HUMAN GENE THERAPY 17:589–600 (June 2006)

Novel *mfgl2* Antisense Plasmid Inhibits Murine *fgl2* Expression and Ameliorates Murine Hepatitis Virus Type 3-Induced Fulminant Hepatitis in BALB/cJ Mice

CHUANLONG ZHU,[1] YI SUN,[1] XIAOPING LUO,[2] WEIMING YAN,[1] DONG XI,[1] and QIN NING[1]

ABSTRACT

Our previous reports, both experimental and human studies, have shown the importance of fibrinogen-like protein-2 (*fgl2*) prothrombinase in the development of fulminant viral hepatitis, a disease with a mortality of more than 80% in cases lacking immediate organ transplantation. To interfere with this potentially effective target, a 322-bp mouse *fgl2* (*mfgl2*) antisense plasmid complementary to the exon 1 sequence of the gene, including the translation initiation site AUG, was successfully constructed. A dose-dependent inhibitory effect on *mfgl2* expression by *mfgl2* antisense plasmid was observed in interferon-γ-treated RAW 264.7 cells. On hydrodynamic delivery, *mfgl2* antisense plasmid significantly reduced *mfgl2* expression *in vivo*; markedly ameliorated inflammatory cell infiltration, fibrin deposition, and hepatocyte necrosis; prolonged the survival time period; and elevated the survival rate among BALB/cJ mice with murine hepatitis virus type 3-induced fulminant hepatitis. This study may provide an effective way to interfere with the potential therapeutic target *fgl2* gene for fulminant viral hepatitis and other diseases with similar pathological characteristics of microcirculation disorders, including acute rejection of xeno- or allograft transplantation and fetal loss syndrome, in which studies show *fgl2* plays an important role.

OVERVIEW SUMMARY

***fgl2* plays an important role in the development of murine hepatitis virus type 3-induced fulminant hepatitis and severe acute chronic hepatitis B. This paper investigates the possibility of interfering with the potential target mouse fibrinogen-like protein-2 (*mfgl2*) gene by means of an *mfgl2* antisense plasmid. The results suggest that therapeutic effects are achievable. The *mfgl2* antisense plasmid significantly ameliorated inflammatory cell infiltration, fibrin deposition, and hepatocyte necrosis; prolonged the survival time period; and elevated the survival rate among BALB/cJ mice with murine hepatitis virus type 3-induced fulminant hepatitis. The same strategy could apply to other diseases such as acute rejection of xeno- or allograft transplantation and fetal loss syndrome, in which studies have shown *fgl2* was also closely involved.**

INTRODUCTION

THERE ARE AN INCREASING NUMBER of patients with chronic hepatitis B who develop acute hepatitis during the chronic phase of the disease and subsequently die of acute hepatic failure (Mondelli and Eddleston, 1984; O'Grady and Williams, 1986; Hoofnagle *et al*., 1995). The hallmark of this severe disease is the extreme rapidity of the necromicroinflammatory process, resulting in widespread or total hepatocellular necrosis in weeks or even days and associated with a marked cellular infiltrate associated with intravascular fibrin deposition (Liu *et al*., 2001). Our previous studies have shown, in both an experimental animal model of fulminant viral hepatitis caused by murine hepatitis virus strain 3 (MHV-3) and in patients with acute or chronic hepatitis B, the importance of macrophage activation and the expression of a unique gene, fibrinogen-like protein-2 (*fgl2*), which encodes a serine protease capable of di-

[1]Laboratory of Infectious Immunology, Department of Infectious Disease, and [2]Department of Pediatrics, Tongji Hospital of Tongji Medical College, Huazhong University of Science and Technology, Wuhan 430030, China.

rectly cleaving prothrombin to thrombin, resulting in intravascular fibrin deposition within the liver and culminating in widespread hepatocyte necrosis (Fung *et al*., 1991; Ding *et al*., 1997; Ning *et al*., 1998; Marsden *et al*., 2003).

Gene silencing has been successfully achieved in a variety of disease conditions including viral infective and inflammatory as well as malignant diseases, using antisense DNA or RNA, ribozymes, and small interfering RNA (siRNA) approaches (Yacyshyn *et al*., 1998; Weiss *et al*., 1999; Caplen *et al*., 2001; Tamm *et al*., 2001). The possible mechanisms by which antisense molecules result in decreased protein expression include the modulation of protein translation by disrupting ribosome assembly, RNase H-mediated cleavage of targeted mRNA, and pretranslational modification of splicing (Weiss *et al*., 1999; Crooke, 2001). Antisense molecules have long since been described but technological advances have led to improvements in pharmacokinetics and pharmacodynamics (Stephenson and Zamecnik, 1978). Expression vectors producing antisense DNA or RNA have major advantages over oligonucleotides, because vectors can synthesize the antisense DNA or RNA continuously inside the cell after a single administration, resulting in a longer duration of action. We have therefore designed an antisense DNA complementary to the 322-bp sequence of exon 1 of the mouse *fgl2* (*mfgl2*) gene, including the translation initiation site AUG. By using this *mfgl2* antisense plasmid in a cell culture system and the MHV-3-induced fulminant viral hepatitis animal model, we have demonstrated a significant reduction in *mfgl2* gene expression both *in vivo* and *in vitro*, marked histological improvement of affected liver tissue, prolongation of animal survival time, and elevation of the survival rate, indicating that the *mfgl2* antisense plasmid may provide an effective way to interfere with *mfgl2* prothrombinase, as a potential therapeutic strategy.

MATERIALS AND METHODS

Virus

MHV-3 was obtained from the American Type Culture Collection (ATCC, Manassas, VA), plaque purified on monolayers of DBT (delayed brain tumor) cells, and titered on L2 cells according to a standard plaque assay (Fung *et al*., 1991; Ding *et al*., 1997; Ning *et al*., 1998).

Animals

All animal experiments were carried out according to the guidelines of the Chinese Council on Animal Care and approved by Tongji Hospital (Tongji Medical College, Huazhong University of Science and Technology, Wuhan, China) Committees on Animal Experimentation. The research protocol was reviewed and approved by the hospital institutional review board of Tongji Hos-

