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Might liver transplantation recipients with primary hepatocellular carcinoma benefit from GVT effect of aGVHD?

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Abstract ***Objective:*** We aimed to access if acute graft-versus-host disease (aGVHD) in liver transplantation recipients of hepatocellular carcinoma (HCC) might develop a graft-versus-tumor effect (GVT) other than immunological damage which would benefit prophylaxis of tumor recurrence. ***Methods:*** Dynamic observation of 3 cases of liver transplantation recipients of HCC and cirrhosis, which developed manifestations of fever, skin rash, watery diarrhea, pancytopenia and were finally diagnosed as aGVHD. Two of which got recovered from intravenously pulse methylprednisolone, high-dose intravenous immunoglobulin, antibiotics administration simultaneously and promptly withdrawal of oral immunosuppressants. Two survivors were follow-up regularly with biological monitoring and imaging surveillance for tumor recurrence thereafter. ***Results:*** Two recipients survived healthily with stable liver graft function and normal serum AFP level and blood routine test. No sign of tumor recurrence was found in repeat imaging examinations for liver graft, lung, brain and other tissue or organs within a period of 96 months and 17 months to date, respectively. ***Conclusion:*** Despite of the fatal damage to according organs and tissue, it suggest that aGVHD in liver recipients of HCC may also develop a GVT effect and benefit prophylaxis of tumor recurrence and result in a long-term healthy recipients survival.

Key words hepatocellular carcinoma (HCC); liver transplantation; acute graft versus host disease; graft versus tumor

Graft versus tumor (GVT) effect has been known as graft versus host allogeneic immune response, which was first described by Bames *et al* in their animal study of bone marrow transplantation in 1956. In clinical practice of allogeneic hematoic stem cell transplantation (allo-HSCT) for treatment of leukemia, Graft-versus-host reactions can lead to multi-organ damage or even death of host, which is so-called graft-versus-host disease (GVHD). On the other hand, graft-versus-host reaction can also remove residual leukemia cells which may cure of leukemia, named GVT effects [1]. Therapeutic effect of allo-HSCT to leukemia mainly depends on the GVT effect. Liver transplantation (LTx) for hepatocellular carcinoma (HCC) accouts for 40% of all LTx recipients in China so far. Incidence of acute graft-versus-host disease (aGVHD) after LTx is relatively rare, only about 1% to 2% as reported. However, multiple organs are often involved in the suffered, with a fatality rate as high as 80%. Does aGVHD after LTx present GVT effect? Here we report three LTx recipients of HCC who developed aGVHD post-operatively. Two recipients got recovered and have survived tumor-freely for 96 months and 17 months, respectively.

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Materials and methods

General clinical data (Table 1)

A total of 137 patients underwent LTx in our center between April 2002 and October 2013. Among them, 53 patients suffered from HCC as primary disease. aGVHD were diagnosed in 3 recipients post-operatively. All 3 cases were from HCC patients who received orthotopic liver transplantation. During surgery, tumor was revealed to break through liver capsule and invade right psoas in case 1. During the perioperative period, case 1 received 12 blood units transfusion, case 2 received 14 units and case 3, with a surgery history of percutaneous transhepatic variceal embolization and splenectomy, underwent laparotomy for hemostasis next day to transplant and 42 RBC units were given, respectively.

Immune suppressive regimen included 500 mg of Methylprednisolone (MP) administrated during surgery,

Table 1 General clinical features

	Case 1	Case 2	Case 3
Surgery date (Y/M)	2006/01	2012/08	2013/05
Gender	Male	Male	Male
Age of donor/recipient (years)	56/26	72/23	61/33
Primary diseases	Cirrhosis and HCC	Cirrhosis and HCC	Cirrhosis and HCC
Hepatitis virus infection	HBV	None	HBV
Child-Pugh class	B	C	C
Serum AFP (umol/L)	3256.7	76.6	63.2
Tumor number	Single	Multifocal	4
Maximum tumor diameter (cm)	14.2	4.0	4.5
Macrovascular invasion	No	No	No
Histological grade of tumor	G2	G3	G2

