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Acute murine cytomegalovirus infection: a model for determining antiviral activity against CMV induced hepatitis

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Abstract

Acute intraperitoneal infection of weanling BALB/c mice with murine cytomegalovirus (MCMV) resulted in an inoculum titer-dependent weight loss, mortality and elevation of plasma transaminases (ALT: alanine transaminase and AST: aspartate transaminase). Three days post infection (p.i.) with $10^{4.85}$ plaque forming units (pfu) there was 90% mortality with a mean death day p.i. of 4.1 ± 0.2. Plasma levels of ALT and AST were elevated 24- and 15-fold, respectively. Organ titers of virus ($\log_{10}$ pfu/g tissue) were 6.16 in the liver, 6.05 in the spleen, 4.0–4.7 in the lung, heart, kidney and intestine and undetectable in the muscle and brain. Organ concentrations (units/g wet-weight) of ALT were highest in the liver, whilst for AST the highest levels were found in the heart. The concentrations of ALT but not AST were reduced (35–55%) in the infected liver; the concentrations of ALT and AST were not changed in other infected organs. There were excellent correlations ($r > 0.95$) between viral titers in the liver, increases of plasma ALT and depletion of liver ALT. HPMPC and ganciclovir administered either p.o. or s.c. reduced mortality, increases in plasma transaminases and viral burdens in the liver and prevented depletion of liver ALT. HPMPC was $\sim$10-fold more potent than ganciclovir. These results strongly suggest that intraperitoneal infection of the BALB/c mouse with MCMV represents an animal model of CMV hepatitis that can be monitored by measuring plasma ALT.

Keywords: Mouse; Cytomegalovirus; Hepatitis; Transaminase

1. Introduction

Cytomegalovirus (CMV) infection is a serious cause of disease and death in neonates and in adult patients immunocompromised as a consequence of either bone marrow transplant, organ transplant followed by immunosuppressive therapy or AIDS (Betts, 1997). Mild CMV infection and rarely, severe CMV infection, have also been observed in immunocompetent individuals (Eddleston et al., 1997; Laing et al., 1997; Leman et al., 1997; Miguelez et al., 1998). CMV infects a wide variety of tissues and manifests in wide

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spectrum disease pathology including pneumonitis, colitis, and retinitis. Hepatitis and infection of the hepatobilliary tract is often observed following solid organ transplantation and is the most severe disease occurring after liver transplantation. CMV hepatitis and acalculous cholecystitis have also been noted in AIDS patients (Drew, 1988; Pollard, 1988; Betts, 1997), while rare severe CMV disease in immunocompetent hosts has been associated with pulmonary and hepatic dysfunction (Eddleston et al., 1997; Miguelez et al., 1998). Whilst CMV infections in these disease states may include many organ systems, the pathogenesis of CMV-induced liver injury is still largely undefined. Approved therapies effective in the control of CMV infection include the nucleoside ganciclovir (Cytovene®), and foscarnet (Foscavir®) (Van der Meer, 1996; Noble and Faulds, 1998) and most recently the nucleotide analogue HPMPC (Vistide®) (Lea and Bryson, 1996).

CMV displays considerable species tropism and various animal models (Staczek, 1990) have been developed in order to investigate CMV disease pathology and responses to antiviral therapy. Both lethal and sublethal acute intraperitoneal (i.p.) infection of the BALB/c mouse with murine cytomegalovirus (MCMV) have been reported to produce disseminated infection and multiple organ involvement (Staczek, 1990; Barnard et al., 1993; Shanley et al., 1993). In particular, MCMV-induced hepatitis, indicated by the elevation of the serological markers alanine transaminase (ALT) and aspartate transaminase (AST), has been implicated as one cause of mortality in the MCMV infected mouse (Shanley et al., 1993). Following i.p. inoculation in the BALB/c mouse, MCMV demonstrates tropism for the liver (Barnard et al., 1993; Shanley et al., 1993) and MCMV-induced liver damage is associated with viral replication (Trgovcich et al., 1997). In this study we have employed the BALB/c mouse acutely infected with MCMV to examine MCMV-induced liver disease. Changes in body weight, plasma and organ ALT and AST and mortality have been measured and their relationship to viral replication in the liver in the absence and presence of therapy with HPMPC and ganciclovir (GCV) has been established.

