has shown promising results in treating the steroid resistant GVHD [78].

Umbilical cord blood is a good source of mesenchymal stem cells [79] and NK cells [80] and can protect the patient from GVHD. Similarly the fetal membrane cells and decidual stromal cells from fetal membrane modulates the allogenic immune response and tissue repair and has been successfully used for the treatment of GVHD after HSCT [81–83]. It has been reported that in HCT, there is a greater risk of GVHD with female donors [84] and this should be considered for study in OLT too. Successful treatment with growth hormone for maintenance of liver function in a child with liver transplant due to NASH has been reported [85] and a multivariate analysis suggest that overall survival and symptom free interval after a transplant depends on tumor type, morphology and biology and not on graft type [86].

Recently Hechinger et al. [70] proposed the therapeutic inhibition of multiple common gamma chain cytokine (CD132) for treatment of acute and chronic GVHD. It has been proposed that CD132 is a subunit of interleukin receptors for IL-2, IL-4, IL-7, IL-9, IL-15 and IL-21, and the level of these cytokines increases in patients with acute and chronic GVHD. Inhibition of CD132 can be a potential therapeutic option in GVHD treatment. It was observed that anti-CD132 monoclonal antibody protects from GVHD by inhibition of granzyme B production in CD8⁺ cells; reduces acute GVHD potently with respect to survival, and reduces production of TNF- α , IFN- γ and IL-6. There is reduced JAK3 phosphorylation from activated T cells and microarray based analysis shows a more naïve phenotype after T cell exposure with anti-CD132 monoclonal antibody. Liver and lung fibrosis and pulmonary dysfunction of bronchitis obliterans of chronic GVHD can be resolved with anti-CD132 monoclonal antibody [87]. Sirolimus, which inhibits the mammalian target of rapamycin essential for T cell proliferation, can be a potential therapeutic agent [38].

It has been proposed by Fu et al. [88] that targeting T-bet (T-box transcription factor), a master regulator for IFN- γ and Th1 differentiation, or regulating its downstream effectors independent of IFN- γ may be a promising strategy to control acute GVHD. It has also been demonstrated in mice models that bacterial lipopolysaccharides (LPS), Toll like receptors (TLR4, TLR5, TLR7 and TLR9) and NOD-like receptors (NLRs) like NOD2 plays a role in pathogenesis of acute GVHD. It has been demonstrated that mutation in TLR4 gene reduces the GVHD risk and ligation of TLR9 with bacterial DNA induces acute GVHD. Similarly polymorphism of TLR 4 and NLRs is associated with higher incidences of acute GVHD and NOD2 and TLR 5 ligand flagellin have an inhibitory effect on GVHD. Thus, the inhibition of MyD88, a downstream molecule on TLR pathways, could play a role in preventing GVHD. There may be a role of regulatory T cells (Tregs) or inducible T-regulatory cells (iTregs) [89] in GVHD inhibition, attenuation and treatment [82, 90]. Damage associated molecular pattern molecules (DAMPs) have also been documented in GVHD and DAMPs inhibition can also be a potential target. Strategies like targeting B cells and B cell depletion with monoclonal antibody, targeting T cells, T cell co-stimulatory molecules inhibition, inhibition of IL-1, IL-6, INF- γ and neutralization of TNF- α can be of potential therapeutic advantage. High level of these pro-inflammatory cytokines plays a crucial role in HSCT GVHD. Activated Janus Kinases (JAK) are required for the response of T-effector cells during inflammation and its inhibition may play a role in reducing the incidence of GVHD. Ruxolitinib, a JAK inhibitor has been successfully used for suppression of inflammatory signaling and can be a potential therapeutic agent for treatment of GVHD [91–96]. All these

strategies have been studied in relation to HCT and need further study and clinical research in relation to OLT [97–98]. Use of lipopolysaccharide antagonists, interleukin-12 inhibitors and use of keratinocyte growth factor are potential aspects for further study to decrease severity of GVHD [13].

