Journal of Medical Virology 24:423–434 (1988)

Occurrence of Cytomegalovirus Hepatitis in Liver Transplant Patients

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The differential diagnosis of liver dysfunction after orthotopic liver transplantation can be difficult. Cytomegalovirus (CMV) hepatitis is one possibility. This report reviews our experience with 17 cases of pathologically proven CMV hepatitis following liver transplantation and demonstrates the need for percutaneous liver biopsies to establish the diagnosis. There were seven pediatric patients (ages 2–11 years, five males, two females) and ten adult patients (ages 17–53 years, eight males, two females). The most common symptoms were prolonged fever (15 patients, with a mean duration of 22 ± 5.5 days), elevation in total bilirubin (14 patients), and elevation in liver enzymes (15 patients); all symptoms were also found in rejection. Leukopenia and thrombocytopenia, reported to frequently occur with CMV infection, were found in only three and five patients, respectively.

Twelve patients with the above symptoms underwent percutaneous biopsy on one or more occasions to differentiate CMV hepatitis from rejection. The diagnosis was made at retransplantation in five patients. CMV hepatitis followed treatment for acute rejection in 14 patients and occurred without additional immunosuppression in three patients. All patients were maintained on cyclosporine and prednisone. Acute rejection episodes were treated with a 5-day tapering dose of steroids (17 courses in 12 patients), OKT3 monoclonal antibody [Ortho (4 patients)] antithymocyte globulin [Upjohn (2 patients)], and azathioprine (1 patient).

CMV was isolated from urine (nine patients), blood (nine patients), throat (seven patients), lungs (two patients), and other organs (two patients). CMV was cultured

Accepted for publication July 7, 1987.

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from the liver biopsy specimens in five of the seven attempts in pediatric patients. When the diagnosis was confirmed in the absence of rejection, immunosuppression was routinely lowered. When rejection occurred concomitantly with CMV hepatitis, therapy had to be individualized. Retrospectively, three patients treated for rejection were noted at retransplantation to have only CMV hepatitis, and all three patients died.

A high index of suspicion and the judicious use of liver biopsies is essential in order to differentiate CMV hepatitis from other causes of posttransplant liver dysfunction.

Key words: liver dysfunction, hepatitis, cytomegalovirus, liver transplant, percutaneous biopsy

INTRODUCTION

The differentiation of liver dysfunction after orthotopic liver transplantation can be difficult. Possibilities include rejection, ischemic harvest injury, vascular thrombosis, bile duct complications, hemolysis, and hepatitis [Starzl et al, 1982; Esquivel et al, 1985]. The treatment is different for each possibility, and therefore there is a critical need to establish the correct diagnosis. One entity that has not received enough attention as both the cause of graft dysfunction, and significant morbidity and mortality is CMV hepatitis. To our knowledge, this is the first report to characterize and describe a series of patients with pathologically proven CMV hepatitis following orthotopic liver transplantation. On the basis of this review, recommendations for the diagnosis and management of CMV hepatitis during liver transplantation are presented.

MATERIALS AND METHODS

Between March, 1980 and September, 1985, after the advent of combined immunosuppression with cyclosporine and prednisone, 553 orthotopic liver transplants in 429 patients were performed at the University of Pittsburgh, with the complete approval of the University's Institutional Review Board.

More specific and aggressive management of rejection has resulted in improved patient and graft survival in an ever increasing patient population. One of the most useful adjuncts contributing to this improvement has been the evolution of a liberal policy of percutaneous liver biopsies. Biopsies are now routinely performed whenever there is clinical suspicion of acute rejection, and to help evaluate fever and biochemical abnormalities when a diagnosis is not clear.

The pathologic specimens from all patients undergoing orthotopic liver transplantation since 1980 were reviewed. Only those patients with pathologically proven CMV hepatitis were included in this analysis.