2. Materials and methods

2.1. Materials

2.1.1. Drugs and chemicals

Both HPMPC and GCV were synthesized in-house according to literature methods. The diagnostic kit for analysis of plasma ALT and AST was obtained from the Sigma, (Procedure # 505, Sigma Diagnostics, a Division of Sigma, Oakville, Ont.). All other reagents were of the highest purity possible and were obtained from standard commercial sources.

2.1.2. Animals and cells

For all studies, 4-week-old female BALB/c (28-days-old, 12–14 g; Charles River, St. Constant, Qué) were used. They were housed under a 12/12 h light–dark cycle and allowed free access to food and water. Mouse embryo fibroblast cells were isolated from the 18-day-old embryos of pregnant Swiss Webster mice according to the procedure of Selgrade and Osborn (1973).

2.2. Methods

2.2.1. Virus and inoculation

MCMV obtained from homogenates of the salivary glands of Nu/Nu mice infected with cell culture virus (Smith strain, American Type Culture Collection) was used in all experiments. BALB/c mice were infected by i.p. injection of 200 μl of a viral inoculum suspended in D-MEM.

2.2.2. Measurement of disease and drug treatment

Mice were weighed daily following infection and monitored for signs of disease and mortality for a period of up to 14 days. When drug therapy was administered, treatment was initiated 3 h post inoculation. GCV was administered subcutaneously (s.c.) once daily for 3 days in a vehicle of phosphate buffered saline. HPMPC was administered s.c. as a single dose in the same vehicle. Both GVC and HPMPC were administered orally (p.o.) three times daily for 3 days in a vehicle of distilled water. The effects of drugs on changes in plasma and organ transaminase levels and organ titers of virus following infection with MCMV

were measured in groups of eight mice. The effect of drugs on mortality induced by lethal infection with MCMV was investigated using groups of 12 mice.

2.2.3. Measurement of plasma and tissue transaminases

The preparation of plasma for measurement of transaminase enzymes was done as follows. On day 3 post-inoculation (or as specified in figure and table legends) mice were anesthetized with halothane, blood was collected by cardiac puncture into heparinized syringes and put on ice. In some experiments, blood was collected by tail clipping and isolating from the tail artery into heparinized capillary tubes. The plasma was separated from whole blood by centrifugation at $3500 \times g$ for 15 min. Plasma samples were kept frozen at -80°C until analysis for transaminase enzymes. Depending on the anticipated levels of transaminase enzymes, thawed plasma samples were diluted from 5–10-fold with physiologic saline prior to enzyme assay. The levels of ALT and AST in mouse plasma were measured using a diagnostic kit available from Sigma. The assay volume of the diagnostic kit was reduced by 12-fold such that the assay sample volume of plasma (either undiluted or diluted) was 16 μl. Enzyme activity was expressed as Sigma Frankel (SF) units/ml plasma.

For measurement of transaminase levels in organs, the liver, spleen, kidney, intestine (ileum), lungs, heart, muscle and brain were rapidly excised and placed into 10 vol. (vol./wt. g) of ice-cold DMEM. Prior to placing the intestine in DMEM, it was placed in ice-cold saline, cut into ~ 5 cm segments and washed to remove the contents. The organs were homogenized by using two 15 s bursts of a tissue homogenizer (Virtis Instruments, Vertishear model). The homogenate was centrifuged at $3500 \times g$ for 15 min and the supernatant retained. A sample volume of 200 μl of the supernatant was mixed with 200 μl of a solution of 10% w/v bovine serum albumin (dissolved in physiologic saline) and frozen at -80°C until analyzed for transaminase levels. The inclusion of bovine serum albumin with the sample was necessary to preserve enzyme activity, especially AST that was sensitive to freezing and thawing. Upon thawing the samples were appropriately diluted with physiologic saline prior to analysis of enzyme activity. The sample dilutions were (fold): liver (50); intestine, brain and heart (10); kidney and muscle (5); spleen and lung (no dilution). Transaminase levels were measured in 16 μl samples as described above for the plasma samples. Enzyme activity was expressed as SF units/g w.w. of tissue.

2.2.4. Measurement of organ titers of MCMV

Organs were homogenized in DMEM (10% w/v) and virus was titered on mouse embryo fibroblast cells (Nedrud et al., 1979) using the limiting viral dilution technique. The limit of detection was 10^3 pfu/g w.w. of tissue.