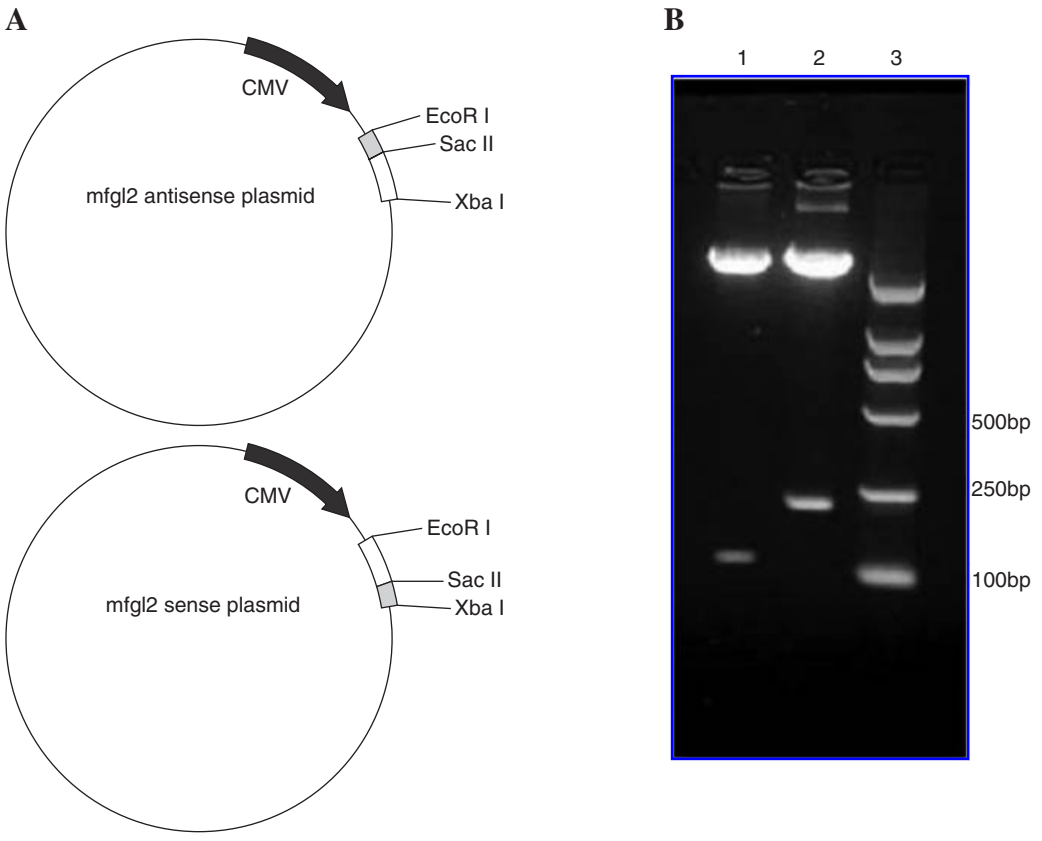


FIG. 1. Construction of *mfgl2* antisense and *mfgl2* sense plasmids. (**A**) The 322-bp antisense and sense DNA was inserted into pcDNA3.0 through *Eco*RI and *Xba*I enzyme sites. (**B**) The *mfgl2* antisense and *mfgl2* sense plasmid was identified by restriction endonuclease digestion with *Xba*I and *Sac*II. Lane 1, *mfgl2* antisense plasmid plus *Eco*RI plus *Sac*II; lane 2, *mfgl2* sense plasmid plus *Eco*RI plus *Sac*II; lane 3, DNA marker.

pital. Female BALB/cJ mice, 8–10 weeks of age and weighing 20–22 g, were purchased from Hubei Provincial Institute of Science and Technology (Wuhan, China). Gene transfection was achieved by hydrodynamic injection, via the tail vein, of 100 μg of *mfgl2* antisense or sense plasmid, or pCMV-LacZ plasmid, dissolved in 2 ml of phosphate-buffered saline (PBS) over 4 to 5 sec (18 animals in each group). The injection was repeated 24 hr later, and 24 hr later mice received 20 plaque-forming units (PFU) of MHV-3 intraperitoneally to develop fulminant viral hepatitis. Liver sections and serum samples from separate sets of animals were sampled at the hours indicated after MHV-3 infection.

Construction of mfgl2 *antisense and sense plasmid*

A 322-bp nucleated fragment of *fgl2* cDNA was amplified from the *mfgl2* cDNA library pBluescript-m166 (pm166) of mouse genomic P1 plasmid (Genome Systems, Inc., St. Louis, MO), which contains the entire mouse *fgl2* gene. The upstream primer was 5′-CTA G**TC TAG A**GC CGC ACT GCA AGG AT-3′ and the downstream primer was 5′-CCG **GAA TTC** TCG TCA GCC TGC AAC TTA C-3′. The boldface, underlined sequences are the restriction enzyme sites for *Xba*I and *Eco*RI, respectively. The amplified fragment (a mouse antisense DNA to the *mfgl2* gene) was then subcloned, at the *Eco*RI and *Xba*I restriction sites, into the pcDNA3.0 expression vector under the control of a cytomegalovirus promoter and bovine growth hormone 3′ processing signals (Patent no. 200610018749.4). By using the same vector, the same 322-bp fragment of *mfgl2* sense plasmid was also constructed as an experiment control (Fig. 1A). Restriction endonucleases *Eco*RI and *Sac*II were used to analyze the orientation of the target gene insert in recombinant plasmid constructs (Fig. 1B). All plasmids were purified with EndoFree Plasmid Maxi kits (Qiagen, Hilden, Germany).

Transfection

Mouse macrophage cell line RAW 264.7 was cultured in six-well plates until 50–80% confluence. *mfgl2* antisense plasmid or *mfgl2* sense plasmid, and pEGFP DNA (as a marker for transfection efficiency; Invitrogen Life Technologies, Carlsbad, CA) in serum-free F12–Dulbecco's modified Eagle's medium were vortexed with Lipofectamine (2 μg/μl) according to the manufacturer's protocol. After incubation at room temperature for 30 min, the mixture was distributed into one of the duplicated wells with RAW 264.7 cells, and transfection was performed at 37°C with 5% CO_2. Because of the toxicity of Lipofectamine, the medium was replaced with fresh complete medium 5 hr after transfection. Interferon-γ (IFN-γ) was added to the cell culture as indicated as the inducer of *mfgl2* gene expression and cells were harvested for various assays. The biological effects of *mfgl2* antisense plasmid were then measured by reverse transcription-polymerase chain reaction (RT-PCR) or quantitative real-time PCR, Western blot, and procoagulant activity (PCA) assay, and a functional assay of *mfgl2* protein was performed.

Real-time fluorescence quantitative RT-PCR

Total RNA was extracted from mouse liver with TRIzol reagent (Invitrogen Life Technologies) according to the manufacturer's standard protocol. Real-time fluorescence quantitative RT-PCR was done with EvaGreen PCR reagents (Biotium, Hayward, CA) according to the manufacturer's standard protocol. The upstream primer was 5′-ACT GTG ACA TGG AGA CCA TG-3′, and the downstream primer was 5′-TCC TTA CTC TTG GTC AGA AG-3′.

Serum aminotransferase measurements

Plasma or serum alanine aminotransferase (ALT) levels were measured by clinical chemistry analyzer (Aeroset system; Abbott Laboratories, Abbott Park, IL).

In situ *hybridization*

The method used here has been described previously (Ding *et al*., 1998; Marsden *et al*., 2003). Digoxigenin-11-UTP (Dig-UTP; Roche, Mannheim, Germany)-labeled cDNA probe was cut with *Eco*RI after subcloning of a 169-bp fragment of *mfgl2* cDNA, representing nucleotides 756 (ACTGTGACA...) to 924 (...GAG-TAAGGA), into pCR2.1 vector (Invitrogen Life Technologies). The Dig-UTP-labeled probe concentration was determined by immunoenzymatic reaction with chemiluminescence detection, and the probes were used for hybridization as reported previously.

