16, 16 Dimethyl Prostaglandin E_2 Prevents the Development of Fulminant Hepatitis and Blocks the Induction of Monocyte/Macrophage Procoagulant Activity after Murine Hepatitis Virus Strain 3 Infection

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Abstract

16, 16 Dimethyl prostaglandin E_2 ($dmPGE_2$), a known cytoprotective agent, was examined for its ability to alter the course of fulminant hepatitis in an experimental model of fulminant viral hepatitis, murine hepatitis virus type 3 (MHV-3). Fully susceptible BALB/cJ mice, infected with 100 50% lethal doses (LD_{50}) of MHV-3 developed histologic and biochemical evidence of fulminant hepatitis, as evidenced by massive hepatic necrosis with hypoglycemia, metabolic acidosis, and a markedly elevated serum alanine aminotransferase (ALT) (mean, 1,402±619 IU/liter). In contrast, animals treated with $dmPGE_2$ either before or after infection (up to 48 h) demonstrated a marked reduction in both histologic and biochemical evidence of liver damage as characterized by normal blood glucose, total CO_2, and ALT determinations (mean ALT, 63±40 IU/liter). Treatment of infected mice with $PGF_{2\alpha}$ demonstrated no cytoprotective effects. High titers of infectious virus were recovered from the livers of both $dmPGE_2$-treated and -untreated animals throughout the course of infection.

In a parallel in vitro study, $dmPGE_2$ (10^{-4}–10^{-8} M) demonstrated a similar cytoprotective effect on monolayers of isolated cultured hepatocytes from fully susceptible BALB/cJ mice infected at a multiplicity of infection of 0.1, 1.0, and 10.0. In addition, splenic macrophages recovered from infected and untreated BALB/cJ mice demonstrated a marked augmentation in procoagulant activity (PCA) from a basal 10±5 mU/10^6 splenic macrophages to a maximum of 615±102 mU/10^6 splenic macrophages, whereas no increase in macrophage PCA was detected in infected animals treated with $dmPGE_2$. These results suggest that $dmPGE_2$, without detectably altering viral replication or infectivity in vivo, confers a marked cytoprotective effect on hepatocytes both in vivo and in vitro, and prevents the induction of macrophage PCA in vivo in fully susceptible BALB/cJ mice after murine hepatitis virus type 3 infection.

Introduction

Prostaglandin is a generic term describing a family of bioactive lipids derived from arachidonic acid via the cyclooxygenase pathway. The biological activities attributed to these compounds are both varied and heterogeneous. A number of investigators have demonstrated the cytoprotective effects of prostaglandins, in particular 16, 16 dimethyl prostaglandin E_2 ($dmPGE_2$),[1] in gastric mucosa (1), in the liver (2), and in the kidney (3) in response to a variety of toxins and other noxious stimuli. However, the cytoprotective role of analogues of prostaglandins of the E series has not been paralleled by $PGF_{2\alpha}$ analogues (4, 5). In addition, prostaglandins have been shown to have varied effects on smooth muscle, particularly changes in the tone of blood vessel musculature (6). Furthermore, it is generally accepted that they exert a predominantly suppressive effect on the immune system, the mechanism of which is poorly understood (7). It has been demonstrated that many macrophage activation parameters are modulated by exogenous PGE_2 (8, 9). More recently both endogenous and exogenous arachidonic acid metabolites (PGE_2 and PGI_2) have been shown to affect the expression of class II histocompatibility molecules by murine macrophages (10). Reports describing the effect of products of arachidonic acid metabolism on viral infection remain controversial. Prostaglandins (PGE_2 and $PGF_{2\alpha}$) have been shown to enhance the spread of herpes simplex virus in cell cultures (11), and they have been implicated indirectly as immunosuppressive agents in murine hepatitis virus type 3 (MHV-3) infection (12). Other investigators have shown that prostaglandins of the A series are potent inhibitors of sendai virus replication in vitro (13). This antiviral action is not specific and is not influenced by the lack of the cell's ability to produce interferon. Furthermore, PGE_1 treatment in vitro reduces the replication of encephalomyocarditis virus (strains mengo and MM) and polio viruses in association with an enhanced yield of interferon, and an inhibition of cell division (14).

MHV-3 is a member of the coronavirus family; it contains single-stranded RNA of positive polarity and produces a strain-dependent spectrum of liver disease in inbred strains of mice. Mice of the A strain are fully resistant, whereas BALB/cJ mice are fully susceptible to acute infection and die of fulminant hepatitis. Although the pathogenic mechanism is not clear, the virus is known to exert a direct cytopathic in vitro effect on hepatocytes isolated from both susceptible and resistant animals (15). In addition, immunologic factors are important determinants of susceptibility to MHV-3 infection (16) and resistance in vivo requires at least three subpopulations of immune cells (17–20).

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1. *Abbreviations used in this paper:* ALT, alanine aminotransferase; DME, Dulbecco's modified Eagle's medium; $dmPGE_2$ and $dmPGF_{2\alpha}$, 16, 16 dimethyl prostaglandins E_2 and $F_{2\alpha}$; Hepes, 3-(*N*-morpholino)-propanesulfonic acid, *N*-Tris (hydroxymethyl) 3 methyl-2 aminoethane sulfonic acid; MHV-3, murine hepatitis virus type 3; PCA, procoagulant activity; PFU, plaque-forming unit; PGE_1, PGE_2, $PGF_{2\alpha}$, and PGI_2, prostaglandins E_1, E_2, $F_{2\alpha}$, and I_2, respectively; p.i., postinfection; WmE, Williams' medium E.

Results of work from our laboratory have shown that strain-dependent susceptibility to MHV-3 correlates directly with the T lymphocyte–controlled spontaneous expression of a monokine that directly activates prothrombin (procoagulant activity [PCA]) (21). Similarly, we have suggested a pathogenic role for this activity as a result of in vivo hepatic microscopic observations during acute MHV-3 infection (22). Abnormalities of the microcirculation consisting of granular blood flow and sinusoidal microthrombi precede in vivo viral replication by 24 h. Subsequently, focal avascular lesions form, which in the susceptible animals progress to confluent necrosis.

