Acta Biochim Biophys Sin 2011, 43: 576–581 | © The Author 2011. Published by ABBS Editorial Office in association with Oxford University Press on behalf of the Institute of Biochemistry and Cell Biology, Shanghai Institutes for Biological Sciences, Chinese Academy of Sciences. DOI: 10.1093/abbs/gmr043.
Advance Access Publication 16 June 2011

Short Communication

Association of human cytomegalovirus viremia with human leukocyte antigens in liver transplantation recipients

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Human cytomegalovirus (HCMV) reactivation is a common complication after liver transplantation (LT). Here, we investigated whether human leukocyte antigen (HLA)-matching was related to HCMV infection and subsequent graft failure after LT for hepatitis B virus cirrhosis. This retrospective study reviewed 91 LT recipients. All the patients were grouped according to HLA-A, HLA-B, and HLA-DR locus matching. Clinical data were collected, including complete HLA-typing, HCMV viremia, graft failure, and the time of HCMV viremia. HLA typing was performed using a sequence-specific primer-polymerase chain reaction kit. HCMV was detected by pp65 antigenemia using a commercial kit. The incidence of HCMV infection post-LT was 81.32%. Graft failure was observed in 16 of 91 (17.6%) patients during the 4-year study. The incidence of HCMV viremia was 100% (5/5), 91.4% (32/35), and 72.5% (37/51) in HLA-A two locus, one locus, and zero locus compatibility, respectively. Nevertheless, the degree of the HLA-A, HLA-B, or HLA-DR match did not influence the time of HCMV viremia, graft failure, or the time of graft failure after a diagnosis of HCMV viremia (all $P > 0.05$). An interesting discovery was that the risk of HCMV viremia tended to be higher in patients with better HLA-A compatibility. Graft failure, time of HCMV viremia, and graft failure after a diagnosis of HCMV viremia appear to be independent of HLA allele compatibility.

Keywords human leukocyte; liver transplantation; cytomegalovirus; hepatitis cirrhosis

Received: November 18, 2010 Accepted: May 11, 2011

Introduction

Human cytomegalovirus (HCMV) is a major pathogen and common opportunistic infection following liver transplantation (LT) [1]. Despite the development of antiviral therapies, HCMV remains one of the most common viral causes of morbidity and mortality following LT, potentially leading to allograft rejection, decreased graft and patient survival, and predisposition to opportunistic infections and malignancies [2–5]. Known risk factors for HCMV infection after LT include the HCMV D+/R− serological state, acute rejection, age, the dose of mycophenolate mofetil or prednisone, and the patient's immune state [6–10]. Cellular immune responses are essential for limiting infection with and replication by HCMV [11]. Through major histocompatibility complex molecule-mediated antigen presentation, T-cells can respond to pp65, immediate-early (IE)1 , and glycoprotein B antigens and then eliminate cells infected with HCMV [12–16]. Previous findings have suggested that human leukocyte antigen (HLA)-A, HLA-B, or HLA-DR match between the donor and recipient increases the incidence of HCMV hepatitis in both primary and secondary HCMV infections [17,18]. However, others hold contrary views that HLA matches have no significant effect on the post-transplantation [19,20].

In this study, we examined the incidence of HCMV viremia and graft failure, the time of HCMV viremia, and graft failure after diagnosis of HCMV viremia in LT recipients. We also investigated whether HLA matching influenced the clinical events after LT for hepatitis B virus (HBV) cirrhosis.

Materials and Methods

Patients

LT recipients with HBV cirrhosis treated at our hospital from July 2004 to September 2008 were enrolled in this study. Some patients were excluded for the following reasons: pp65-positive before LT, ABO blood type incompatibility, death within 1 month of transplantation, or missed follow-up visit. Finally, 91 recipients (78 males, and 13 females) were included. These patients had a median age of 44 years [inter-quartile range (IQR) 12]. There were 57 living and 34 deceased donors. The recipient operation was performed using orthotopic LT [21]. The donors and recipients were selected after considering age, blood type, graft size, HCMV status, and liver function. There was no ABO-incompatible transplant or HCMV-seropositive donor in our series. Every organ donation and transplantation in our center strictly followed the guidelines of the Ethics Committee of our hospital and the Declaration of Helsinki.

Follow-up study

Patients were examined at regular intervals in our outpatient clinic for liver enzymes, HBV DNA, hepatitis B antigen and antibody, and HCMV viremia. Follow-up was conducted once a week for the first month, twice a month during the second month, once a month thereafter during the first year post-transplantation, and at least bimestrially in subsequent years. The length of the follow-up period ranged from 1 to 48 months.