Immunohistochemical staining

Immunohistochemical staining was performed with a rabbit polyclonal antibody made against the *fgl2* prothrombinase or with a polyclonal antibody against fibrinogen (Dako, Glostrup, Denmark) as described previously (Ding *et al*., 1997; Marsden *et al*., 2003). Affinity-purified polyclonal antibody to both murine and human *fgl2* prothrombinase was produced in rabbits by repeated injections with a 14-amino acid hydrophilic peptide (CKLQADDHRDPGGN) from the exon 1-encoded portion of *fgl2* prothrombinase, which had been coupled to keyhole limpet hemocyanin. Immunohistochemical staining was carried out as described previously.

Western blot analysis

Cellular extracts were obtained from treated RAW 264.7 cells posttransfection. IFN-γ-treated and nontreated cells were used as positive and negative controls, respectively. Twenty micrograms of cellular extract was boiled for 5 min in 2× sodium dodecyl sulfate (SDS) buffer containing 20% dithiothreitol (DTT) and resolved by sodium dodecyl sulfate–polyacrylamide gel electrophoresis (SDS–PAGE). The resolved proteins were transferred to a nitrocellulose membrane. The membrane was blocked in 5% nonfat milk–0.05% Tween 20–PBS for 2 hr at room temperature with shaking and then probed for 1 hr at room temperature with shaking, using polyclonal antibody against *mfgl2* prothrombinase (1:500 dilution). The membranes were rinsed twice with PBS and washed six times with 0.05% Tween 20–PBS for 5 min with shaking. The membrane was probed with a horseradish peroxidase-labeled goat anti-rabbit secondary antibody (Santa Cruz Biotechnology, Santa Cruz, CA) for 1 hr with shaking before being rinsed twice with PBS and washed six times in 0.05% Tween 20–PBS for 5 min. The blots were developed by chemiluminescence for 2 min and exposed to X-Omat blue film (PerkinElmer Life and Analytical Sciences, Boston, MA) for 1 sec to 30 min.

PCA assay

Treated RAW 264.7 cells were evaluated for functional *mfgl2* prothrombinase activity in a one-stage clotting assay, as

previously described (Ning *et al*., 1998). Briefly, after incubation, samples were washed three times with unsupplemented RPMI 1640 and resuspended to a final concentration of 10^6 cells/ml. After being frozen and thawed three times, samples were assayed for the ability to shorten the spontaneous clotting time of normal citrated human platelet-poor plasma. Milliunits of activity were assigned by reference to a standard curve generated with serial log dilutions of a standard of rabbit brain thromboplastin (Dade Behring, Deerfield, IL).

Statistical analysis

Quantitative data were expressed as means ± SD. Statistical analysis was carried out by one-way analysis of variance, and a p value less than 0.05 was considered statistically significant.

RESULTS

Construction of mfgl2 *antisense and sense plasmid*

The *mfgl2* antisense plasmid and *mfgl2* sense plasmid were successfully constructed as evidenced by the restriction enzyme mapping shown in Fig. 1 and further confirmed by sequence analysis.

Efficient transfection of plasmid into RAW 264.7 cells and liver

To evaluate transfection efficiency, the pEGFP plasmid (Clontech, Palo Alto, CA) was cotransfected with other constructs as indicated. Fluorescent signal was detected by fluorescence microscopy. Fluorescence-positive cells and total cells were counted in 10 randomly selected fields. Transfection efficiency was calculated to be more than 90% as showed in Fig. 2A and B.

To evaluate transfection efficiency and the location of DNA plasmid in liver *in vivo*, 100 μg of pCMV-LacZ plasmid (Invitrogen Life Technologies) was delivered twice by hydrodynamic injection via the tail vein at time 0 and 24 hr later. Liver samples were collected 12, 24, 48, and 96 hr after the second injection and stained with 5-bromo-4-chloro-3-indolyl-β-D-galactopyranoside (X-Gal). A time course study (data not shown) displayed the transfection efficiency peaks 48 hr after the second injection, and the estimated transfection efficiency was about 40% as shown by blue-colored cells in Fig. 2C and D. The uptake of plasmid by hydrodynamic injection was randomly distributed and mainly within the endothelial cells, hepatocytes, and Kupffer cells around hepatic sinusoid. A trace amount of pCMV-LacZ expression was also detected in kidney, brain, and cardiac tissue (data not shown). pcDNA vectors