The following studies were undertaken to determine the effects of the synthetic long-acting analogues, $dmPGE_2$ and $dmPGF_{2\alpha}$, on the course of MHV-3 infection in fully susceptible BALB/cJ mice.

Methods

Cells. The origin and growth of 17 CL 1, DBT, and L2 cells has been described previously (23–25). Cells were propagated in Dulbecco's modified Eagle's medium (DME) (Flow Laboratories Inc., Rockville, MD) supplemented with 10% fetal calf serum (FCS) (Flow Laboratories Inc.) and 25 μg/ml chlortetracycline hydrochloride grade II (Sigma Chemical Co., St. Louis, MO) and buffered with 15 mM *N*-2-hydroxyethylpiperazine-N'-2-ethanesulfonic acid (Hepes), 3-(*N*-morpholino)-propanesulfonic acid (MOPS), *N*-Tris (hydroxymethyl) 3 methyl-2 aminoethane sulfonic acid (TES), and 4 mM glutamine (Sigma Chemical Co.).

Virus. MHV-3 was obtained from the American Tissue Type Culture Collection, Rockville, MD (ATTCC VR 262) and plaque purified on monolayers of DBT cells as previously described (21). Stock virus was grown to a titer of 1.2×10^7 plaque-forming units (PFU) per milliliter in 17 CL 1 cells. The virus was harvested by one cycle of freeze thawing and clarified by centrifugation at 4,500 *g* for 1 h at 4°C. The virus was then assayed on monolayers of L2 cells in a standard plaque assay. Aliquots of stock virus were stored at −70°C.

Viral dilutions for inoculation were prepared in DME supplemented with 2% FCS and 25 μg/ml chlortetracycline hydrochloride grade II, and buffered with 15 mM Hepes and 4 mM glutamine.

Prostaglandins. $dmPGE_2$ and $dmPGF_{2\alpha}$ were kindly provided by the Upjohn Co., Kalamazoo, MI, and were reconstituted in absolute ethanol to a stock concentration of 10 mg/ml. Dilutions were then carried out using a 0.1-M phosphate buffer (pH 7.4) and the freshly prepared solution was injected intraperitoneally. Vehicle controls were prepared using an identical amount of absolute ethanol diluted in 0.1 M phosphate buffer (pH 7.4), and this resulted in an equivalent final concentration of ethanol. A dose titration was performed to establish the highest non–toxic dose of both prostaglandins. A dose > 2 μg/kg body weight of $dmPGF_{2\alpha}$ or 10 μg/kg $dmPGE_2$ resulted in a dose-dependent incidence of mortality, whereas a dose between 2 and 10 μg/kg of $dmPGE_2$ resulted in a dose-dependent incidence of diarrhea and dehydration (weight loss, elevated blood urea nitrogen). The selected dosage of 2 μg/kg body weight for both agents was used in all subsequent experiments, and this resulted in no detectable diarrhea and no mortality in control animals (i.e., prostaglandin alone).

Mice. BALB/cJ mice, 6–8 wk of age, were obtained from Jackson Laboratories, Bar Harbor, ME. Animals were divided into seven groups (10 animals per group): group 1, uninfected, untreated animals; group 2, uninfected, treated ($dmPGE_2$) animals; group 3, uninfected, treated ($dmPGF_{2\alpha}$) animals; group 4, infected (MHV-3) animals; group 5, infected (MHV-3), treated (vehicle); group 6, infected (MHV-3), treated ($dmPGE_2$); group 7, infected (MHV-3), treated ($dmPGF_{2\alpha}$). Animals were infected intraperitoneally with 100, 1,000 or 10,000 LD_{50} (1 PFU = 10 LD_{50}) of MHV-3.

In our initial experiments, the animals were pretreated with prostaglandin or vehicle 30 min before infection and 30 min after infection. They were then treated every 2 h for 24 h and every 8 h thereafter. In subsequent experiments, pretreatment with prostaglandin was omitted and the aforementioned protocol was initiated at 30 min, 24 or 48 h after infection.

Mice were killed at 24, 48, 72, and 96 h after infection, blood was obtained by axillary bleeding, and tissues were studied for viral titers and histology.

Biochemistry. The sera from all animals were analyzed quantitatively for serum alanine aminotransferase (ALT) using a Worthington Statzyme GPT kit obtained from Cooper Biomedical, Inc., Malvern, PA, as previously described (26). Blood glucose, electrolytes, blood urea nitrogen, creatinine, amylase, bilirubin, calcium, and phosphorus were analyzed on an Ektachem 700 analyzer in the Department of Biochemistry at Mount Sinai Hospital, Toronto, Canada.

Histological examination and quantitation of necrosis. Samples of liver were sliced into 1 × 0.2-cm blocks and were fixed by immersion into 10% formalin (Fischer Scientific Co., Fairlawn, NJ) in 0.1 M phosphate buffer, pH 7.4. After fixation, the tissues were dehydrated in graded alcohols and xylene, were embedded in paraffin, and a total of three 4-μm sections were cut and stained with Harris' hematoxylin for 4 min, and were then counterstained with eosin Y for 30 s. The sections were then washed with distilled water, dehydrated in graded alcohols and xylene, and were mounted with Permount. To quantitate the effect of the prostaglandins on liver histology, a digitalized image analysis system HP-88 (Hewlett-Packard Co. Ltd., Mississauga, Canada) with customized software was used. This constitutes a modification of a technique described previously (27). The areas of necrosis were encircled, as well as the entire section, yielding a percentage figure representing the proportion of diseased liver present in that particular section. Three sections from different parts of the liver were assayed in this fashion for each animal (n = 10/group), and the mean±standard deviation calculated.

Viral titers. Frozen liver tissue (−70°C) was homogenized in DME supplemented with 2% FCS and 4 mM glutamine as a 10% homogenate at 4°C. Viral titers were then determined on monolayers of L2 cells in a standard plaque assay (21).

