HUMAN HERPESVIRUS-6 INFECTION IN LIVER TRANSPLANTATION

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Academic dissertation

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TABLE OF CONTENTS

LIST OF ORIGINAL PUBLICATIONS

This thesis is based on the following original publications referred to in the text by their Roman numerals:

I Lautenschlager I, Härmä M, Höckerstedt K, Linnavuori K, Loginov R, Taskinen E. Human herpesvirus-6 infection is associated with adhesion molecule induction and lymphocyte infiltration in liver allografts. J Hepatol 2002;37(5):648-54.

II Härmä M, Höckerstedt K, Lautenschlager I. Human herpesvirus-6 and acute liver failure. Transplantation 2003;76(3):536-9.

III Härmä M, Höckerstedt K, Krogerus L, Lautenschlager I. Pre-transplant HHV-6 infection of patients with acute liver failure is a risk factor for post-transplant HHV-6 infection of the liver. Transplantation 2006;81(3):367-372.

IV Härmä M, Höckerstedt K, Lyytikäinen O, Lautenschlager I. HHV-6 and HHV-7 antigenemia related to CMV infection after liver transplantation. J Med Virol 2006;78(6):800-805.

ABBREVIATIONS

AIH	Autoimmune hepatitis
ALT	Alanine aminotransferase
ALF	Acute liver failure
ALP	Alkaline phosphatase
APC	Antigen presenting cell
AST	Aspartate aminotransferase
BIL	Bilirubin
CNI	Calcineurin-inhibitor
CNS	Central nervous system
CMV	Cytomegalovirus
CR	Chronic rejection
CyA	Cyclosporine A
EBV	Epstein-Barr virus
EGFR	Epidermal growth factor receptor
ELAM-1	Endothelial leukocyte adhesion molecule-1
γ-GT	Gamma-glutamyl transpeptidase
GVHD	Graft versus host disease
HAV	Hepatitis A virus
HBV	Hepatitis B virus
HCC	Hepatocellular carcinoma
HCV	Hepatitis C virus
HHV-6	Human herpesvirus-6
HHV-7	Human herpesvirus-7
HHV-8	Human herpesvirus-8
HLTR	Helsinki Liver Transplant Registry
HSV	Herpes simplex virus
ICAM-1	Intercellular adhesion molecule-1
IL-1-12	Interleukin 1-12
IL-2-R	Interleukine-2-receptor
LFA-1	Leukocyte function antigen-1
MHC	Major histocompatibility complex
ORFs	Open reading frames
PBC	Primary biliary cirrhosis
PBMC	Peripheral blood mononuclear cell
PSC	Primary sclerosing cholangitis
sLex	Sialyl-LewisX-molecule
TCR	T cell receptor
VBDS	Vanishing bile duct syndrome
VCAM-1	Vascular cell adhesion molecule-1
VLA-4	Very late antigen-4
VZV	Varicella zoster virus

ABSTRACT

Rejection and infections are the two most common complications during the first six months after liver transplantation. Human herpesvirus-6 (HHV-6) belongs to the betaherpesviruses, together with its close relatives cytomegalovirus (CMV) and human herpesvirus-7 (HHV-7). Although the role and impact of cytomegalovirus in liver transplant patients and their morbidity is well characterized, the roles of the other two betaherpesviruses have been acknowledged only recently. All three betaherpesviruses are closely related with large genomic overlapping, and interactions between the viruses have been suggested.

Although, HHV-6 reactivation after transplantation is usually asymptomatic, the virus may infect the liver transplant and cause an intragraft lymphocyte dominated inflammatory reaction. HHV-6 is also associated with liver allograft rejection but the mechanisms behind the association of HHV-6 and rejection are unclear.

The aim of this study was to investigate the intragraft immunological processes associated with HHV-6, the involvement of HHV-6 in acute liver failure (ALF) of unknown origin and the hepatic HHV-6 infection of the same patients after transplantation. In addition, the occurrence of HHV-6 and HHV-7 was investigated in liver transplant patients with symptomatic CMV infection.

HHV-6 infection of the liver graft was associated with portal lymphocyte infiltration and with a significant increase of adhesion molecules (ICAM-1 and VCAM-1) and the number of cells expressing their ligand molecules (LFA-1, VLA-4) and class II antigens. HHV-6 infection was associated with significant immunological changes, but the immune response was limited to lymphocyte infiltration and the adhesion molecule level. However, one third of these patients developed chronic rejection during the follow-up.

Of the patients with ALF of unknown origin, most patients demonstrated HHV-6 antigens in the liver, whereas the opposite was seen in ALF patients with a known disease. After transplantation, HHV-6 recurrence was found in the liver transplant in half of these patients with pre-transplant HHV-6 infection of the liver, whereas no post-transplant HHV-6 infection of the liver was seen in patients without pre-transplant HHV-6.

Our studies further demonstrated that both HHV-6 and HHV-7 antigenemia often appeared in association with CMV disease in liver transplant patients. The time-related occurrence of the viruses differed, as HHV-6 appeared early after transplantation and regularly preceded CMV whereas HHV-7 often appeared concurrently with CMV.

In conclusion, these results indicate that all three betaherpesviruses are common after liver transplantation, often associated with each other. The immunological events caused by HHV-6 in the liver transplant may be involved in, or trigger rejection. In addition, HHV-6 could be one of the causes of ALF, and pre-transplant HHV-6 infection in ALF patients

may be a risk factor for post-transplant HHV-6 infection of the graft. These results strongly support the clinical significance of HHV-6 in liver transplantation. Even though the reactivation is usually asymptomatic, in some individuals HHV-6 infection may lead to severe manifestations, such as liver failure or in transplant patients, graft dysfunction and rejection.

REVIEW OF THE LITERATURE

1. Liver transplantation

The first liver transplantation was performed in 1963 in Denver, USA by Tom Starzl. Today liver transplantation is the most important treatment for acute liver failure, chronic end-stage liver diseases and certain metabolic diseases (Wilson disease, hemochromatosis, alpha-1-antitrypsin deficiency etc.) (Consensus statement Paris 1994, Hoofnagle et al. 1995). In addition patients with primary hepatic malignancies can be eligible for treatment with liver transplantation (Pichlmayr et al. 1994, Bruix and Sherman 2005).

Since 1982, more than 650 livers have been transplanted in Finland, and approximately 50 liver transplantations are currently performed annually (Isoniemi and Höckerstedt 2006). Pediatric liver transplantations in Finland commenced in 1987, and today more than 90 children have undergone liver transplantation. In Finland all liver transplants are harvested from cadaveric donors, and the technique used is orthotopic transplantation. A major advance in pediatric liver transplantation was the development of reduced sized liver transplantation, and split liver transplantation, in which one liver is used for transplants for two recipients. Almost all liver transplantations are performed between patients with identical or compatible blood type. Negative cross match or HLA matching are not required (Matinlauri et al. 2005). Approximately 8% of the patients transplanted in Finland end up with re-transplantation. Five year patient survival is nowadays 85% and more than 70% of the patients survive longer than 10 years (Isoniemi and Höckerstedt 2006).

1.1 Immunosuppression

After transplantation, lifelong immunosuppressive medication is necessary to prevent rejection. The basic immunosuppression consists of the calcineurin-inhibitors (CNI) cyclosporine A or tacrolimus, which are often combined with antimetabolites azathioprine or mycophenolic acid and methylprednisolone. Triple drug treatment with cyclosporine A (CyA), azathioprine and corticosteroids has been the standard regimen in Finland for years, although tacrolimus and mycophenolate acid are also used increasingly (Neuberger 2004, Isoniemi and Höckerstedt 2006). Adjustment of the oral CyA and tacrolimus dose is based on serum trough measurements during postoperative weeks, months and years.

Cyclosporine A was the basic immunosuppressive drug in all transplantations for 20 years in the 1980's and 90's. Its effect is based on selective suppression of cell-mediated immunity via inhibition of T-cell activation. The major side effects of CyA-therapy are nephrotoxicity, metabolic abnormalities, hypertension, gingival hyperplasia and hirsutism. In addition drug interactions are common (Ojo et al. 2003, Post et al. 2005). Tacrolimus was introduced in the late 80's, and a slightly reduced incidence of acute rejection and increased graft survival has been demonstrated in some studies (Wiesner 1998, Mühlbacher 2001). In general the side effects and drug interactions of tacrolimus are similar to CyA but a higher frequency of diabetes mellitus and neurological symptoms are found (John and

Thuluvath 2002). Corticosteroids have been a mainstay since the early days of liver transplantation. The immunosuppressive effect is based on blocking T-cell derived and antigen-presenting cell-derived cytokine expression. Side effects are numerous and include hypertension, hyperglycemia, osteoporosis, hyperlipidemia and increased risk of gastric ulcer (Hay 2003, Post et al. 2005). Azathioprine was the first antimetabolite used in liver transplantation but its use has since decreased. It antagonizes purine metabolism, which inhibits synthesis of DNA, RNA and proteins in lymphocytes. Side effects include significant myelosuppression and hepatotoxicity. Mycophenolic mofetil (mycophenolate acid) is the most recent addition to the antimetabolites and is used instead of azathioprine, especially in patients with side effects from other drugs (CNI). It seems to be more effective in preventing acute rejections than azathioprine (Schlitt et al. 2001, Wiesner et al. 2001). The common side effects of all antimetabolites are in addition to myelosuppression nausea, vomiting and diarrhea (Post et al. 2005). A new immunosuppressive drug is sirolimus, which is used particularly long-term in patients with CNI nephrotoxicity. Its main side effect is hyperlipidemia (Post et al. 2005). Anti T-cell antibody therapy such as OKT3 is used in Finland rarely to treat steroid-resistant rejection. It inhibits T-cell activation resulting in a rapid fall in the number of mature lymphocytes. Usage of OKT3 is nowadays quite low as the side effect is cytokine-release syndrome including fever and tachycardia (Post et al. 2005). Two monoclonal antibody preparations to the interleukin 2 receptor (IL-2R) have been introduced recently: daclizumab and basiliximab (Hirose 2002, Boillot et al. 2005) and are being used increasingly (Pascual et al. 2001, Nashan 2005).

1.2 Indications

In Europe over 65 000 liver transplantations were performed by the end of year 2005, and the most common indications are virus-related (hepatitis B and C viruses, HBV, HCV) cirrhosis and alcoholic cirrhosis (Adam et al. 2005). However, in Finland the most common indications are acute liver failure (ALF), primary biliary cirrhosis (PBC) and primary sclerosing cholangitis (PSC) (Figure 1). These indications include over half of all liver transplantations in Finland (Isoniemi and Höckerstedt 2006). However, alcohol induced liver cirrhosis has become more common in Finland as also in the other Nordic countries (Björö et al. 1999, NLTR 2006). End-stage liver disease caused by hepatitis B and C are increasing but still rather rare in our country.

PBC is the most common chronic liver disease leading to liver transplantation in Finland and usually the time-point of transplantation is not difficult to decide by the gradual appearance of severe, life-threatening complications. PSC is also a common reason for transplantation with a more insidious course. Alcoholic cirrhosis is the most common reason for liver transplantation in Europe. In Finland approximately 700 patients die due to this disease annually, and only 6-8 patients were transplanted because of alcoholic cirrhosis. The fourth largest group of chronic diseases is patients with autoimmune hepatitis (AIH) (Isoniemi and Höckerstedt 2006).

By the end of year 2006, altogether 652 liver transplantations were performed in Finland. Approximately 10 % of these transplantations are made on children, and the most common

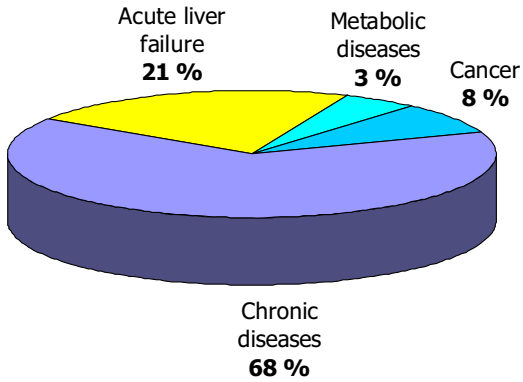
indications were biliary atresia (in about 50%), metabolic diseases, acute liver failure and primary hepatic malignancy (Isoniemi and Höckerstedt 2006). Of all these indications only acute liver failure is here discussed, because of its relevance in this study.

1.3 Acute liver failure (ALF)

Acute liver failure, synonymous with fulminant hepatic failure, is a syndrome characterized by a sudden onset, jaundice, and hepatic encephalopathy in the absence of preexistent liver disease (Trey and Davidson 1970, O'Grady and Williams 1993). Acute liver failure is thought to affect approximately 2000 persons per year in United States (Polson et al. 2005). It often causes multisystem organ failure, and usually nonspecific symptoms, such as malaise, anorexia, nausea, abdominal pain, fever and jaundice, progressing to the development of encephalopathy (Shakil et al. 2000). The syndrome is remarkable for its high morbidity and mortality, until recently widely reported to be as high as 80% in patients with grade III-IV encephalopathy (Polson et al. 2005). However, with an improved understanding of the underlying pathophysiology, new innovative treatment modalities and increased experience of OLT as a treatment option, survival rates have improved. In Europe, one year patient survival with transplantation is 70% (Ostapowicz et al. 2002, Isoniemi and Höckerstedt 2006).

ALF is a common indication for hepatic transplantation, in 10% of the patients in Europe (ELTR 2006) and in 20% in Finland, which makes it primary indication in our country (Isoniemi and Höckerstedt 2006) (Figure 1). Almost half of the patients in Finland have an acute disease of unknown origin (i.e. non-A to non-E hepatitis) (Isoniemi and Höckerstedt 2006), whereas drug toxicity (especially paracetamol), vascular disorders and viral hepatitis (HBV, HCV) are more common in other countries (Shakil et al. 2000, Brandsäter et al. 2002, Ostapowicz et al. 2002). The prognosis is worst in patient with ALF of unknown origin, hepatitis B and Wilson's disease and disulfiram toxicity, as more than 80% of patients die without transplantation.