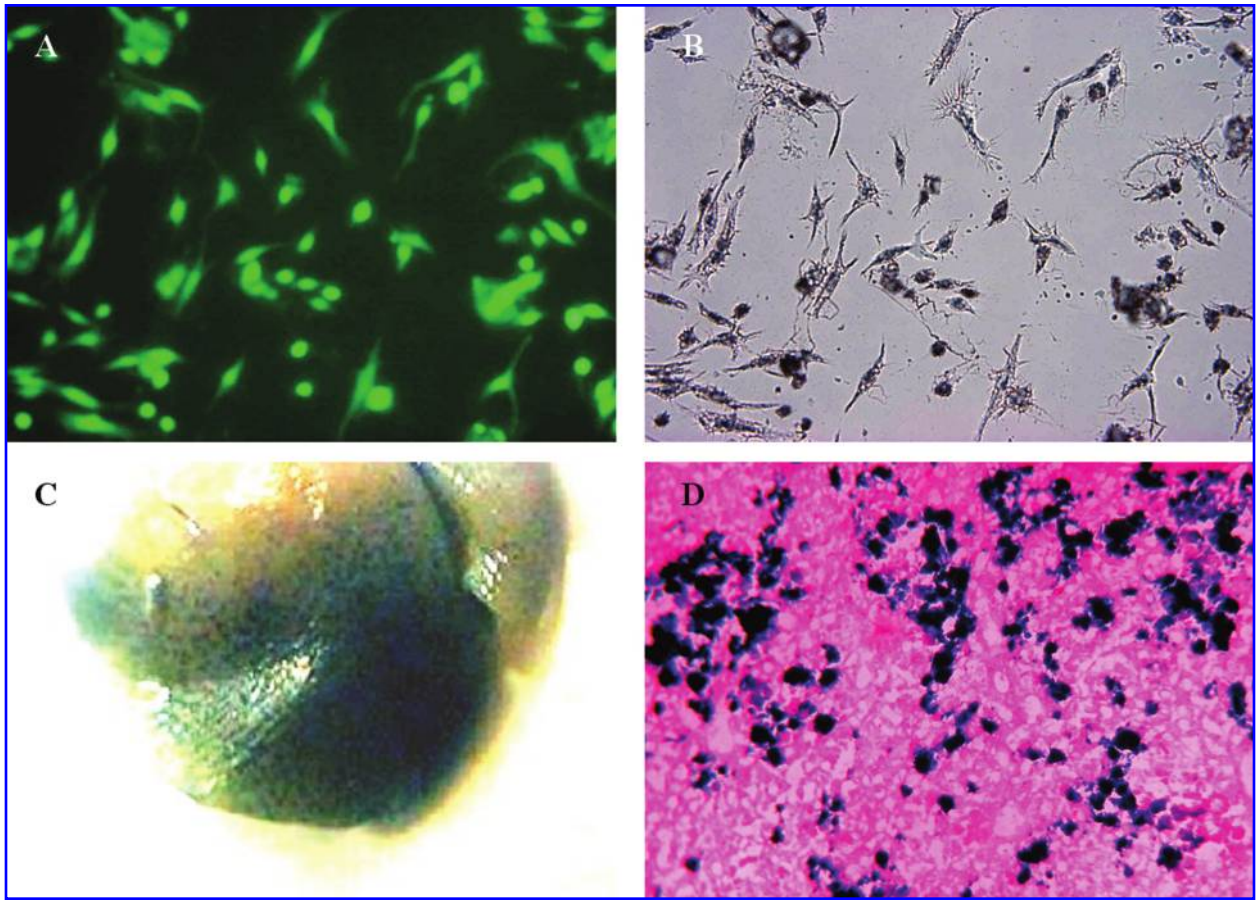


FIG. 2. Evaluation of transfection efficiency by pEGFP plasmid expression *in vitro* and pCMV-LacZ *in vivo*. pEGFP plasmid-transfected cells were examined by fluorescence microscopy (**A**) and light microscopy (**B**) within the same visualized area. (**C** and **D**) Bulk liver (**C**) and a liver tissue section (**D**) from pCMV-LacZ-transfected BALB/cJ mice, stained with X-Gal.

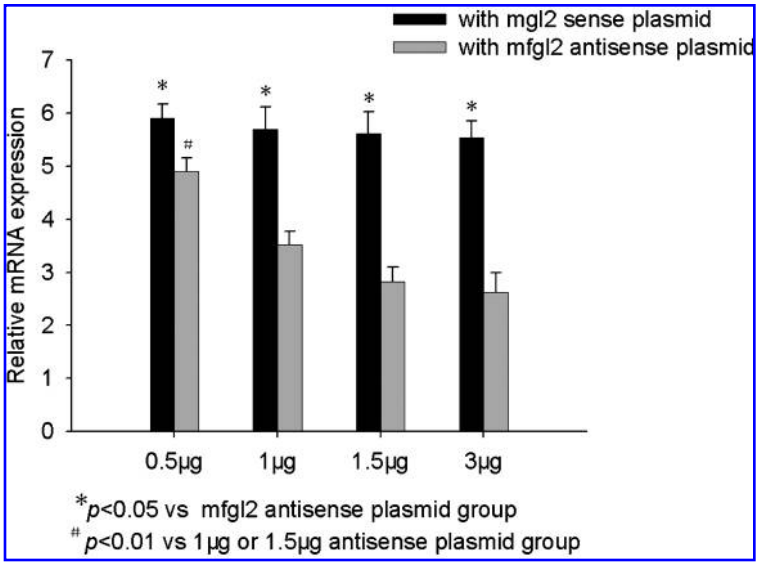
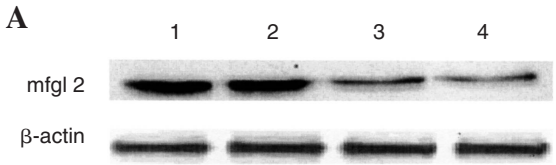


FIG. 3. Dose-dependent inhibitory effect of *mfgl2* antisense plasmid on *mfgl2* expression *in vitro*, determined by quantitative real-time PCR. *mfgl2* antisense plasmid or sense plasmid (0.5, 1.0, 1.5, and 3.0 μg), with Lipofectamine according to the manufacturer's standard protocol, was added to RAW 264.7 cells at 50–70% confluence. IFN-γ (100 μg/ml) was added to induce *mfgl2* gene expression. Twenty-four hours later cells were harvested and measured for *mfgl2* expression by quantitative real-time PCR. Values represent the mean and SD of three separate experiments done in triplicate. $^{*}p < 0.05$ compared with *mfgl2* antisense plasmid group; $^{\#}p < 0.01$ compared with 1.0 or 1.5 μg of *mfgl2* antisense plasmid group.

are suitable for transfection of mouse liver, and the vector itself induced no immune response on the basis of lymphocyte influx (Yin *et al*., 2002; Christ *et al*., 2004).

Effect of mfgl2 *antisense plasmid on* mfgl2 *expression* in vitro

Studies were undertaken with *mfgl2* antisense plasmid for its ability to interfere with IFN-γ-induced *mfgl2* expression in a cell culture system. A dose titration for IFN-γ and a time course study were performed and indicated that IFN-γ at 100 μg/ml gave the maximal *mfgl2* mRNA level by RT-PCR, and by 72 hr *mfgl2* mRNA levels decreased (data not shown). The *mfgl2* antisense plasmid significantly and maximally inhibited IFN-γ-induced *mfgl2* expression at 24 hr posttransfection (data not shown). A study of the dose-dependent inhibitory effect on *mfgl2* expression by *mfgl2* antisense plasmid was performed and the results show that 1.5 μg of *mfgl2* antisense plasmid can maximally inhibit IFN-γ-induced *mfgl2* expression (Fig. 3).



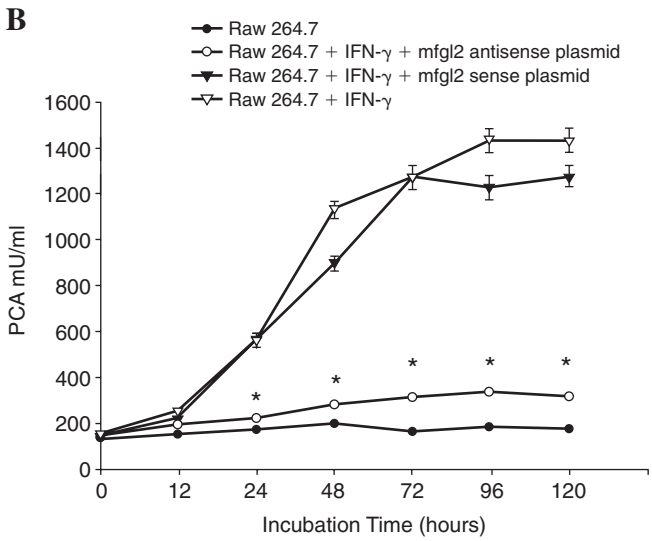


FIG. 4. Effect of *mfgl2* antisense plasmid on *mfgl2* protein expression in RAW 264.7 cells. (**A**) Western blot analysis. Lane 1, RAW 264.7 cells plus IFN-γ (100 μg/ml); lane 2, RAW 264.7 cells plus IFN-γ (100 μg/ml) plus *mfgl2* sense plasmid; lane 3, RAW 264.7 cells plus IFN-γ (100 μg/ml) plus *mfgl2* antisense plasmid; lane 4, RAW 264.7 cells. (**B**) *mfgl2* prothrombinase functional assay (PCA). Treated RAW 264.7 cells were incubated for the indicated times as described in Materials and Methods and harvested for measurement of PCA activity. Values represent means ± SD of three separate experiments done in triplicate. $^{*}p < 0.01$ compared with the *mfgl2* sense plasmid group and with the no-plasmid control group.