Infectivity assay. Susceptibility of mice to lethal infection with MHV-3 was determined. Liver homogenates were assayed for viral titers as described in the preceding section, appropriately diluted, and then injected intraperitoneally. After infection, the animals were observed for 10 days and mortality data obtained. A total of 84 BALB/cJ mice were infected intraperitoneally in groups of seven with 0.1, 1.0, 10, or 1,000 PFU of MHV-3, derived from one of three sources: stock MHV-3; liver homogenates of infected and untreated animals; liver homogenates of infected but $dmPGE_2$-treated animals.

Isolation of spleen cells. Cells were isolated from the spleens of BALB/cJ mice and resuspended in DME. Mononuclear cells were isolated over Ficoll-Hypaque (Pharmacia Co. Ltd., Montreal, Canada) (density: 1.034 g/ml) gradients by centrifugation at 22°C at 2,200 *g* for 12 min. Cells at the interface were harvested, yielding a cell population consisting of > 98% mononuclear cells by cytologic examination using Giemsa stain. Cells were washed twice and resuspended in DME at a concentration of 2×10^6 mononuclear cells per milliliter.

PCA. Samples of cells, after three cycles of freeze thawing and sonication were assayed for the capacity to shorten the spontaneous clotting time of normal citrated human plasma in a one-stage clotting assay (28). To 0.1 ml of cellular homogenate at 4°C, 0.1 ml of citrated normal human platelet-poor plasma was added, followed by 0.1 ml of 25 mM $CaCl_2$ to start the reaction. The time for the appearance of a fibrin gel was recorded. Clotting times were converted to units of PCA by comparison to a rabbit brain thromboplastin standard (Dade Div., American Hospital Supply, Miami, FL) where 36 mg dry weight per milliliter were assigned a value of 100,000 mU PCA. The assay was utilized over the range of 1 to 100,000 mU or 10^2 to 10^8 cells, this range being linear with normal plasma substrate. Media and buffers were all without activity.

Endotoxin contamination. DME, virus preparations, prostaglandins, and spleen cell preparations were assayed for endotoxin contamination by standard limulus assay (E. toxate; Sigma Chemical Co.) and

contained < 0.1 ng/ml of endotoxin, which constitutes lower limits of this test (29, 30).

Preparation and culture of hepatocytes. Liver cells were isolated using a modification of the technique described by Seglen (31). Male BALB/cJ mice, 8–10 wk old, were anesthetized using inhalational ethyl ether (Caledon Laboratories, Georgetown, Canada). Using sterile technique, the abdominal cavity was opened and the inferior vena cava ligated just below the liver. The potal vein was then cannulated with a 22-gauge catheter and the liver perfused in situ with 0.05 M EGTA (J. T. Baker Chemical Co., Phillipsburg, NJ) in Ca^{++}-free Hanks' balanced salt solution (Gibco/BRL Life Technologies Inc., Burlington, Canada) at 37°C. After the perfusion was initiated, the suprahepatic inferior vena cava was transected, allowing free outflow of the perfusate. After 4.5 min, the perfusion solution was changed to 0.05% collagenase (Sigma Chemical Co.) in Williams' medium E (WmE) (Gibco/BRL Life Technologies Inc.) supplemented with 25 mM Hepes for 8–10 min. The liver was excised and placed in a 60-mm plastic Petri dish (Falcon Labware, Div. of Becton-Dickinson & Co., Oxnard, CA) containing a small amount of the collagenase solution and the liver was gently combed with forceps to obtain isolated hepatocytes. This suspension was filtered through a nylon mesh and washed with WmE containing 10% heat-inactivated FCS, 2 mM glutamine, and 100 mU/ml insulin (Connaught Laboratories, Toronto, Canada). The cells were allowed to settle for 20 min and the supernatant aspirated. The cells were resuspended in the 10% FCS solution and the suspension centrifuged at 400 rpm for 3 min at 4°C. Cell count and viability was determined in a hematocytometer using the trypan blue exclusion technique. Mean viability was 75±5%. The cells ($7 \times 10^4/cm^2$ in 2.0 ml) were plated onto 3-cm diam tissue culture dishes (Falcon Labware, Div. of Becton-Dickinson & Co.) previously coated with 0.2 ml of a 10% type 1 collagen solution (Collagen Co., Palo Alto, CA), dried, and subsequently washed with 2 ml of WmE. The plated cell cultures were incubated at 37°C and 5% CO_2 for 2 h before experimental manipulation.

The medium in the plates was either changed to serum-free WmE (in order to minimize contamination with Kuppfer cells) containing glutamine and insulin alone, or the same medium containing in addition $dmPGE_2$, at concentrations of 10^{-4}–10^{-8} M or vehicle at equivalent concentrations of ethanol, and the cells were incubated under identical conditions. The medium was changed every 24 h, and at 48 h, when the hepatocyte monolayer was confluent, MHV-3 was added to the plates at a multiplicity of infection of 0.1, 1.0, and 10. All plates were examined serially for 48 h for determination of cytopathic effect. Neither $dmPGE_2$ nor vehicle were cytotoxic to the hepatocyte cultures.

Statistical analysis. Statistical analysis was carried out using analysis of variance and the Wilcoxon ranked sum test, and a P value of 0.05% or less was considered statistically significant.

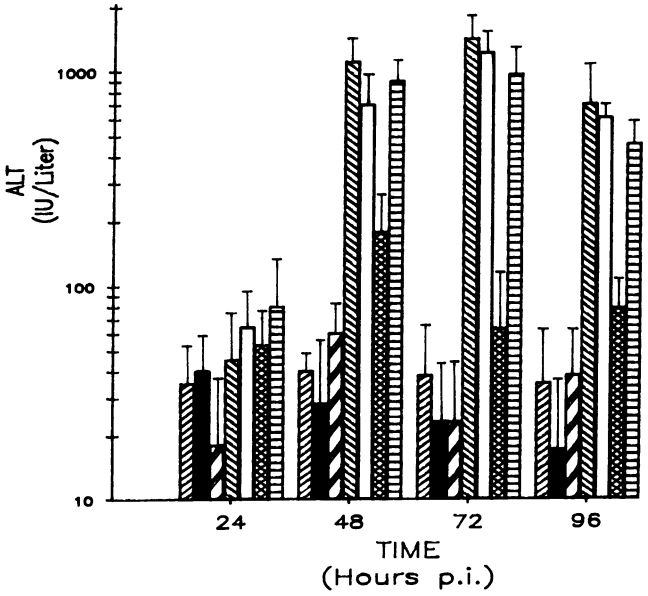

Figure 1. The levels of serum ALT after MHV-3 infection. There is a statistically significant difference between the level of ALT from MHV-3–infected mice treated with $dmPGE_2$ and infected animals untreated, treated with vehicle, or with $dmPGF_{2\alpha}$ ($P < 0.01$). (■) No treatment; (■) PGE_2; (□) $PGF_{2\alpha}$; (▨)MHV-3; (□) MHV-3 + vehicle; (■) MHV-3 + PGE_2; (■) MHV-3 + $PGF_{2\alpha}$.