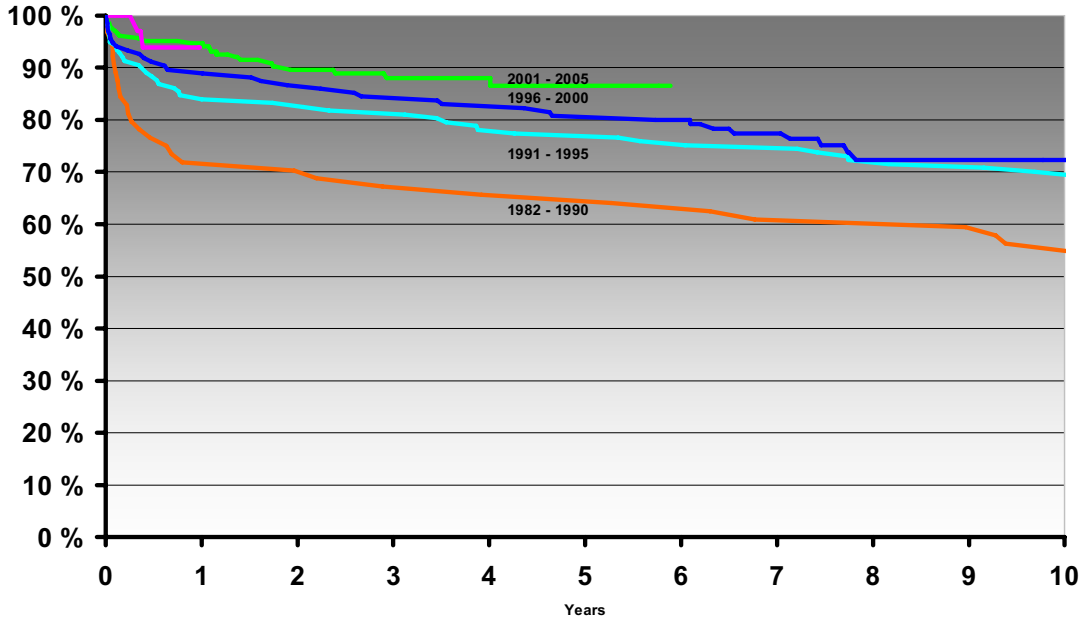
Figure 1 Indications for liver transplantation in Finland.



1.3 Outcome of liver transplantations

In patients with ALF the one year patient survival after liver transplantation is currently 85% in Finland (Isoniemi and Höckerstedt 2006), 80% in Nordic countries (ELTR 2006), and 74% in Europe (Adam et al. 2005, ELTR 2006). When all indications are considered, patient survival rates in Europe are 70% at 5 years, and 61% after 10 years (Adam et al. 2005). In Finland the patient survivals are 85% at 5 years and more than 70% in 10 years (Isoniemi and Höckerstedt 2006). Patient survival time has improved regularly as shown in (Isoniemi and Höckerstedt 2006) (Figure 2).

Figure 2 Patient survivals after liver transplantation in Finland 1982-2006.



What has not changed with time, however, is the importance of the first year in determining the final outcome of the transplant recipient; more than two thirds of deaths and more than three quarters of re-transplantation occurred within the first year after liver transplantation (Adam et al. 2005). Primary dysfunction and thrombosis of the hepatic or the portal vein are the most common reasons for re-transplantation during the early phases. Later, up to three years post-transplantation chronic rejection is the main cause for retransplantation. For long-term results, recurrent disease, particularly recurrent HCV, is a problem worldwide, and new effective therapies are needed (Berenguer et al. 2002, Adam et al. 2005). Also PBC, PSC and AIH may recur in the new liver but in most cases only after 5 to 15 years after transplantation and even then they seldom lead to end stage disease. In 5

years, 30-50% of patients transplanted due to hepatocellular carcinoma (HCC) experience recurrences (Ringe 2002, ELTR 2006).

Most complications after liver transplantation occur during the first months postoperatively, and are usually treatable. Complications in the immediate postoperative period include pulmonary and renal problems, bleeding, hepatic artery and portal vein thrombosis, biliary stricture and bacterial infections (Bhattacharjya et al. 2001, Adam et al. 2005). Primary non-function (0.4 % of the Finnish patients) leads to the death or retransplantation, whereas primary dysfunction (15%) is usually treatable, although it induces other complications in the graft, which lead to increased morbidity of the patients (Isoniemi and Höckerstedt 2006).

Infections and acute rejections occur mainly during the first three months. Chronic rejection is rare but may hit the graft during the first two years, seldom later (Demetris et al. 2000). The biliary tree is considered the Achilles heel of liver transplantation. The bile ducts have a very vulnerable vascularity, which makes them prone to ischemia and hence complications, such as strictures and leaks in the biliary tree. It has been shown that biliary complications as well as VBDS occurs significantly more often in connection with CMV infection (Lautenschlager et al. 1997, Martelius et al. 1998, Halme et al. 2003).

1.4 Infections

Due to immunosuppressive therapy, the transplant recipients with their pre-existing severe liver disease are at high risk of infections (Patel and Paya 1997). More than two-thirds of liver transplant recipients have infections in the first year after transplantation and infection is the leading cause of death in these patients (bacterial, fungal and viral infections) (Blair and Kusne 2005, ELTR 2006, Isoniemi and Höckerstedt 2006, Kusne and Blair 2006) (Figure 3). In addition, infections may have indirect effects, including allograft injury, opportunistic superinfection, and malignancy (Rubin 2002). The spectrum of infections in transplant patients appears to be evolving. Infections caused by gram-positive bacteria are becoming more frequent, while a decline in the incidence of *Pneumocystis jirovecii* (previously known as *Pneumocystis carinii*) and cytomegalovirus (CMV) infection has been recorded, largely as a result of effective prophylaxis (Singh 1998). However, it has been shown, that the frequency and type of infections in children with liver transplant are in long-term quite similar to those in age-matched healthy children (Their et al. 2000).

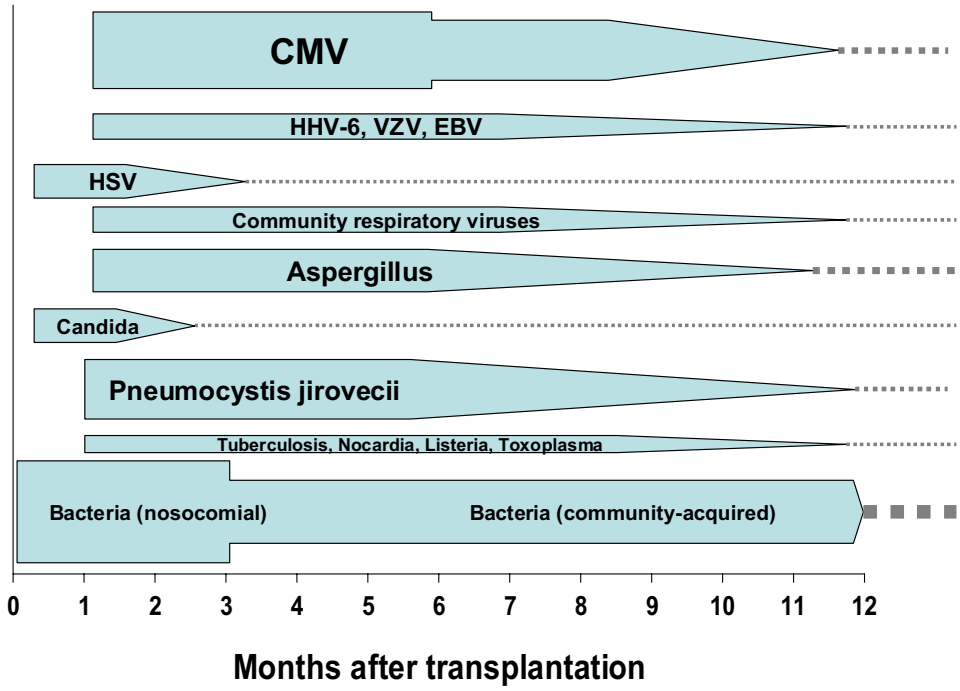


Figure 3 Appearance of the most significant infections after liver transplantation.
Bars indicate the common period for the onset of infection. Dotted lines show the continued risk of infections. Weight of the bars and lines indicate the significance of infection during different tine periods. (Modified from Juho Lehto with permission.)
CMV; cytomegalovirus, HHV-6, Human herpesvirus-6; VZV, varicella zoster virus; EBV, Epstain-Barr virus; HSV, herpes simplex virus

The majority of bacterial infections occur within two months post-transplantation (Figure 3) and are frequent and serious complications after liver transplantation (Blair and Kusne 2005). Fungal infections are also demonstrated after transplantation, with *Candida* spp. and *Aspergillus* spp. being the most common causes (Tollemar 2003).

1.4.1. Viral infections

Viral infections in liver transplant recipients are important to recognize and treat early because of their association with substantial morbidity and even mortality (Patel and Paya 1997). In addition to the classical hepatitis viruses (hepatitis B virus (HBV) and hepatitis C virus (HCV)), cytomegalovirus (CMV) has long been recognized as an important virus in transplantation. Also some other viruses (HHV-6, VZV, EBV, adenovirus and other respiratory viruses) have received attention in the medical literature because of their

association with particular clinical post-transplant syndromes or being frequently detected in transplant patients (Kusne and Blair 2006).

Recurrence of HBV and HCV infection after transplantation are common complications in liver transplant recipients worldwide and considered together the most common indication for liver transplantation in Europe and the U.S. (ELTR 2006). Reactivation of HBV and HCV after liver transplantation accounts for a significant proportion of the morbidity, mortality and costs (Samuel et al. 1997, Paya 2001, Berenguer et al. 2002). In Finland, however, end-stage liver disease caused by hepatitis B and C is increasing too but is still rather rare, also after transplantation. The insidence of HBV and HCV in Finnish population is low, and no transmission via blood products occurs in our country. Adenovirus may cause infection in pediatric liver transplant recipients but is less common in adult recipients (Kusne and Blair 2006). Also other community respiratory viruses, such as respiratory syncytial virus, influenza viruses, parainfluenza viruses, rhinoviruses, enteroviruses and coronaviruses may infect transplant recipients (Englund and Whimbley 2003). In addition, norovirus infections (Mattner et al. 2006) and rotavirus infections (Stelzmüller et al. 2006) may also complicate post-transplant period.

The human herpesviruses that may cause infection after liver transplantation are herpesvirus-1 (HSV-1) and -2 (HSV-2), varicella zoster virus (VZV), cytomegalovirus (CMV), Epstein-Barr virus (EBV), human herpesvirus-6 (HHV-6) and human herpesvirus-7 (HHV-7) (Singh 2000, Kusne and Blair 2006). Although a rare occurence, human herpesvirus-8 (HHV-8) may cause Kaposi sarcoma (Singh 2000). The primary infections of these herpesviruses are rare in adult liver transplant patients due to the high seroprevalence already in early childhood. The herpesvirus family is divided into three subfamilies. HSV-1, HSV-2 and VZV belong to the alphaherpesviruses, whereas CMV, HHV-6 and -7 are betaherpesviruses and Epstein-Barr (EBV) virus and HHV-8 are gammaherpesviruses (Zhou et al. 2006). Primary varicella zoster virus infection causes chickenpox (varicella) but the recurrent form is varicella zoster in immunosuppressed and elderly persons. EBV is the cause of the majority of mononucleosis cases. Although uncommon with a low incidence in solid-organ transplantations, the EBV-related PTLD is an aggressive disease, with high mortality rates of up to 80% (Paya et al. 1999). The main characteristic of human herpesviruses is the capacity to remain in a latent state in a host after primary infection and reactivate later.

Of the betaherpesviruses, in addition to CMV, the role of which in post-transplantation clinical illness and outcomes are well characterized (Paya and Razonable 2003) also human herpesvirus-6 (HHV-6) infection has been found after transplantation implicated in encephalitis and allograft dysfunction (Lautenschlager et al. 1998, Singh 2000, Ljungman 2002a, Kusne and Blair 2006). The role of human herpesvirus (HHV-7) in transplant complications remains unclear. Some viruses, such as CMV and HHV-6, also have immunomodulatory properties and can facilitate other infections (Kusne and Blair 2006).

1.5 Liver allograft rejection

1.5.1. Acute rejection

Transplant rejection occurs when the immune system of the recipient of a transplant attacks the transplanted organ or tissue. The rejection types are traditionally charactirized as hyperacute (within a few hours), acute (within some days or months) or chronic (within months or years) rejection. Hyperacute rejection, caused by preformed antibodies against donor antigens (for example, ABO blood type antibodies), where the organ fails immediately following revascularization, is extremely rare in liver transplant patients (Batts 1999). Instead, acute cellular rejection is a common T-cell mediated response against the liver allograft characterized by portal inflammation, endothelialitis and cholangitis (Demetris 1997). Acute rejection develops in approximately 20-60% of liver transplant patients (Batts 1999, Neuberger 1999, ELTR 2006). Most acute rejections occur during the first month after transplantation (Wiesner et al. 1998, Batts 1999). Acute allograft rejection may cause clinical symptoms, such as fever and malaise (Wiesner et al. 1998). The biochemical changes include an increase in AST (aspartate aminotransferase), ALT (alanine aminotransferase) and γ-GT (γ-glutamyltransferase) and eventually also in increasing levels of serum bilirubin and ALP (alkaline phosphatase) (Neuberger 1999). However, the diagnosis is always based on a combined evaluation of clinical, biochemical and histological parameters.

In graft histology, acute liver allograft rejection is characterized by intense portal inflammation (Demetris 1997). A histopathological diagnosis of acute rejection is based on a triad of; 1) mixed but predominantly mononuclear portal inflammation, containing mainly activated lymphocytes, neutrophils and frequently eosinophils; 2) bile duct inflammation/damage; and 3) subendothelial inflammation of portal veins or hepatic venules. These three can be semiquantitatively scored on a scale of 0 to 3 and added together to arrive at a final Rejection Activity Index (RAI) (Demetris 1997). Late acute rejection occurs mainly when patients are under immunosuppressed, usually due to non-compliance.

1.5.2. Chronic rejection

Chronic rejection (CR) of the liver is an irreversible condition characterized by immune mediated destruction of bile ducts and obliterative arteriopathy (Vierling and Fennell 1985, Ludwig et al. 1987, Demetris et al. 2000), also known as vanishing bile duct syndrome (VBDS), and is one of the causes of late dysfunction. The histological features of chronic liver allograft rejection are well documented (Demetris et al. 2000) and the minimal diagnostic criteria CR are: 1) the presence of bile duct atrophy, affecting the majority of bile ducts, with or without bile duct loss; 2) convincing foam cell obliterative arteriopathy; or 3) bile duct loss affecting more than half of the portal tracts. Since bile duct loss is such an important feature in CR, the term VBDS is often used as a synonym for CR of the liver. The final diagnosis of CR is based on a combination of the clinical, laboratory and histopathological findings.