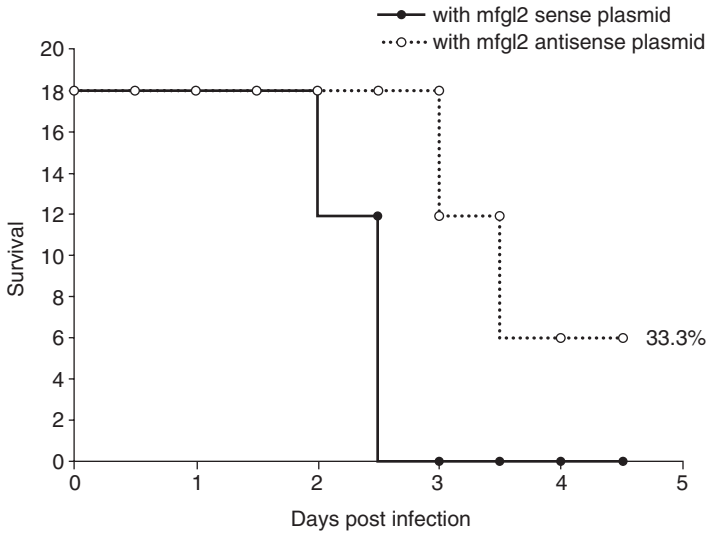


FIG. 5. Effect of *mfgl2* antisense plasmid on the survival of MHV-3-infected BALB/cJ mice. *mfgl2* antisense plasmid or *mfgl2* sense plasmid was introduced into BALB/cJ mice; the mice were then infected as described in Materials and Methods. Survival data are presented.

To further confirm that the *mfgl2* antisense plasmid inhibitory effect was also seen at the *mfgl2* prothrombinase protein level, we performed Western blot and functional clotting assays. IFN-γ-induced and cotransfected RAW 264.7 cells were harvested 24 hr posttreatment and the data showed that *mfgl2* antisense plasmid resulted in a marked decrease in *mfgl2* protein (Fig. 4A). A time course study of the inhibition of functional activity of *mfgl2* prothrombinase as shown by clotting assay of procoagulant activity (PCA) indicated a significant diminishment of protein activity at 24 hr posttransfection; the inhibitory effects lasted until 120 hr (end of observation) (Fig. 4B). *mfgl2* sense plasmid alone failed to inhibit *mfgl2* expression (Fig. 4B).

Reduced lethality and improved liver pathology in MHV-3-induced fulminant hepatitis on treatment with mfgl2 *antisense plasmid*

To further investigate the biological effect of *mfgl2* antisense plasmid *in vivo*, *mfgl2* antisense plasmid, and as a control *mfgl2* sense plasmid, were delivered twice by hydrodynamic injection into 18 MHV-3-infected BALB/cJ mice. All 18 mice with *mfgl2*

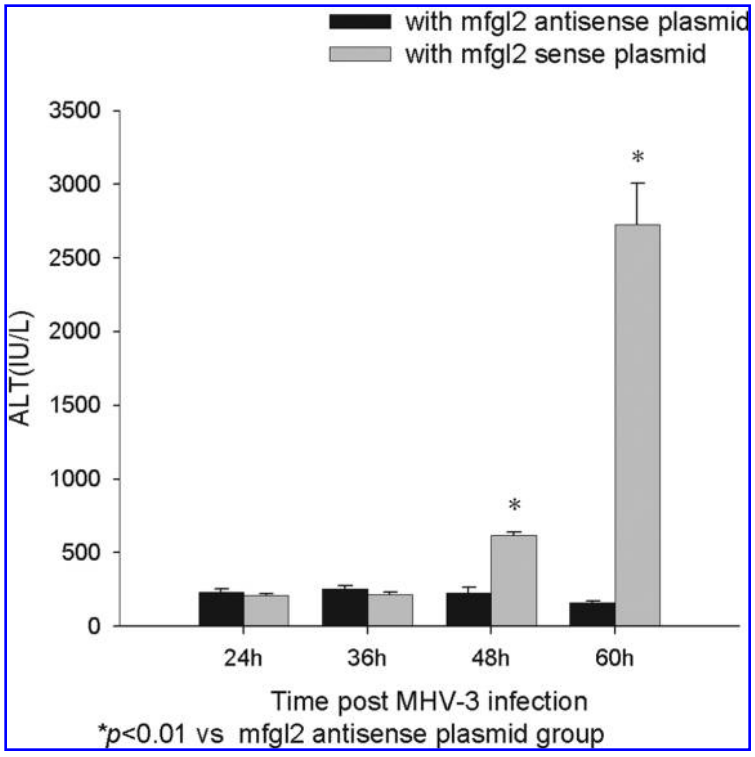


FIG. 6. Effect of *mfgl2* antisense plasmid on the serum ALT level of MHV-3-infected BALB/cJ mice. *mfgl2* antisense plasmid and *mfgl2* sense plasmid were each dissolved in 2 ml of PBS, and introduced into BALB/cJ mice by hydrodynamic injection twice. Mice then received 20 PFU of MHV-3 intraperitoneally to develop fulminant viral hepatitis. We determined ALT levels in the two groups of mice. Values represent means and SD of three separate experiments done in triplicate. *$p < 0.01$ compared with *mfgl2* antisense plasmid group.

antisense plasmid were alive on day 3 postinfection, and 6 of 18 mice (33.3%) recovered from fulminant viral hepatitis (Fig. 5). In contrast, no mice in the *mfgl2* sense plasmid-treated group survived to day 3 (Fig. 5), nor did mice in the nontreatment group (data not shown).

To better understand the mechanism behind the biological effects of the *mfgl2* antisense plasmid, a time course study of liver function (serum ALT level) and liver histology of MHV-3-infected mice was applied. There was no significant difference in serum ALT level between the *mfgl2* antisense and sense plasmid groups 24 and 36 hr after MHV-3 infection. Serum ALT levels started to increase 48 hr postinfection both in *mfgl2* sense plasmid-treated mice (Fig. 6) and nontreated MHV-3-infected BALB/cJ mice (data not shown). However, ALT levels in *mfgl2* antisense plasmid-treated MHV-3-infected BALB/cJ mice were significantly decreased compared with the *mfgl2* sense plasmid-treated group 48 and 60 hr postinfection. To determine the influence of hydrodynamic injection on liver function, a separate group of MHV-3-infected BALB/cJ mice was injected with saline; elevation of the ALT level during the first 24 hr returned to nearly normal levels 48 hr after MHV-3 infection (data not shown).