Table 2 Clinical manifestations and laboratory tests

	Patient #1	Patient #2	Patient #3
Early syndrome	High fever	High fever	High fever
Diarrhea	Watery 8–12 times/day	Watery 15–20 times/day	Watery 10–16 times/day
Appearance of skin rash	Erythemaous maculopapular rash	Erythemaous maculopapular	Erythemaous maculopapular rash to exfoliative dermatitis
Distribution of skin rash	Trunk to limbs	Head, neck and chest	Trunk to limbs
Low value of WBC (× 10^9/L)	0.5	0.6	0.1
Low value of PLT (× 10^9/L)	12.0	18.0	1.0
Chimerism	Peripheral blood (+)	Peripheral blood (+)	Unknown
Tc population (CD4+/CD8+)	1:4	1:4	1:8
Bone marrow biopsy	Hemophagocytosis	Mild suppression	Mild suppression deteriorate to aplastic anemia

20 mg basiliximab given intravenously after portal vein anastomosis finished and 300 mg to 500 mg of MP given once daily within 2 days after surgery. Maintenance dose of oral MP (16 mg/day) were given thereafter. The 500 mg mycophenolate mofetil (MMF) was taken twice daily since the first day after surgery. The initial dose of tacrolimus was 2 mg twice daily on post-operative day 2 and the dosage was adjusted to a maintenance trough whole blood concentration between 8 and 12 ng/mL thereafter.

Clinical manifestations and treatments of aGVHD

In these three patients, aGVHD was developed at day 16, day 14 and day 11 post-operatively. Fever, skin rash, diarrhea and hematocytopenia were typical clinical presentations in them. Progressive skin rash and watery diarrhea usually appeared 3–5 days after fever, followed with deteriorated leukopenia and thrombocytopenia and mild anemia could also be observed (Table 2). No specific infectious agents could be identified in sputum, blood, urine or abdominal fluid samples. The tests for CMV DNA and antibody, EB virus and serum galactomannan antigen were negative. Case 1 received bone marrow biopsy and mononuclear phagocytes appeared to be hyperplasia, accounting for more than 30% of karyocytes in the bone marrow slides. Hemophagocytosis, which was phagocytosis by macrophages of erythrocytes, leukocytes, platelets and their precursors in bone marrow, could be observed. Skin biopsy of case 3 suggested presence of superficial interface dermatitis with a lichenoid pattern with lymphocytic inflammation and predominately-vacuolar change in the basilar layer. When patients developed typical aGVHD manifestations, peripheral blood lymphocytes were isolated for HLA typing and which was repeated 3 months after aGVHD recovery. Then HLA typing of donors were taken into comparative analysis, which showed that donor specific HLA locus could be detected in peripheral blood lymphocytes of 2 survivors within progression of disease. The HLA typing results also confirmed aGVHD diagnosis retrospectively. Liver function and tomography findings, however, remained normal in the course of aGVHD arising.

Treatment of aGVHD was initiated by pulse use of MP. Initial dose of MP at 5mg/kg/d was given intravenously once or in two times daily when recipients were highly suspected of aGVHD with characteristic manifestations including fever, diarrhea and rash appeared and MP dosage reduced to half 3 days later. Body temperature should be maintained below 37.5 ℃, but body temperature of case 3 rebounded after MP tapering. So MP was restored