Most cases of chronic rejection occur within first year, and the prevalence does not appear to increase with time after transplantation as is the case with all other transplant organs (Demetris et al. 2000). The incidence of CR of the liver is much lower than in other transplanted organs, and seems to be declining; rates of 2% to 3% have nowadays been reported (Demetris et al. 2000, Neil and Hübscher 2002), whereas in earlier series, rates of 15% to 18% were reported (van Hoek et al. 1992). Risk factors for chronic rejection include the underlying diseases, acute rejections, low levels of immune suppression and viral infections, especially CMV infection (Neuberger 1999, Demetris et al. 2000). The only possible therapy for CR is re-transplantation.

1.5.3. Mechanisms of rejection

The major histocompatibility complex (MHC) is a large gene family. The best-known genes in the MHC region are the subset that encodes cell-surface antigen-presenting proteins. In humans, these genes are referred to as human leukocyte antigen (HLA) genes and the MHC is divided into three regions: Class I, II and III. The A, B and C genes belong to MHC class I, whereas the six D genes belong to class II. The class III genes encode proteins with a variety of different functions but do not themselves stimulate T cells in the same way as class I and II proteins.

The inflammatory pathology described above is generated by the process of alloimmune response, whose key events are T-cell recognition of foreign antigens and later T-cell activation. After transplantation, immune cells of the recipient recognize donor MHC antigens. T cells carry different T cell receptors (TCRs), which recognize foreign epitopes. The TCR expressed on T lymphocytes has the potential to recognize MHC antigens expressed in the allograft in many different forms (Dallman 2001). MHC class I antigens are expressed by most nucleated cells, while class II antigens are expressed mainly by antigen-presenting cells (APC), such as dendritic cells, B-cells and some endothelial cells. In the liver, class II antigens are faintly expressed in the sinusoidal but not in vascular endothelium or bile duct epithelial cells (Daar et al. 1984, Lautenschlager et al. 1984). However, MHC class II expression can be induced by cytokines in endothelial and parenchymal cells of the graft. In the liver, bile duct cells, endothelial cells and also hepatocytes have been shown to express strongly class II molecules in rejection (Demetris et al. 1985, Steinhoff 1990).

Allorecognition is traditionally divided into direct and indirect pathways. In direct allorecognition, recipient T cells recognize foreign MHC antigens on the surface of donor APC (Lechler et al. 1990). Direct allorecognition induces an early strong alloimmune response and thought to be mainly driven by the class II mismatch (Gould and Auchincloss 1999, Womer et al. 2000). In indirect allorecognition, donor antigen is taken up by recipient APC and presented to recipient CD4+ T cells and the response is mainly driven by indirect allorecognition, and mainly class I difference (Gould and Auchincloss 1999, Womer et al. 2000). The interaction of TCR with MHC class II-peptide complex is alone not capable to initiate the immune activation but costimulation is also needed. The first signal is provided by interaction of the TCR with the MHC molecule/antigen complex on the APC (Dallman 2001), strengthened by CD28/B7 mediated costimulation (Sayegh and Turka 1998). Also

intercellular adhesion molecule-1 (ICAM-1) expression on APC is necessary for T-cell activation (Altmann et al. 1989). Activation of the CD4+ T-helper cells leads to a cascade of immune effector functions, leading to destruction of the graft. Unlike cytotoxic T-cells, the natural killer (NK) cells do not need to interact with antigen to become lytic to target cells (Dallman 2001).

The production of cytokines by the inflammatory cells is crucial, as the type of cytokine response determinates the type and intensity of the immune response. Inflammatory cytokines, such as IL-1, TNF-alpha and IFN-gamma produced within the allograft can increase the local expression of adhesion molecules and thereby promote the entry of specific and nonspecific cells. IL-1 and TNF-alpha have been shown to be upregulated in liver allografts rejection (Hoffmann et al. 1993). These cytokines are also essential in T-cell activation. T cell activation leads to the expression of IL-2 receptors on the surface of T-cells and production of IL-2, an interleukin which is central to allograft rejection (Kirkman et al. 1985, Dallman 2001). IL-2 induces T cell growth and differentiation of CD4+ T cells to either Th1 or Th2 type cytokine production. In allograft rejection, Th1 cytokines activate CD8+ T cells to become cytotoxic T lymphocytes, which increase the secretion of TNF-α and IL-12, which activates macrophages. Th2 cytokines include IL-4 and IL-10, and are thought to direct the immune response towards antibody production and inhibition of Th1 responses (Mosmann and Coffman 1989). A major cytokine in alloresponse is IFN-γ, produced by activated T cells, induces MHC class I and especially class II antigen expression in the graft and also stimulates the expression of adhesion molecules on endothelial cell surface (Dinarello and Mier 1987).

1.5.4. Adhesion molecules

Vascular adhesion molecules are important in the early phase of T-cell activation and leukocyte extravasation in the graft, serving as adhesion receptors for inflammatory cells in the endothelium (Springer 1990). The expression of adhesion molecules is up-regulated by various cytokines, such as IFN-γ, IL-1 and TNF-α, produced by inflammatory cells (Dustin et al. 1988, Springer 1995). Three major structural groups of adhesion molecules are traditionally recognized: selectins, integrins and immunoglobulin superfamily (Table 1). From selectin family, P- and E-selectin (ELAM-1) can be induced from the activated endothelium. The integrin, very late antigen-4 (VLA-4, ligand for VCAM-1) is expressed on endothelium as well as another integrin, LFA-1 (ligand for ICAM-1), and both are responsible for the invasion of leukocytes into tissues. The immunoglobulin superfamily includes e.g. VCAM-1, ICAM-1 and -2, platelet-endothelial cell adhesion molecule (PECAM-1) and its ligand CD31 (Heemann et al. 1994). In addition there are some adhesion molecules not belonging to these groups, such as vascular adhesion protein-1 (VAP-1) (Salmi and Jalkanen 1997).

Adhesion molecule on endothelium	Structural family	Ligand on lymphocyte	Structural family
P-selectin	selectin	PSGL-1	sialomucin
E-selectin	selectin	sLex	oligosaccharide
ICAM-1	immunoglobulin	LFA-1	integrin
ICAM-2	immunoglobulin	LFA-1	integrin
VCAM-1	immunoglobulin	VLA-4	integrin
PECAM-1	immunoglobulin	CD31	immunoglobulin

Table 1 Molecules involved in lymphocyte adhesion to endothelium.

Leukocyte adhesion to endothelium proceeds in a cascade-like manner: weak contacts by selectins and their ligands lead to rolling of leukocytes on endothelium. When in closer contact, chemoattractants on endothelial surface activate integrins (LFA-1, VLA-4) on the surface of leukocytes, leading to firm adhesion (binding to endothelial ICAM-1 and VCAM-1). This is followed by transmigration through endothelium towards the site of inflammation (Springer 1995). (Figure 4)

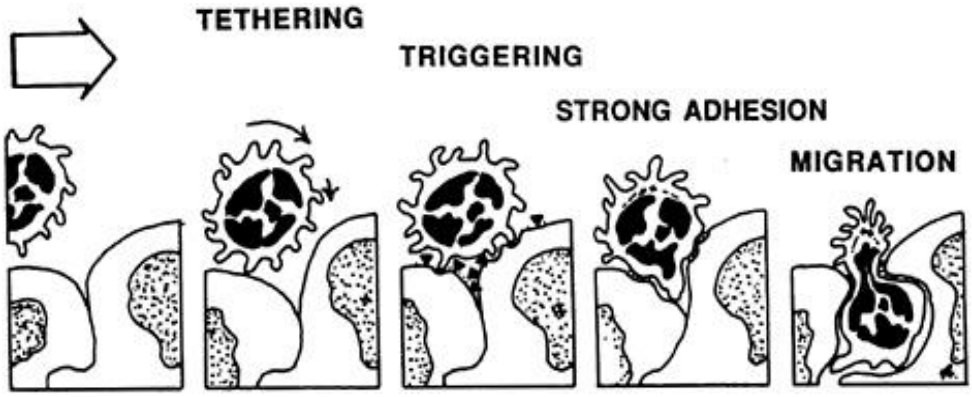


Figure 4 Diagram of the adhesion cascade. *Reversible contacts between endothelial selectins and their ligands on lymphocyte lead to rolling. Chemokines on endothelial surface activate integrins leading to high affinity binding followed by transmigration to the tissue. Adapted from Anderson and Shaw (1993).*

Adhesion molecules also bring APCs and responding T cells to closer contact promoting recognition of antigen and MHC via the TCR/CD3 complex (Adams 1995). This leads to activation of integrins thereby strengthening the adhesion and also provides crucial costimulatory signals that are required for optimum T cell activation (Springer 1990).

Sequental adhesive interactions between the leukocyte and the endothelium lead to the influx of inflammatory cells into the graft (Adams and Shaw 1994, Springer 1995, Butcher et al. 1999). The expression of adhesion molecules has been correlated with episodes of allograft rejection (Heemann et al. 1994, Adams 1995). In allograft rejection, adhesion molecules, such as ICAM-1, VCAM-1 and ELAM-1 are broadly upregulated in organ transplants (Adams et al. 1989, Taylor et al. 1992, Ferran et al. 1993, Gibbs et al. 1993, Koskinen et al. 1993, Lautenschlager and Höckerstedt 1993, Steinhoff et al. 1993b, Herskowitz et al. 1994, Solez et al. 1997), participating in T cell activation and leukocyte extravasation.

In addition, viral infections may be involved in the rejection cascade. Especially CMV has been demonstrated to cause lymphoid immune response, increase MHC class II molecules and induce adhesion molecules in the graft (von Willebrand et al. 1986, Steinhoff et al. 1993b, Lautenschlager et al. 1996). CMV also induces the production of various cytokines, e.g. IL-1 and especially TNF-a, which may lead to other immunological events and trigger rejection (Iwamoto et al. 1990, Smith et al. 1992, Lautenschlager et al. 1999). The involvement of other betaherpesviruses, especially HHV-6, in allograft rejection has also be suggested (Okuno et al. 1990, Hoshino et al. 1995, Lautenschlager et al. 1998, Griffiths et al. 1999, Lautenschlager et al. 2000, Humar et al. 2002a, Feldstein et al. 2003, Neurohr et al. 2005). Whether HHV-6 induces the same immunological events as CMV and triggers transplant rejection remains still to be investigated.

2. Betaherpesviruses

2.1 General properties of betaherpesviruses

Betaherpesviruses are a subfamily of human herpesviruses that share architectural features of their virion, including a core containing a linear double-stranded DNA, an icosahedral capsid with 162 capsomeres, an amorphous tegument and an envelope (Landolfo et al. 2003). Human herpesvirus-6 (HHV-6) and human herpesvirus-7 (HHV-7) belong to the betaherpesvirus family together with its close relative cytomegalovirus (CMV). All three viruses are ubiquitous and infect the majority of humans. The betaherpesviruses are more closely related to each other than to other herpesviruses with large genomic overlapping (Efstathiou et al. 1988).

The betaherpesviruses use the cellular transcription and translation machinery to produce three kinetic classes of viral proteins; immediate early (IE), early (E) and late (L). IE proteins are synthesized within a few hours after infection and regulate the expression of other genes, E genes proteins are mainly involved in DNA metabolism and replication. Late proteins serve as components of the mature virus particles. The total time from infection to release of new virions takes approximately 72h (Landolfo et al. 2003). Following primary infection, these viruses, as well as other herpesviruses, maintain latency in the human host by integrating in the host cell chromosomes or by maintaining subclinical low level viral replication that is adequately controlled by a functioning immune system. The latency is lifetime long, and viruses may reactivate during immunosuppression in transplanted patients.

HHV-6 causes *exanthema subitum,* febrile seizures and other infectious syndromes of early childhood (Yamanishi et al. 1988). In the adult population, there is a high seroprevalence of 90-95% and primary infections are uncommon (De Bolle et al. 2005). Two variants of HHV-6 have been categorized: HHV-6A and HHV-6B (Ablashi et al. 1991, Schirmer et al. 1991). HHV-6 and HHV-7 are lymphotropic viruses, though HHV-6 in particular, which uses CD46 molecule as its cellular receptor, may also infect other cell types (De Bolle et al. 2005, Santoro et al. 1999). HHV-6 and HHV-7 are related and share a limited sequence homology (Berneman et al. 1992). After first infection, HHV-6 and HHV-7 persist for life, being shed in saliva and transmitted to others via this route, and may reactivate during immunosuppression (Clark 2000, Ward 2005).

HHV-7 was first isolated from the CD4+ lymphocytes of a healthy young adult in 1990 (Frenkel et al. 1990). Only few acute HHV-7 infections have been identified, even though HHV-7 is highly seroprevalent (>90%) worldwide (Fujiwara et al. 2000, Ward 2005), already in early childhood (Wyatt et al. 1991, Black and Pellett 1999). HHV-7 viremia could represent primary or reactivated infection and may be affected by the interaction with HHV-6 (Hall et al. 2006). The median age of patients with primary HHV-7 has been shown to be older than patients with HHV-6 (Caserta et al. 1998). Little is known about the clinical and virological characteristics of primary infection with HHV-7 but case reports

and small patient series have associated primary infection with *roseola infantum* (Tanaka et al. 1994), hepatitis (Hashida et al. 1995), neurological manifestations (van den Berg et al. 1999, Ward et al. 2005) and possibly transplant complications (Emery 2001). HHV-7 binds to the cellular CD4 molecule and uses this protein as a receptor (Lusso and Gallo 1994). Both HHV-6 and HHV-7 initially infect CD4+ T cells (Lusso et al. 1991, Lusso and Gallo 1994).

CMV is a widespread pathogen responsible for generally asymptomatic and persistent infections of healthy people. The seroprevalence of adult population ranges from 30% to 97%, being approximately 70-80% in Finland and Scandinavian countries, whereas in United Kingdom the seroprevalence of CMV is only 40% (Ho 1990). Primary infection with CMV in early childhood is usually asymptomatic but in young adults, CMV may cause mononucleosis-like clinical symptoms. Furthermore it represents the major infectious cause of birth defects (Malm and Engman 2007). The less clearly characterized receptor(s) for CMV is widely distributed among host cell types and contributes to the broad viral tropism observed (Landolfo et al. 2003). Viral entry is the result of a cascade of interactions; CMV attach EGFR (epidermal growth factor receptor) on permissive cells (Wang et al. 2003), and other interactions between CMV envelope glycoproteins and cellular integrins promote receptor clustering (Feire et al. 2004).