Patients

Seventeen patients, 13 males and 4 females, ranging in age from 2 years to 53 years, were identified. There were seven pediatric patients and ten adult patients. Eight of the patients had more than one transplant. Table I lists chronologically all of the patients diagnosed as having CMV hepatitis. A wide spectrum of pretransplant pathologic diagnoses existed in this group of patients, but the preoperative diagnosis was not prognostically significant (biliary atresia—four; primary biliary cirrhosis—two; alpha-1-antitrypsin deficiency—two; sclerosing cholangitis—two; idiopathic cirrhosis—two;

TABLE I. Patients With Pathologically Proven Diagnosis of Cytomegalovirus (CMV) Hepatitis*

OLTx	Sex	Age (years)	No. of Tx	Method of diagnosis and pathologic findings	Infestation viral inclusions/mm²	No. of rejection episodes	Treatment of rejection	Management of CMV hepatitis	Survival
203	M	19	3	ReTx/CMV-Isch		1	1 steroid recycle	ReTx	Died
213	M	2	1	Bx/CMV-Rej	0.9	1	1 steroid recycle	CyA, Pred.	Alive
257	M	21	2	ReTx/Isch-CMV	0.5	1	1 steroid recycle	ReTx	Died
291	M	45	2	ReTx/Isch-Rej-CMV	0.1	2	1 steroid recycle, azathioprine	ReTx	Died
292	M	4½	1	Bx/CMV-Rej CMV-Rej	5.5 7.5	1	1 steroid recycle	CyA, Pred.	Died
310	M	26	3	Bx/CMV-Infarct ReTx/Infarct-CMV-Rej	2.5 1.3	3	3 steroid recycles	ReTx	Alive
334	M	33	3	Bx/CMV-Rej ReTx/CMV-Rej	2.4 1.8	1	1 steroid recycle	None	Died after 2 yr
348	M	28	2	ReTx/Isch-CMV-Rej	4.1	3	2 steroid recycles, ATG	ReTx	Died
390	F	53	2	Bx/Isch-CMV-Rej	0.3	4	3 steroid recycles, ATG	CyA, Pred.	Alive
393	M	26	1	Bx/CMV	0.3	1	1 steroid recycle	CyA, Pred.	Alive
394	M	7	3	Bx/CMV-Rej	0.4	2	1 steroid recycle, OKT3	None	Alive
454	M	6	1	Bx/CMV	0.2	1	1 steroid recycle	Withdraw CyA, Pred.	Died (of hepatoma)
472	F	7	1	Bx/CMV-Rej	10	2	1 steroid recycle, OKT3	CyA, Pred.	Alive
480	F	2½	1	Bx/CMV-Rej CMV-Rej	1 23	0	None	CyA, Pred.	Alive
481	M	34	1	Bx/CMV-Rej CMV-Rej	0.5 2	1	OKT3	None	Died
514	M	11	1	Bx/CMV-Rej and rejection	4	0	None	Recycled for rejection	Alive
556	F	17	1	Bx/CMV		0	None	Pred.	Alive

*Tx, transplants; ReTx, retransplant; CyA, cyclosporine; OLTx, orthotopic liver transplantation; Bx, biopsy; Rej, rejection; Pred., prednisone; Isch, ischemic injury.

Clinical specimens (throat, urine, buffy coat, biopsy, or autopsy tissue) were inoculated into tube cultures of human foreskin fibroblasts (F7000, Flow Laboratories, McLean, VA) for isolation of CMV as previously described [Dummer et al, 1983]. These specimens are routinely cultured for CMV in all patients every 2 weeks during hospitalization.

RESULTS

Evaluation of Serology and Culture of CMV Infections (Table II)

CMV infection is diagnosed by serologic changes and/or isolation of the virus. Primary CMV infections are diagnosed by seroconversion. Eight of the patients in this series had primary CMV infection. Three patients (OLTx #310, 472, 480) were seronegative prior to transplant and seroconverted after transplantation; pretransplant serology was not available on one patient (OLTx #481), but he was seronegative posttransplant and later seroconverted. Of the remaining four patients, all were seronegative either pre- or posttransplants; later serum samples were not available to confirm seroconversion.

Reactivation infection is diagnosed by a four-fold or greater rise in antibody titer. One patient had reactivation infection confirmed by a diagnostic rise. Five patients had "probable" reactivation infection, as indicated by seropositivity prior to and/or posttransplant, although a diagnostic rise could not be demonstrated because samples were not available. In three patients, the presence of primary or reactivated CMV infection could not be determined due to a lack of appropriate serum samples.

CMV was isolated from one or more specimens (i.e., blood, urine, throat) in 15 of the 17 patients. The associated bacterial and fungal infections found in these patients are also listed in Table II.

Evaluation of Clinical Signs and Symptoms

Table III summarizes the fever and hepatic function profiles for these patients. Fifteen of 17 patients had fever for more than 5 days, with a range of 5–68 days and a mean of 22 ± 5.5 days. One patient was afebrile throughout the course of the disease, and one patient had fever for a single day. The highest temperatures ranged from 38.5°C to 40.5°C, and in some patients, fever persisted after the resolution of biochemical abnormalities.