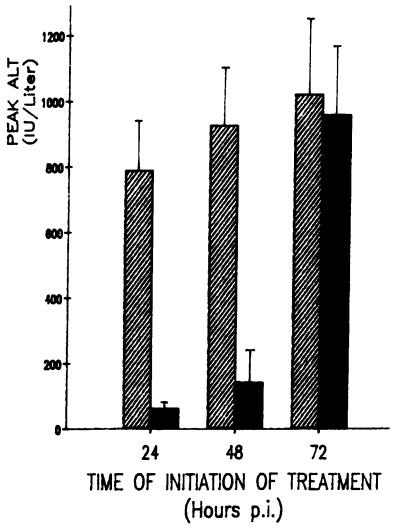

Figure 2. The effect of delay of $dmPGE_2$ treatment on ALT determinations after MHV-3 infection. The rise in ALT after MHV-3 infection is markedly reduced even when treatment with $dmPGE_2$ is initiated 24 or 48 h after infection with MHV-3 ($P < 0.01$). No beneficial effect was observed when treatment was initiated 72 Hours p.i. (not significant). (▨) MHV-3 + vehicle; (■) MHV-3 + PGE_2.

Results

Biochemistry. Treatment with either $dmPGE_2$ or $dmPGF_{2\alpha}$ alone had no apparent hepatotoxic effects as determined by normal biochemistry (ALT) when compared with uninfected and untreated animals. MHV-3–infected but untreated mice developed a marked elevation in ALT by 48 h, which rose until their demise at ~ 96 h. Serum ALT remained within normal limits in infected mice treated with $dmPGE_2$ beginning 30 min after viral inoculation (Fig. 1). Similar results were observed when treatment with $dmPGE_2$ was delayed by 24 or 48 h after infection (Fig. 2). However, a delay in treatment of 72 h resulted in a peak elevation in ALT comparable with that of untreated, infected animals. In contrast, infected animals treated with either vehicle or $dmPGF_{2\alpha}$ demonstrated ALT

Table I. Effect of $dmPGE_2$ on Blood Glucose and TCO_2 during MHV-3 Infection in BALB/cJ Mice

MHV-3 (10 PFU)	dmPGE (2 μg²/kg)	Blood glucose	Total CO_2
		mmol/liter	*mmol/liter*
–	–	6.8±1.8	27±3
+	–	2.5±1.4	18.2±5
+	+	5.5±2.1	26±3

There were 10 mice in each group. Biochemical determinations were obtained 96 h after infection.

