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World J Gastroenterol 2004;10(13):1857-1861
World Journal of Gastroenterology
 ISSN 1007-9327

• REVIEW •

Cytomegalovirus and chronic allograft rejection in liver transplantation

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Received: 2004-02-20 **Accepted:** 2004-03-12

Abstract

Cytomegalovirus (CMV) remains one of the most frequent viral infections and the most common cause of death after liver transplantation (LT). Chronic allograft liver rejection remains the major obstacle to long-term allograft survival and CMV infection is one of the suggested risk factors for chronic allograft rejection. The precise relationship between cytomegalovirus and chronic rejection remains uncertain. This review addresses the morbidity of cytomegalovirus infection and the risk factors associated with it, the relationship between cytomegalovirus and chronic allograft liver rejection and the potential mechanisms of it.

Gao LH, Zheng SS. Cytomegalovirus and chronic allograft rejection in liver transplantation. *World J Gastroenterol* 2004; 10(13): 1857-1861

http://www.wjgnet.com/1007-9327/10/1857.asp

INTRODUCTION

Chronic allograft liver transplantation, also termed vanishing bile duct syndrome (VBDS), develops slowly over a period of months or years and is a main cause of late graft loss. In fact, the onset is usually within several months after transplantation. Diagnostic criteria for chronic rejection are (1) the presence of bile duct atrophy/pyknosis, affecting the majority of bile ducts, with or without bile duct loss; (2) convincing foam cell obliterative arteriopathy; or (3) bile duct loss affecting greater than 50% of the portal tracts[1]; (4) total fibrous obliteration of main portal vein and portal foam cell venopathy[2]. Risk factors for chronic liver rejection include transplantation for primary sclerosing cholangitis (PSC)[3], primary biliary cirrhosis (PBC)[4], certain patterns of HLA match between donor and recipient[5-7], positive lymphocyte cross-match[8], cytomegalovirus infection, transplantation between donor and recipient of different ethnic origins[9], sex mismatch[10], and absence of azathioprine from the immunosuppressive regimen[11]. Not all these risk factors have subsequently been confirmed. Cytomegalovirus infection is one of the suggested risk factors for chronic allograft liver rejection. Some results showed there was no direct correlation between them, others demonstrated CMV infection somehow implicated in mechanisms of chronic rejection and played a key role in the pathological changes of atrophy of bile duct and generation of graft arteriosclerosis, characteristic of chronic rejection.

The review addresses several questions. First, CMV infection and risk factors associated with it in liver transplantation. Second, CMV infection and cytokines. Third, relation between CMV infection and chronic liver rejection, potential etiological mechanism of CMV infection in chronic liver transplantation. Is the actual incidence of CMV infection a cause of VBDS?

CMV INFECTION AND RISK FACTORS

Human cytomegalovirus (HCMV) infection occurred in 30-65% of liver transplantation recipients, of which 18-40% were symptomatic infection and mostly developed 1 to 3 mo after transplantation. HCMV infection has two pathways: primary infection and infection activated by latent infection. Many factors are involved in HCMV infection. A prospective study of 218 LT recipients by Paya CV showed that 55% of patients developed CMV infection during the 1st year post-transplantation[12]. Symptomatic CMV infection developed in 25% of all patients, being a major cause of death (21% of all deaths). Of the 62 episodes of documented organ invasion, liver was the major site (38 episodes), followed by lung, gastrointestinal tract and retina. Multivariate statistical analysis of risk factors indicated that the R-/D+ group was the main risk factor for CMV infection and symptomatic infection. Use of antilymphocyte preparations, retransplantation, donor CMV seropositivity, use of antilymphocyte preparations, and retransplantation were risk factors for the development of CMV diseases following liver transplantation[13]. A higher incidence of cytomegalovirus infection was seen in the liver recipients of alcoholic sclerosis[14]. Intraoperative hypothermia during liver transplantation increased the risk of CMV infection in the 1st month postoperation and active warming seemed to reduce this risk[15]. Early application of OKT-3 was the risk factor for development of spreading HCMV diseases; FK506 could reverse rejection effectively, but increased the incidence of HCMV diseases[16]. Others found that immunosuppressant FK506 after liver transplantation augmented inducible NK cell activity and alleviated CMV infection[17]. Among immunosuppressive drugs, only anti- interleukin-2Rab was proved to significantly reduce the incidence of CMV[18]. The role of antirejection therapy may be particularly important, since it could suppress CMV specific cytotoxic T-cell responses and result in prolonged viraemia, which in turn could cause a prolonged alloreactive cytotoxic response. HCMV infection is associated with human herpesviruses (HHV) 6 and 7. Lautenschlager *et al*.[19] analyzed it in consecutive 34 adult liver allograft recipients, CMV disease was diagnosed in 12 patients, in which 10 patients had concurrent HHV-6 infection and 9 had HHV-7 infection. A prolonged prothrombin time, acute fulminant hepatitis diagnosed as the underlying liver disease and hepatic artery thrombosis were found to be significant risk factors for CMV infection[20]. Total number of units of blood transfusion and transfusion of seropositive CMV blood had no effect on primary CMV infection after liver transplantation, though it had an influence on the severity of CMV infection and seropositive CMV recipients. The study about the effect of cytomegalovirus infection status on the first-year mortality among orthotopic liver transplantation recipients showed[21]:

seronegative donors and recipients (11%), seronegative donors and seropositive recipients (22%), seropositive donors and recipients (30%), and seropositive donors and seronegative recipients (44%). Multivariate analysis showed that retransplantation, total number of units of blood products administered during transplantation, CMV infection and bacteremia were associated with higher mortality rates. Thus donor and recipient CMV serologic status is a significant pretransplantation determinant for death in liver transplant recipients.

HCMV INFECTION AND CYTOKINES

The significance of some cytokines highly expressed in grafts and blood serum after liver transplantation with HCMV infection remains unknown. Vascular adhesion molecules and their ligands are important both in leukocyte-endothelial cell interactions and in T-cell activation of rejection cascade. A significant induction of intercellular adhesion molecule-1 and vascular cell adhesion molecule-1 was seen in vascular and sinusoidal endothelium associated with both CMV and rejection, and induction of endothelial leukocyte adhesion molecule-1 in vascular endothelium was seen in rejection only. In both cases, the number of leukocytes expressing leukocyte function antigen-1 was significantly increased, but very late antigen-4-positive cells were more characteristic for CMV[22]. IL2-receptor (IL2R) positivity was practically seen in rejection only, but both IL2R and CD8 were increased in cytomegalovirus hepatitis. Simultaneously increased IL2R and CD8 may mean the development of cytomegalovirus hepatitis on the basis of acute rejection. Vascular adhesion protein-1 (VAP-1), an adhesion molecule involved in lymphocyte adhesion, was up-regulated in acute liver rejection of sinusoids, hepatocytes in bile duct and this up-regulation was prolonged by RCMV infection[23]. Thus the severity of acute rejection was intensified. Tumor necrosis factor-alpha (TNF-a) plays a key role in regulating reactivation of CMV infection. TNF-a could activate CMV-IE enhancer and result in high CMV-IE antigen expression in peripheral blood mononuclear cells particularly in monocytes. Increased tumor necrosis factor-a (TNF-a) could lead to occurrence of cachexy after CMV infection and mediate development of vanishing bile duct syndrome. Inhibition of TNF-a release or action might be an alternative strategy for preventing CMV-associated morbidity in allograft recipients[24].

CMV-IE protein could activate transforming growth factor-β1 (TGF-β1) promotor during CMV infection and result in early high expression of TGF-β1 mRNA[25]. In chronic human allograft rejection, increased infiltrated macrophages and up-regulated platelet-derived growth factor (PDGF), fibroblast growth factor (FGF) could lead to transformation of lipocytes to myofibroblast-like cells, which would lead to increase secretion of extracellular matrix and were engaged in hepatic fibrosis[26]. TGF-β1 increased levels of FGF and FGF receptor mRNAs in myofibroblast cells and expression levels of PDGF mRNA[27]. Thereafter CMV infection may implicate in chronic rejection by secretion of chronic fibroblast factors. Whether by regulating some of the cytokines which were thought to be involved in chronic rejection, skewing of immunity towards Th2 cytokines (TGF-β, IL-4, IL-10) and humoral response, expression of adhesive molecules and antigens can be induced in graft, to mediate occurrence of chronic rejection, needs further research.