Bilirubin was elevated in 14 of the 17 patients. When the diagnosis was established at retransplantation, bilirubin was substantially higher than when the diagnosis was established by biopsy. The highest values of 44 mg/dl and 20.4 mg/dl occurred in patients undergoing retransplantation with simultaneous CMV hepatitis and ischemic injury.

SGOT was elevated in 16 of 17 patients at diagnosis and continued to rise after diagnosis in 8 patients. Most elevations were in the 100–300 IU range, but in three patients, values were greater than 700 IU. SGPT was elevated in 16 of 17 patients and continued to rise in 6 patients after the diagnosis was established. Most values were in the 50–200 IU range, and five values were greater than 400 IU. It is of that note only three patients developed leukopenia (white blood cell count of less than 3,000), and only five patients developed thrombocytopenia (platelet count less than 100,000).

Evaluation of Management

The management of CMV hepatitis has been altered by our increasing use of percutaneous liver biopsies. In our series of 17 patients, 5 of the first 8 patients having

TABLE III. Clinical Signs and Symptoms of Cytomegalovirus Hepatitis*

OLTx no.	Fever		Bilirubin (mg/dl)		SGOT (I.U.)		SGPT (I.U.)	
	Duration (days)	Highest (°C)	On Bx date	Highest	On Bx date	Highest	On Bx date	Highest
203	35	38.5	4.4	4.4	157	157	49	49
213	22	39.4	4.9	4.9	282	282	486	486
257	20	38.5	20.4	30	188	441	263	802
291	17	39.8	—	—	—	—	—	—
292	20	39.3	1.7	1.7	27	321	131	131
310	35	39.2	4.6	4.6	1103	1103	470	470
334	22	39	6.2	12.4	274	759	333	405
348	5	39	9.0	9.0	266	443	111	292
390	7	39	8.6	27.2	121	423	89	89
393	Afebrile		2.7	4.6	203	203	140	140
394	68	39.5	0.7	1.3	160	185	67	76
454	11	39	0.1	0.7	58	58	40	40
472	25	40.5	2.7	5.3	745	745	588	588
480	24	39	0.9	16.8	72	746	58	332
481	1	38.8	2.5	5.4	78	153	63	112
514	23	40	0.7	0.7	163	163	159	159
556	14	39.5	2.1	2.3	72	85	56	69

*OLTx no., orthotopic liver transplant; Bx, biopsy.

CMV hepatitis were only diagnosed at the time of retransplantation; therefore only 3 of these first 8 patients were diagnosed by biopsy. However, all of the last nine patients to be diagnosed as having CMV hepatitis were diagnosed by percutaneous biopsy. The use of percutaneous biopsy significantly facilitates the establishment of the diagnosis of CMV hepatitis and favorably influenced patient survival in this series. Only 4 of the 12 patients diagnosed by biopsy have died, whereas 4 of 5 patients who were unexpectedly diagnosed at retransplantation have died.

When the diagnosis of CMV hepatitis was established by biopsy and no rejection was present, the management was the reduction of cyclosporine and/or prednisone (eight patients). In one other such patient, there was no reduction in maintenance immunosuppression. In most patients, cyclosporine was lowered in order to achieve RIA levels of 300–400 ng/per dl. Maintenance prednisone doses were reduced to 5, 10, or 15 mg/day.

Difficulty in patient management arose when a biopsy revealed concurrent CMV hepatitis and rejection. This occurred in four patients (OLTx 291, 394, 481, and 514), and it required careful individualization of management. It was always necessary to treat the rejection component, and it was not uncommon to find worsening of the CMV hepatitis after therapy for rejection. Following resolution of rejection, immunosuppression had to be closely monitored and conceivably lowered to allow resolution of the CMV hepatitis.

The worst outcome was evident in a group of patients who underwent retransplantation with the diagnosis of endstage rejection but who in fact had CMV hepatitis with no rejection at the time of retransplantation. All three of these patients (OLTx 203, 257, 348) ultimately expired.

Three patients (OLTx 480, 514, and 556) were recognized as having CMV hepatitis prior to receiving therapy for acute rejection. Therefore, 3 of 17 patients developed CMV hepatitis with no treatment for rejection, and 14 of 17 patients had from one to four courses of treatment for acute rejection prior to developing CMV hepatitis. These are summarized in Table I.