to initiating dose and was then tapered carefully by 1/3 daily. Additionally, intravenous immunoglobulin (IVIG) was given initially. Case 1 and case 2 received high dose of IVIG (0.4g/kg/d) with a 5-day course of treatment. Repeating courses of treatment was depending on progression of the disease. Unfortunately, case 3 could not receive full dosage of IVIG. Despite MMF was routinely used after transplantation, withdrawal of MMF was necessary when aGVHD arised with high fever, which may also lead to diagnosis of infection. Immunosuppressive treatments were continued with recombinant humanized anti-CD25 monoclonal antibody (50 mg every 5 days) when case 1 was diagnosed with aGVHD and when case 2 and case 3 had typical manifestations. Then patients were transferred to isolation ward for intensive nursing for dermal and oral cavity, pharynx, larynx and anoperineal. When hematocytopenia was observed or myelosuppression was suggested in bone marrow biopsy, granulocyte colony-stimulating factor or recombinant human interleukin-11 was given subcutaneously. Low dose of vancomycin was administered as prophylactic for infections. Ornidazole and fluconazole might also be given depending on individual needs. Proton-pump inhibitor (Losec, 40 mg, Bid) and probiotic supplements were routinely used. Patients received low dose transfusion of isolated red blood cells, fresh plasma and cryoprecipitate to prevent internal bleeding. Parenteral nutrition provided nutrients through peripherally inserted central venous catheters (PICC). Bone marrow biopsy from case 3 suggested secondary aplastic anemia, which warranted ATG-F administration for 100 mg/day. Unfortunately, patients died of fatal septicemia 3 days later.

Long-term treatments and follow-up

Immiunosuppressive agents included tacrolimus, MMF and MP were used for maintenance therapy. Tacrolimus dosage was adjusted to maintain whole blood trough concentration at 8 ng/mL thereafter. MMF was taken 0.5 g twice daily. Oral MP was withdrawal at 6 months after transplant. Case 1 received chemotherapy (Epirubicin 60 mg/m^2/d on days 1 and 2; cis-DDP 75 mg/m^2/d on day 1 to 3, totally 2 courses with an interval of 1 month), 1 month after aGVHD got cured with stable results of blood routine tests, liver function tests and tacrolimus concentration. Case 2 did not receive chemotherapy.

Monitoring of blood routine tests, liver function tests, tacrolimus concentration and serum α-fetoprotein (AFP) levels was undertaken regularly during follow-up in outpatient department. Liver ultrasound scanning, CT scanning of lung and brain were also conducted periodically.

Results

Normal results of blood routine tests and liver function tests (serum bilirubin level, AST and ALT levels, serum albumin level) were observed in 2 survivors 3 months after transplant. Serum AFP levels stayed under 3.5 μmol/L. Liver ultrasound imaging, CT scan of lung and brain showed negative image findings. Abnormal liver function tests with ascending AST and ALT levels was then found in case 1 five years after surgery. Further seropositivity of hepatitis B surface antigen (HBsAg) and raise of hepatitis B virus (HBV) DNA suggested HBV recurrence. This patient got recovered after administration of antiviral treatment (entecavir) and hepatoprotective medicine and effective suppression of HBV replication also achieved according to HBV DNA tests [2].

Two survivors have longer tumor-free survival time to date (96 months and 17 months, respectively). Serum AFP levels stay normal. Abdominal ultrasound examination and CT scan of lung and brain show neither signs of HCC recurrence nor organs metastasis. Liver grafts also work well so far. Case 1 had been back to daily work since 2 years after surgery. Case 2, 74 years old now, is able to perform activities of daily living.

Discussion

China is one of the countries with highest incidence of HCC. Owning to cirrhotic liver, large or multifocal tumor, many patients have lost opportunity to receive curative hepatectomy. Therefore, a considerable number of liver transplant recipients are from patients with HCC. However, recurrence of HCC after transplantation becomes one of the most important factors threatening lives of recipients. How to prevent HCC recurrence after LTx? Firstly, restrictive criteria of transplantation indication, which aims at selecting optimal patients with HCC for LTx is of most important. Milan criteria (single tumor of up to 5 cm in diameter or 2–3 masses with diameter less than 3 cm) are most frequently accepted. The 4-year recurrence-free survival rate of patients fulfilling Milan criteria was 83% after transplant. Nowadays some institutions tend to expand the Milan Criteria in order to benefit more patients to LTx. Hangzhou Criteria, which consider the AFP level and tumor histological grade besides larger tumor volume, shows no significant difference in survival rate compared with Milan criteria [3]. Hence, more Chinese patients may benefit from Hangzhou Criteria. Secondly, favorable adjunctive therapies are thought to be associated with increased survival after LTx for HCC. For instance, sirolimus-based immunosuppression has proved with unique effects in HCC patients leading to improved survival [4]. Several trials of adjuvant