Prognostic indicators

Development of pancytopenia is a poor prognostic indicator and indicates the onset of inevitable decline [10, 30, 54]. It has been suggested that engraftment of bone marrow stem cells from the liver graft may be a good clinical sign in patients with GVHD [3].

Conclusion

Graft versus host disease after orthotopic liver transplant poses many challenges in diagnosis and treatment. Though, delay in diagnosis may influence the prognosis of disease, early treatment is also not a determinant of outcome. Non-specificity of symptoms and infections after the immunosuppressive therapy poses further problems. Measures should be taken to prevent post-OLT GVHD and careful donor selection, pre-transplant depletion of donor lymphocytes by irradiation or with lytic monoclonal antibodies against lymphocyte cell surface protein and reduced immune-suppression may be fruitful [13]. The chances of a better outcome can be increased by having a high index of suspicion, making an early diagnosis and starting early treatment before the onset of severe pancytopenia. It is also important to note that, as reported by some authors, GVHD can occur after a long period post transplant, it has been suggested that cell pellets from transplant recipient as well as of donor should be stored to facilitate the diagnosis [14]. Therefore, any patient with fever, diarrhea and rash on body should be thoroughly investigated and GVHD should be a differential diagnosis. Supportive medical therapy, prophylaxis for opportunistic infections, isolation and nutritional support can improve the outcome and decrease the incidence.

Expert commentary and five-year view

Considering the complexity and delay in diagnosis of graft versus host disease after liver transplant, no definitive treatment and very low survival rate due to multi-organ failure and sepsis, it is very important to make GVHD as a differential diagnosis in a liver transplant patient with symptoms of fever, rash or diarrhea. Assessment of pre-transplant risk factors and post-transplant prognostic indicators along with Keystone criteria for classification of GVHD may be of potential help in improving the patient outcome. Though reduction or amplification of immunosuppressive therapy with immunosuppressant and corticosteroids remains the main treatment modality till date, the results are not satisfactory and hence no definitive treatment has been advised. Recently cytokine antagonists, removal of the rejected graft, removal of the inflamed bowel, re-transplantation, graft T cell depletion and inhibition of gamma chain cytokine have been tried for GVHD treatment. As activation and clonal expansion of the immunocompetent donor T lymphocyte mounts the cellular response resulting in GVHD, anti-T-cell antibody seems to be a promising treatment modality. At present stem cell therapy is an evolving area of research for treatment of various diseases; further research is needed for using stem cell therapy in GVHD. The role of toll like

receptors, NOD-like receptors, DAMPs and PAMPs in pathogenesis of GVHD has been discussed; the specific inhibitors for these molecules as well as inhibitors for downstream signaling molecules may be a novel therapy for GVHD. Also the serum analysis of these markers may help in early diagnosis of GVHD.

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Reference annotations

* *Of interest*

** *Of considerable interest*

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Key issues

- GVHD after liver transplant is a rare disease with very high mortality.
- Immunoreactions between immune-competent donor T-lymphocyte and recipient surface antigens result in GVHD.
- Post transplant GVHD involves skin, liver, intestine and bone marrow of the patient.
- Fever, rashes, diarrhea and pancytopenia are the main clinical symptoms of GVHD.
- Rashes of GVHD should be differentiated from skin rash of TEN and CMV and HSV infection.
- Early diagnosis of GVHD after liver transplant can improve the outcome.
- Immunosuppressive treatment with immunosuppressant and corticosteroids is the commonly used treatment for GVHD.
- Assessment of risk factors before transplant and prognostic markers after transplant is very important for better outcome.

Figure 1.

