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High-dose acyclovir and intravenous immune globulin reduce the incidence of CMV disease after liver transplantation

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Abstract We attempted to prevent cytomegalovirus (CMV) disease in liver transplant (LTx) recipients by means of a combined prophylaxis regimen consisting of high-dose acyclovir (HDA) and immune globulin (IVIG). In 259 consecutive patients, HDA was given for 3 months post-LTx; recipients seronegative for CMV also received IVIG. The previous 94 patients comprised our control group; in this group, low dose acyclovir was given to prevent herpes, and prophylaxis of CMV consisted of IVIG given only to seronegative recipients of seropositive donors. The overall incidence of CMV disease was lower in the HDA group (10.8 %) than in the control group (27.6 %); ($P < 0.001$). The CMV disease rate associated with primary exposure was 26.3 % in the HDA group and 83.3 % in the control group ($P < 0.001$). The incidence of CMV disease occurring after acute rejection was 9.5 % in HDA patients and 24.6 % in controls ($P < 0.005$) The HDA protocol was associated with a trend toward a lower incidence of CMV in patients requiring OKT3 therapy (16.7 % vs 29 %). High-dose acyclovir/IVIG thus reduces the incidence of CMV disease in seronegative recipients after LTx and lowers the risk of CMV disease associated with therapy for rejection.

Key words Cytomegalovirus, liver transplantation · Acyclovir, CMV, liver transplantation · Immune globulin, acyclovir, CMV, liver transplantation

Introduction

Cytomegalovirus (CMV) is associated with significant morbidity and rare mortality in solid organ transplant recipients [4, 22]. In addition, bacterial and fungal superinfection and graft rejection have been associated with CMV infection and may further increase morbidity and mortality [4, 11].

Primary exposure, or transmission of the virus from seropositive donors to seronegative recipients, is associated with a 60 %–75 % infection rate; the majority of infected individuals develop clinical CMV disease after transplantation [22]. The donor organ is thought to be the most important source of the virus [9]. Another important route of transmission is transfusion of blood products [17]. In addition, the potent immunosuppressive agents used to treat rejection, primarily the antilymphocytic monoclonal antibody preparation OKT3, have been associated with a significantly increased risk of infection with CMV as well as with bacteria and fungi [16].

Several regimens have been employed to prevent CMV infection, including passive immunization with CMV hyperimmune globulin or nonspecific immunoglobulins [19, 23], active immunization with attenuated CMV [18], administration of antiviral agents such as oral acyclovir [1, 15] and, more recently, intravenous ganciclovir [10, 13]. We report here on the results of CMV prophylaxis in liver transplant recipients using high dose oral acyclovir, combined with intravenous immune globulin in seronegative recipients.

Table 1 Demographics, primary disease, blood loss, and CMV serology distribution in the control and prophylaxis groups

	Control ($n = 87$)	Prophylaxis ($n = 240$)
Age	46.6 + 13.5	44.1 + 17.2
Sex		
Male/female	45/42	124/116
Primary disease		
Primary biliary cirrhosis	12	40
Primary sclerosing cholangitis	10	18
Chronic hepatitis	27	61
Alcoholic cirrhosis	14	47
Cryptogenic cirrhosis	10	22
Fulminant failure	3	13
Biliary atresia	2	15
Other	9	24
Blood transfusion RBC	16.1 ± 17.3	15.7 ± 16.7
FFP	18.6 ± 17.6	18.6 ± 18.4
Cryo	9.2 ± 14.3	5.1 ± 10.1
Platelets	19.1 ± 17.8	12.2 ± 12.5*
CMV serology D − R −	20 (21.3 %)	26 (10.2 %)**
D − R +	29 (30.9 %)	75 (29.5 %)
D + R −	12 (12.8 %)	38 (15.0 %)
D + R +	26 (27.6 %)	101 (39.8 %)

* $P < 0.001$; ** $P < 0.01$

Materials and methods

Between August 1988 and August 1992, 408 liver transplantations (LTx) were performed in 348 patients (22 children and 326 adults) at the Mount Sinai Medical Center in New York. CMV prophylaxis consisting of high-dose acyclovir (HDA), 800 mg p.o. q.i.d., was given for 3 months post-LTx in 254 consecutive patients (from August 1990 to August 1992). Seronegative recipients also received IVIG (Venoglobulin-I, Alphatherapeutic, Los Angeles, Calif.), 4 gm/kg i.v. in eight divided doses: the first four doses weekly and the last four doses biweekly. The previous 94 patients (from August 1988 to July 1990) comprised our control group. In this group, a similar IVIG protocol was given only to seronegative recipients of seropositive donors; all patients also received low-dose acyclovir, 200 mg p.o. b.i.d., given to prevent herpes. Patients who died within the first 14 days post-LTx were excluded from analysis (14 in the prophylaxis group, 7 in the control group).

During the entire study period, donor and recipient serologic status was determined pretransplantation by CMV enzyme-linked immunosorbent assay (Diamedix ELISA, Florida). For each patient in both groups, 20 units of CMV-negative blood was made available pretransplantation.