HCMV INFECTION AND CHRONIC REJECTION IN TRANSPLANT LIVER

Many studies have demonstrated a close relationship between HCMV infection and chronic liver rejection. Analysis of ten liver transplants whose graft was lost due to histologically confirmed chronic rejection showed[28] that there was at least one episode or many times of rejection early after transplantation. All patients had a history of CMV infection usually following acute rejection. Persistent CMV-DNA was found in all of those grafts examined by DNA-hybridization *in situ*, CMV-DNA was strongly expressed in the remaining bile ducts and moderately expressed in endothelial cells of the vascular structures. Persistent CMV genome was found in those structures that were the major targets of chronic rejection process in the liver. These findings support the suggestion that CMV infection is one of the risk factors for chronic allograft rejection. Arnold *et al*.[29] found that CMV- DNA was identified in hepatocytes in 10 of 12 patients with VBDS, of whom 1 had no serological evidence of CMV infection, 9 developed cytomegalovirus infection at 1 wk until death or retransplantation. Cytomegalovirus DNA was identified in hepatocytes and never identified in either biliary or endothelial tissue. CMV-DNA was identified in all 18 patients with HCMV infection but no bile duct was injured. However in those with uncomplicated cytomegalovirus, infection occurred earlier but was eliminated more quickly, and the number of infected hepatocytes was greater when compared with those with vanishing bile duct syndrome. The data indicated that vanishing bile duct syndrome was associated with persistent cytomegalovirus replication within hepatocytes. Further study showed[30] that interferon-alpha (IFN-α) was identified more frequently and patients developed VBDS after a longer period in the bile duct cytoplasm compared with those with acute HCMV infection without evidence of VBDS. These indicate that persistent CMV infection of bile duct cells resulting in increased IFN-α is likely a co-factor linked to progression to VBDS. Martelius *et al*.[31] performed liver transplantations in a rat strain combination with PVG (RT1c) → BN (RT1n). One group of animals was infected with RCMV intraperitoneally. They found in liver allografts undergoing acute rejection, CMV significantly increased portal inflammation and caused more severe bile duct damage linked to the induction of VCAM-1 in endothelial cells. The ongoing infection was found to vary over time in different structures of liver grafts. These results support an association between CMV infection and the immunological mechanisms of rejection, as well as the role of CMV in the development of bile duct damage in liver allografts. In the same rat strain combination, Martelius *et al*.[32] examined CMV infection of the graft at various time points and found that rat cytomegalovirus (RCMV) caused an active infection in the graft from 5 d to 2 wk after transplantation. Thereafter the cultures were negative. RCMV antigens and DNA were found in hepatocytes, endothelial, inflammatory, and bile duct cells during the active infection. At 4 wk, RCMV DNA positive cells decreased. IE-1 mRNA expression was, however, only detected during the active infection, but not at 4 wk postinfection. They concluded the CMV-induced graft damage did not require the continued expression of IE-1. Halme *et al*.[33] demonstrated that CMV infection was a risk factor for development of biliary complication after liver transplantation.

A variety of risk factors for VBDS have been postulated, but they are controversial. O' Grady *et al*.[34] confirmed A 1-2 antigen matched for HLA DR antigens, a zero matched for HLA A/B antigens, and active CMV infection were independently associated with an increased risk of VBDS. Hoffmann *et al*.[35] examined 120 liver transplants retrospectively and analyzed the risk factors for VBDS. Ten patients (8.3%) developed VBDS. Seventeen patients had hepatitis C virus infections after liver transplantation. In this group, the incidence of VBDS was the highest (4 of 17, or 23.5%) and reached statistical significance. They found hepatitis C infection predisposed one to the development of VBDS after OLT.

The potential mechanisms of CMV cause VBDS. (1) Virus itself directly destroys or liquefies the infected structure. (2) Cytotoxic T lymphocyte plays a role in inducing VBDS. CMV infection may trigger an immune response by inducing MHC antigens and adhesion molecules on the bile ductal cell surface and make the ductal cells a target for immunological attack. For example, a cross-reaction between the viral protein and MHC molecules is possible because CMV has been shown to code a protein homologous to MHC class I antigen[36], and a CMV IE_2 protein has been found to share an epitope with the HLA-DRβ chain[37]. CMV is known to increase expression of class II human leukocyte antigens on bile duct epithelial cells. After immune recognition of these foreign antigens by host antigen-presenting cells, CD_4^+T would release cytokines and stimulate differentiation and proliferation of cytotoxic T cells (CD_8^+T). The activated CD_8^+T then plays a immune killing role. CMV has been shown to induce proinflammatory cytokines, such as IFN-γ and TNF-α, which could lead to other immunological events. (3) Dystrophy and ischemic sequelae caused by obliterative arteriopathy. Although CMV-DNA can not be detected on some bile duct epithelial cells in VBDS, CMV might play a pathogenetic role in the development of VBDS[38]. The sequelae of clearing infection, host immune response would selectively kill these bile duct epithelial cells with CMV infection. CMV infection of hepatocytes would in some way up-regulate the expression of HLA antigens on biliary epithelial cells. CMV viral antigens are present and bound to HLAs on the surface of bile duct cells. So even though CMV was cleared *in vivo*, they could exhibit their episode role.