Schematic diagram showing pathogenesis of GVHD in liver transplant. The first phase includes mucosal damage of recipient due to chemotherapy, irradiation, infections or liver disease causing an influx of bacterial endotoxin like lipopolysaccharide (LPS) from the intestinal lumen into the blood. Macrophages secrete cytokines and chemokines, including interleukin-1 (IL-1) and tumor necrosis factor-alpha (TNF- α) leading to up-regulation of major histocompatibility complex I and II (MHC-I, MHC-II) expression and adhesion molecules, which cause further increase in antigen presenting capacity. Association of antigen presenting cells (APCs) and donor T cells causes activation, proliferation and clonal expansion of T lymphocytes via cytokines like Interleukin-2 (IL-2) and Interleukin-12 (IL-12), and these activated T cells will further secrete IFN-gamma (IFN- γ) leading to proliferation of effector cells like natural killer cells (NK cells) and cytotoxic T lymphocytes (CTLs). These effector cells migrate to target organ and cause symptoms of GVHD. Both specific effector cells (CTL) and nonspecific effector cells (NK cells and macrophage) contribute to tissue damage. CTLs cause apoptosis through release of granzyme or perforins or by Fas-Fas ligand interaction [13].

Figure 2.

Schematic diagram showing existing and potential novel treatment modalities for post-OLT GVHD. —|— Indicate negative regulatory role of the drug; Post-OLT GVHD-post-orthotropic liver transplant Graft versus Host Disease; IL (interleukin)-1, 2,12; TNF (tumor necrosis factor)-alpha; IFN (interferon)-gamma; TLRs-toll like receptors; NLRs- NOD-like receptors; DAMPs- damage associated molecular pattern; LPS-lipopolysaccharide; T-bet - T cell-Bromodomain and Extra-Terminal motif; OKT3-Purified Anti-human CD3 Antibody Anti-CD3; APC-antigen presenting cells; TCR-T-cell receptor; MHC- major histocompatibility complex. (For the description of mechanism of action of drugs and inhibitors, please see the text).

Table 1

Three phases of development of GVHD after an organ transplant. Cytokine-injury-cytokine cycle will result in development of inflammation and finally give rise to GVHD [38].

Phase-I Pre-treatment/ Conditioning	Phase-II Activation phase/ proliferation phase	Phase-III Evolution phase/ Effector phase
Chemotherapy, radiotherapy, release of endotoxin following infection, blood transfusion, treatment for the disease, prior liver disease ↓ Affect endothelial and epithelial cells ↓ Release of inflammatory cytokines like IL-1, IL-6, TNF-α ↓ Upregulation of antigen and adhesion molecules on histiocytes of target organ and immunoreaction with T lymphocyte	-Activation of T lymphocyte -MHC-I antigen inconsistency of donor and recipient →CD8+ cytotoxic cell proliferation -MHC-II antigen inconsistency of donor and recipient→CD4+ cytotoxic cell proliferation -Polarization of T cells into CD4+ T helper-1 cells -Secretion of IL-2 and IFN-γ from Th-1 cells ↓ T cell proliferation and NK cell activation	Donor mononuclear cells activation by IL-2 and IFN-γ ↓ Secretion of large amount of IL-2 and TNF-α ↓ Development of GVHD and onset of symptoms

IL-1: Interleukin-1; IL-2: Interleukin-2; IL-6: Interleukin-6; TNF-α; tumor necrosis factor-alpha; IFN-γ: interferon gamma; Th-1: T helper cell-1; MHC-I/II: major histocompatibility complex I and II.

Table 2

Keystone criteria for classification of acute GVHD.

Stage-I	Stage-II	Stage-III	Stage-IV
Rash area <25% Hemoglobin 24.2–51.3µmol/L Diarrhea >500ml or Persistent nausea	Rash area – 25–50% Hemoglobin 51.3–102.6µmol/L Diarrhea >1,000 ml or Persistent nausea	Rash area >50% Hemoglobin 102.6–256.5µmol/L Diarrhea >1500ml or	Extensive erythrodermia with blister formation, Hemoglobin >256.5µmol/L and Severe abdominal pain with or without intestinal obstruction.

Table 3

Treatment modalities used for treatment of post liver transplant GVHD.