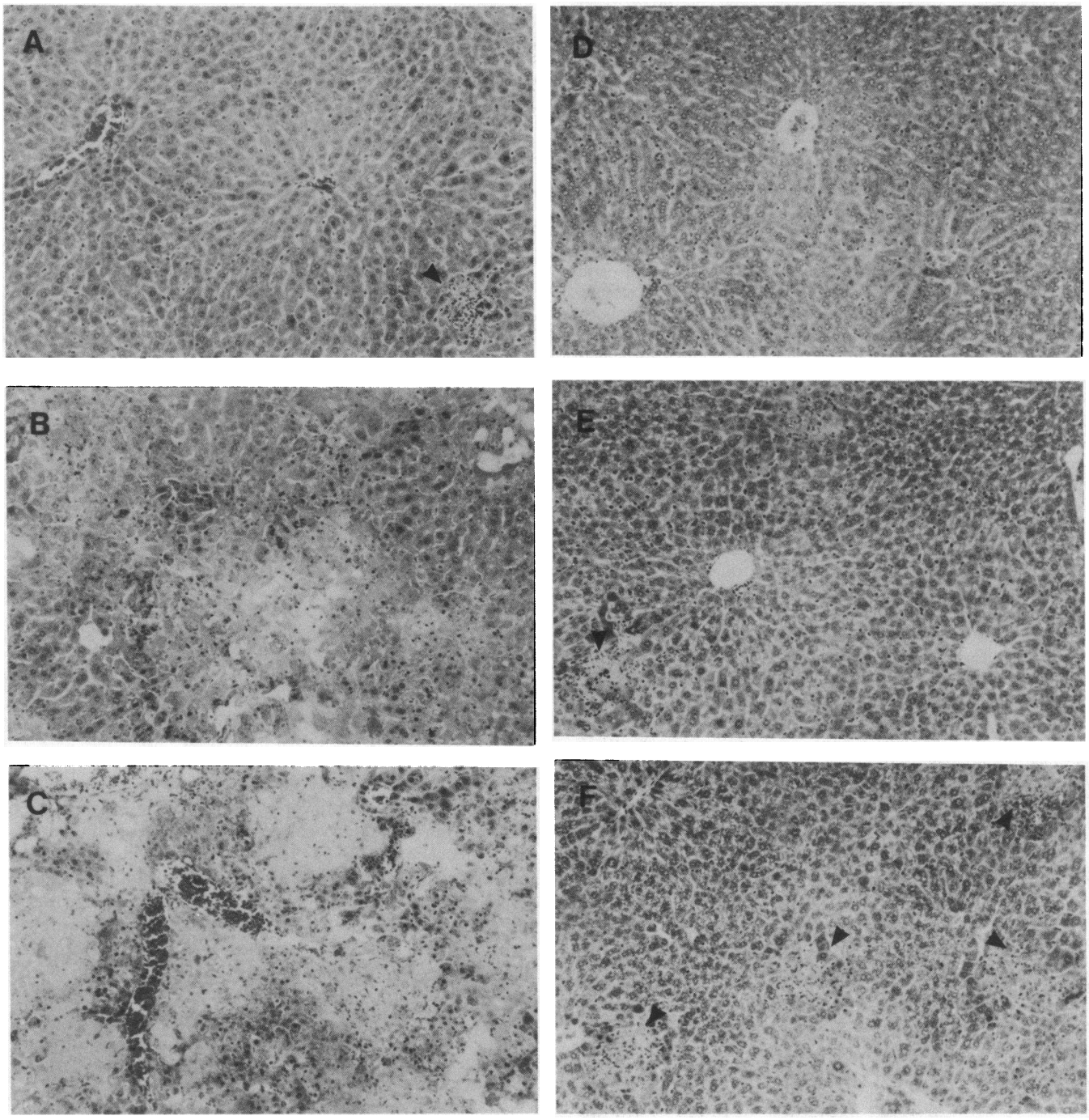

Figure 3. The effect of $dmPGE_2$ on liver histology after MHV-3 infection. Liver hematoxylin and eosin (× 600) sections after infection demonstrate scattered, small foci of inflammatory cells (*arrow*) at 48 h (*A*), widespread necrosis at 72 h (*B*), and confluent necrosis at 96 h (*C*). In contrast, concomitant treatment with $dmPGE_2$ results in normal histology at 48 h (*D*), only an occasional focus of inflammation at 72 h (*E*), and scattered foci at 96 h (*F*).

levels comparable with those of mice infected but not treated with $dmPGE_2$ (Fig. 1). In MHV-3–infected mice, there was a marked hypoglycemia and metabolic acidosis consistent with hepatic failure. In contrast, no abnormalities in blood glucose or TCO_2 were seen in $dmPGE_2$-treated and infected animals (Table I). Similar results were obtained when mice were infected with increasing concentrations of MHV-3 (to 1,000 PFU) (data not shown).

Liver histology. Mice infected with MHV-3 developed histologic evidence of liver disease. By 24 h postinfection (p.i.), small, discrete foci of necrosis with a sparse polymorphonuclear leukocyte infiltrate could be seen. At 48 h, these lesions became both more pronounced and more numerous, and by 72–96 h, confluent liver necrosis was apparent. In contrast, mice infected with MHV-3, but treated with $dmPGE_2$ 30 min later showed a marked reduction in liver pathology, with only

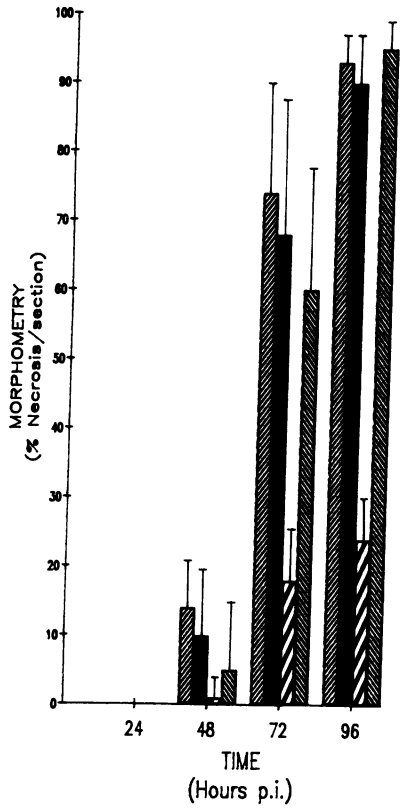

Figure 4. Morphometric analysis of liver histology. A marked difference in the proportion of diseased to normal liver is seen at 48 h ($P < 0.05$) and at 72 and 96 h ($P < 0.01$) between infected animals treated with $dmPGE_2$ and infected animals either untreated, treated with vehicle, or with $dmPGF_{2\alpha}$. (▨) MHV-3; (■) MHV-3 + vehicle; (□) MHV-3 + PGE_2; (▧) MHV-3 + $PGF_{2\alpha}$.

a few foci observed even at 72 and 96 h p.i. The confluent necrosis observed in the untreated, infected animals was not seen in the $dmPGE_2$-treated, infected mice (Fig. 3). Morphometric studies by image analyzer showed that the proportion of diseased to normal liver expressed as a percentage was significantly different between the infected, $dmPGE_2$-treated and -untreated groups at 48, 72, and 96 h (Fig. 4). A similar protective effect was seen when initiation of treatment with $dmPGE_2$ was delayed for 24 or 48 h, but not 72 h (Fig. 5). In contrast to the infected, $dmPGE_2$-treated mice, those treated with $dmPGF_{2\alpha}$ demonstrated as marked liver disease as untreated, or vehicle-treated infected animals. (Fig. 4). Treatment with $dmPGE_2$ or $dmPGF_{2\alpha}$ alone resulted in no detectable liver disease.

Initially, the protective effect of $dmPGE_2$ was demonstrated using an inoculum of 10 PFU (100 LD_{50}) of MHV-3. A similar cytoprotective effect was observed even when animals were infected with either 100 or 1,000 PFU of MHV-3 (data not shown).

PCA. In untreated but infected animals, a sharp increase in macrophage PCA was noted at 24 h p.i. Maximal PCA was seen at 48 h and PCA remained elevated until the animals' death. In contrast, in infected but $dmPGE_2$-treated animals, no elevation of PCA could be detected at any time during the course of the infection, and the values obtained were comparable with those of uninfected, unreated animals. Uninfected animals treated with either $dmPGE_2$ or $dmPGF_{2\alpha}$ had PCA values comparable with control mice. Infected mice treated with either vehicle or $dmPGF_{2\alpha}$ demonstrated an elevated PCA similar to that of untreated infected mice. (Fig. 6).