Liver histology was examined by hematoxylin and eosin (H&E) staining at the indicated times. More than 10 low-power field microscopic examinations were carried out for each slide. There were rare or noninfiltrating inflammatory cells, and rare or nonhepatocyte necrosis, in the liver of both groups 24 hr after MHV-3 infection (data not shown), and in *mfgl2* antisense plasmid-treated mice 36 hr after MHV-3 infection (Fig. 7B). However, by 36 hr after MHV-3 infection, mild to moderate inflammatory cell infiltration and spotty necrosis were evident in the hepatic lobules of mice treated with *mfgl2* sense plasmid (Fig. 7A) or nontreated mice (data not shown). By 48 to 60 hr after MHV-3 infection only mild inflammatory cell infiltration (Fig. 7D), or noninfiltration of inflammatory cells and no evidence of hepatocyte necrosis (Fig. 7D and F), were shown in *mfgl2* antisense-treated mice compared with the display of focal, even bridge necrosis with massive inflammatory cell infiltration within the liver of *mfgl2* sense plasmid-treated mice (Fig. 7C and E). These data strongly suggested that *mfgl2* antisense plasmid improved the pathology in MHV-3-induced fulminant viral hepatitis; in some cases the pathological process was reversed, allowing the animal to recover from this disease.

Effect of mfgl2 *antisense plasmid on* mfgl2 *expression and related fibrin deposition* in vivo

To evaluate the inhibitory effects of *mfgl2* antisense plasmid on *mfgl2* expression *in vivo*, a time course study of the effects was performed by real-time fluorescence quantitative RT-PCR, using liver samples from MHV-3-infected mice 24, 36, 48, and 60 hr postinfection. It was evident that the inhibitory effect of *mfgl2* antisense plasmid began 24 hr postinjection, with increasing effect (to 68%) until 60 hr postinfection (Fig. 8A). These data were further confirmed by *in situ* hybridization, using a 169-nucleotide sequence from the conserved carboxyl end-encoding region of the gene, and by immune histochemistry, using a specific polyclonal antibody against *mfgl2* protein. Results indicated none or rare *mfgl2* expression in the liver, with rare or mild inflammation in antisense plasmid-treated mice compared with *mfgl2* sense plasmid-treated mice, with *mfgl2*-expressing cells in areas of focal or even bridging necrosis and liver vasculature easily visible (Fig. 8B and C). There was significant reduction of fibrin deposition in liver tissues from mice with *mfgl2* antisense plasmid treatment compared with that from *mfgl2* sense plasmid-treated mice (Fig. 8D).

DISCUSSION

Chronic infection with hepatitis B virus affects an estimated 350 million people on a global basis, especially in Asia, where there is an HBV chronic carrier rate of 10–15% (Pawlotsky, 2002; Cheng *et al*., 2003; Higuchi and Gores, 2003; Nakamoto and Kaneko, 2003). Although most carriers do not develop hepatic complications from chronic hepatitis B, 15–40% will develop serious sequelae during their lifetime. The mechanisms for virus-induced hepatocyte injury are still not clearly known (Coleman, 2003; Higuchi and Gores, 2003; Nakamoto and Kaneko, 2003; Rhoads, 2003). This disease is characterized by recurrent flares of hepatocellular injury. Flares of marked chronic hepatitis are postulated to reflect T lymphocyte-mediated immune responses to the virus (Guidotti and Chisari, 2001). Intrahepatic production of IFN-γ is thought to figure prominently in how viral hepatitis B-specific T cells mediate hepatocellular injury, resulting in widespread hepatocyte necrosis (Hong *et al*., 2002). A number of investigators have described fibrin deposition in the livers of patients with chronic hepatitis (Kakimi *et al*., 2001; Leifeld *et al*., 2002). Early reports demonstrated that fibrin deposition was located mainly in the sinusoids of rats and mice after dimethylnitrosamine (DMN) intoxication, endotoxin administration, and liver transplantation and in the necrotic areas of animals after CCl_4 intoxication (Fujiwara *et al*., 1988; Mochida *et al*., 1990). It has been reported that sinusoidal fibrin deposition is characteristic in patients with fulminant viral hepatitis, but the deposition exists exclusively in necrotic areas of the liver in patients with chronic hepatitis (Rake *et al*., 1970). Our previous studies demonstrated that expression of human *fgl2* (*hfgl2*)/fibroleukin correlated highly with disease severity and fibrin deposition in severe acute or chronic hepatitis B or marked chronic hepatitis B, and in mice with fulminant hepatitis induced by MHV-3, but not in patients with mild chronic hepatitis B or HBV carriers. Fibrin deposition was evident both in sinusoids and in necrotic areas (Ding *et al*., 1997; Marsden *et al*., 2003). We further reported that the nucleocapsid protein of MHV-3 induced *mfgl2* gene expression and that the liver-specific transcription factor hepatocyte nuclear factor-4 (HNF4) is essential for this gene activation (Ning *et al*., 1999, 2003). Taken together with results reported from our group and many others, we propose that the pharmacological blockade of *fgl2*/fibroleukin may offer an important new therapeutic approach in hepatitis virus-induced disease.

In this paper, we examined whether antisense methodology could be employed to modulate *mfgl2* expression both *in vitro* and *in vivo* and eventually provide novel therapeutic strategies for fulminant viral hepatitis. To begin this direction of study, an *mfgl2* antisense plasmid was successfully constructed. A 322-bp fragment of antisense DNA under the control of the CMV promoter (pcDNA3.0) was designed to match the trans-