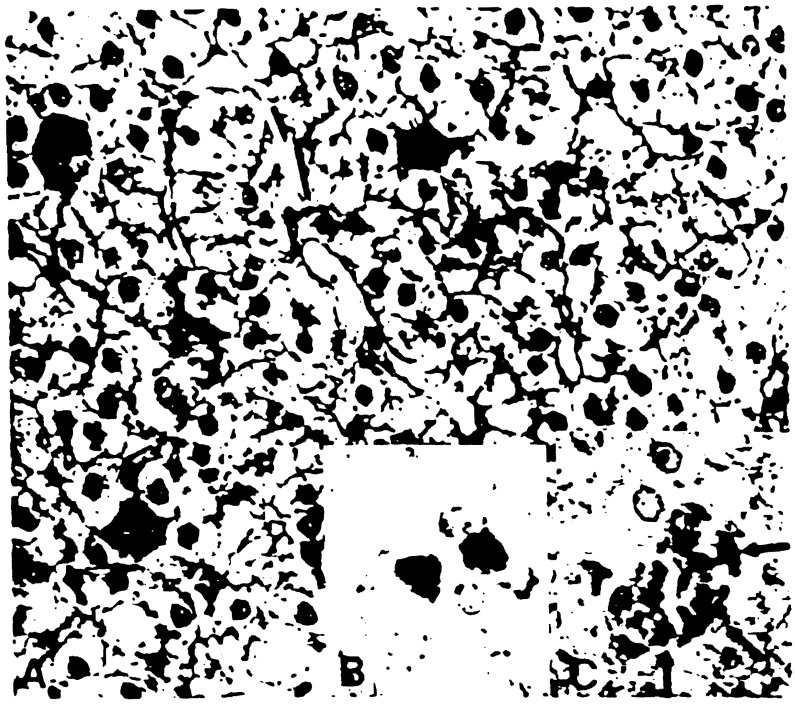

Fig. 1. A) Virally transformed cells are large, with nuclear inclusions noted in some sections, but cytoplasmic changes are visible (arrow) even where nuclear inclusions are not evident (H/E ×198). B) Immunoperoxidase staining using anti-CMV antibody reveals the presence of cytoplasmic antigen, the nuclear inclusion being unstained (DAB ×257). C) CMV antigen can be detected in degenerating cell fragments engulfed by neutrophils, seen here as dark precipitate (arrows) (DAB ×265).

Evaluation of Biopsies and Differentiation from Rejection (Table I)

Clusters of neutrophils, often forming a microabscess within the lobule, were a clue to the presence of CMV, and their presence sparked a search for the virus. Virus was found almost entirely within hepatocytes, more rarely in Kupfer cells, endothelial cells, or biliary epithelium. In adults, where concomitant ischemic changes were more common, virus was noted to be in periportal hepatocytes and Kupfer cells as well as in biliary epithelial cells. Rapidly dividing cells in granulation tissue around abscesses or at anastomotic lines appeared to be particularly vulnerable. These cells were often heavily involved, while surrounding tissues were devoid of CMV. Neutrophil aggregates were less prominent in adults, in whom mononuclear cells were more conspicuous.

Infestation varied markedly, from 0.2 to 23 infected cells per mm^2 of tissue. Hematoxylin-eosin proved as sensitive for the diagnosis as anti-CMV antibody. Staining of viral antigen using the anti-CMV antibody demonstrated cytoplasmic virus only in those cells displaying cytopathic effect. In no instance was unsuspected infection demonstrated by use of the antibody alone. However, the neutrophil clusters often surrounded cell debris in which CMV antigen was detectable, even through no inclusions were noted (Fig. 1).

The presence and severity of other concomitant processes could be assessed independently of the CMV hepatitis. Inflammation in the hepatitis was limited to neutrophils and mononuclear cells in close contact with virally infected cells. Rejection could be independently evaluated and was absent in five patients and present with varying