Following CMV infection the virus is excreted in body fluids for months or even years and reactivation of the virus is common, especially in immunosuppressed patients (Landolfo et al. 2003). CMV infection occur in the majority of solid organ transplant patients, primarily in the first months when immunosuppression is most intense, CMV disease incidence ranges from 10% to 50% (Seehofer et al. 2002, Paya and Razonable 2003). Most CMV infections are caused by reactivation of latent virus of either recipient or donor origin. CMV infection and disease are recognized as the predominant clinical problem among opportunistic infections causing fever, hepatitis, neutropenia, thrombocytopenia, pneumonitis, gastrointestinal ulcerations and retinitis, and being important causes of morbidity and even mortality among transplant recipients (Ljungman et al. 2002).

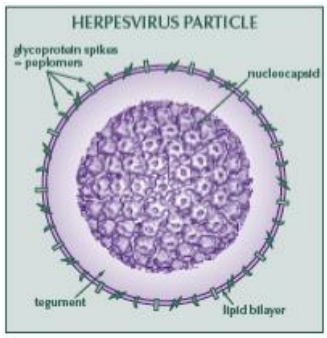
In addition to clinical disease, awareness of CMV-associated indirect effects, such as increased risk of allograft rejection and the development of other infections are increased (Cainelli and Vento 2002, Ljungman 2002). CMV is thought to be a risk factor for invasive fungal and bacterial infections in recipients of liver transplants (Ljungman 2002, Husain et al. 2003) and associations between CMV and other viral infections have been reported (Razonable et al. 2002a, Singh et al. 2005). More severe forms of recurrence HCV have been associated with CMV (Razonable et al. 2002a) and CMV and HHV-6 infections (Humar et al. 2002b). Concurrent betaherpesvirus activations are frequently found after transplantation and transactivations have been suggested (Humar et al. 2002b, Razonable and Paya 2002, Yoshikawa 2003). CMV infection has been shown a risk factor for acute graft rejection in renal transplantation (Pouteil-Noble et al. 1993, Reinke et al. 1994, Sageda et al. 2002), and an association between CMV chronic allograft nephropathy (chronic rejection) has been reported (Humar et al. 1999, Tong et al. 2002, Helanterä et al. 2003, Sagedal et al. 2004). CMV is associated with chronic rejection and allograft

vasculopathy in heart transplantation (Grattan et al. 1989, Loebe et al. 1990, Koskinen 1993, Valantine 2003). CMV is also suggested as being involved in acute liver rejection and an association with developing VBDS and chronic rejection has been reported (Arnold et al. 1992, Lautenschlager et al. 1997, Evans et al. 1999).

CMV is an immunomodulatory virus and is thought to be involved in alloresponse (Lautenschlager 1998, Koskinen et al. 1999, Soderberg-Naucler and Emery 2001). CMV triggers the inflammation in the graft by upregulation of cytokines, MHC antigens and adhesion molecules (Iwamoto et al. 1990, Smith et al. 1992, van Dorp et al. 1993, Lautenschlager et al. 1999) and induces various chemokines and growth factors (Fietze et al. 1994, Helanterä et al. 2005). CMV induces the production of central cytokine TNF-α, which may lead to other immunological events and enhance alloresponse. On the other hand, TNF-α has been shown to stimulate the activity of the CMV-IE promoter leading to reactivation of the virus (Smith et al. 1992, Lautenschlager et al. 2003). CMV upregulates the production of IL-1 by mononuclear cells (Iwamoto et al. 1990). CMV increases the expression of class II molecules and certain adhesion molecules, such as ICAM-1 and VCAM-1, known to be important in leukocyte extravasation and T-cell activation (von Willebrand et al. 1986, Koskinen 1993, Steinhoff et al. 1993a, Lautenschlager et al. 1996, Helanterä et al. 2005). CMV is suggested to enhance the development of chronic rejection probably via development of transplant vasculopathy and fibrosis (Lautenschlager 1998, Cainelli and Vento 2002, Rubin 2002).

Whether HHV-6, as a close relative of CMV, may trigger these immunological cascades and is involved either in acute or chronic rejection is not yet known.

Figure 5 Herpesvirus particle.



2.2 Human herpesvirus-6 (HHV-6)

2.2.1. The virus

HHV-6 was first isolated by Salathuddin et al. (Salathuddin et al. 1986) using peripheral blood lymphocytes obtained from immunocompromised patients. Human herpesvirus-6 is a member of the Roseolavirus genus of the betaherpesvirus subfamily. Like all herpesviruses, it is a large DNA-virus of 200nm in diameter consisting of a linear double-stranded DNA of approximately 160 to 162 kb inside an icosahedral nucleocapsid. The nucleocapsid is coated with a tegument enclosed in an envelope, in which the viral glycoproteins are embedded (Biberfeld et al. 1987) (Figure 5). The HHV-6B genome contains 119 open reading frames (ORFs), 9 of which are absent in HHV-6A. An additional cluster of genes, the so-called US22 gene family, is conserved among betaherpesviruses only. Several genes are unique to HHV-6. The overall nucleotide sequence identity between HHV-6A and B variants is 90%. HHV-6 is a close relative to CMV, and the genetic homology is as high as 67%, and 21% with all other herpesviruses (De Bolle et al. 2005).

Both the A and B variants of HHV-6 enter the cell through interaction with CD46, which is present on the membrane of all nucleated cells and is physiologically involved in complement regulation (Santoro et al. 1999). Both HHV-6A and HHV-6B replicate in lymphocytes (Takahashi et al. 1989) but the virus infects also monocytes, natural killer cells, fibroblasts, epithelial and endothelial cells, astrocytes, oligodendrocytes and microglia (De Bolle et al. 2005). The host tissue range of HHV-6 in vivo is broad including brain tissue, liver, tonsils, salivary glands and vascular endothelium (De Bolle et al. 2005, Chen and Hudnall 2006). Like the other human herpesviruses, also HHV-6 establishes latency and is capable of persisting in the host after primary infection. According to experimental data, this persistence involves both a true latent state (with no production of infectious virus) and a low-level chronic replication, whereas salivary glands and brain tissue are suspected of harboring persistent HHV-6 infection (Fox et al. 1990, Chan et al. 2001). Candidate sites for latency are monocytes and early bone marrow progenitor cells (Luppi et al. 1999, Kondo et al. 2003). HHV-6 DNA integration to the peripheral blood mononuclear cell (PBMC) genome and genetic transmission of integrated HHV-6 from parent to child and through stem cell transplantation has been described (Luppi et al. 1993, Daibata et al. 1999, Clark et al. 2006).

2.2.2. Epidemiology

HHV-6 is ubiquitous in human populations, with more than 95% of adults being seropositive (Clark 2000). The geographic differences in HHV-6 prevalence vary between 70 and 100% (De Bolle et al. 2005). HHV-6 infects over 90% of people within the first two years of life (Okuno et al. 1989, Hall et al. 1994). Two genetically distinct variants of the virus exist, HHV-6 A and B (Ablashi et al. 1991, Aubin et al. 1991, Schirmer et al. 1991), of which the variant B is most common (Dewhurst et al. 1993). The virus is believed to be spread by salivary contact (Fox et al. 1990, Sada et al. 1996, Clark 2000), possibly also perinatal transmission is possible (Hall et al. 1994). Congenital HHV-6 infection has been estimated to occur in 1-2% of children (Adams et al. 1998, Hall et al. 2004). One possible mode of congenital transmission of HHV-6 is by chromosomal integration (Daibata et al. 1999).

2.2.3. Clinical manifestations

HHV-6B is the causative agent to *Exanthema subitum* (Yamanishi et al. 1988), a childhood disease characterized by high fever and a mild skin rash, occasionally complicated by seizures or encephalitis (Asano et al. 1991, Yamanishi et al. 1992). Primary HHV-6 infection usually takes place in children between 6 and 12 months old (Hall et al. 1994), and is almost exclusively caused by HHV-6B, not HHV-6A (Schirmer et al. 1991, Dewhurst et al. 1993), although occasional primary infections with HHV-6A in an infant with acute roseola has been described (Hidaka et al. 1997). Infection with HHV-6A appears to be much less frequent but is suggested to be more neurotropic than HHV-6B (Hall et al. 1998). HHV-6A has been detected in fetuses with hydrops (Ashshi et al. 2000) and critically ill immunocompetent individuals (Razonable et al. 2002b).

Most HHV-6 infections are asymptomatic or very mild, and about 80% of them without any clinical symptoms (Krueger et al. 1988). Even those infections characterized by high fever usually have a benign and self-resolving course, although the most common complications of *exanthema subitum* are malaise, otitis and gastrointestinal and respiratory tract symptoms (Hall et al. 1994). Also an association with febrile seizures and convulsions has been confirmed for HHV-6B (Asano et al. 1994, Hall et al. 1994, Ward et al. 2005) but only a few fatal cases of disseminated HHV-6 infection have been reported (Asano et al. 1992, Hoang et al. 1999, Yoshikawa et al. 2001c). Nonetheless, case reports highlight the versatile pathogenicity of HHV-6, with cases of fulminant hepatitis (Asano et al. 1990, Ishikawa et al. 2002), liver dysfunction (Tajiri et al. 1990, Tajiri et al. 1997, Takikawa et al. 1992), thrombocytopenia (Yoshikawa et al. 1993) and hemophagocytic syndrome (Sugita et al. 1995). HHV-6 has also been proposed as a cofactor in the etiology of MS and other neurological disorders (Challoner et al. 1995). Given the high seroprevalence in the population, primary HHV-6 infection in adults is very rare. In a few cases, a more severe outcome, such as infectious lymphadenitis or fulminant hepatitis has been reported in the immunocompetent adults (Steeper et al. 1990, Ishikawa et al. 2002, Maric et al. 2004, Cacheux et al. 2005). In immunocompromised patients, the consequences of primary HHV-6 infection may be more severe.

2.3 HHV-6 in transplant recipients

HHV-6 infection is frequently detected in immunosuppressed patients (Dockrell et al. 1997, Ihira et al. 2001). Considering the high seroprevalence in the population, infection is likely to result from reactivation of recipient virus or from the donor. However, those are mainly asymptomatic reactivations of the virus and in adult patient population primary infections are rare. In a recent study, a high HHV-6 seroprevalence (96.4%) was reported in adult solid organ transplant patients, and only one patient developed a symptomatic primary infection (Cervera et al. 2006). HHV-6B is commonly detected in transplant patients but HHV-6A occurs only occasionally (Wang et al. 1999). The incidence peaks at 2 to 4 weeks after transplantation (Singh and Carrigan 1996, Griffiths et al. 1999, Ljungman et al. 2000, Ihira et al. 2001, Humar et al. 2002a, Yoshikawa et al. 2002). The incidence of HHV-6 infection varies between 48% (28 to 75%) for stem cell transplant patients and 32% (0 to 82%) for solid organ (especially liver and kidney) transplant recipients (Ljungman et al. 2000, Razonable and Paya 2002, De Bolle et al. 2005). HHV-6 infection in transplant patients is usually asymptomatic but complications have also been reported. HHV-6 may cause fever and other clinical symptoms, such as neurological disorders, graft dysfunction, pneumonitis and hepatitis (Herbein et al. 1996, Humar et al. 2002a, Lautenschlager et al. 1998, Ljungman et al. 2000, Singh et al. 1997, Yoshikawa et al. 2002, Zerr et al. 2001). In addition to the direct effect of HHV-6, indirect effects also have been recorded. HHV-6 is considered an immunomodulatory virus that may facilitate superinfections with other opportunistic infections (Flamand et al. 1995, Singh et al. 1997). HHV-6 reactivations are often associated with rejections and CMV infections (Dockrell et al. 1997, Griffiths et al. 1999, Lautenschlager et al. 1998). For HHV-6 infection in transplant patients there are no definitions developed, as published for CMV (Ljungman et al. 2002).

After stem cell or bone marrow transplantation, the most frequently reported clinical diseases are encephalitis or encephalopathy (Drobyski et al. 1994, Wang et al. 1999, Yoshida et al. 2002), pneumonitis (Cone et al. 1993) and delayed engraftment (Johnston et al. 1999), whereas only a few reports mention fever and skin rash as a result of HHV-6 infection (Tanaka et al. 2000, Volin et al. 2004, Wang et al. 2006). HHV-6 infection has also been associated with bone marrow suppression (Carrigan and Knox 1994, Hentrich et al. 2005) and graft versus host disease (GVHD) (Appleton et al. 1995, Hentrich et al. 2005, Zerr et al. 2005). In a recent study acute GVHD manifested (statistically significantly) earlier in patients with HHV-6B antigenemia compared to those negative after SCT (Volin et al. 2004).

In solid organ recipients, clinical manifestations of HHV-6 reactivation are rare. They manifest as fever and/or rash, encephalitis or encephalopathy (Sutherland et al. 1991, Yoshikawa et al. 2001b, Montejo et al. 2002), hepatitis (Ward et al. 1989, Humar et al. 2002a), graft dysfunction or rejection (Okuno et al. 1990, Acott et al. 1996, Lautenschlager et al. 1998, Griffiths et al. 1999), bone marrow suppression or pneumonitis (Singh et al. 1997). In renal transplantation, the frequency of HHV-6 DNAemia/antigenemia varies between 38 and 82% (Morris et al. 1989, Okuno et al. 1990, Merlino et al. 1992, Kidd et

al. 2000). The presence of HHV-6 antigens in renal biopsy material has been found in association with other pathological conditions of kidney allografts, such as acute and chronic rejection and cyclosporine-related nephropathy (Okuno et al. 1990, Hoshino et al. 1995). In heart, heart-lung and lung transplantation, HHV-6 activation has been detected in 31-66% of the recipients, usually without clinical symptoms (de Ona et al. 2002, Jacobs et al. 2003, Neurohr et al. 2005). However, some case reports describe also encephalitis, gastroduodenitis and pancreatitis (Randhawa et al. 1997, Nash et al. 2004). In a longitudinal study of lung transplant patients, Jacobs et al. (Jacobs et al. 2003) reported a significantly higher mortality rate in patients with HHV-6 early after transplantation. In lung transplant patients, there is some evidence of the association of detection of HHV-6 in bronchoalveolar lavage fluid and *bronchiolitis obliterans* syndrome (Neurohr et al. 2005).