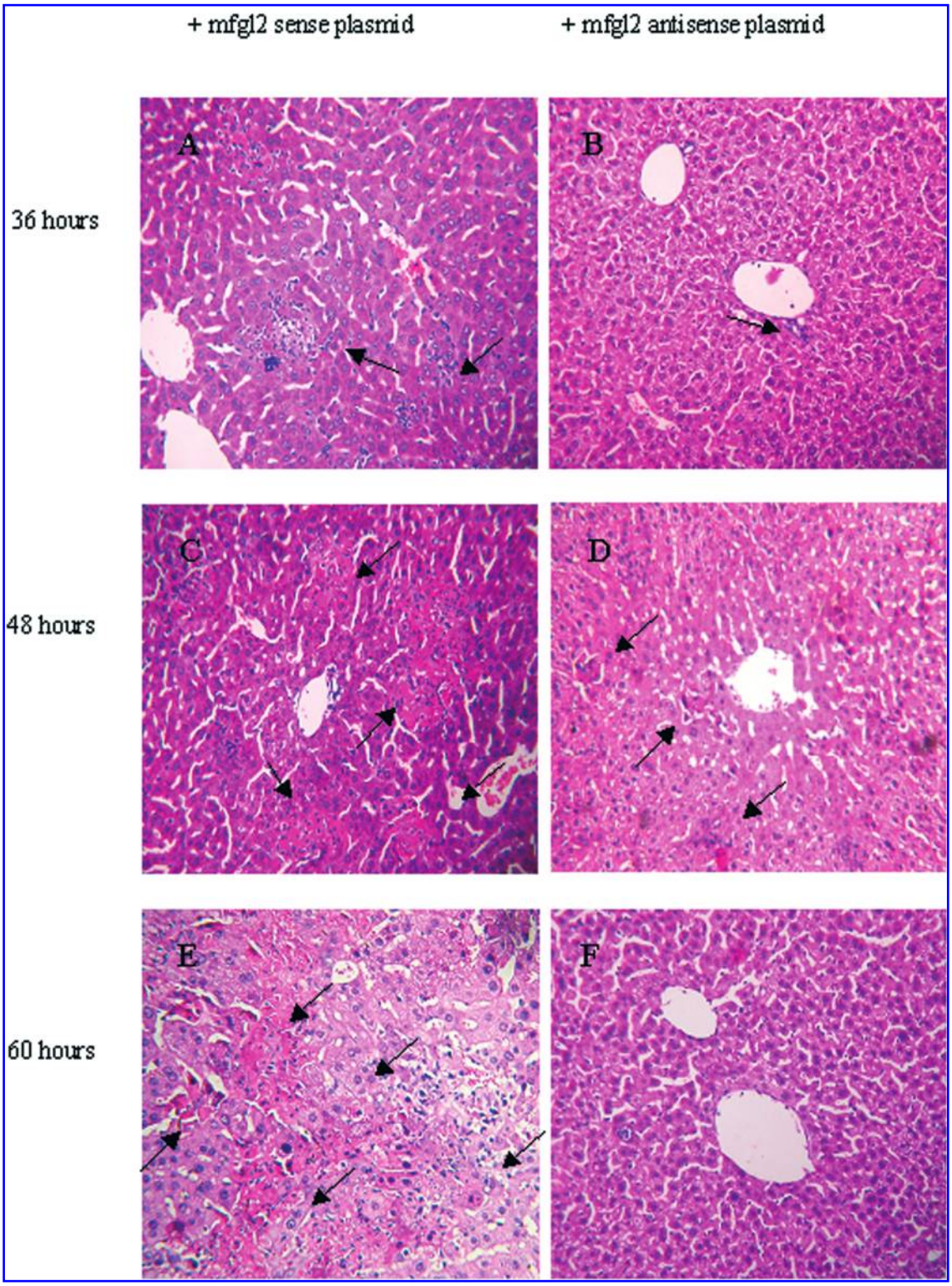


FIG. 7. Effect of *mfgl2* antisense plasmid on liver pathology in MHV-3-infected BALB/cJ mice. Livers were collected from *mfgl2* sense plasmid-treated (**A**, **C**, and **E**) and *mfgl2* antisense plasmid-treated (**B**, **D**, and **F**) BALB/cJ mice 36 hr (**A** and **B**), 48 hr (**C** and **D**), and 60 hr (**E** and **F**) after MHV-3 infection and pathologic characteristics were investigated by H&E staining. Original magnification, ×400. Arrows represent the necroinflammatory areas.

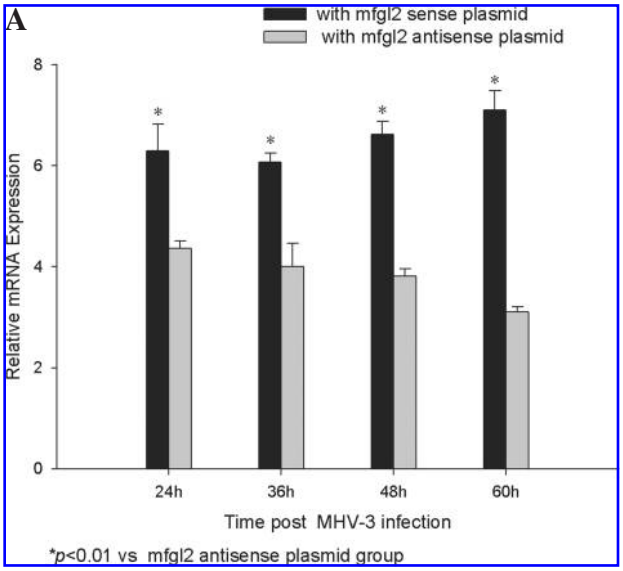
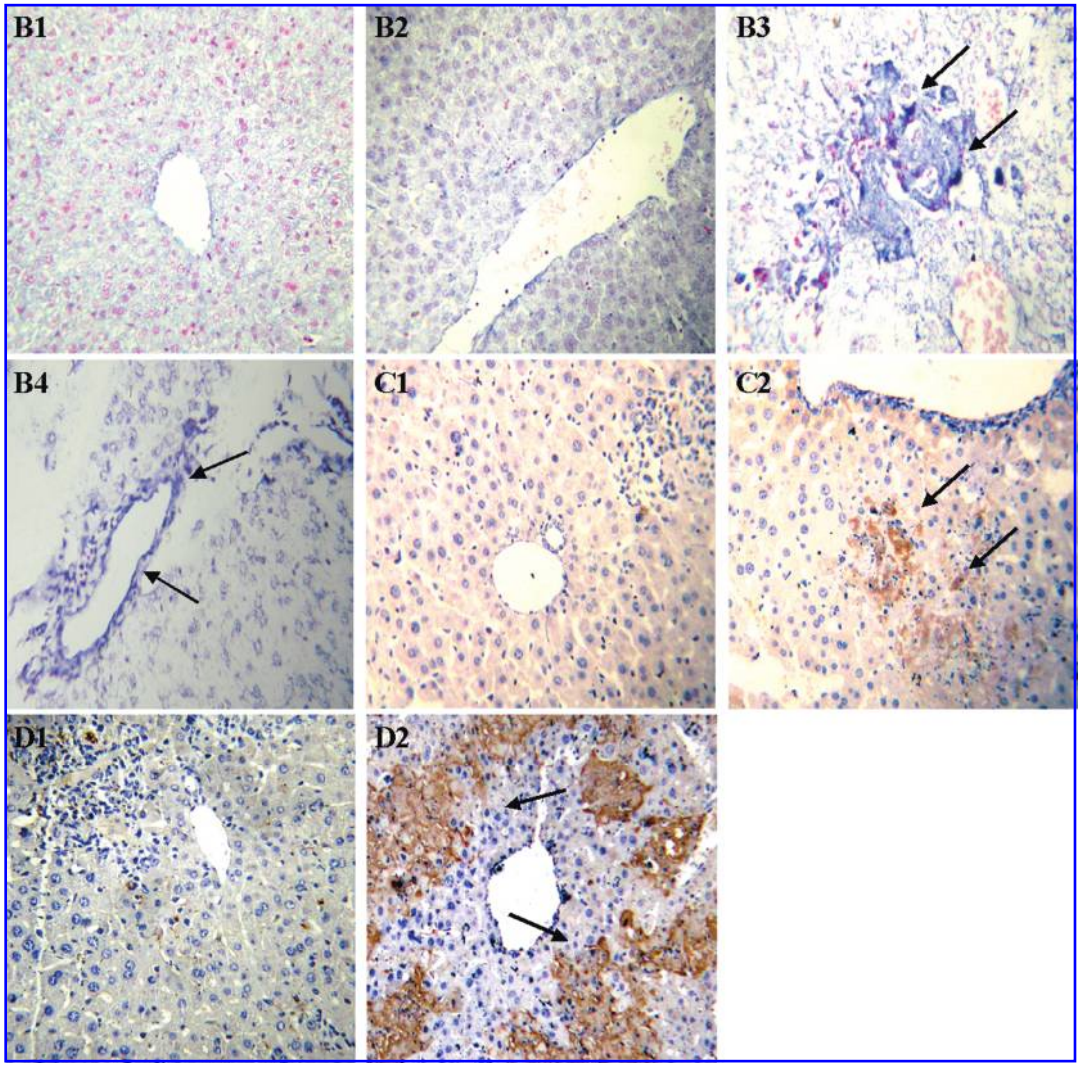