Viral titers. By 24 h p.i., high viral titers were recovered

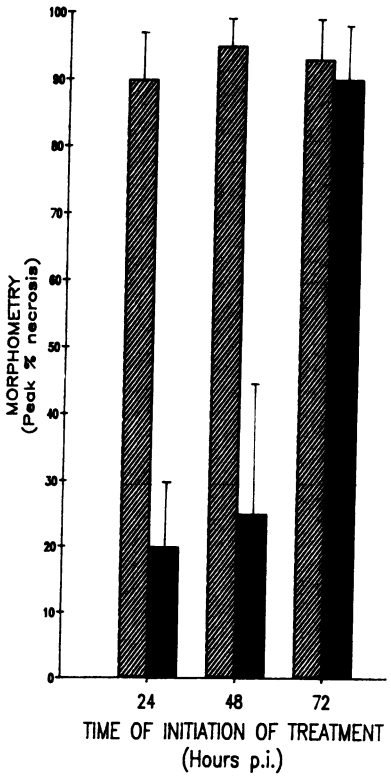

Figure 5. The effect of delay of $dmPGE_2$ on morphometric analysis of liver histology. The protective effect of $dmPGE_2$ on liver histology was maintained even when treatment was initiated up to 48 h p.i. ($P < 0.05$). This effect was not observed when treatment was started 72 h p.i. (not significant). (▨) MHV-3 + vehicle; (■) MHV-3 + PGE_2.

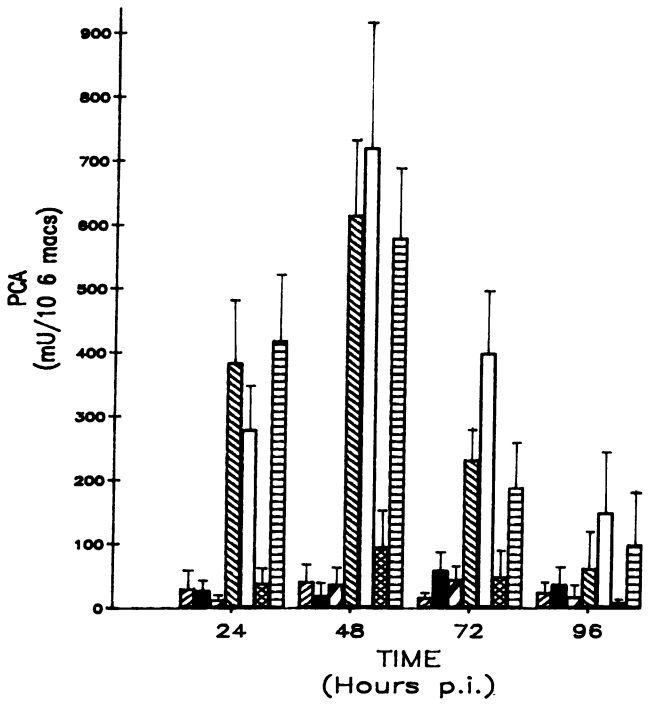

Figure 6. The effect of $dmPGE_2$ on MHV-3 induction of macrophage PCA in vivo. Treatment with $dmPGE_2$ abrogated the induction of PCA after infection with MHV-3 at 24 and 48 h ($P < 0.01$), and at 72 h ($P < 0.05$) as compared with the PCA of infected animals untreated, or treated with either vehicle, or with $dmPGF_{2\alpha}$. (▨) No treatment; (■) PGE_2; (□) $PGF_{2\alpha}$; (▧) MHV-3; (□) MHV-3 + vehicle; (■) MHV-3 + PGE_2; (■) MHV-3 + $PGF_{2\alpha}$.

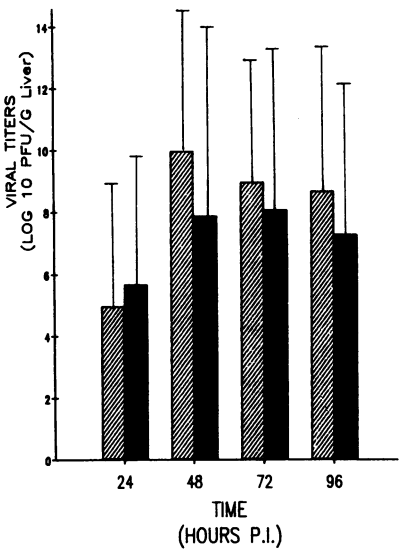

Figure 7. The effect of $dmPGE_2$ on viral titers from liver homogenates. High titers of virus were recovered from both $dmPGE_2$-treated and -untreated infected mice at 24, 48, 72, and 96 h after infection (not significant). (▨) MHV-3 + PGE_2; (■) MHV-3.

from liver homogenates of both $dmPGE_2$-treated and -untreated animals, and these persisted until the death of the animals (Fig. 7). There was no statistically significant difference between treated and untreated groups ($P > 0.05$).

Infectivity assay. Using the method of Reed and Muench (32), an LD_{50} for BALB/cJ of < 1–5 PFU was established for MHV-3 from all three sources assayed. MHV-3 recovered from infected and $dmPGE_2$-treated animals was as pathogenic as stock MHV-3 or virus recovered from infected and untreated mice, and thus resulted in equivalent mortality. (Fig. 8). Furthermore, maximal PCA from BALB/cJ mice that were infected with MHV-3 derived from liver homogenates of either infected and $dmPGE_2$-untreated (745±115 mU/10^6 macrophages) or infected and $dmPGE_2$-treated mice (785±95 mU/10^6 macrophages) was comparable with that from mice infected with stock MHV-3 (730±100 mU/10^6 macrophages).