2.2.5. Statistics

Group comparisons were checked for significance employing, where appropriate, either a two-tailed unpaired Student's t-test or ANOVA followed by a SNK post-hoc analysis and accepting the $P < 0.05$ level of significance. ED_{50} values were calculated by nonlinear regression (BMDP Statistical Software, Los Angeles, CA).

3. Results

3.1. Infection of BALB/c mice with MCMV

Infection of mice with MCMV via the intraperitoneal route resulted in a general malaise (marked by lethargy and piloerection) commencing 2 days p.i. and an inoculum titer-dependent weight loss (Fig. 1). Mortality was observed at inoculum titers ranging from $10^{3.95}$ to $10^{4.85}$ pfu with the mean death day ranging from 4.4 to 6.8 days. At an inoculum of $10^{3.65}$ pfu there was a resolution of disease between 10 and 14 days characterized by weight gain similar to non infected controls (Fig. 1).

A number of organs were assessed for titers of MCMV 3 days following infection with $10^{4.85}$ pfu. Organ burdens of virus were highest in the spleen and liver ($\sim 10^6$ pfu/g w.w.) and approx. 1–2

orders of magnitude lower in the lung, heart, kidney and intestine. Virus was not detected in the muscle and brain (Table 1). The inoculum dependence of organ titers of virus was investigated in

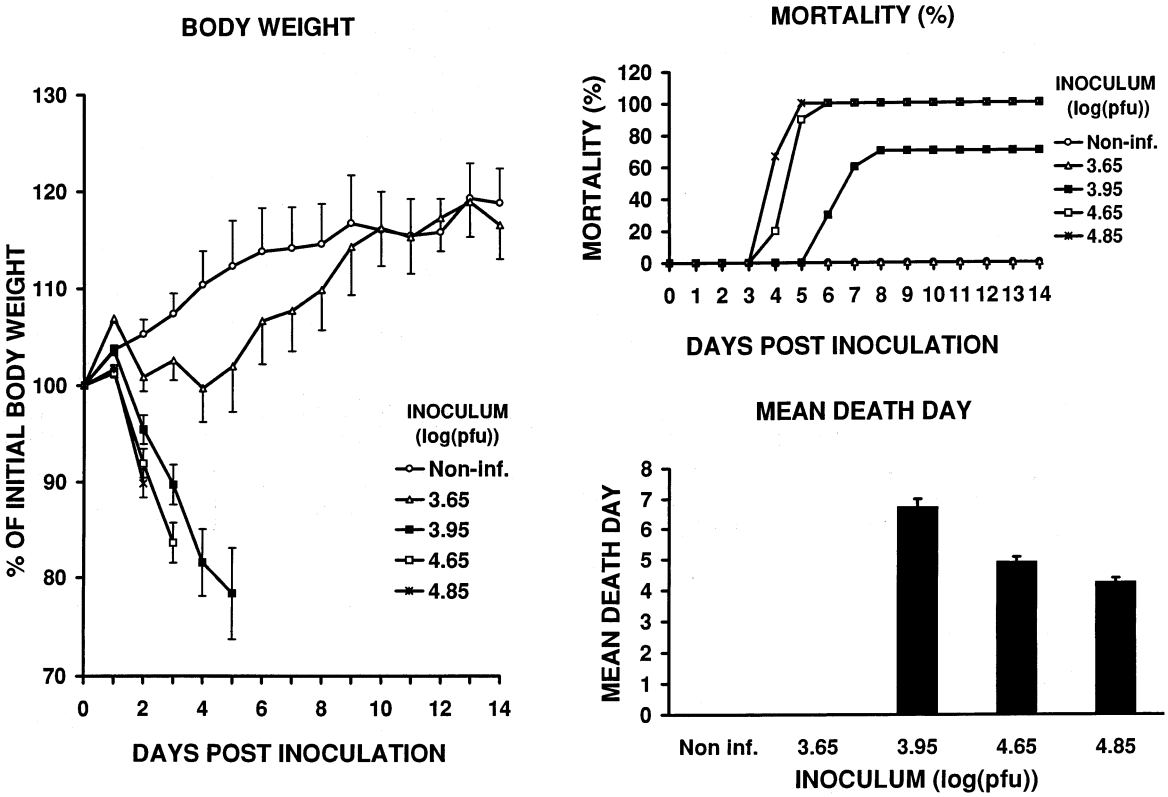

Fig. 1. MCMV infection in mice. Mice were either sham infected (non-inf.) or infected with different inoculums of virus i.p. Changes in body weight and mortality were measured over a period of 14 days. Each experimental group contained 12 mice. The data is presented ± SEM.