Few studies about the relation of CMV infection and angiopathy are available. Grefte *et al*.[39] demonstrated that in patients with active CMV infection, distinctive large cells were present in peripheral blood. Moreover, these cells were shown to express CMV antigens and to have endothelial origin with immunologic staining, indicating an association between CMV infection and widespread occult vascular damage. CMV-induced endothelial damage may be a potent antigenic stimulus, leading to the production of anti-endothelial cells autoantibodies. Anti-endothelial cell autoantibodies may represent not only a marker of cell injury but also contribute to the progression of inflammatory response leading to the exposure of tissue-privileged self-antigens and induction of other autoantibodies such as SMA. These would further aggravate pathological damages. Analysis of autoantibody was carried out in sequential sera from 40 liver transplantation patients by Varani *et al*.[40]. Ten out of 23 antigenemia-positive and none of antigenemia-negative patients developed serum autoantibodies. Anti-endothelial cell autoantibodies were found in 9 cases and SMA in 4 patients. Antinuclear antibodies were detected in 1 autoantibody-negative patient. All but 1 case of autoantibody positivity were observed in the high antigenemia group and detected in blood during the antigenemia phase and in most cases in coincidence with or after the antigenemia peak.

In the arteries of an allografted organ, endothelial injury may arise from immune injury, ischemia/reperfusion injury, and injuries due to dyslipidemia, hypertension, or infectious agents. The injured endothelial cells can elaborate small molecules and cytokines that can activate macrophages and smooth muscle cells to express functions that may contribute to arterial lesion formation. The precise immunological mechanisms underlying chronic vascular rejection are unknown. Three potential effector mechanisms have been implicated in allograft rejection[41]: alloreactive $CD4^+$ cytokine-producing "helper" T (TH) lymphocytes, alloreactive $CD8^+$ cytolytic T lymphocytes (CTL), and alloreactive antibodies (produced by B lymphocytes). Chronic delayed-type hypersensitivity mediated by host $CD4^+$ T cells activated by graft alloantigens presented directly by graft endothelial and dendritic cells or indirectly by host dendritic cells, is likely a candidate. All of which contribute to atherosclerotic vascular disease, and chronic vascular rejection.

CMV infection might directly increase MHC antigens on the surface of graft cells through the induction of release of mediators such as interferon and may activate cytotoxic T cells, which can trigger acute rejection in association with concurrent alloantigen stimulation. Acute rejection results in a generalized inflammatory response. Kas-Deelen and colleagues have postulated that the occurrence of acute rejection at the allograft site could sensitize the endothelial surface of the host to CMV-induced damage[42]. These endothelial cells then became a target for alloreactive T cells. According to these *in vitro* studies, even a few CMV-infected endothelial cells in a transplanted organ might trigger autoreactivity[43]. CMV infection enhances several steps, with ensuing chronic rejection. Endothelial adhesion molecule expression, in particular, could provoke influx of inflammatory cells and smooth muscle cell proliferation. In addition, CMV infection could induce vascular wall changes resembling fatty streaks reported in the early stages of classic atherosclerosis[44].

There is still a controversy concerning the relationship between CMV infection and chronic allograft rejection. Paya *et al*.[45] studied 81 liver transplant recipients and found that cytomegalovirus infection developed in 46 recipients (57%), and VBDS occurred in 9 recipients (11%). CMV infection developed in only 5 of the 9 patients with VBDS. Univariate analysis of pretransplantation recipient/donor CMV serological tests and human leukocyte antigen typing showed they were not significant risk factors for the development of VBDS. The data indicated no association was found between CMV infection alone or in relation to class I or II human leukocyte antigen match and the subsequent development of VBDS. van den Berg *et al*.[46] in a retrospective study confirmed there was no association among CMV infection, HLA-DR and VBDS. Wright TL in an editorial postulated that CMV was indeed an innocent bystander rather than a culprit. In the pathogenesis of VBDS, it is the immune responsiveness of the patient that is important. It is quite possible that patients with VBDS have an inherent defect in immune response that allows persistence of CMV infection, and CMV is unrelated to destruction of bile ducts. On the other hand, if CMV infection is really an etiological factor for VBDS, antiviral therapy would be effective in decreasing incidence of the chronic rejection. Unfortunately, many studies about antiviral therapy for CMV failed to show an association between the development of CMV disease and the occurrence of rejection[47].

CONCLUSION

Many studies have demonstrated a close association between CMV infection and chronic allograft liver transplantation, but it did not prove an etiological role for the virus in this syndrome. CMV infection may be one of the risk factors for development of VBDS. A better understanding of the etiologic role of CMV in VBDS, is important for designing effective therapeutic strategies to ameliorate this process.

ACKNOWLEDGMENTS

We thank Dr. Ran Tao, Department of Starzl Transplantation Center, University of Pittsburgh, for critically reading this paper and discussion.

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Edited by Wang XL **Proofread by** Chen WW and Xu FM