2.4 HHV-6 in liver transplantation

2.4.1. Direct effects

In liver transplant patients, detection of HHV-6 in the blood by culture or PCR has been reported in 28-54% of cases (Ward et al. 1989, Herbein et al. 1996, Schmidt et al. 1996, Singh et al. 1997, Griffiths et al. 1999, Humar et al. 2002a). HHV-6 infection in liver transplant patients is usually asymptomatic, but HHV-6 was reported as a cause of fever in liver transplant recipients (Chang et al. 1998). Liver dysfunction and even hepatitis may occur with HHV-6 infection and other direct effects include encephalitis, bone marrow suppression, leucopenia, pneumonitis and neurological complications (Ward et al. 1989, Herbein et al. 1996, Singh et al. 1997, Lautenschlager et al. 1998, Griffiths et al. 1999, Humar et al. 2002a). HHV-6 has been shown to infect the graft after liver transplantation and cause graft dysfunction (Lautenschlager 1998).

2.4.2. Indirect effects

The indirect effects of HHV-6 appear to be clinically significant in organ transplant recipients. HHV-6 may trigger reactivation of other infections and increase the risk of opportunistic infection in liver transplant recipients by 3.68-fold (Humar et al. 2002a). Bacterial opportunistic infections have been detected in liver transplant patients with HHV-6 infection (Humar et al. 2002a). HHV-6 infection has been reported as an independent significant predictor of invasive fungal infections in liver transplant patients (Dockrell et al. 1999, Rogers et al. 2000). HHV-6 has also been shown to have a contributory role in the pathogenesis of hepatitis C infection (Humar et al. 2002b, Singh et al. 2002).

HHV-6 reactivation is often associated with CMV after liver transplantation (Lautenschlager et al. 1998, Dockrell et al. 1999, Humar et al. 2000, Mendez et al. 2001, Razonable et al. 2003). HHV-6 (alone or with HHV-7) seems to influence the CMV reactivation rate and alter the course of CMV disease in transplant patients (Dockrell et al. 1997, DesJardin et al. 1998, Humar et al. 2000, DesJardin et al. 2001, Humar et al. 2002b). In addition, a temporal pattern of reactivation of the three betaherpesviruses was found to underlie clinical symptoms, with HHV-6 being reactivated first, followed by HHV-7 and CMV (Griffiths et al. 1999, Ihira et al. 2001). HHV-6 and HHV-7 may interact with each

other and may cause reactivation of CMV from latency or, conversely, CMV infection may trigger HHV-6 or HHV-7 reactivation (Mendez et al. 2001, Humar 2006). HHV-6 may be a risk factor for CMV infection in liver transplant patients (Herbein et al. 1996, Humar et al. 2000).

2.4.3. HHV-6 and liver allograft rejection

An association with liver allograft rejection has been recorded (Lautenschlager et al. 1998, Griffiths et al. 1999, Humar et al. 2002a, Feldstein et al. 2003). Griffiths et al. (Griffiths et al. 1999) demonstrated that both HHV-6 and CMV were independently associated with biopsy-proven graft rejection. In another prospective study HHV-6 and rejection association was recorded in a subgroup of patients with acute rejection later than 30 days after transplantation, following HHV-6 infection (Humar et al. 2002a). In a study on pediatric liver transplant recipients, primary HHV-6 infection was found in association with acute graft rejection (Feldstein et al. 2003). HHV-6 was first shown to infect the liver transplant and cause lymphocyte infiltration and graft dysfunction (Lautenschlager et al. 1998). HHV-6 antigens were mainly located in the inflammatory infiltrate on the portal areas. In biopsy histology five patient demonstrated signs of mild to moderate acute rejection and some were associated with CMV antigens in the liver biopsy indicating graft infection with both of the viral agents. In a prospective study from Lautenschlager et al. (Lautenschlager et al. 2000), HHV-6 antigenemia was detected in 22% of patients during the first year after liver transplantation, all caused by the variant HHV-6B. Signs of liver dysfunction were recorded in 74% of patients in association with the diagnosis of HHV-6. In addition to antigenemia, HHV-6B antigens were detected in the liver biopsies of some patients indicating an intragraft HHV-6 infection. The intragraft HHV-6 infection was associated with mild to moderate portal inflammatory infiltration with lymphocytic predominance indicating an ongoing immunological process in the transplant.

The possible involvement of HHV-6 in the immunomechanims of rejection has been suggested. HHV-6 is known to be an immunomodulative virus (Flamand et al. 1991, Flamand et al. 1995, Krueger and Ablashi 2003, De Bolle et al. 2005) and shown to down-regulate e.g. CD3 and suppress T cell functions including reduced IL-2 synthesis (Flamand et al. 1995). On the other hand, HHV-6 also upregulates and stimulates inflammatory pathways. Infection induces up-regulation of TNF-α, IL-1-β and IFN-α (Kikuta et al. 1990, Flamand et al. 1991). HHV-6 was shown to increase expression of genes for IL-18, IL-2R and members of the TNF-α superfamily receptors (Mayne et al. 2001). Whether the production of TNF-α stimulates back the activity of HHV-6, as has been shown to CMV, is not known. HHV-6, like CMV, induce the release of inflammatory cytokines, such as TNF-α and IL-1 (Kikuta et al. 1990, Flamand et al. 1991), which are important mediators in the early phase of rejection cascade. This may lead to other immunological events, such as T cell activation and induction of adhesion molecules, as well as triggering rejection also in the case of HHV-6. The immunological effects of HHV-6 in transplant patients need to be explored further.

2.5 Diagnosis and therapy of HHV-6

2.5.1. Diagnosis

There is no reference diagnostic test for HHV-6 infection. Viral culture of HHV-6 is possible in lymphoid cell lines, but the technique is slow and laborious, and not routinely used in diagnostic laboratories. The culture techniques described already decades ago (Salahuddin et al. 1986) have been earlier used for the isolation of the virus. Many investigators regard isolation of infectious HHV-6 from PBMC of individuals valid for the diagnosis of primary infection (Yamanishi et al. 1988, Hall et al. 1994). In bone marrow and organ transplant patients, virus isolation has also been used (Carrigan and Knox 1994, Singh and Carrigan 1996). Although, rapid culture methods have been developed (Singh and Carrigan 1996), demonstration of viral antigens and nucleic acids are today more common.

There are several serological methods available to diagnose the seroresponse against HHV-6. In addition to immunofluorescence based techniques, more sensitive specific EIA-methods are commonly used (Singh and Paterson 2000). Serological examinations are useful in diagnosing primary infections, when both rise of IgG and especially presence of IgM indicate active infection (Linnavuori et al. 1992, Ward et al. 2001). The same elevations can be seen in reactivation of HHV-6 in transplant recipients, but the seroresponse is slow and does not indicate the temporal infection (Suga et al. 1992, Ihira et al. 2001, Yoshikawa et al. 2001a).

Virus specific antigens can be demonstrated in the tissues and cellular specimens by immunostaining (Hoshino et al. 1995, Singh and Carrigan 1996, Lautenschlager et al. 1998, Luppi et al. 1998, Knox et al. 2000). Some of the monoclonal antibodies are used on formalin fixed tissue sections also (Drobyski et al. 1993). Commercially available monoclonal antibodies can be used for detection of HHV-6 proteins, some of the antibodies even can differentiate HHV-6 variants A and B. In the HHV-6 antigenemia test the virus specific proteins are demonstrated from peripheral blood mononuclear cells (PBMC) by monoclonal antibodies and immunoperoxidase staining (Lautenschlager et al. 2000) or immunofluorescence (Nishimura et al. 2005). The HHV-6 antigenemia test has also used to detect an active HHV-6 infection in transplant patients (Lautenschlager et al. 2000, Volin et al. 2004, Savolainen et al. 2005). HHV-6 antigenemia test is even suggested to be one of the most important methods to diagnose HHV-6 in the future (Tomoiu and Flamand 2006). HHV-6 antigenemia correlates with quantitative PCR (Härmä et al. 2005), but the method itself is rather qualitative, than quantitative.

Nucleic acids can be detected either by hybridization in situ or by PCR. The most sensitive methods for the detection of HHV-6 are those employing PCR-based techniques. However, it is not always possible to distinguish between latent and active infection with qualitative PCR (Carrigan 1995, Ihira et al. 2001, Kondo et al. 2003). More recently, quantitative PCR-tests have been developed to monitor the viral load either in plasma, whole blood or peripheral blood mononuclear cells (PBMC) of transplant patients (Clark et al. 1997,

Locatelli et al. 2000, Gautheret-Dejean et al. 2002). Some of these PCR-tests even are able to differentiate HHV-6a and HHV-6B (Safronetz et al. 2003, Boutolleau et al. 2006). However, there is no agreement which blood component should be used and the methods are not standardized. In addition, diagnostic results may be affected by the fact that high HHV-6 DNA levels in whole blood and serum are found in individuals with viral chromosome integration (Ward et al. 2006).

In situ hybridization has been used in the tissue and cellular specimens to detect viral DNA (Yadav et al. 1996, Arivananthan et al. 1997, Tanaka-Taya et al. 2004, Loginov et al. 2006). In situ hybridization has been used in numerous studies to demonstrate HHV-6 infection of liver, brain, kidney, lung and other organs (Tajiri et al. 1997, Cuomo et al. 2001, Ishikawa et al. 2002, Goodman et al. 2003, Vuorinen et al. 2004, Sebekova et al. 2005).

2.5.2. Therapy

No controlled studies of antiviral therapy against HHV-6 have been performed, and no pharmaceutical compounds have been formally approved for the treatment of HHV-6 infections. Therefore, the drugs clinically used against HHV-6 are the same as those used in CMV therapy or prophylaxis. Ganciclovir, foscarnet, and cidofovir all have in vitro activity (De Clercq et al. 2001) against HHV-6 and reports of their clinical effect on HHV-6 have been published (Ljungman et al. 2000, Zerr et al. 2002, Ljungman et al. 2007).

For the nucleoside analogs acyclovir and ganciclovir, inhibition of viral DNA synthesis is carried out by their triphosphate metabolite, which is formed in three consecutive phosphorylations, and inhibits the herpesvirus DNA polymerase by competition with the natural substrate dGTP (De Bolle et al. 2005, Naesens et al. 2006). The selectivity of the nucleoside analogs relies most importantly on their selective phosphorylation by a viral kinase. The nucleoside phosphonate analog cidofovir does not need to be activated by viral kinase, explaining its broad-spectrum anti-virus activity. Cidofovir diphosphate acts as a competitive inhibitor of dCTP and an alternative substrate for the herpesvirus DNA polymerase (Naesens et al. 2006). The pyrophosphate analog foscarnet inhibits the herpesvirus DNA polymerase by binding to the pyrophosphate-binding site of the enzyme. The selectivity of both foscarnet and cidofovir is based on their higher affinity for the viral DNA polymerase compared to cellular DNA polymerase (Naesens et al. 2006).

In vitro studies from several groups suggest that nucleoside analogs ganciclovir (and valganciclovir), but not so clearly to acyclovir (or valaciclovir), the nucleotide analogue cidofovir and the pyrophosphate analogue foscarnet all are effective for the treatment of HHV-6 infection (Reymen et al. 1995, Manichanh et al. 2000, De Clercq et al. 2001, De Bolle et al. 2005). Foscarnet was consistently shown to have a clear effect on HHV-6 replication with relatively high selectivity (Manichanh et al. 2000, De Clercq et al. 2001). Cidofovir displays strong activity against HHV-6 in vitro (Reymen et al. 1995).

Clinical experience of the antivirals in the treatment of HHV-6 infection in transplant patients is also limited, since controlled trials of antiviral therapy against HHV-6 have not yet been conducted. Thus, conclusions on the efficacy of antiherpetic drugs rely on a compilation of case studies (Yoshikawa 2004, De Bolle et al. 2005). Antiviral therapy with ganciclovir or foscarnet has been shown to lead to reduction in HHV-6 viral load in CSF (Mookerjee and Vogelsang 1997, Bethge et al. 1999, Yoshida et al. 2002, Zerr et al. 2002), blood (Mendez et al. 2001, Zerr et al. 2002) or saliva (Ljungman 2001, Ljungman et al. 2007), although some cases of fulminant HHV-6 infection showed no response to ganciclovir (Tiacci et al. 2000, Rossi et al. 2001). Both ganciclovir and foscarnet have been reported to be effective against HHV-6 meningoencephalitis after transplantation (Singh and Paterson 2000, Yoshida et al. 2002, Zerr et al. 2002). When the response to antiviral therapy against CMV was investigated in liver transplant patients, reduced viral loads of HHV-6 was also recorded after ganciclovir therapy (Mendez et al. 2001). Also, prophylactic therapy with ganciclovir proved effective in preventing HHV-6 reactivation in stem cell or BMT transplant recipients (Tokimasa et al. 2002). Recently, valganciclovir has become available and is currently indicated for the prevention of CMV disease. Only one single-center study has been published showing safety and effectivity of valganciclovir in CMV disease therapy after renal transplantation (Babel et al. 2004). Valganciclovir prophylaxis was first reported to be effective preventing CMV in solid organ transplant patients (Paya et al. 2004) and later even lower HHV-6 viral loads were recorded in the patients receiving valganciclovir (Razonable et al. 2005). Acyclovir seems to be clinically ineffective against HHV-6 (Rapaport et al. 2002).

Foscarnet appears to be effective against HHV-6 but it may cause renal toxicity. Some successful use of ganciclovir and foscarnet for HHV-6 encephalitis in bone marrow transplant recipients in vivo has been reported (Bethge et al. 1999, Zerr et al. 2002). Clinical use of cidofovir for CMV infections has been limited to second-line therapy of resistant virus strains (De Bolle et al. 2005, Biron 2006). Clinical reports on cidofovir for the treatment of HHV-6 infection are limited to few case reports in patients with HHV-6 encephalopaties (Denes et al. 2004, Astriti et al. 2006). As the studies mentioned above consider therapies initiated at the onset of HHV-6 disease, the benefits of HHV-6 antiviral prophylaxis remain less evident.