Table 1
Organ titers ($\log_{10}$ pfu/g w.w.) of virus 3 days p.i.[a]

Organ	Inoculum (pfu)			
	$10^{3.65}$	$9.0 \times 10^{3.95}$	$10^{4.65}$	$10^{4.85}$
Liver	4.92 ± 0.12	5.82 ± 0.14	6.12 ± 0.06	6.28 ± 0.02
Spleen	6.13 ± 0.07	5.95 ± 0.16	5.62 ± 0.13	6.07 ± 0.12
Lung	2.74 ± 0.07	3.65 ± 0.12	3.90 ± 0.08	4.18 ± 0.10
Heart	n.d.	n.d.	n.d.	4.31 ± 0.15
Kidney	n.d.	n.d.	n.d.	4.70 ± 0.21
Intestine	n.d.	n.d.	n.d.	4.00 ± 0.14
Muscle	n.d.	n.d.	n.d.	Below limit of detection[b]
Brain	n.d.	n.d.	n.d.	Below limit of detection[b]

[a] Values are presented as the mean ± S.E.M. of organs obtained from eight animals; n.d. means not determined.
[b] The limit of detection for the limiting dilution viral titration assay was 1000 pfu/g w.w.

Table 2
Inoculum-dependent elevation of serological markers and liver titers of virus in infected mice[a]

Inoculum (pfu)	Plasma ALT (SF units/ml)	Plasma AST (SF units/ml)	Liver titers of virus log (pfu/g w.w.)
$10^{2.18}$	34 ± 3	91 ± 5	<3
$10^{2.48}$	38 ± 4	107 ± 10	3.58 ± 0.16
$10^{3.18}$	57 ± 5	281 ± 10	3.90 ± 0.23
$10^{3.65}$	167 ± 20[b]	322 ± 54	4.93 ± 0.09
$10^{3.95}$	351 ± 56[b]	827 ± 116[b]	5.82 ± 0.14
$10^{4.65}$	649 ± 75[b]	1151 ± 94[b]	6.12 ± 0.06
$10^{4.85}$	845 ± 124[b]	1725 ± 232[b]	6.16 ± 0.06

[a] The data is presented as the mean $\pm$ SEM of plasma samples from eight animals. The plasma levels of transaminases and viral titers in the liver were measured 3 days p.i.
[b] Significantly different from control, $P<0.05$, ANOVA followed by S.N.K.

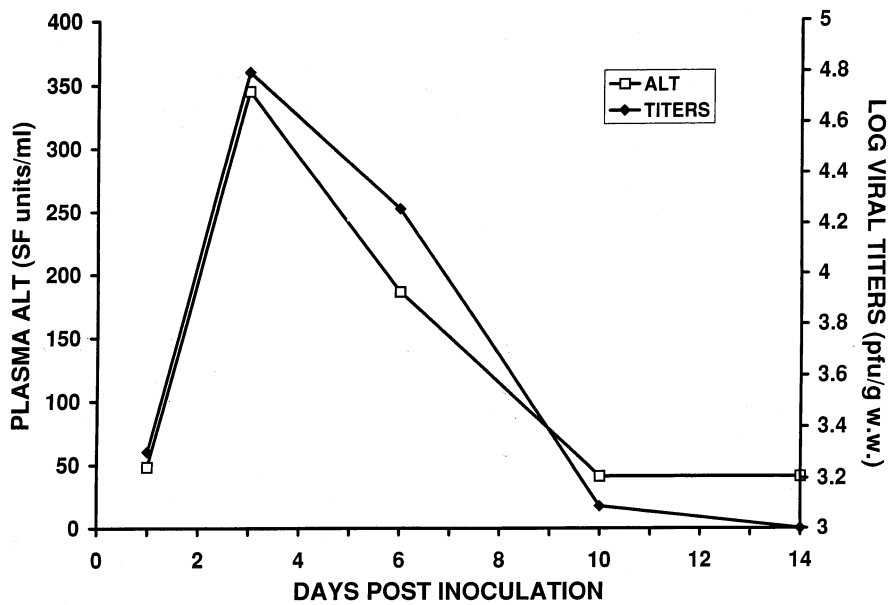

Fig. 2. Time-course for changes in plasma ALT and viral titers in the liver. Mice were either sham infected (Control) or infected with $10^{3.65}$ pfu of virus and killed at various times p.i. for the measurement of plasma ALT and viral titers in the liver. The data are presented $\pm$ SEM of six determinations.

the liver, spleen and lung. Inoculum dependent organ viral titers were observed in the liver and lungs but not in the spleen (Table 1).