HCMV viremia was deemed asymptomatic if the recipient was pp65 antigenemia-positive, despite a lack of clinical signs or symptoms or laboratory abnormalities. Positive HCMV antigenemia was defined as the detection of >1 positive cell per 50,000 peripheral blood leukocytes (PBLs) in a blood sample.

The onset time point of HCMV infection and the time to graft failure were all calculated from LT.

HCMV detection

An HCMV antigen assay was performed as described previously with minor modifications [21]. A standard two-step immunohistochemical method was used to assay CMV antigen expression in PBLs . Briefly, leukocytes were separated from EDTA-anticoagulated blood and spread on slides. An anti-CMV-pp65-Ag monoclonal antibody (DAKO, Glostrup, Denmark), the EnVision+ System, and a peroxidase DAB kit (DAKO) were used in the analysis. Stained samples were analyzed under an optical microscope with an image recording system (BH-2; Olympus, Tokyo, Japan).

Immunoprophylaxis and antiviral therapy

All patients received prophylaxis, both intramuscularly with hepatitis B immunoglobulin (HBIg) and orally with lamivudine. Lamivudine (100 mg/day) was administered to post-LT. HBIg was administered to all patients using an unfixed dosing schedule involving 2000 IU of HBIg in the anhepatic phase, followed by 800 IU daily for the next 6 days, and weekly for 3 weeks, and then 800 IU monthly thereafter [22].

All pp65 antigenemia-positive recipients received prophylactic treatment with ganciclovir for 3 months, which consisted of 14 days of intravenous ganciclovir (5 mg/kg/day), followed by an unfixed period of from 2 weeks to 3 months, according to the number of pp65-positive cells. When the number of pp65-positive cells exceeded 10 per 50,000 PBLs, the method of administration was switched from oral (1000 mg ganciclovir thrice a day) to intravenous (5 mg ganciclovir/kg everyday).

Immunosuppression and management of rejection

The immunosuppressive regimen consisted of a triple-drug regimen of tacrolimus, mycophenolate, and prednisone. The dose of tacrolimus was adjusted to maintain a blood drug level of 7–10 ng/ml for the first post-operative month and 5–7 ng/ml thereafter. The dose of mycophenolate was 500 mg twice a day (bid) orally. Prednisone was given at 1000 mg during the anhepatic phase, and decreased by 20 mg bid per week until discontinued.

Rejection was confirmed by graft biopsy in cases with rising liver enzyme levels, reduced bile flow, altered bile color, fever, and clinical deterioration. Episodes of acute cellular rejection were treated intravenously by steroid pulse therapy with methylprednisone (1000–1500 mg) for 4–8 days. The dose of oral prednisone was increased to 30 mg/day, and then tapered by 5 mg/day every 14 days to a maintenance dose of 10 mg/day.

Statistical analysis

All statistical analyses were performed using the SPSS software (version 16.0 for Windows; SPSS, Chicago, USA). Quantitative variables were expressed as the median or IQR (IQR; number of pp65-positive cells, the time of HCMV viremia, and graft failure). These data were compared using the two independent-samples test between the two groups (0 matching or >0 matching of HLA-A, HLA-B, HLA-I, and HLA-DR group) (Z and P, **Table 1**). Qualitative variables were expressed as a percentage of the positive results (HCMV viremia and graft failure), and differences between these variables were evaluated using the χ^2 between the two groups (0 matching or >0 matching of HLA-A, HLA-B, HLA-I, and HLA-DR group) (**Table 1**). P value<0.05 was considered to indicate statistical significance.

Table 1 Incidence of HCMV recurrence in difference groups with HLA matching

HLA-compatibilities		Number of HCMV infection	Onset time of HCMV infection post-LT (months)	Number of graft failure	Time point of graft failure (months)
A-locus					
0[a]	($n = 51$)[b]	37[c] (72.5%[d])	2[e] (1.25[f])	9[g] (17.6%[h])	16[i] (12[j])
1 or 2	($n = 40$)	37 (92.5%)	2 (1)	7 (17.5%)	12 (11)
χ^2 or Z		5.847	0.233	0.000	1.534
P		0.015	0.631	0.985	0.235
B-locus					
0	($n = 52$)	40 (76.9%)	2 (2.5)	9 (17.3%)	16 (10)
1 or 2	($n = 39$)	34 (87.2%)	2 (1)	4 (17.9%)	12 (12)
χ^2 or Z		1.543	1.574	0.006	0.083
P		0.214	0.214	0.937	0.777
HLA-I allele (A + B locus)					
0	($n = 35$)	25 (71.4%)	2 (2.25)	5 (14.3%)	16 (8.0)
1, 2, or 3	($n = 56$)	49 (87.5%)	2 (2.50)	11 (19.6%)	12 (12.0)
χ^2 or Z		3.662	0.150	0.427	0.006
P		0.056	0.700	0.514	0.941
DR-locus					
0	($n = 53$)	41 (77.4%)	2 (3)	9 (17.04%)	16 (9)
1	($n = 38$)	33 (86.8%)	2 (1)	7 (18.0%)	10 (12)
χ^2 or Z		1.310	1.036	0.179	0.036
P		0.252	0.309	0.859	0.309

χ^2, the chi-squared test; Z, two independent-samples test.