CMV disease was defined as an invasive or symptomatic infection with a positive CMV culture or viral cytopathic inclusions seen on histologic sections or cytologic samples. When CMV hepatitis was suspected, immunohistochemical staining of biopsies for detection of viral antigen was performed. CMV disease not affecting the liver was suspected based upon one or more clinical symptoms (fever, malaise, diarrhea, pneumonia, or gastrointestinal bleeding) associated with leukopenia and was confirmed by culture or cytology. In a few patients, disease was diagnosed on the basis of IgM seroconversion or a significant increase in IgG titers (at least fourfold) in the setting of clinical CMV syndrome.

Treatment was begun upon diagnosis and consisted of ganciclovir (Cytovene, Syntex Laboratories, Palo Alto, Calif.), 5 mg/kg i.v. every 12 h for 14–21 days. The dose was adjusted according to renal function. Treatment was extended beyond 21 days in cases of persistent infection.

A triple immunosuppressive regimen including cyclosporin (CyA), azathioprine, and steroids was employed in most patients. OKT3 (Orthoclone OKT3, Ortho Biotech, Raritan, N.J.) was used for induction in 54 patients in the control group as part of a randomized study comparing OKT3 induction with standard triple immunosuppression. In both groups, OKT3 was also employed in patients with preoperative renal insufficiency and in those undergoing retransplantation for acute rejection. Maintenance steroid and CyA doses were reduced and azathioprine was maintained in most cases during CMV disease. Immunosuppression was discontinued in a few patients with disseminated disease associated with fungal or bacterial superinfection.

Liver biopsies were performed when acute rejection was suspected on the basis of liver function test abnormalities. Acute rejection was treated first with augmented doses of steroids. OKT3 was employed for steroid-resistant rejection (5 mg i.v. for 10–14 days); FK 506 was instituted as a rescue therapy for patients who failed OKT3 treatment.

The prophylaxis and control groups were compared for incidence of CMV disease within the first 6 months post-transplantation. Effects of associated risk factors (i.e., serologic status, blood transfusion, steroid treatment, and OKT3 therapy for rejection) in each group were also evaluated. Statistical analysis was done using chi-square testing for parametric variables and Student's t-test or log-rank test for continuous variables. P values below 0.05 were considered statistically significant.

Results

CMV disease occurred in 26 of 240 patients (28 episodes) in the prophylaxis group and in 24 of 87 patients (26 episodes) in the control group (10.8 % versus 27.6 %, $P < 0.001$). The mean interval from transplant to diagnosis was 49 ± 62 and 45 ± 26 days in the prophylaxis and control groups, respectively (P = NS). Mean platelet use was higher in the control group; otherwise, the two groups were comparable for age, sex, primary disease, and intraoperative blood product use (Table 1). A greater percentage of patients in the control group were seronegative recipients of seronegative organs. There was no difference between the groups, however, in the percent of patients with primary exposure (seropositive donor to seronegative recipient).

Characteristics of patients in both groups who developed CMV disease are summarized in Table 2. There was no difference in mean operative blood product use, site of CMV disease, or simultaneous occurrence of severe fungal or bacterial infection. There was no difference in mean operative blood loss between patients with and without CMV disease. Of those who developed CMV disease, four patients in the prophylaxis group and three in the control group received more than 20 units of blood intraoperatively.

In both groups, the incidence of CMV disease was highest among seronegative recipients of grafts from seropositive donors (Table 2). In the presence of this

Table 2 Characteristics of patients with CMV disease in the control and prophylaxis groups

		Control ($n = 24$)	Prophylaxis ($n = 26$)
Mean blood product use	RBC	15.1	15.9
	FFP	16.3	14.3
	Platelets	18.5	7.8
Serostatus	D − R −	5/20 (25 %)	1/26 (3.8 %)*
	D − R +	4/29 (13.8 %)	5/75 (6.7 %)
	D + R −	10/12 (83.3 %)	10/38 (26.3 %)**
	D + R +	5/26 (19.2 %)	10/101 (9.9 %)
	Total	24/87 (27.6 %)	26/240 (10.8 %)**
CMV disease site	Liver	15	16
	Lung	2	4
	GI tract	3	2
	Retinitis	–	1
	Multiple sites	4	3
CMV with severe bacterial and fungal infection		4	3

* $P < 0.05$; ** $P < 0.001$

Table 3 CMV disease associated with rejection therapy and OKT3 induction in the control and prophylaxis groups

		Prophylaxis group ($n = 240$)	Control group ($n = 87$)
Treatment for rejection	Steroid Rx	Patients with CMV: 17 (9.5 %)* Group total: 179	Patients with CMV: 15 (24.6 %) Group total: 61
	OKT3 Rx	Patients with CMV: 8 (16.7 %) Group total: 48	Patients with CMV: 7 (29.2 %)[a] Group total: 24
OKT3 induction		Patients with CMV: 3 (13.0 %) Group total: 23	Patients with CMV: 10 (18.5 %) Group total: 54

* $P < 0.005$

[a] 6/7 patients who required OKT3 received the standard induction triple immunosuppression with CyA

combination, however, the incidence of CMV disease was significantly lower in the prophylaxis group than in the control group (26.3 % versus 83.3 %, $P < 0.001$). When both the donor and recipient were seronegative, the incidence of CMV was 3.8 % in the prophylaxis group and 25 % in the control group ($P < 0.05$). There was a trend toward a reduction in the CMV disease rate among seropositive recipients of organs from seropositive donors.