AIMS OF THE STUDY

Previous studies have shown that HHV-6 may infect the liver graft. Acute liver failure (ALF) is a significant reason for liver transplantation. The cause of acute liver failure is often unknown, but viral infections are believed to be involved. After transplantation reactivations of all three betaherpesviruses are common.

The specific aims of this study were:

1. To investigate the HHV-6 induced immunological events in the liver allograft in detail. The expression of vascular adhesion molecules and their ligands on graft infiltrating lymphocytes, as well as lymphoid activation markers, were studied in HHV-6 infected transplants (I).

2. To study the involvement of HHV-6 in acute liver failure and also the prevalence of post-transplant HHV-6 infection in the liver graft of the ALF patients with pre-transplant HHV-6 infection (II, III).

3. To investigate the post-transplant occurrence of HHV-6 and HHV-7 antigenemia in relation to symptomatic CMV infection in adult liver transplant patients (IV).

MATERIALS AND METHODS

1. Patients

Between January 1996 and September 2002 liver transplantation was performed on 250 patients at the Department of Surgery, Transplantation and Liver Surgery Clinic in Helsinki University Central Hospital. The patients included in this study have been characterized in detail in the original communications I-IV and are outlined here only briefly. This thesis is based on two independent studies and two studies concerning the same patient population, with clinical data and material obtained from adult patients of this time period.

(I) The first communication deals with intragraft immunological events associated HHV-6 infection of the liver transplant. HHV-6 infection of the hepatic allograft was diagnosed in 19 recipients in the population of 139 adult liver transplant patients under regular post-transplant follow-up during the years 1996-2000. As patients with concomitant acute rejections or other infections were excluded from the study, a pure HHV-6 infection of the liver was detected in the biopsies of eight grafts.

(II-III) In the second communication the 32 liver allograft recipients with acute liver failure and representative biopsies of the explanted livers, who underwent transplantations from May 1996 to September 2002, were included in the study. These patients were not consecutive, as alltogether up to 60 patients were transplanted due to ALF during this time-period. Non-A to non-E hepatitis (unknown) ALF was the reason for transplantation in 15 cases, whereas 17 patients with known cause of ALF served as controls. The aim of the study was to investigate whether HHV-6 antigens or DNA could be found in the explanted livers or in the post-transplant liver biopsies of ALF-patients. For the patients in the control group, ALF was attributed to toxic causes in 14 patients and Budd-Chiari syndrome in four. The male to female ratio was 6 to 9 in the study group and 4 to 13 in the control group. The mean ages of the patients were 45 years and 47 years in the study and control groups, respectively. In the third communication the same 32 liver allograft recipients transplanted due to ALF were monitored after transplantation, and HHV-6 antigens and DNA were examined from the blood samples and liver biopsies.

(IV) In the fourth communication 64 consecutive adult liver transplant patients from 1999-2001 were included to investigate the occurrence of both HHV-6 and HHV-7 antigenemia in relation to CMV infection. The male to female ratio was 32 to 32, and the mean age of the patients 46 years. The basic immunosuppression (triple drug therapy) consisted of, like in all patients in these studies (I-IV), various combinations of steroids, azathioprine and cyclosporine, or tacrolimus.

1.1 Pre- and post transplant examinations

In addition to biopsy histology, the explanted livers were examined for hepatitis viruses A to E and other possible viral agents, especially herpesviruses, such as EBV, CMV, and HHV-6 and –7. The routine pre-transplant microbiological screening of the patients also included diagnostic procedures for other infections, i.e. fungal, bacterial and viral infections. Serological screening of hepatitis A to E, CMV and EBV was performed according to standard pre-transplant laboratory routine. The pre-transplant HHV-6 or HHV-7 status of the recipients (or donors) was not determined, as all the patients were adults and supposed to be seropositive for these viruses (Yanagi et al. 1990, Krueger et al. 1994).

Post-operatively, the patients received as basic immunosuppression triple drug therapy of various combinations of steroids (methylprednisolone 2 mg/kg/d at day one rapidly tapering to 0.25 mg/kg/d), azathioprine (2-1 mg/kg/d) and cyclosporine or tacrolimus (according to trough level), and high doses of methylprednisolone (3mg/kg/d for 5 days) were used as antirejection treatment. The liver biopsy histology was performed in the case of graft dysfunction only, and no protocol biopsies were obtained. To monitor graft function, the following serial liver function tests were performed: serum alanine aminotransferase (ALT), alkaline phosphatase (ALP), gamma-glutamyl transpeptidase (g-GT) and bilirubin (BIL).

The pre and post-transplant monitoring of the patients included diagnostical tests for fungal, bacterial and viral infections, in addition to those for hepatitis A to E infections. Antifungal prophylaxis was given in combination with antibacterial prophylaxis to all liver transplant patients during the first three postoperative days and thereafter if necessary. *Pneumocystis* prophylaxis was given to all patients starting from second week post-transplantation and continuing 3-4 months. In addition, prophylaxis for gastric ulcers was given to all patients during the first month after liver transplantation. Serological screening of CMV and EBV was also performed. CMV infections were diagnosed by the frequent monitoring of CMV pp65-antigenemia (van den Berg et al. 1989, The et al. 1995) and by viral cultures. CMV infections were treated with intravenous ganciclovir. No routine antiviral prophylaxis was given at the timepoint of this study, besides intravenous ganciclovir prophylaxis during increased immunosuppression due to acute rejection. Nowadays CMV prophylaxis is used in Helsinki in D+/R- liver transplantations.

2. Diagnosis of HHV-6 infection and the other betaherpesviruses

2.1 Betaherpesvirus antigenemia

2.1.1. HHV-6 and HHV-7 antigenemia

HHV-6 infections were diagnosed by the HHV-6 antigenemia test, which detects the virus spesific antigens in blood mononuclear cells. The patients were monitored weekly during the patients' hospital stay up to 4 weeks, and thereafter at each visit during the first post-transplant year and in any case of suspected viral infection, for HHV-6 antigenemia in parallel with CMV pp65 antigenemia. Briefly, the peripheral blood mononuclear cells (PBMC) were isolated by Ficoll-Paque density gradient centrifugation and cytocentrifuged onto microscope slides. The presence of viral antigens were demonstrated by indirect immuperoxidase staining and by monoclonal antibodies against both variants A and B (MAB8533 and MAB8535, Chemicon Inc., Temecula, CA) as decribed in (Lautenschlager et al. 2000). Also HHV-6 serology was performed as described in detail in (Linnavuori et al. 1992). Concomitantly, HHV-7 specific antigens were demonstrated in the cytocentrifuge preparations of PBMC by using two monoclonal antibodies (Biodesign International, Saco, ME) to detect the early and late antigens of HHV-7 respectively.

2.1.2. CMV antigenemia

The standard CMV-pp65 antigenemia test was used for detecting the viral proteins in the leukocytes as described previously (van den Berg et al. 1989, The et al. 1995). In short, the viral proteins in the cellular specimens were demonstrated by using a monoclonal antibody against CMV pp65 antigen (Biotest, Frankfurt, Germany), mainly located in the polymorphonuclear leukocytes. In every staining series known negative and positive samples were used as controls. The positive results were quantified by counting the number of pp65 expressing cells per 50000 leukocytes on the cytocentrifuge preparation, as described for the original CMV pp65-antigenemia assay (van den Berg et al. 1989, The et al. 1995).

2.2 Demonstration of betaherpesviruses from the biopsies

The same immunohistochemical methods and the monoclonal antibodies were used on the liver biopsy frozen sections to demonstrate the possible presence of HHV-6 in explanted livers and occasionally obtained biopsy material, as described above for PBMC and for tissue sections previously (Lautenschlager et al. 1998, Lautenschlager et al. 2000). For detection of HHV-6/HHV-7 antigens, the liver biopsy material was snap-frozen, sections were cut, and acetone-fixed. The presence of HHV-7 in the biopsies was also detected by immunohistochemistry as described in (Lautenschlager et al. 2002). From each biopsy of the explanted livers and post transplant liver biopsies, CMV was demonstrated by immunohistochemistry and viral cultures (Lautenschlager et al. 1989). CMV antigens were demonstrated in snap-frozen, 3-um-thick cut, sections of liver biopsies by immunoperoxidase staining, using the same monoclonal antibody against CMV-spesific

antigens as in CMV pp65 antigenemia test described above (Halme et al. 2003). The presence of HHV-6 DNA was demonstrated by in situ hybridization (ISH) using the biotinylated oligoprobe (Qiagen, Cologne, Germany) detecting both variants A and B. The HHV-6 ISH was performed using the rabbit anti-biotin antibody (Enzo, Farmingdale, USA), followed by a second biotinylated anti-rabbit-IgG (Vector Lab, Burlingame, CA, USA), and streptavidin-alkaline phosphatase conjugate (Zymed, San Francisco, CA, USA). 4-Nitro blue tetra-zolium chloride, 5-bromo-4-chloro-3-indonyt-phosphate, toluidine salt (NBT-BCIP) (Roche Diagnostics GmbH, Mannheim, Germany) was used as a substrate and hemalum was used as a counter stain. The histopathological findings, such as inflammation, were correlated against the presence of the virus.

2.3. Liver histology (I-III)

Biopsies of the explanted livers and post-transplant liver allograft biopsies were formalin-fixed and embedded in paraffin wax, two-micron-thick sections were stained to evaluate the histological changes in the liver samples. Standard liver histology was performed on explanted livers. Especially, the possible histological and histopathological findings associated with HHV-6, HHV-7 or CMV or other viral infections were observed. The international Banff histological criteria for acute and chronic rejection were followed to diagnose rejection in the post-transplant biopsies (Demetris 1997, Demetris et al. 2000). The Banff criteria for acute rejection were defined as a triad with significant portal lymphocytic inflammation, cholangitis and endothelialitis. The criteria for chronic rejection included portal fibrosis and mild lymphoid infiltration, vanishing of bile ducts and occasional thickening of the arteries in the portal area.

2.4. Immunohistochemistry

The expression of vascular adhesion molecules, such as ICAM-1, VCAM-1 and ELAM-1 and their ligand molecules Leukocyte function antigen-1 (LFA-1), Very late antigen-4 (VLA-4) and Sialyl-LewisX-molecule (sLex), as well as the lymphoid activation markers MHC class II antigens and IL-2 receptor were demonstrated in frozen sections of liver biopsies by indirect immuperoxidase stainings as described above. Commercial monoclonal antibodies were used for ICAM-1, VCAM-1, ELAM-1 (R&D Systems, Abingnon, UK), LFA-1 (Dako, Glostrup, Denmark), VLA-4 (Immunotech, Marseilles, France), sLex (Becton Dickinson Immunocytometry Systems, San Jose, USA), class II (Dako) and IL-2-R (Becton Dickinson). The intensity of the vascular adhesion molecule expression was scored semiquantitatively on a scale from 0 to 3. The number of leukocytes positive for the ligand molecules (LFA-1, VLA-4, sLex) or the lymphocyte activation markers (class II and IL-2-R) were counted per high power visual field.

Five biopsies obtained previously from patients with normal graft function and without rejection or infection were used as negative controls for immune activation. Ten biopsies from other liver transplant patients who had demonstrated a biopsy proven acute rejection, but no infection, served as positive controls for measurements of immune activation.

2.5 Statistical methods

All data are expressed as mean +- 1 standard deviation, unless otherwise indicated. Pearson chi-square statistic or Fisher's exact test were used to compare frequencies between patient groups (II-IV). Student t test was used to compare continuous data between groups of patients (I, IV). Differences between two groups in the distribution of continuous variables were analyzed with the nonparametric Mann-Whitney U-test (IV). Nonparametric test was chosen because all distributions were not normal and because of small sample size (IV). The calculations were performed with SPSS statistical software (version 12.0.1., SPSS Inc, Chicago, IL). Two tailed P-values lower than 0.05 were considered statistically significant.

2.6 Ethical considerations

All specimens were obtained due to clinical indications only. All procedures were approved by the Ethical Committee of Helsinki University Hospital and in accordance with the Helsinki Declaration of 1975.

RESULTS

1. Immunological events linked with HHV-6 in the liver allograft (I)

HHV-6 may infect the liver transplant and cause lymphocytic inflammation in the graft. The possible involvement of HHV-6 in the immunological events and alloresponse in the liver allograft was investigated in detail. HHV-6 infection of 19 hepatic allografts was diagnosed in the population of 139 adult liver transplant patients. HHV-6 antigens were demonstrated in the biopsies of liver allografts after liver transplantation. A pure HHV-6 infection, without the presence acute rejection or other infections, such as CMV, was detected in the biopsies from eight liver grafts. All patients demonstrated concurrently also a HHV-6 antigenemia (7-95 days after transplantation). As the patients were HHV-6 seropositive before transplantation, all HHV-6 infections were reactivations. A concomitant increase in the liver function markers without other clear causes indicated that the infection caused graft dysfunction. In most patients the increase in the liver functions tests could only be linked to HHV-6 infection. However, in two of the patients with a prolonged (>80 days) HHV-6 antigenemia other clinical complications occurred, which were succesfully treated. Even in these patients the HHV-6-infection continued.

HHV-6 was located in the inflammatory mononuclear cells of the portal areas, and the predominant histological finding associated with intragraft HHV-6 was mild to moderate lymphocyte infiltration of the portal areas. No evidence of cholangitis or endothelialitis indicative of acute rejection was observed. Hence, the inflammatory response was seen in the graft and the main finding of the intragraft HHV-6 infection was lymphocyte dominated inflammatory infiltrate.

HHV-6 significantly increased the vascular expression (scored from 0 to 3) of ICAM-1 and VCAM-1 in the graft, when compared to the normal liver (Table 2). ICAM-1 was strongly expressed in the endothelium of all types of vascular structures and in the sinusoids, as well as in the epithelial cells, in the HHV-6 infected graft. An intense VCAM-1 induction was recorded in the vascular, but to a lesser extent in sinusoidal endothelia in the case of HHV-6. ELAM-1 was slightly, but not significantly, induced and located in the vascular endothelium only. In allograft acute rejection, all three adhesion molecules were significantly increased in the liver transplant.

Table 2 Expression of adhesion molecules ICAM-1, VCAM-1 and ELAM-1 in the vascular and sinusoidal endothelium in liver allografts during acute rejection (RX), in HHV-6 infected grafts (HHV-6) and in normal graft function without rejection or infections.