3.2. Plasma and tissue transaminase levels

Infection with MCMV resulted in elevation of the plasma levels of ALT and AST (Table 2). This elevation was demonstrated to be inoculum titer-dependent (Table 2) with fold elevations compared to uninfected mice of up to 25 for ALT and 15 for AST. In a non-lethal infection paradigm (inoculum of $10^{3.65}$ pfu) the elevation of plasma transaminases was also demonstrated to be time-dependent (Fig. 2). The time and inoculum-dependent changes in plasma ALT correlated well with changes in the liver titer of MCMV during both progression and resolution of disease (Fig. 2).

Since transaminases are ubiquitously distributed amongst tissues, tissue damage due to

viral replication could cause their release contributing to the total pool of plasma transaminases. As such the effect of MCMV infection on tissue transaminase levels was determined. The distribution of ALT and AST amongst various mouse tissues is shown in Fig. 3. For ALT, the liver contained the highest concentration of this enzyme. The intestine contained approx. 5-fold less ALT with the remaining organs containing considerably less ALT. In the case of AST, the liver contained enzyme concentrations comparable to other tissues such as the kidney, brain and muscle and approximately one half of that in the heart. The concentration of AST was higher than ALT for the spleen, kidney, heart, lung, brain and muscle. However, in the liver and intestine ALT was present at higher concentrations than AST with the ratio (ALT/AST) varying from 1.5 to 2.5. When measured 3 days p.i., an inoculum titer-dependent reduction of ALT, but not AST was observed in the liver and not in other organs. The inoculum-dependent changes in the liver content of ALT correlated well with both the extent of viral replication in the liver and the elevation of plasma ALT (Fig. 4).

3.3. Effects of GCV and HPMPC on markers of MCMV infection

Administration of HPMPC and GCV reduced the severity of MCMV infection (employing an inoculum of $10^{4.85}$ pfu) in a dose-dependent manner as measured by a reduction of infection-induced mortality, elevation of plasma transaminases, viral titers in the liver and decreases of liver ALT (Tables 3 and 4). The therapeutic effects of HPMPC and GCV manifested themselves following dosing subcutaneously (s.c.) and orally (p.o.). In either case HPMPC was more potent than GCV. For HPMPC, there was a good agreement between the ED_{50} values for reduction of mortality and the elevation of plasma transaminases with somewhat higher doses required to reduce liver titers of virus (Table 4). For GCV, while there was a good agreement amongst the ED_{50} values for reduction of elevated plasma transaminases and liver titers of virus, the ED_{50} for reduction of mortality was lower (Table 4).

To evaluate whether or not the antiviral effects of HPMPC and GCV were inoculum titer-dependent, the effects of administration of each drug