Therapy	Reference	Age/ sex	GVHD onset	Symptoms	Outcome	Cause of death
Abatacept	Elfeki et al [7]	60 yr/M	POD 26	Skin rash, diarrhea, fever	Pt. survived	
MP, ATG	Chaib et al [6]	64 yr/M	POD 31	Skin rash, fever, maculopapular eruptions, diarrhea leukopenia,	Pt. died POD 36	Multiple organ failure
ATG Alefacept Rituximab HCT	Rogulj et al [9]	53 yr/F	POD 38	Tremors, headache, forgetfulness, difficulty in word finding	Pt. died POD 86	Septicemia, Aspergillosis, RDS
ATG	Perri et al [4]	59 yr/F	POD 24	Fever, rash	Pt. died POD 38	Invasive Aspergillosis
ATG Basiliximab	Perri et al [4]	66 yr/F	POD 35	Fever, rash	Pt. died POD 126	Respiratory failure
MP	Perri et al [4]	55 yr/M	POD 23	Fever, rash	Pt. died POD 41	Sepsis
ATG	Perri et al [4]	48 yr/F	POD 34	Fever, rash, pancytopenia	Pt. died POD 385	Aspiration pneumonia
ATG Basiliximab	Perri et al [4]	64 yr/M	POD 27	Fever, diarrhea, leukopenia	Pt. died POD 293	Invasive Aspergillosis
Tacrolimus, MP ATG	Calmus et al [45]	48 yr/M	POD 35	Fever, diarrhea	Pt. died POD 61	Pneumonia
HCT	Pollack et al [14]	52 yr/M	8 months after transplant	Oral thrush, skin rash, fever, diarrhea	Pt. died 5 days after HCT	<i>Candida krusei</i> infection, multi-organ failure
Basiliximab, bowel resection	Sudhindram et al [30]	45 yr/M	POD 24	Fever, rash and vesicular lesion	Pt. survived	
Basiliximab	Sudhindram et al [30]	56 yr/M	POD 31	Maculopapular rash, diarrhea	9 months after OLT	Recurrent fibrosing hepatitis

Therapy	Reference	Age/ sex	GVHD onset	Symptoms	Outcome	Cause of death
Reduced immunosuppression	Chinnakotla et al [47]	58 yr/M	10 weeks post OLT	Skin rash	Pt. Survived	
Reduced immunosuppression	Chinnakotla et al [47]	52 yr /M	18 weeks post OLT	Fever, neutropenia	Pt. survived	
Reduced immune-suppression, ATG	Chinnakotla et al [47]	62 yr /M	2 weeks post OLT	Generalized rash	Pt. died POD 63	Bacterial sepsis
Etanercept	Thin et al [10]	65 yr/M	Pod 20	Rigors, pancytopenia	Pt. survived	
FK506, MP	Zhang et al [38]	53 yr/M	POD 26	Fever, rash, diarrhea, pancytopenia	Pt. died POD 46	Septicemia, RDS, multiple organ failure
Basiliximab Cyclosporine A ATG, OKT3	Kohler et al [8]	30 yr/M	POD 42	Fever, skin rash	Pt. died POD 54	Cerebral hemorrhage
MP, ATG, OKT3, plasmapheresis	Kohler et al [8]	44 yr/F	POD 20 after re- transplant	Skin rash	Pt. died POD 31 after re- transplant	Sepsis, multi-organ failure
MP, ATG	Kohler et al [8]	61 yr/M	POD 60	Skin rash, fever	Pt. died POD 88	Multi-organ failure
None	Kohler et al [8]	64 yr/F	POD 35	Intracranial bleeding, skin rash	Pt. died POD 47	Pancytopenia, multi-organ failure, septic shock
MP	Kohler et al [8]	67 yr/M	POD 34	Skin rash, hypotension, weakness	Pt. died POD 39	Sepsis, multi-organ failure
Tacrolimus, mycophenolate	Yilmaz et al [60]	49yr/ M	POD 22	Elevated liver enzyme, skin rash	Pt. died POD 8 months	Multi-organ failure

Note: In most post-transplant cases initial immunosuppression was done with tacrolimus, mycophenolate mofetil and corticosteroid. Sepsis and multiple organ failure are the main reason for patient death. ATG: Anti-thymocyte globulin; MP: methylprednisolone; RDS: Respiratory distress syndrome; HCT: Hematopoietic Stem Cell transfusion; OLT: orthotopic liver transplant; POD: post-operative day.