[a]The number of HLA matching, such as HLA-A 0 indicates zero HLA-A matching; [b]the number of patients with different HLA matching; [c]the number of HCMV-infected patients post-liver transplantation; [d]the percentage of HCMV-infected patients in each different HLA matching; [e]the median time of the onset time of HCMV infection post-liver transplantation; [f]the inter-quartile range of the onset time of HCMV infection post-liver transplantation; [g]the number of HCMV-infected patients with graft failure post-liver transplantation; [h]the percentage of HCMV-infected patients with graft failure in different HLA matching; [i]the median time of the time point of graft failure; [j]the inter-quartile range of the time point of graft failure.

$P < 0.05$ was considered statistical significance.

Results and Discussion

After LT, the patients were followed for a median of 17 (IQR 24.5) months. Seventy-four patients (81.3%) had HCMV infection, at a median of 2.0 (IQR 2.0) months post-LT; the median number of pp65-positive cells in this group was 6 (IQR 3.25) per 50,000 PBLs. Graft failure after a diagnosis of HCMV viremia occurred in 16 of 91 (17.6%) patients during the 4-year study period.

The incidence of HCMV viremia was 72.5, 92.5% in patients with zero, one or two HLA-A compatibility groups ($\chi^2 = 5.847$, $P = 0.015$). The median numbers of pp65-positive cells were 5.0 (IQR 2.0), 6.0 (IQR 4.0) per 50,000 PBLs in the two groups ($Z = 0.610$, $P = 0.8518$). The median time of HCMV viremia, the incidence of graft failure after a diagnosis of HCMV viremia, and the time of graft failure were not significantly different in patients with zero, one or two HLA-A compatibility groups (**Table 1**).

The incidence of HCMV viremia was 76.9, 87.2% ($\chi^2 = 1.543$, $P = 0.214$) between the zero, one, or two HLA-B compatibility groups, respectively. The median number of pp65 cells was 4.0 (IQR 3.0), 7.0 (IQR 3.0) per 50,000 PBLs in the two groups ($Z = 1.315$, $P = 0.063$). The median time of HCMV viremia and the incidence of graft failure after a diagnosis of HCMV viremia were

independent in patients with zero, one or two HLA-B compatibility groups (**Table 1**).

The incidence of HCMV viremia was 71.4, 87.5% in patients with zero, one or two, or three HLA-I compatibility groups ($\chi^2 = 3.662$, $P = 0.056$). The median number of pp65-positive cells was 4.5 (IQR 2.5), 6.5 (IQR 4.0) per 50,000 PBLs in the two groups, respectively ($Z = 1.106$, $P = 0.173$). The median time of graft failure and the incidence of graft failure after a diagnosis of HCMV viremia were independent in patients with zero, one or two, or three HLA-I compatibility groups (**Table 1**).

The incidence of HCMV viremia was 77.4 and 86.8% in patients with zero and one HLA-DR matching groups ($\chi^2 = 1.310$, $P = 0.252$). The median number of pp65 cells was 2.0 (IQR 3.0) and 2.0 (IQR 1.0) per 50,000 PBLs in the respective groups ($Z = 1.036$, $P = 0.309$). The median time of graft failure and the incidence of graft failure after a diagnosis of HCMV viremia were independent in patients with zero or one HLA-DR compatibility (**Table 1**).

The use of prophylactic and preemptive antiviral therapies significantly reduces the incidence of HCMV infection post-transplantation. Nevertheless, HCMV infection is a severe, frequent complication in LT patients. Some clinical studies have suggested an association between HLA mismatches and a high incidence of HCMV infection after LT. We evaluated this risk factor in 91 patients with LT for HBV cirrhosis, with complete HLA typing and follow-up over a 4-year period, and found that the risk of HCMV viremia tended to be higher in patients with better HLA-A compatibility. Graft survival, the time of HCMV viremia, and graft failure after a diagnosis of HCMV viremia may be independent of HLA allele compatibility.

Some of the risk factors for HCMV infection after LT are HCMV D+/R− serological state, patient age, and the state of immunosuppression [6–10]. In this study, we avoided these potential risk factors before analyzing the association between HLA and HCMV viremia, because all HCMV-seropositive donors were excluded, all the recipients received a uniform triple-drug immunosuppressive regimen, consisting of tacrolimus, mycophenolate, and prednisone, and there was no significant difference in age among the different groups divided by HLA matching (0.220, 0.415, 0.931, and 0.752 for respective groups).