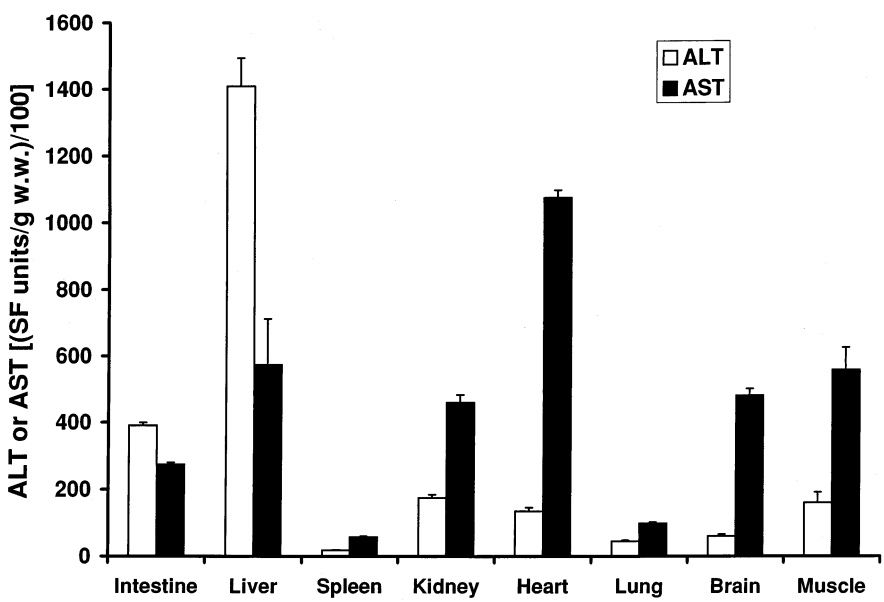

Fig. 3. Organ distribution of ALT and AST in mice. The organ distribution of ALT and AST was determined in non-infected control mice. Organs were isolated and processed (see Section 2.1) for determination of ALT and AST. The results are presented as the mean ± SEM of six determinations.

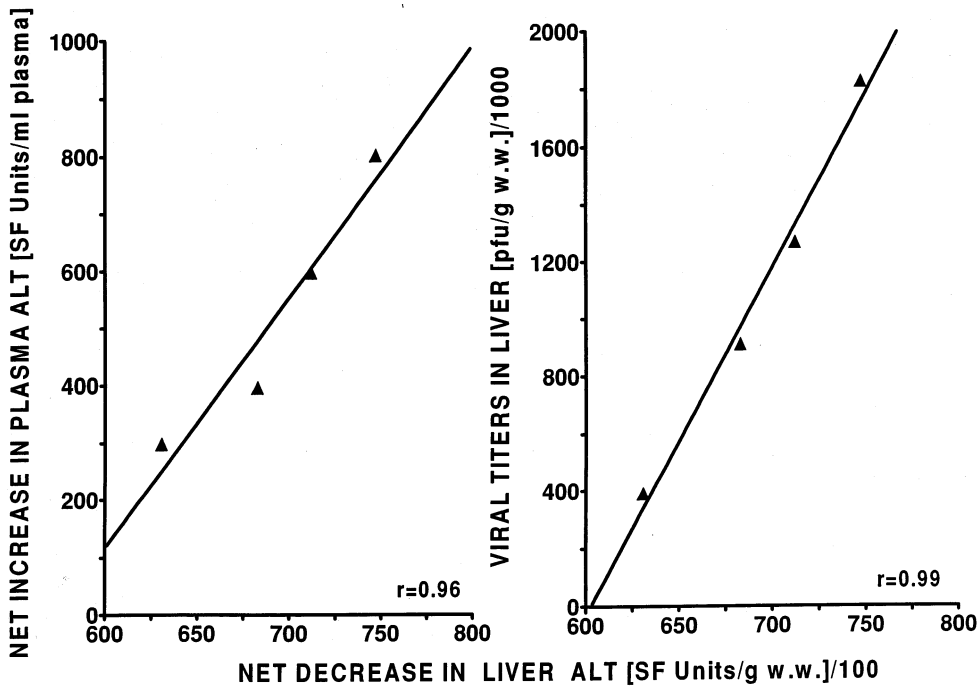

Fig. 4. Correlation between decreases in liver ALT, increases in plasma ALT and viral titers in the liver. The correlation between infection mediated decreases in liver ALT at 3 days p.i. with $10^{3.65}$, $10^{3.95}$, $10^{4.65}$ or $10^{4.84}$ pfu of virus and either plasma ALT or liver titers of virus is illustrated. Each data point represents the average data from six determinations.

p.o. was evaluated on comparable parameters of disease induced by inoculating mice with $10^{3.65}$ and $10^{4.85}$ pfu of MCMV. For each drug there was an increase in the ED_{50} for reduction of elevated plasma ALT and AST and liver titers of virus (Table 5).

4. Discussion

The use of the BALB/c mouse infected with MCMV as an animal model of CMV hepatitis and the association between disease markers such as weight loss, mortality, serological markers of hepatitis (ALT and AST) and viral replication was investigated. CMV disease manifests itself as a disseminated infection in humans, involving many organs and dependent on the state of immunocompetence of the host (Osborn, 1982; Alford and Britt, 1990; Stinski, 1990). The same is also true of MCMV (Lussier, 1975; Stinski, 1990; Staczek, 1990). The inoculum titer-dependent

Table 3
Liver concentration of ALT following infection and subsequent therapy with HPMPC and GCV[a]

Group and treatment	ALT (units/g w.w.)
Group 1	
Sham infected (vehicle)	110162 ± 5687
Infected (vehicle)	69219 ± 3647[b]
Infected (HPMPC 5 mg/kg)	115639 ± 11387
Group 2	
Sham infected (vehicle)	140918 ± 8453
Infected (vehicle)	95323 ± 2889[b]
Infected (GCV 150 mg/kg)	143460 ± 8310

[a] The results are presented as the mean ± SEM of ALT concentration of liver samples from six animals.