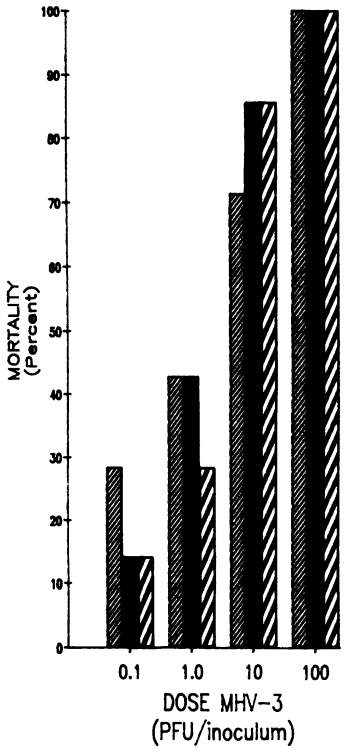

Figure 8. Susceptibility of BALB/cJ mice after infection with MHV-3. BALB/cJ mice were infected intraperitoneally with 0.1, 1.0, 10, or 100 PFU of stock MHV-3, or MHV-3 recovered from liver homogenates of infected, untreated BALB/cJ mice, or from liver homogenates of infected, $dmPGE_2$-treated BALB/cJ mice. Each group contained seven mice. Mortality was equivalent for all three sources of the virus (not significant). (▨) MHV-3 (stock); (■) homogenate (MHV-3); (□) homogenate (MHV-3 + PGE_2).

Survival data. There was no difference in survival between infected animals, and those infected and treated with either $dmPGE_2$, $dmPGF_{2\alpha}$, or vehicle. All animals died within 5 d after infection whether or not they received prostaglandin.

Isolated hepatocyte cultures. Hepatocyte monolayers were established. In the infected (MHV-3) but not treated ($dmPGE_2$) cell cultures, cytopathic effect was observed by 18 h, as demonstrated by the formation of syncitia representing the fusion of hepatocytes with degradation of the outer cell membrane. By 48 h, syncitial formation was complete, and cell lysis occurred with the destruction of the hepatocyte monolayer (Fig. 9). In contrast, in the infected cells treated with $dmPGE_2$, there was a marked reduction in cytopathic effect, although occasional syncitia were still evident. Optimal cytoprotection was achieved at 10^{-4} M $dmPGE_2$ (Fig. 9).

Discussion

Fulminant hepatitis remains a major therapeutic problem and to date there exists no satisfactory treatment (33). The present studies demonstrate a cytoprotective role for $dmPGE_2$ both in vivo and in vitro in a murine model of fulminant hepatitis (MHV-3). The mechanism for the observed hepatic protection from the effects of MHV-3 by $dmPGE_2$ is probably multifaceted and quite complex. It is evident from our results that $dmPGE_2$ does not affect viral replication in vivo as demonstrated by the recovery of high titers of virus from the liver homogenates of both $dmPGE_2$-treated and -untreated mice, suggesting that viral replication alone does not account for the cytopathic effect of MHV-3 on hepatocytes. Similarly, $dmPGE_2$ does not interefere with the infectivity of MHV-3, as MHV-3 extracted from liver homogenates from both groups revealed equal pathogenicity when inoculated into fully susceptible BALB/cJ mice.

The lack of a detectable protective effect by $dmPGF_{2\alpha}$ in our studies is consistent with previous reports by others (4, 5), in which this analogue was unable to confer a protective effect similar to that of $dmPGE_2$ and points to the heterogenous properties of these agents. The cytoprotective effect of $dmPGE_2$ on hepatocyte cultures infected with MHV-3 in vitro suggests that $dmPGE_2$ acts, at least in part, directly at the level of the hepatocyte. Although others have reported that certain prostaglandins alter the fluidity of the lipid bilayer of mouse liver membranes as evidenced by changes in membrane viscosity using fluorescence probes (34), it is unlikely that this effect of PGE_2 interferes with either budding of the virus or expression of a viral receptor as virus was isolated in equal titers from the livers of both $dmPGE_2$-treated and -untreated mice.

Our data suggests that in addition to acting directly at the hepatocyte level, $dmPGE_2$ may also influence the immune response to MHV-3. Sitrin et al. have demonstrated that the addition of PGE_2 or PGE_1 reverses the suppressive action of indomethacin on PCA after stimulation in vitro by bacterial lipopolysaccharide of rabbit alveolar macrophages (35). They concluded from these studies that prostaglandins of the E series were required for the augmentation of PCA in this system. In contrast, Edgington et al. (36) have reported that the T