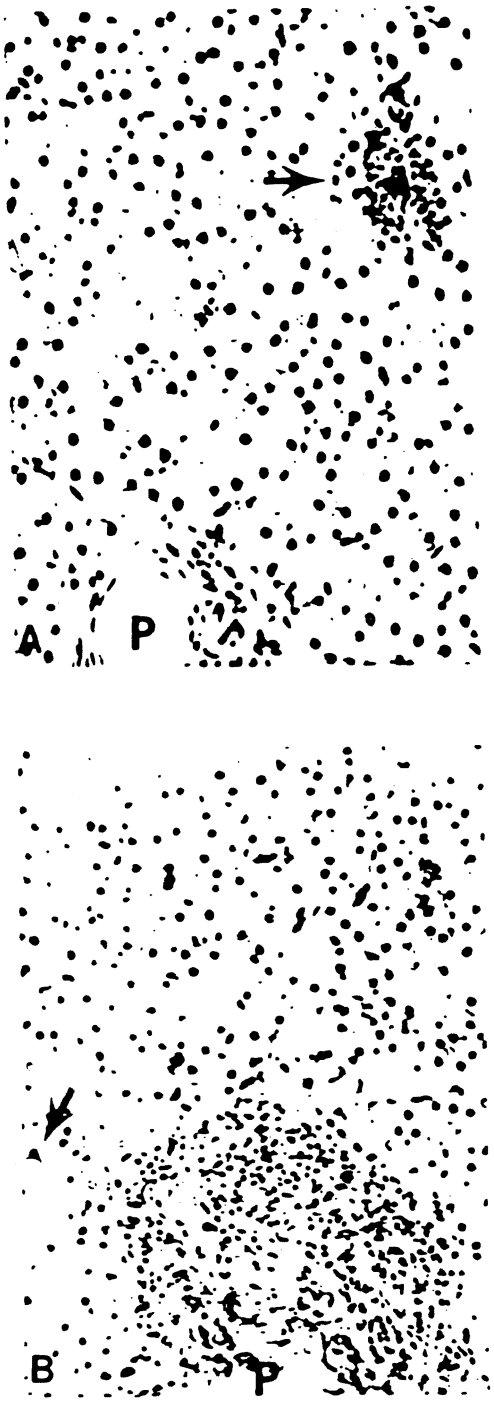

Fig. 2. CMV is independent of the amount of rejection. A) The portal area (P) shows complete absence of inflammatory cells. A lobular microabscess surrounds a virally infected cell (arrow) (H/E ×120). B) Expanded and infiltrated portal area (P) characteristic of rejection is present in this biopsy, in which virally transformed cells are visible in the lobule (arrow) (H/E ×114).

degrees of severity in 12. There was no relationship between the extent of the CMV infestation and the severity of the rejection changes (Fig. 2).

DISCUSSION

Although CMV hepatitis has been well recognized in both the normal and the immunocompromised host, this is the first report to describe this entity as a recurrent problem in orthotopic liver transplantation. While hepatitis is common in CMV infections, it is usually not severe, even in other types of transplant patients [Ten Napel et al, 1984]. Unique concerns arise when CMV infection associated with abnormalities in bilirubin and liver enzymes occur following liver transplantation. The differential of liver dysfunction after orthotopic liver transplantation includes rejection, ischemic injury, vascular thrombosis, bile duct complications, hemolysis, and hepatitis.

CMV hepatitis arises relatively late after liver transplantation (15–132 days, with a mean of 44 ± 6 days) and virtually always is accompanied by prolonged fever (15 of 17 patients, with a mean duration of fever of 22 ± 5.5 days). Practically, this means that the critical differentiation is between hepatitis and rejection. The quickest and most definitive means of discriminating between these two entities is a percutaneous liver biopsy. We established the diagnosis of CMV hepatitis in the last nine patients by percutaneous biopsy, while we established the diagnosis at retransplantation in five of the first eight patients. This difference arose in our series because we have liberalized our policy concerning percutaneous biopsies in the last 1 to 2 years. When the diagnosis was first established at retransplantation, four of the five patients diagnosed in this manner died, while only 4 of 12 patients diagnosed on percutaneous biopsy have died. Other reports have attempted to document CMV hepatitis in immunocompromised patients, such as kidney, heart, heart-lung, and bone marrow transplant recipients [Dummer et al, 1983; Ho 1983]. But few of these reports have histopathologic confirmation of the diagnosis of CMV hepatitis, whereas the histologic confirmation of this diagnosis was obtained in all of the patients in this series. In addition to histologic confirmation, the CMV virus was cultured in five pediatric and one adult liver specimens. CMV was isolated on culture of other sites in 15 of the 17 patients. The timing of these isolations, however, was not always clinically useful, as viral cultures can take 2 to 3 weeks, and frequently the cultures were not positive until after we had established the diagnosis on biopsy or retransplant. Infection is frequently asymptomatic [Ho et al, 1983]. Hence these cultures are insufficient evidence for the diagnosis of CMV hepatitis.