	ICAM-1		VCAM-1		ELAM-1	
	Vascular	Sinusoidal	Vascular	Sinusoidal	Vascular	Sinusoidal
RX	1,9±0,7*	2,4±0,5*	1,4±0,8*	1,3±1,3*	1,3±0,6*	0,0±0,0
HHV-6	2,1±0,9*	2,3±0,7*	1,1±0,5*	0,4±0,5	0,7±0,7	0,0±0,0
NOR	0,8±0,4	1,2±0,4	0,2±0,4	0,4±0,5	0,0±0,0	0,0±0,0

The number of portal lymphocytes positive for the ligand molecules LFA-1 and VLA-4 for ICAM-1 and VCAM-1 was significantly increased (Table 3). The number of lymphocytes positive for the sLex-molecule, the ligand for ELAM-1 was not significantly increased. As the number of cells expressing the lymphoid activation markers, characteristic for alloresponse, the class II expressing lymphocytes were significantly increased in the graft not only in acute rejection but also in HHV-6 infection. However, only a few IL-2R-positive cells were seen in the case of HHV-6 and the number was not significantly increased in the similar manner as in the case of rejection.

Table 3 The number of lymphoid cells positive for the ligand molecules (LFA-1, VLA-4, sLex) and activation markers (class II expression, IL-2-R) in liver allografts during acute rejection (RX), in HHV-6 infected grafts (HHV-6) and in normal graft function without rejection or infections (NOR) counted per high power visual field (mean±SD)

	LFA-1	VLA-4	SleX	Class II	IL-2-R
RX	35±6*[a]	12±5*	20±5*	28±8*	11±7*
HHV-6	39±16*	32±12*	9 ±8	26±12*	3 ±3
NOR	12±2	4 ±4	2 ±1	5 ±3	1 ±1

HHV-6 infection was associated with significant immunological changes in the graft without acute rejection, but the immune response was limited to lymphocyte infiltration and the adhesion molecule level. However, two of the patients developed a mild acute rejection on days 5 and 19 after onset of the HHV-6 episode, and were treated with high doses of steroid, one additional patient received antirejection treatment because of clinical suspicion of acute rejection. Later on, three patients developed chronic rejection (without preceding clinical acute rejection), one of these had a persistent HHV-6 infection and in another patient HHV-6 antigens were still found in the explanted graft. In addition, later four patients developed biliary strictures. These results demonstrate that HHV-6 infection of the liver allograft is associated with increased expression of vascular adhesion molecules and lymphocyte infiltration of the graft.

2. HHV-6 infection in acute liver failure patients (II-III)

2.1 Pre-transplant HHV-6 infection

Of the 15 patients transplanted due to non-A to non-E hepatitis ALF, 12 (80%) demonstrated HHV-6 antigens in the explanted liver. Most of these patients (10/12) also showed HHV-6 antigenemia and one patient had a serological evidence of recent HHV-6 infection. In the control patients with a known cause of ALF, 4 of 17 demonstrated HHV-6 antigens in the liver, together with pre-transplant HHV-6 antigenemia. In addition, one patient had a serological evidence of HHV-6 infection. Alltogether 18 patients had a pre-transplant HHV-6 infection. The other control patients demonstrated no HHV-6 antigens in the liver or in the blood. The difference between the control patients and the patients with ALF of unknown origin was also statistically significant ($p<0.05$).

No CMV, HHV-7 or other possible viral agents were found in the blood samples of the patients before transplantation. With the exception of one patient with EBV reactivation, HHV-6 remained the only infectious agent found in the patients with non-A to non-E hepatitis patients. Hepatic HHV-6 infection was also retrospectively confirmed by detecting HHV-6 DNA in the liver by in situ hybridization. In explanted livers the predominant finding of the HHV-6 infection in addition to fulminant parenchymal necrosis was a moderate to severe lymphocyte infiltration of the portal areas. The HHV-6 antigen positivity was usually seen in the portal areas, located mainly in lymphocytes and other mononuclear cells, but in some livers with numerous HHV-6 positive inflammatory cells even a few hepatocytes demonstrated a positive staining. The HHV-6 finding in the liver was confirmed by DNA hybridization in situ demonstrating HHV-6 DNA also in the same cellular structures.

Altogether 18 of 32 patients had some pre-transplant evidence of HHV-6 and 16 of these patients also had HHV-6 antigenemia preoperatively.

2.2 Post-transplant HHV-6 infection

Nine (50%) of the 18 patients described above demonstrated HHV-6 infection of the liver during the first 6 months after transplantation (mean 19 days, range 6-38 days) (Figure 6a). During the first six months after transplantation, HHV-6 antigenemia was detected in 16 of these 18 patients. All nine patients with post-transplant HHV-6 infection of the liver also demonstrated HHV-6 antigenemia concurrently. None (0/14) of the patients without pre-transplant HHV-6 infection showed HHV-6 antigens in the post-transplant biopsies. The HHV-6 antigenemia was demonstrated for some time in 11 of these patients during the first six months after transplantation. All cases were of variant B. The HHV-6 finding was retrospectively confirmed by in situ hybridization also by detecting HHV-6 DNA in the liver (Figure 6b). With the exception of the one patient with CMV, HHV-6 remained the only infectious agent found at the timepoint of these liver biopsies.

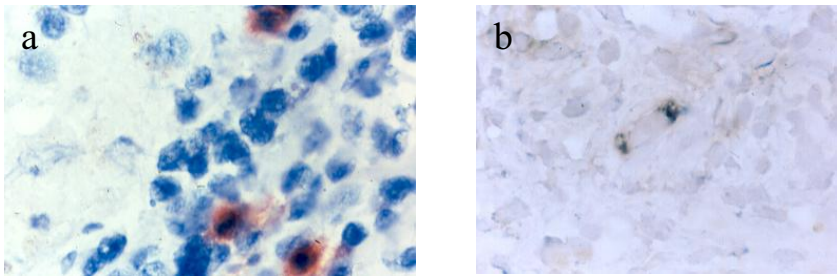


Figure 6 HHV-6 positive cells in the portal area demonstrated by immunoperoxidase staining (a) and in situ hybridization (b) (original magnification x 1000).

The HHV-6 antigens were usually located in the mononuclear cells of the portal areas and also in a few hepatocytes. As mentioned before, none (0/14) of the patients without pre-transplant HHV-6 evidence showed HHV-6 antigens in the post-transplant biopsies. The HHV-6 infection of the liver was significantly more frequent in the ALF patients with pre-transplant evidence of HHV-6 when compared to those without pre-transplant HHV-6 ($p<0.05$).

The total number of biopsies taken during the first 6 months after transplantation was 54. In biopsy histology, 78% (7/9) of intragraft HHV-6 relapse patients demonstrated a clinical picture suggestive of viral infection with portal inflammation and hepatocellular damage without endothelialitis or clear damage to the bile ducts. In addition, one patient had a mild acute rejection and one a suspicion of acute rejection at the timepoint of hepatic HHV-6 infection. Besides in one of the HHV-6 positive livers, hepatic arterial thrombosis was also confirmed in histology. One patient had no diagnostic findings in histology. The histological findings associated with post-transplant intrahepatic HHV-6 infection were mild to moderate portal lymphocytic inflammation and fibrosis, as well as a few parenchymal point necrosis.

All nine post-transplant HHV-6 infections of the liver were associated with graft dysfunction. On the day of the first HHV-6 positive liver biopsy, impaired liver function was seen by serum liver function tests in all nine patients. Post-transplant HHV-6 infection had no effect on patient or graft survivals when compared with patients without HHV-6.

3. HHV-6 and HHV-7 antigenemia related to CMV infection after liver transplantation

Of the 64 patients, 57 demonstrated HHV-6 and 46 HHV-7 antigenemia after liver transplantation (Figure 7 and 8). Nineteen patients developed symptomatic CMV infection during the first 3 months after transplantation. In addition, 25 asymptomatic patients demonstrated a temporary low-level CMV pp65 antigenemia. Two patients had CMV antigens also in the liver biopsy. No other causes of graft dysfunction; infections or complications could be found in these patients, with the exception of five patients with a concomitant episode of acute rejection.

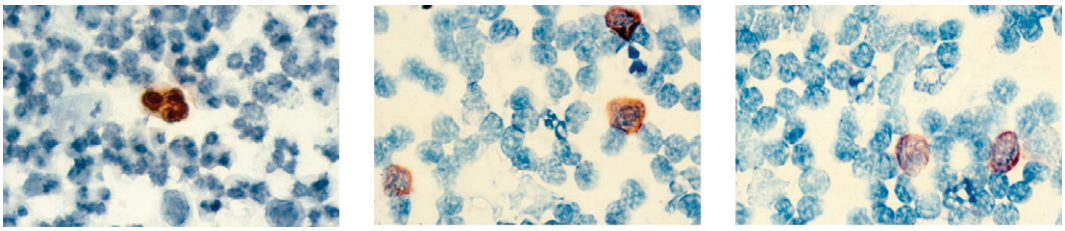


Figure 7 A CMV pp65 positive leukocyte, HHV-6 positive lymphocytes and HHV-7 positive lymphocytes demonstrated by immunoperoxidase staining in cytocentrifuge preparations of peripheral blood leukocytes (original magnification 1000x).

The incidence of HHV-6 and HHV–7 antigenemia was nearly the same in patients with and without CMV infection (Figure 8). However, the time-related appearance of HHV-6 and HHV-7 antigenemia differed significantly ($p<0.05$) (Figure 9).

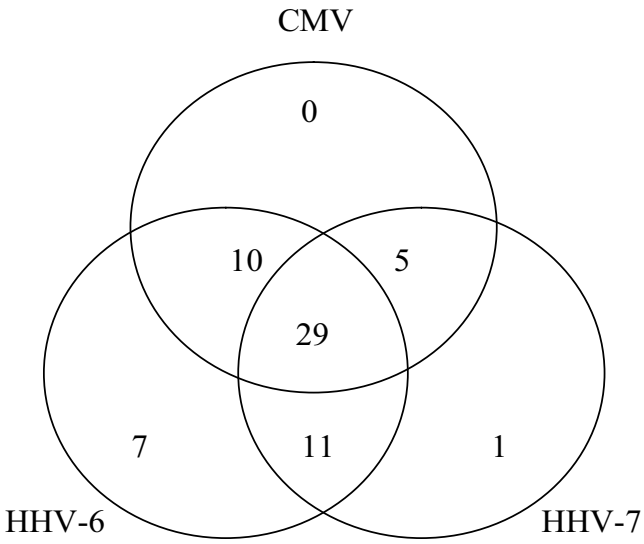


Figure 8 Number of patients positive for HHV-6, HHV-7 and CMV antigenemia.

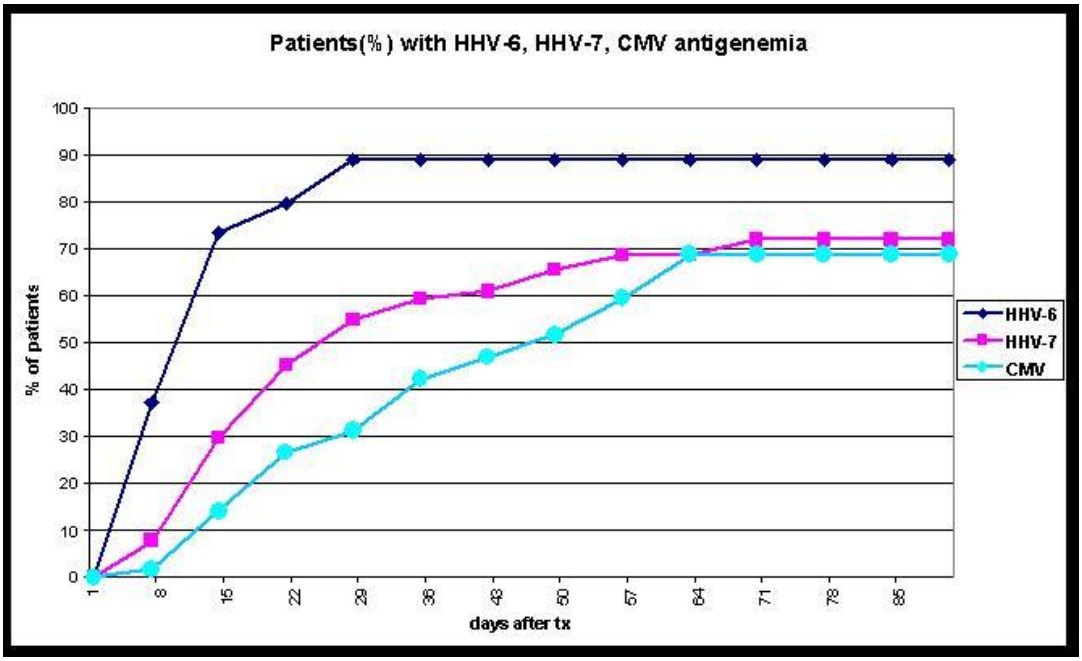


Figure 9 Time-related appearance of HHV-6, HHV-7 and CMV antigenemia.

Concurrent antigenemia with CMV was detected in 16/19 HHV-6 and in 15/19 HHV-7 patients. HHV-6 and HHV-7 antigens were located mainly in the lymphocytes, but also some monocytes were positive for HHV-6 antigens. HHV-6 antigenemia was in every case caused by variant B. HHV-6 appeared significantly earlier than CMV, HHV-7 more closely with CMV. In 7 patients with HHV-6 preceding CMV disease, an HHV-6 related graft dysfunction was seen. No symptoms could be linked with HHV-7. All symptomatic CMV infections were succesfully treated with ganciclovir. In most cases a response to ganciclovir therapy was also seen in HHV-6 and HHV-7 antigenemia but less clearly, during several weeks or even months.

DISCUSSION

Rejection and infections are the two most essential complicating factors after liver transplantation. Viral infections, especially those of the herpesvirus group, have also been suggested to be involved in the mechanisms of rejection. The involvement of viral infection in rejection is thought to be due to immunologic virus-host interactions. Previous studies have shown that HHV-6 may infect the liver transplant, because an intragraft lymphocyte dominated inflammatory reaction and be associated with rejection (Lautenschlager et al. 1998, Griffiths et al. 1999, Lautenschlager et al. 2000, Humar et al. 2002a, Feldstein et al. 2003). The purpose of this study was to study the HHV-6 induced immunological events in the liver transplant in detail.