Of the 179 patients in the prophylaxis group who received steroids for rejection, 17 (9.5 %) developed CMV disease after treatment. Among the 61 control patients treated with steroids, 15 (24.6 %) developed CMV disease ($P < 0.005$; Table 3). OKT3 was administered for steroid-resistant rejection to 48 prophylaxis patients and 24 control patients; CMV disease developed in 8/48 patients (16.7 %) in the prophylaxis group and 7/24 patients (29.2 %) in the control group (P = NS). OKT3 induction was employed in 23 patients in the prophylaxis group and 54 in the control group; 3/23 (13.0 %) in the prophylaxis group and 10/54 (18.5 %) in the control group developed CMV disease (P = NS).

Most patients treated with ganciclovir experienced a complete response. Two patients in each group relapsed, but all improved after a second course of ganciclovir. The most common side effect of ganciclovir therapy was leukopenia. Immunosuppression was discontinued only in patients with associated severe bacterial and fungal infections.

Seven patients in each group died. CMV-related mortality was recorded in three patients in the prophylaxis group and four in the control group; all seven died of severe CMV disease associated with invasive opportunistic *Candida* infection.

Discussion

The reported incidence of CMV infection in liver transplant recipients ranges from 35 % to 60 %, with more than half of these patients developing clinical CMV disease [6, 20, 21]. Most studies have evaluated the rate of CMV infection, which is often asymptomatic. Our study focused instead on the rate of CMV disease, which accounts for morbidity and mortality among

transplant recipients. Indeed, our combined prophylaxis protocol significantly reduced the CMV disease rate, primarily among patients at highest risk, namely, those with primary exposure.

Several studies have been conducted in LTx recipients of single-agent CMV prophylaxis using IVIG, hyperimmune globulin (CMVIG), or acyclovir. All but one nonrandomized study [2] have failed to reduce the overall incidence of CMV disease after transplantation [5, 6, 8, 24]. However, in a large, randomized trial of CMV prophylaxis with CMVIG, Snydman et al. did achieve a significant reduction in severe CMV-associated disease but no effect on overall CMV infection and disease rates [24].

Better results have been achieved when two antiviral agents have been employed in combination in patients with primary exposure [15, 25]. Accordingly, use of IVIG alone among our patients with primary exposure resulted in a CMV disease rate of 83.3 %, whereas a combination of IVIG and high-dose acyclovir in the second group significantly reduced the CMV disease rate to 26.3 %.

More recently, a short course of intravenous ganciclovir followed by oral acyclovir has been employed in the prophylaxis of CMV [8, 13]. Although initial results are promising, the need for i. v. administration of ganciclovir, its potential bone marrow suppression, and the risk of developing resistant strains [7] may limit its appeal for prophylaxis.

Use of seronegative donor organs does not insure an acceptable risk. Although throughout our study period we used a single protocol for serologic testing and screening of blood products, the rate of CMV disease among seronegative recipients of seronegative organs in our control group was relatively high (25 %).

It is possible that in patients with low CMV titers who are considered seronegative, immunosuppression may enhance viral replication. In fact, it has been recognized that polymerase chain reaction studies, which enable detection of DNA, may identify viral sequences in the blood of patients shown to be seronegative by ELISA [3]. Alternatively, transmission from donors with low CMV titers may occur via the allograft. Our prophylaxis regimen, in which all seronegative recipients were given IVIG in addition to acyclovir, significantly lowered the CMV disease rate to 3.8 % in these patients.

An association between CMV infection and the use of augmented immunosuppression for rejection, primarily OKT3, has been reported [12, 16, 21]. It has been suggested that intense suppression of the immune response during rejection permits reactivation of the virus. When a combination of high-dose acyclovir and IVIG was begun only upon initiation of OKT3 therapy, the incidence of CMV infection was not changed [26], whereas our prophylaxis protocol diminished the risk of CMV disease associated with OKT3 treatment for rejection. In accordance with previous studies, we found that OKT3 induction is not associated with an increased risk of CMV infection [14].

In summary, our retrospective review of protocols to prevent CMV disease in two noncontemporaneous patient populations shows that high-dose oral acyclovir combined with immune globulin effectively reduced the incidence of CMV disease among liver recipients at high risk, namely, those with primary exposure. This protocol was also associated with a reduction in the rate of CMV disease in patients treated for rejection.

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