HLA includes HLA classes I (HLA-I) and II (HLA-II): HLA-I contains mainly HLA-A and HLA-B, and HLA-II contains HLA-DR.

Previous findings have suggested that HLA-A, HLA-B, or HLA-DR match between the donor and recipient increases the incidence of HCMV hepatitis in both primary and secondary HCMV infections [17,18]. However, others hold contrary views that HLA matches have no significant effect on the post-transplantation [19,20]. Manez *et al*. [17] and Seehofer *et al*. [18] suggest that an HLA-DR match between donor and recipient increases the incidence of CMV hepatitis in both primary and secondary CMV infections. However, Navarro *et al*. [19] and Jakab *et al*. [20] have revealed that HLA matching has no clinically significant impact on the outcome after LT. While we found that HLA has an effect on HCMV infection, which is similar to previous reports [17,18] to some extent, but not the same. In this study, we found it was not HLA-DR matching, but HLA-A matching that correlated with HCMV viremia. The difference is interpreted as follows: cellular immunity (HLA-I medicated) may play an essential factor in the control of infection and the recurrence with HCMV, while humoral immunity medicated by HLA class II has no effect. Therefore, it is the HLA-I (HLA-A or HLA-B) matching that has an effect on HCMV infection, not the HLA-DR matching. HLA-A-medicated escape mechanism was the advantage of HLA-A-medicated clear mechanism after HCMV infection, and thus it is reasonable to observe that the more compatibility of HLA-A, the higher risk of HCMV infection in LT recipients.

Compared with humoral immunity mediated by HLA class II (primarily HLA-DR), Cellular immunity (HLA-I mediated) is more essential in the control of HCMV infection [23], because HCMV infection is an intracellular infection. Thus, it is not surprising that the HLA-DR match has almost no influence of HCMV infection, as we found. Furthermore, HLA-DR-mediated humoral immunity consists primarily of the B-cell response to HCMV, which is largely dominated by an anti-glycoprotein B immunity response; most neutralizing antibodies are also directed against this glycoprotein [24,25]. HCMV infection may block interferon-alpha signal transduction and inhibit HLA II expression by disrupting the Jak/Stat pathway [26,27]. As a result, the humoral immunity response to HCMV involving B cells is limited, and HCMV infection may be independent of HLA-DR compatibility.

The specific mechanism of cellular immunity mediated by the HLA class I pathway is as follows: in patients with HCMV infection, the HLA class I pathway involves the internal processing of pp65 or IE Ag peptides within cells, leading to their display on the surface of HCMV-infected cells [11,28,29]. These antigen peptides on the surface of infected cells are recognized by HLA class I-restricted $CD8^+$ T-cells, leading to direct cell killing by $CD8^+$ cytotoxic T lymphocytes (CTLs) [12,30,31].

In our study, HLA-A was the factor affecting HCMV infection after transplantation in LT recipients, and not HLA-B. A possible reason is that HLA-A is the dominant locus, although this should be investigated further.

The higher risk of HCMV infection in LT recipients with greater HLA-A compatibility may be accounted for by the fact that the HLA-A-mediated escape mechanism is

an advantage in the HLA-A-mediated clearance mechanism after HCMV infection. HCMV may develop mechanisms to restrict $CD8^+$ CLTs reactivity to the receptors. That is, $CD8^+$ CTLs are unable to recognize different HLA class I antigens on the cell surface, which may lead to increased, faster HCMV replication. A possible escape mechanism for restricting $CD8^+$ CTL's reactivity is that some cells infected with HCMV may inhibit the presentation of certain antigen peptides, which are recognized easily when two HLA-A factors are compatible in the receptor and donor.

The immunoprophylaxis used in our hospital should reduce liver injury and graft failure by suppressing the CTL response. Furthermore, lamivudine antiviral therapy may block HBV and reduce damage to the graft. In this study, we found that HLA mismatch did not influence graft survival, the time of HCMV viremia, or graft failure after HCMV viremia, which is consistent with a previous study [32].

Although it is too early to draw final conclusions based on a study in 91 patients, some of our findings regarding the incidence of HCMV infection, the time of HCMV infection, and the incidence of graft failure are noticeable. Further studies are needed to clarify the role of HLA matching in LT.

Funding

This work was supported by grants from the National Basic Research Program of China (2007CB513005), the Science Research Foundation of Health Department of China (WKJ2009-2-023), the National Natural Science Foundation of China (30872239), and the Independent Research topics of State key Laboratory for Diagnosis and Treatment of Infectious Diseases.

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