[b] Significantly different from sham infected, $P<0.05$ ANOVA followed by S.N.K. GCV was administered p.o. up to day 2 p.i. commencing 3 h p.i., the total daily dose administered as three separate doses at 4 h intervals. HPMPC was administered as a single s.c. dose 3 h p.i. The liver was isolated 3 days p.i. and assessed for its concentration of ALT.

Table 4
Reduction of disease parameters by HPMPC and GCV[a]

Drug	ED_{50} (mg/kg per day)			
	Mortality	ALT	AST	Log viral titers (liver)
HPMPC				
p.o.	2.7 ± 0.5	3.3 ± 0.5	8.4 ± 1.3	24.5 ± 3.8
s.c.[b]	0.3 ± 0.2	0.3 ± 0.05	0.5 ± 0.03	1.3 ± 0.5
GCV				
p.o.	34 ± 17	126 ± 35	118 ± 35	117 ± 18
s.c	3.1 ± 1.6	30 ± 7	21 ± 8	21 ± 8

[a] Mice were inoculated with $10^{4.85}$ pfu of virus and viral titers and plasma transaminases were measured 3 days p.i. The ED_{50} values are presented as the parameter estimates $\pm$ asymptotic S.E. obtained from nonlinear regression of dose-response curves containing at least five data points. Mortality was measured using the same inoculum of virus but in a separate protocol employing 12 mice. Drugs were administered up to day 2 p.i. commencing 3 h p.i. For administration of HPMPC and GCV p.o. and GCV s.c. the total daily dose was administered as three separate doses at 4 h intervals.

[b] For HPMPC s.c. drug was administered as a single dose 3 h p.i.

MCMV induced phenomenon of weight loss, mortality and development of organ burdens of virus observed in these studies are consistent with previous reports (Neyts et al., 1992; Smee et al., 1992; Shanley et al., 1993; Barnard et al., 1993; Collins et al., 1993). Three days p.i., viral titers were highest in the liver and spleen, evidence that these organs are major sites of MCMV replication, consistent with previous observations (Katzenstein et al., 1983; Trgovcich et al., 1997) and the proposal that MCMV displays a tropism for the liver (Trgovcich et al., 1997). However, viral titers in the liver and lung, but not in the spleen displayed an inoculum dependence. The lack of inoculum dependence for viral titers in the spleen, may be due to either the time point chosen for measurement of organ viral titers, the rather large extent of virally induced necrosis associated with viral replication (Mims and Gould, 1978) or that splenic macrophages serve as a major site for accumulation of virus (Katzenstein et al., 1983).

Elevated plasma transaminases have been employed, but not fully evaluated as markers of the liver pathology associated with MCMV disease (Shanley et al., 1993; Trgovcich et al., 1997). Plasma levels of both ALT and AST were elevated in an inoculum titer-dependent manner in infected mice. The presence of elevated plasma ALT and AST levels is consistent both in occurrence and magnitude with previous findings in MCMV infected mice, their elevation a consequence of hepatocellular necrosis (Barnard et al., 1993; Shanley et al., 1993). There existed a good correlation between the elevation of plasma transaminases and viral titers in the liver. However, such a correlation does not confirm that the liver is the major source of the elevated plasma transaminases, especially since MCMV is a disseminated infection and both ALT and AST are

Table 5
Inoculum dependence of the antiviral effects of GCV and HPMPC[a]

Response	Inoculum $10^{3.65}$ pfu	Inoculum $10^{4.65}$ pfu
	ED_{50} (mg/kg per day)	ED_{50} (mg/kg per day)
HPMPC		
ALT	0.9 ± 0.2	3.3 ± 0.5
AST	0.7 ± 0.1	8.4 ± 1.3
Viral titers (liver)	6.0 ± 1.3	24.5 ± 3.8
GCV		
ALT	31 ± 13	126 ± 35
AST	30 ± 12	118 ± 35
Viral titers (liver)	34 ± 11	117 ± 18