of neoadjuvant systemic chemotherapy in LTx with HCC have been performed trying to limit the proliferation of already established microscopic spreading of the tumor cells in circulatory system. However, the chemotherapy regimens have not reached consensus. Further, trials of chemotherapy have failed to show any survival benefit. A randomized clinical trial from Soderdahl *et al* suggests that neoadjuvant treatment with systemic low-dose doxorubicin seems not to improve either survival or tumor-free survival in patients with HCC undergoing LTx [5]. It also has been reported that sorafenib administration after LTx can improve overall survival of HCC patients [6–7].

Transplantation immunology associated mechanisms may contribute to tumor elimination. With respect to allo-HSCT most often performed for patients with malignant cancers of the blood or bone marrow, patients with HCC are the most common to receive solid organ transplantation. Allo-HSCT is often performed after high-dose radiation and chemotherapy to treat hematological malignancy and aGVHD is a major complication after surgery. Nevertheless, aGVHD is a double-edged sword. On one hand, it attacks host tissues and organs. On the other hand, it may also turn against leukemia cells residual in the host. This beneficial aspect of aGVHD has come to be known as the GVT effect and it is a very important reason to perform allo-HSCT in patients with hematological malignancy. However, achieving a balance between aGVHD and GVT is difficult. Major efforts of researchers have been directed toward molecular immunological strategies that may prevent clinically significant aGVHD more effectively and enhance the GVT effect to control progression of malignant disease early after transplantation [8–9].

With large volume and widespread microvessel network, donor liver always contains large amount of nucleated cells include lymphocytes and phagocytic-histiocytic reticular cells, which can migrate to peripheral blood of recipient leading to a state of chimerism after LTx. T lymphocytes carried by donor liver were regarded as critical factor contributing pathogenesis of aGVHD. aGVHD after LTx, with atypical clinical manifestations, progresses quickly. Therefore, when clinical diagnosis is built, most of patients have already been in critical condition. Recipients fatality rate of aGVHD after LTx are usually more than 80% [10], most of which die of sepsis or fatal internal bleeding. To date, most researches in aGVHD treatments have focused on improvement of survival rate. Nevertheless, whether aGVHD hinder recurrence of HCC after LTx or not has rarely been discussed. In our case report, three HCC patients after LTx with secondary aGVHD have received treatments including early withdrawal of immunosuppressive drugs, high dose of MP and IVIG administration. The treatments bring rapid improvement and stronger immunity. Lower risk of complication including infection and internal bleeding has also achieved so that 2 patients got over the hump. Impressively, they have remarkable long tumor-free survival time though they are not meeting Milan criteria or even Hangzhou criteria (case 1 suffered from very large tumor and with high level of serum AFP, case 2 had poor differentiation tumor involving both left and right lobe). It is possible that residual hepatocellular carcinoma tumor cells in peripheral blood and lymph nodes, which express host MHC alloantigens, may have been destroyed by migrated graft alloreactive T cells, which results in a GVT effect and may therefore present protective role in tumor recurrence. The exact molecular mechanisms of GVT effect remain a largely elusive goal. The hypothesis may make researchers probe into brand new approach to prevent recurrence of HCC after LTx by taking advantage of transplantation immunity.

Conflicts of interest

The authors indicated no potential conflicts of interest.

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