Interestingly, patients with both primary and reactivation infections developed CMV hepatitis, and the patients' pretransplant serologic status was not prognostically significant. Eight patients with CMV hepatitis had primary CMV infection, and in six patients, the hepatitis followed CMV reactivation. We were unable to interpret the serologic data in three patients. The rate of primary CMV infections among liver transplant recipients is reported to be 28% [Ho et al, 1983]. As 57% of our patients developed hepatitis following a primary CMV infection, this suggests that patients with a primary infection are at greater risk for developing hepatitis. In eight patients with primary CMV infection, there were three deaths, and five patients survived. In the six patients with reactivated disease, there were three deaths and three survivors. Also, the site of CMV isolation, namely in the blood, has been reported to be an indicator of more severe or life-threatening infection [Armstrong et al, 1971; Pass et al, 1980]. This did not prove to be the case in the analysis of the present patient group.

The management of these patients has evolved as we have come to be more aware of the entity. In at least three cases, patients underwent retransplantation for an incorrect diagnosis. The liver function abnormalities were thought to represent endstage rejection in these patients, whereas the pathology demonstrated the absence of rejection and the presence of CMV hepatitis. In most instances, CMV hepatitis is reversible with the reduction of immunosuppression.

The diagnosis of CMV hepatitis was made when a single viral inclusion was identified. Infestation was seen to increase markedly in some instances when concomitant rejection was aggressively treated. Because the inflammatory response provoked by the CMV was localized to the infected cells, there was very little overlap with other simultaneous processes. In particular, the portal inflammatory profile characteristic of rejection was not provoked by the CMV hepatitis alone. Severe hepatitis with heavy infestation was noted without portal infiltrate of any degree, even though it must be recognized that antirejection therapy can alter the cellular profile of the rejection response.

Starr et al [1984] have shown that the natural killing against CMV-infected target cells was depressed in kidney recipients for 2 years after transplantation but that a reduction in immunosuppression in these patients resulted in temporal association with resolution of CMV disease. In addition, CMV is felt to be an immunosuppressive agent on its own, and therefore, if full-maintenance immunosuppression is maintained during the course of the disease, the patient may in fact be over-immunosuppressed and susceptible to additional bacterial or opportunistic infections.

At present, when a diagnosis of CMV hepatitis is established in a liver transplant recipient in whom there is no evidence of rejection, our management is to lower the doses of cyclosporine and/or prednisone. With the confirmation of CMV hepatitis, the therapeutic levels of cyclosporine of 700–1,000 ng/dl are usually lowered to 300–400 ng/dl. When there is clinical resolution of the hepatitis, cyclosporine is then returned to therapeutic levels. Prednisone is usually lowered from maintenance at 20 mg/day to 5, 10, or 15 mg/day depending on the clinical severity of the disease (in children, the dose is frequently lowered even further). A problem arises in the management of patients who have concomitant CMV infection and rejection, as occurred in four patients. In this group of patients, our initial concern is directed toward the treatment of rejection. However, as we treat rejection, it is not uncommon that the CMV hepatitis worsens, and after resolution of rejection, we frequently have to deal with the CMV hepatitis. This may require the reduction in the maintenance levels of immunosuppression until the CMV disease has resolved; full-maintenance immunosuppression is then resumed.

CMV hepatitis after orthotopic liver transplantation is a definite clinical entity with specific signs and symptoms, serologic and culture findings, and pathologic features; it is being recognized with increased frequency in both adult and pediatric patients. The differentiation of CMV hepatitis from other causes of liver dysfunction is essential in order to institute appropriate management. Currently it is best achieved by a high index of suspicion and the judicious use of liver biopsies.

We have, for the past two years used a monoclonal antibody to early intermediate CMV antigen (Chemicon, El Segundo, CA), which can, after trypsin digestion, demonstrate nuclear antigen before infected cells are morphologically transferred. It is thus possible to make the diagnosis of CMV on biopsy of an apparently uninvolved specimen. This has not changed any of the conclusions presented in this paper.

ACKNOWLEDGMENTS

This work was supported by Research Grants from the Veterans Administration and Project Grant No. AM-29961 from the National Institutes of Health, Bethesda, Maryland. Leonard Makowka is the recipient of a Centennial Fellowship from the Medical Research Council of Canada.

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