[a] The ED_{50} values are presented as the parameter estimate $\pm$ the asymptotic S.E. obtained from nonlinear regression of dose-response curves containing at least five data points. HPMPC and GCV were administered orally and according to the treatment schedule outlined in the legend to Table 4.

ubiquitously distributed amongst tissues (Bogin et al., 1996). Thus, organs that were evaluated for MCMV replication were also assessed for their concentration of ALT and AST. The liver clearly contained the highest concentration of ALT, considerably higher than any other of the organs surveyed. The extent of the decrease in the liver concentration of ALT upon MCMV infection was correlated with viral titers in the liver and the extent of the increase in plasma ALT. Such observations confirm the direct link between putative liver damage induced by MCMV infection and elevation of plasma ALT. This finding is consistent with the predominant use of ALT as a diagnostic serological marker for assessment of disease and drug induced hepatitis (Shindo et al., 1992; Abe et al., 1992; Karwinski et al., 1994; Houghton, 1996; Williams-Blangero et al., 1996). Thus, measurement of plasma ALT in the mouse by either sampling blood by cardiac puncture or by tail artery (tail clipping) represents a simple way to monitor the progress of MCMV induced hepatitis.

AST was present in a number of organs in amounts either comparable to (i.e. liver and intestine) or considerably higher than ALT. Indeed, elevations of plasma AST have been used to diagnose myocardial infarction (Reitman and Frankel, 1957; Amador et al., 1967). The lack of a significant change in the liver content of AST upon MCMV infection was surprising to us. It is possible that this is attributable to the up-regulation of AST in the infected liver or perhaps to immune cell invasion (Sissons, 1986; Trgovcich et al., 1997) which also might contribute to the liver content of AST. In support of the latter proposal, preliminary studies in immunodeficient SCID mice infected with MCMV revealed significant decreases in the liver content of both ALT and AST (data not shown). Therefore, the good relationship existing between plasma AST and titers in the liver is likely a consequence of the liver's weight, AST content and extent of viral replication relative to other organs. The possibility however, that the total plasma AST is due to contributions from other infected tissue sources and the necrosis of both hepatocytes and infiltrating immune cells in the liver cannot be ruled out.

Therapy with the effective anti-CMV drugs HPMPC and GCV further revealed the relationship between MCMV disease markers and viral replication. The superior potency of HPMPC and GCV following s.c. compared to p.o. administration is consistent with previous literature reports indicating that the poor oral availability of these drugs limits their pharmacodynamic activity (Collier and Corey, 1992; Smee et al., 1992; Lea and Bryson, 1996; Noble and Faulds, 1998) and that HPMPC is $\sim$10–40-fold more potent than GCV (Neyts et al., 1992, 1993). There was reasonable agreement between the ED_{50}'s of HPMPC for reduction of MCMV induced mortality, elevated plasma transaminases and liver viral titers. This observation supports the proposal that lethal hepatitis is the major disease associated with acute MCMV infection (Katzenstein et al., 1983; Shanley et al., 1993; Trgovcich et al., 1997). However, the results with GCV would tend to refute such a proposal as GCV was $\sim$4–10 times more potent at reducing mortality. It is known that i.p. infection with MCMV can produce severe adrenalitis and pancreatitis (Shanley and Pesanti, 1986; Shanley et al., 1993) and infect other organs such as the heart, kidney, intestine and lung. It is quite possible that GCV has antiviral activity at target organs other than the liver to prevent mortality. In the SCID mouse infected with MCMV it was noted that mortality most closely correlated with virus replication in the lung (J. Duan, personal communication).

The therapeutic effect of both HPMPC and GCV against MCMV induced hepatitis was dependent on the titer of the infecting inoculum demonstrating that increases in the extent of the initial infection require greater amounts of drug to elicit a similar therapeutic response. The good agreement between the ED_{50}'s for reduction of elevated plasma transaminases and viral titers in the liver at low and high titer inoculums further illustrates the close association between plasma transaminases and the extent of viral infection in the liver.

In summary, acute MCMV infection in the BALB/c mouse produced weight loss, mortality, and disseminated infection with a major involvement of the liver and spleen. MCMV induced

hepatitis was monitored by measuring plasma ALT and AST. It has now been established that plasma ALT represents a specific marker of liver disease as a consequence of viral infection. Thus, the monitoring of hepatitis in the BALB/c mouse by measurement of elevated plasma ALT represents a good method for the evaluation of candidate anti-CMV drugs against CMV induced hepatitis.

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