1. Immunological events induced by HHV-6 infection of the graft (I)

In rejection, the influx of inflammatory cells into the graft is an essential step, involving sequential adhesive interactions between the leukocyte and the endothelium. Rolling of the lymphocytes and monocytes (/macrophages) is followed by reversible and later firm adhesion, when endothelial activation by inflammatory cytokines takes place. This complex process is coordinated by several adhesion molecules (Springer 1995, Butcher et al. 1999). In liver rejection, endothelium is induced to express adhesion molecules such as ICAM-1, VCAM-1 and ELAM-1, and their ligands LFA-1, VLA-4, sLex, is greatly increased in rejection (Steinhoff et al. 1993a, Lautenschlager et al. 1996). Adhesion molecules play a role in many other cell-cell interactions, such as antigen presentation and T-cell activation in the rejection cascade.

According to the results, HHV-6 infection of the liver allograft was associated with significantly increased expression of ICAM-1 and VCAM-1, and portal infiltration of lymphocytes in the graft. ICAM-1 was also induced in the epithelial cells of the liver, as described previously for acute rejection and viral infection (Lautenschlager and Höckerstedt 1993). However, there was not a significant increase of adhesion molecule ELAM-1 or the cells expressing its ligand sLex as described for rejection (Steinhoff et al. 1993a). Although, a mild to moderate lymphocyte response, with class II increase, was seen in the graft, the histological criteria for acute rejection were not fullfilled. In addition, a very low number of IL-2R expressing lymphocytes were in agreement with the absence of rejection in the HHV-6 positive biopsies.

The significant induction of vascular adhesion molecules in the allograft can be explained by the powerful cytokine production induced by HHV-6 (Kikuta et al. 1990, Flamand et al. 1991, Flamand et al. 1995). In general, the findings are very similar to that found for its close relative CMV, which induces a lymphocyte mediated immune response and production of various cytokines, such as IL-1, TNF-α and INF-γ. CMV upregulates vascular adhesion molecules and MHC antigens (Iwamoto et al. 1990, Smith et al. 1992,

van Dorp et al. 1993). The immunological events associated with HHV-6 are similar to those described for CMV, which increases expression of ICAM-1 and VCAM-1 and the ligand molecules LFA-1 and VLA-4, as well as MHC class II molecules in the liver transplant (Steinhoff et al. 1993a, Lautenschlager et al. 1996, Lautenschlager et al. 2006). HHV-6, like CMV, induces the release of inflammatory cytokines, such as TNF-α and IL-1-β, which are important mediators in the early phase of rejection cascade and in the induction of adhesion molecules (Kikuta et al. 1990, Flamand et al. 1991, Flamand et al. 1995).

Although HHV-6 induced a significant immunological activation in the transplant, the lymphoid infiltration was less intense during HHV-6 infection than in acute rejection, and no vascular or biliary changes occurred. However, later two of the patients with a pure HHV-6 infection developed a mild acute rejection and one-third ended up with chronic rejection. The general prevalence in our liver transplant patients is less that 5%. Thus, it is possible that the immunological response in the graft caused by HHV-6 may have triggered the processes leading to chronic rejection.

In this study (I), triggered immunological processes associated with a HHV-6 infection of the liver in eight patients were recorded and more than half of these patients developed later acute or chronic rejection, suggesting some association between HHV-6 infection and rejection processes. Some association with liver allograft rejection has been recorded also in previous clinical studies (Lautenschlager et al. 1998, Griffiths et al. 1999, Humar et al. 2002a, Feldstein et al. 2003). Griffiths et al. (Griffiths et al. 1999) demonstrated that both HHV-6 and CMV (DNAemia) were significantly and independently associated with liver graft dysfunction and biopsy-proven rejection. In another, prospective study HHV-6 infection and high viral load was significantly associated with biopsy-proven rejection in the subgroup of patients with rejection later than 30 days (Humar et al. 2002a). In a study on pediatric liver transplant recipients, no other infections but primary HHV-6 infection was found with biopsy-proven liver graft rejection in two infants (Feldstein et al. 2003). There are no articles published recently on the association of HHV-6 and graft rejection.

Probably the most important mechanism associated of the virus induced immunopathology is the triggering of inflammation by the upregulation of cytokines, MHC antigens and adhesion molecules. These processes may lead to other intragraft immunological events, such as T cell activation and induction of other adhesion molecules and trigger rejection also in the case of HHV-6. On the other hand, this phenomenon may be also reverse, and HHV-6 could be carried to the liver by passenger leukocytes which are always present in an allograft. In any case, HHV-6 infection seems to interact with the immunological events in liver graft.

2. HHV-6 infection in acute liver failure patients

2.1. HHV-6 and acute liver failure

HHV-6 was found in the study in most explanted livers of patients with ALF of unknown origin ending-up with liver transplantation. HHV-6 antigens were demonstrated in the explanted livers with numerous positive inflammatory cells and even a few hepatocytes. This indicates that the epithelial cells of the liver may be infected, and that ALF and hepatocyte necrosis may be caused by HHV-6. On the other hand, it is possible, that the hepatocyte necrosis seen in the current and other's studies, is not caused by HHV-6 directly, but the immunoresponse triggered by the virus leads to destruction of hepatocytes.

HHV-6 has been demonstrated to cause acute fulminant hepatitis as a complication of primary infection in immunocompromised patients (Dubedat and Kappagoda 1989, Asano et al. 1990, Sobue et al. 1991, Mendel et al. 1995, Tajiri et al. 1997, Aita et al. 2001, Ohashi et al. 2004, Nobili et al. 2006). Ozaki et al. (Ozaki et al. 2001) reported that HHV-6 DNA is frequently detected in hepatocytes of liver of children with various liver diseases by in situ hybridization. Aita et al. (Aita et al. 2001) demonstrated the histopathology of HHV-6 infection of the liver, and described HHV-6 DNA expression in portal vein endothelial cells. HHV-6 DNA has been detected also in retrospective analysis of explanted livers of ALF patients (Ishikawa et al. 2002), in a recent study on non-A-to-E hepatitis (unknown) patients, HHV-6 was detected by DNA hybridization in the liver tissues in 9 of 22 patients with acute fulminant hepatitis (Ishikawa et al. 2002). In this study, viral DNA and RNA were localized in hepatocytes, and viral antigens were also demonstrated in the hepatocytes by immonohistochemistry. Another study gave more contradictory results, as HHV-6 was not found by PCR of the liver specimens of non-A-to-E fulminant hepatic failure patients (Mason et al. 1996). In a more recent study, two cases of HHV-6-related ALF in immunocompetent adults have been reported (Cacheux et al. 2005). Viral origin was evidenced in this paper by the detection of high amounts of HHV-6 DNA in liver tissue by PCR, the decrease of intrahepatic viral load was also recorded after therapeutic intervention.

No other herpesviruses but HHV-6 (or any other viruses) were found in the explanted livers of non-A-to-E ALF patients, whereas only a few control patients displayed HHV-6 in the liver. Although we have now detected one possible agent, the actual pathogenic role of HHV-6 in ALF remains still suggestive only, as a viral reactivation could alternatively be induced by an unknown factor, which leads to ALF and the occuring of HHV-6 that enters the scene after the onset of liver failure. On the other hand, the impact of HHV-6 infection on the immunological events of the liver and triggering inflammation could be one of the mechanisms of HHV-6 to enhance acute liver failure. Despite the lack of definitive evidence of causality of HHV-6 in fulminant hepatitis, these observations strongly support its involvement in some cases, and a systematic research in case of unexplained ALF could be recommended.

2.2. HHV-6 in the post-transplant period of the ALF patients

In the second ALF-study (III), half of the patients with pre-transplant HHV-6 evidence showed HHV-6 antigens in the post-transplant biopsies, whereas none of the patients with no evidence of pre-transplant HHV-6 demonstrated HHV-6 antigens in the liver after transplantation. All the cases of post-transplant intrahepatic HHV-6 infection were associated with graft dysfunction, and most patients showed a picture of viral infection in histology. No other viral agents were found in patients before or after the transplantation, except in one patient post-transplant CMV was found concurrently with HHV-6 in the liver transplant.

These findings indicate that pre-transplant HHV-6 infection of the liver in ALF patients may be a risk factor for HHV-6 infection of the new transplant. This phenomenon could be either patient or virus strain dependent. However, post-transplant HHV-6 did not cause any severe liver failure in these patients, and the 1-year graft and patient survivals were not affected by HHV-6 infection.

In general, the post-transplant HHV-6 infection is usually asymptomatic, but may also be associated with clinical symptoms (De Bolle et al. 2005). On the other hand, intrahepatic HHV-6 infection occurs only in a minority of grafts after liver transplantation (Lautenschlager et al. 1998) and even though impaired liver function has been found, long-term effects on the graft or patient survivals are rare. However, to definitively exclude the negative influence of HHV-6 infection relapse in the long-term course of liver transplantation, a longer follow-up of the recipients is needed.

3. HHV-6 and HHV-7 antigenemia related to CMV infection after liver transplantation (IV)

In addition to CMV, the other betaherpesviruses HHV-6 and HHV-7 are frequently found in transplant patients. Primary infections with significant clinical symptoms have been described in pediatric transplantation for both HHV-6 and HHV-7 (Feldstein et al. 2003, Yoshikawa 2003). The clinical significance of HHV-7 reactivation in transplant patients is not clear, interactions with other betaherpesviruses and associations with transplant complications have been suggested (Emery 2001, Razonable et al. 2003). However, primary infections in the adult population are rare, and the reactivations are usually asymptomatic. Although most of the HHV-6 associated clinical manifestations are mild and self-limiting, HHV-6 infection related graft dysfunction and encephalitis have been described (Lautenschlager et al. 1998, De Bolle et al. 2005).

CMV infection occurs in the majority of solid organ transplant patients, primarily in the first months, and CMV disease incidence ranges from 10% to 50% (Seehofer et al. 2002, Paya and Razonable 2003). CMV infection and disease are recognized as a major clinical problem among opportunistic infections being important causes of morbidity and mortality among transplant recipients (Ljungman et al. 2002). CMV, HHV-6 and HHV-7 are all closely related and share a limited sequence homology (Berneman et al. 1992). Concurrent

betaherpesvirus activations are frequently found after transplantation and transactivations have been suggested (Razonable and Paya 2002, Yoshikawa 2003). Thus, CMV associated syndromes such as fever or graft dysfunction may also be caused by one or another of the betaherpesviruses (Razonable and Paya 2002).

We demonstrated the time-related appearance of HHV-6 and HHV-7 antigenemia in the patients with symptomatic CMV infection. HHV-6 antigenemia preceded CMV antigenemia, and probably caused the symptoms (fever/graft dysfunction) in some patients. Instead, HHV-7 antigenemia appeared only a few days before or concurrently with CMV antigenemia, and could not be linked with any spesific symptoms. This temporal pattern of reactivation of the three betaherpesviruses has been demonstrated by others, too (Griffiths et al. 1999, Ihira et al. 2001). Interactions between HHV-6 and CMV has been previously suggested both in a case of CMV primary infection and reactivations (Herbein et al. 1996, Humar et al. 2000, DesJardin et al. 2001). HHV-6 reactivation is considered as a risk factor, predisposing the patients to severe CMV infection (Dockrell et al. 1997). Also HHV-7 is suggested to be a co-factor for CMV disease progression (Tong et al. 2000). However, in the current study, HHV-6 and HHV-7 appeared almost equally in patients with and without CMV.

When CMV episodes subsided with intravenous ganciclovir treatment, also HHV-7 antigenemia disappeared. HHV-6 antigenemia, in contrast, tended to last longer, but subsided in several weeks after antiviral treatment was stopped. On the other hand, the antigenemia test used (Lautenschlager et al. 2000) is rather qualitative than quantitative making the interpretation of the response more difficult. However, at the DNA level ganciclovir treatment significantly decreased the HHV-6 viral load in peripheral blood leukocytes of liver transplant patients (Loginov et al. 2006). In vitro, ganciclovir, foscarnet and cidofovir all have antiviral activity against CMV, HHV-6 and HHV-7 (De Clercq et al. 2001), but no controlled study has been performed for the prevention or treatment of HHV-6 or HHV-7. In a recent study by Humar (Humar 2006), low incidences of HHV-6 and HHV-7 viremia in patients receiving CMV prophylaxis (valganciclovir) were recorded, suggesting that CMV prophylaxis may have some effect on HHV-6 and HHV-7 reactivation also.

4. Summary and conclusions

It was demonstrated, that intragraft HHV-6 infection is associated with portal lymphocyte infiltration and an increased expression of vascular adhesion molecules ICAM-1 and VCAM-1, and the number of lymphocytes expressing their ligand molecules and class II antigens in liver allografts. Thus, HHV-6 infection was associated with significant immunological changes in the graft, without acute rejection. However, three out of eight of these patients later developed chronic rejection suggesting that the immunological stimulation caused by the virus may be involved in, or trigger the alloresponse.
HHV-6 was found in most of the livers of patients with ALF of unknown etiology ending up with liver transplantation, whereas the opposite was seen in patients with a known

disease. These findings suggest that HHV-6 may be one of the causes or risk factors for ALF. Intrahepatic HHV-6 was also found in half of the patients with pre-transplant evidence of HHV-6, whereas no post-transplant HHV-6 infection of the liver was seen in the patients without pre-transplant HHV-6. Thus, the pre-transplant HHV-6 infection of the liver in patients with ALF is a risk factor for postransplant HHV-6 infection of the transplant.

All three betaherpesviruses are commonly reactivated after liver transplantation, but the time-related appearance of the viruses differed. HHV-6 antigenemia preceded CMV, but HHV-7 appeared together with CMV. CMV regularly responded to ganciclovir treatment, but HHV-7 and HHV-6 less clearly.

Overall, these results suggest that all the betaherpesviruses are common after liver transplantation. Although HHV-6 reactivations are usually asymptomatic, the virus may infect the liver transplant and cause an intragraft lymphocyte dominated inflammatory reaction and graft dysfunction. Immunological events caused by HHV-6 in the liver transplant may be involved in, or trigger rejection. In addition, HHV-6 could even be one of the causes of ALF and pre-transplant HHV-6 infection in ALF patients may be a risk factor for post-transplant HHV-6 infection of the graft. These results strongly support the clinical significance of HHV-6 which in some individuals leads to severe manifestations.

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"You caan't see ze peak rhait now. Thiz is the rhesult of thrree yeahrs of phreparation." –Horst Nussbaum

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