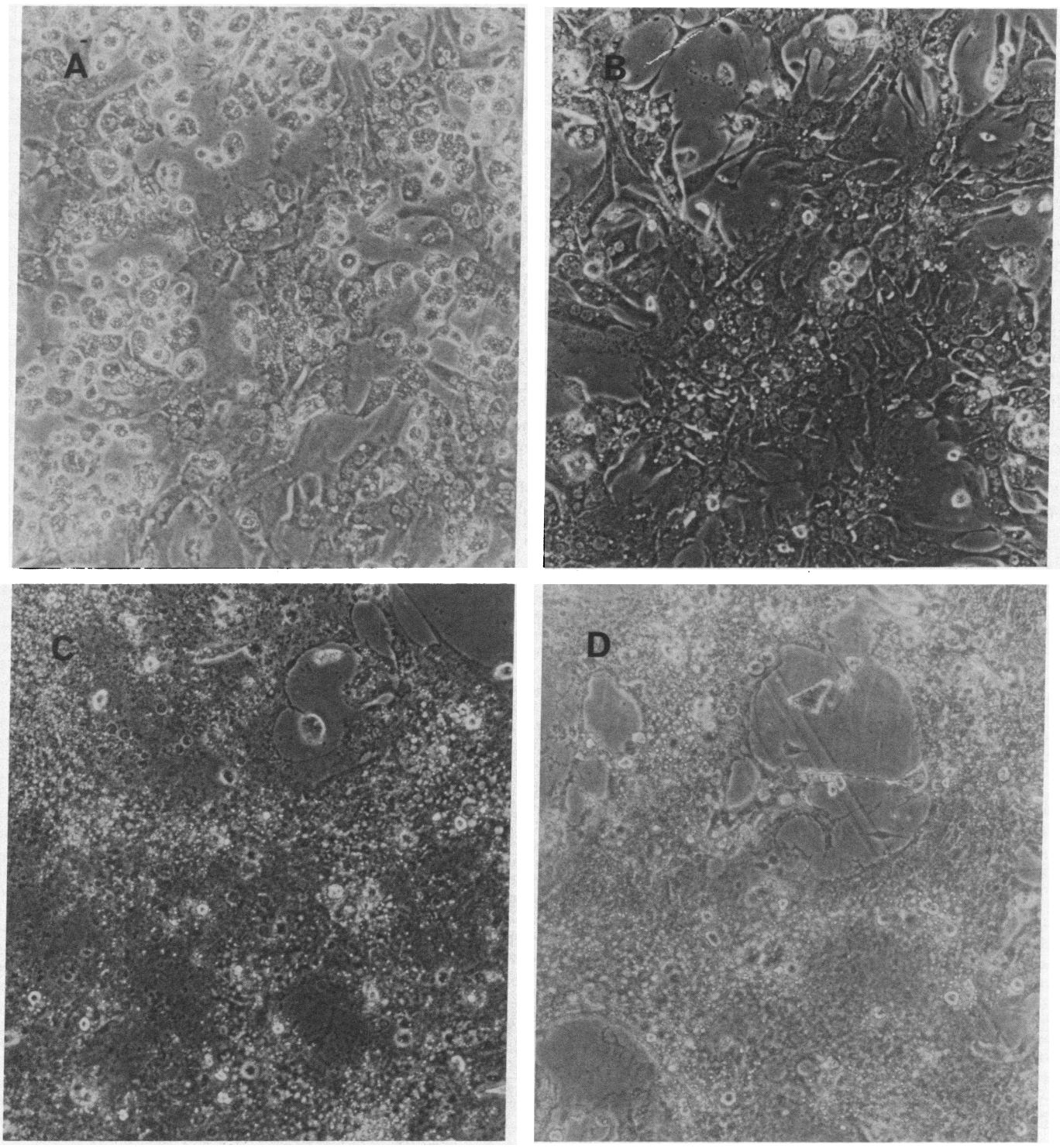

Figure 9. The effect of $dmPGE_2$ on MHV-3–infected hepatocyte cultures. Little or no cytopathic effect was observed in cultured hepatocytes (× 200) infected with MHV-3 (multiplicity of infection, 10) and treated with $dmPGE_2$ (10^{-4} M) at (*A*) 48 h and (*B*) 72 h after infection, whereas marked syncitial formation was noted in $dmPGE_2$-untreated but MHV-3–infected cells at 48 h (*C*) with ensuing cell and monolayer lysis occurring at 72 h (*D*).

cell–instructed PCA response to LPS by human peripheral blood mononuclear cells in vitro was modulated by arachidonic acid metabolites, exhibiting a-dose dependent suppression of the PCA response. We have shown in this report that treatment in vivo with $dmPGE_2$ abrogated the enhanced induction of macrophage PCA, which as we have previously documented correlates with susceptibility to MHV-3 and to disease activity (21). The association of the coagulation cascade with cell-mediated immunity is well known, and the enhanced expression of monocyte/macrophage PCA in association with

fibrin deposition and intravascular coagulation is a feature of a number of diseases including experimental autoimmune encephalomyelitis, systemic lupus erythematosus, and allograft rejection (37–41). The induction of monocyte/macrophage PCA is the product of complex cellular interactions involving lymphocytes, lymphokines, and macrophages with the resultant expression of at least three PCAs (42). Although the significance of an elevated PCA in the pathogenesis of MHV-3 infection is not known, there are a number of possible mechanisms by which it may contribute to tissue injury. Sturman and Holmes (43) have proposed that the mechanism for the development of cytopathic effect after coronavirus infection is enhanced by the proteolytic cleavage of the E_2 glycoprotein, which is expressed on the membrane of the virion. Cleavage of the E_2 glycoprotein from a 180-kD to two 90-kD subunits promotes cell fusion (syncitia) and cell lysis. Since monocyte PCA induced by MHV-3 has been shown to have characteristics consistent with a serine protease (44), it may play a role in the cleavage of the E_2 glycoprotein, hence mediating the cytopathic effect of MHV-3.

Our laboratory has previously shown a deleterious effect of MHV-3 on the microcirculation of the liver early in the course of infection (22). This is characterized by granular blood flow, sinusoidal microthrombi, distortion of sinusoids by edematous hepatocytes, and the formation, as early as 12 h after infection, of localized avascular foci of necrosis with fibrin deposition. It has been proposed that these effects are secondary to induction of PCA, which results in alterations in the immune coagulation system.

Prostaglandins, particularly those of the E and I types, are known to relax circular smooth muscle of various animal species and lead to vasodilatation (6). Thus, the observed cytoprotective effect of $dmPGE_2$ could be the result of improved microcirculatory flow in the livers of treated mice.

Despite the remarkable hepatic cytoprotection afforded by $dmPGE_2$ in our murine system, mortality was unchanged. The failure of $dmPGE_2$ treatment to prolong survival, despite the absence biochemically and histologically of liver disease, demonstrates the complexity of fulminant systemic viral infection. It is known that members of the mouse hepatitis virus family are not exclusively hepatotropic (45, 46), and infections with these viruses result in demyelinating neurological lesions which have been well characterized. However, this group of viruses form a spectrum within which MHV-3 represents one of the more hepatovirulent (47). Nevertheless, it has been established that MHV-3 replicates in other organs including brain, kidney, pancreas, spleen, lung, and lymph nodes (48). Hence, mortality that occurs during fulminant viral hepatitis may be attributed to infection of other organ systems. The absence of hypoglycemia and of a metabolic acidosis in infected animals treated with $dmPGE_2$ precludes the possibility of disruption of hepatic synthesis and metabolism.

We have shown in this report a hepatic cytoprotective effect both in vivo and in vitro by the synthetic $dmPGE_2$ in a murine model of fulminant hepatitis. The ability of $dmPGE_2$ to prevent both the induction of PCA and the development of liver disease in susceptible animals is similar to the course of MHV-3 infection in resistant A/J mice in which viral replication proceeds in the absence of induction of PCA and liver injury (21, 49). This constitutes additional evidence for a role of PCA in the pathogenesis of MHV-3 infection. Further studies are ongoing in our laboratory in an attempt to elucidate the mechanism of hepatic protection of $dmPGE_2$ in fulminant murine viral hepatitis infection.

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