FIG. 8. Effect of *mfgl2* antisense plasmid on *mfgl2* expression and related fibrin deposition *in vivo*. (**A**) Real-time PCR analysis of *mfgl2* expression. Livers from *mfgl2* antisense plasmid-treated and *mfgl2* sense plasmid-treated BALB/cJ mice were collected 24, 36, 48, and 60 hr after MHV-3 infection. Values represent means and SD of three separate experiments done in triplicate. $*p < 0.01$ compared with *mfgl2* antisense plasmid group. (**B**) *In situ* hybridization analysis of *mfgl2* expression. Livers from *mfgl2* antisense-treated and *mfgl2* sense plasmid-treated BALB/cJ mice were collected 48 hr after MHV-3 infection, and *in situ* hybridization was performed for *mfgl2* expression. Rare or no *mfgl2* expression was evident in hepatic lobules (**B1**) and vasculature (**B2**) of liver tissue from *mfgl2* antisense-treated mice compared with marked *mfgl2* expression in hepatocyte necrosis area (**B3**, arrows) and vasculature (**B4**, arrows) in liver tissue in *mfgl2* sense plasmid-treated mice. Original magnification, ×200. (**C** and **D**) Immunohistochemistry analysis of *mfgl2* and fibrin. Livers from *mfgl2* antisense-treated (**C1** and **D1**) or *mfgl2* sense plasmid-treated (**C2** and **D2**) BALB/cJ mice were collected 48 hr after MHV-3 infection, respectively. Immunohistochemistry was performed for *mfgl2* expression (**C1** and **C2**) and fibrin deposition (**D1** and **D2**) as described. Original magnification, ×200. Arrows indicate the *mfgl2* markedly expressed cells (**C2**) and fibrin-stained cells (**D2**).



lation initiation site and part of exon 1 of *mfgl2*, which encodes the active site of this protein (Chan *et al*., 2002). To test the biological effect of *mfgl2* antisense plasmid both *in vitro* and *in vivo*, Lipofectamine-mediated transfection and tail vein hydrodynamic injection were performed, respectively. Efficient transfection (more than 90% in our hands) was achieved by Lipofectamine-mediated transfection, as observed in a cell culture system with plasmid pEGFP, which encodes enhanced green fluorescent protein. Approximately 40% gene transfection efficiency was obtained *in vivo* within seconds by the method of tail vein hydrodynamic injection of target gene(s), as shown by the X-Gal staining of pCMV-LacZ plasmid-transfected liver sections.

Activated macrophages have been reported to play a role in the pathogenesis of fulminant viral hepatitis both in humans and in mice (Ding *et al*., 1997; Marsden *et al*., 2003). It was demonstrated the activated macrophages highly expressed Fgl2 prothrombinase, which in turn cleaves prothrombin to thrombin, eventually leading to fibrin deposition. Our previous reports have shown that IFN-γ is a potent inducer of *mfgl2* both in primary macrophages and in the transformed RAW 264.7 cell line, which normally expresses only low basal levels of this gene (Liu *et al*., 2003). *In vitro* we were able to decrease IFN-γ-induced *mfgl2* expression by 78% with *mfgl2* antisense plasmid. This promising result led us to investigate its effects *in vivo*. Gene transfer of *mfgl2* antisense *in vivo* also resulted in a significant reduction of *mfgl2* expression and fibrin deposition, improvement of liver function and histology, prolongation of the survival time period, and elevation of the survival rate in BALB/cJ mice with MHV-3-induced fulminant hepatitis. The possible mechanisms behind these results are still an open question. It should be noted that activation of the coagulation cascade is an integral component of host inflammation. Experimental evidence in animal models also indicates that the coagulation cascade plays a crucial role in the outcome of inflammatory insults. Fibrin deposition simultaneously causes microvascular thrombosis, leukocyte accumulation, and upregulation of inflammatory responses. Silencing of the gene encoding Th1 immune coagulant *mfgl2* leads to a significant decrease in fibrin deposition in liver; this negative feedback in turn could result in the diminishment of inflammation or lymphocyte influx. Experiments need to be performed to provide the details of mechanisms leading to the biological effects described, including the function of macrophages and endothelium within the liver. A cytokine profile study, both local (liver) and systemic (serum), is under way in our laboratory. Gene delivery procedures will be optimized and a combination of antiviral medicine along with gene delivery of more than one cytokine antisense sequence is under consideration.

A crucial consideration in the design of an antisense inhibitor is specificity (Bestor, 2000; Cordeiro *et al*., 2003; Furling *et al*., 2003). One parameter that affects specificity is the relative accessibility to hybridization of the target sequence versus the accessibility of targets with undesired homology to the intended target. The target site for the 322-bp antisense plasmid was chosen because of its proximity to the AUG start codon and *in vitro* mapping had shown that this site was accessible to hybridization. To test the specificity of the 322-bp antisense plasmid, two additional reporter constructs were used. The first was pEGFP, which, like the basic plasmid pcDNA3.0, encodes a green fluorescence protein under the control of the CMV promoter. The second construct was *mfgl2* sense plasmid, which is identical to our antisense construct except that the latter carries the 322-bp *mfgl2* antisense DNA. These two constructs failed to inhibit *mfgl2* expression either at the level of transcription or translation (some of the data are shown in Figs. 4–7). These data provided additional evidence of the specificity of the 322-bp antisense DNA and indicated that *mfgl2* expression was specifically targeted by the *mfgl2* antisense DNA.

It should be noted that data are now emerging to support the hypothesis that, irrespective of the etiology of fulminant hepatic failure, the host immune response (including production of proinflammatory cytokines and mediators) contributes to microcirculatory disturbances that result in hypoxic injury and cell death (apoptosis). Given the complexity of this multidimensional disorder, the challenge is to provide a rational basis for treatment. This might include enhancement or suppression of immune responsiveness by manipulation of multiendogenous cytokine synthesis or by cytokine administration and, at the same time, use of strategies to increase hepatic regeneration. In our current study the survival rate at 60 hr posttreatment was as high as 66.7% and the overall survival rate was 33.33% after *mfgl2* antisense administration in MHV-3-induced fulminant hepatitis. The mice that died between 60 and 72 hr posttreatment displayed a later break of fulminant viral hepatitis with similar biochemistry and pathology when compared with mice in the control group (data not shown). This result indicated there are other target factors that need to be considered, and appropriate investigation is under way.

There has been significant interest in the use of RNA interference (RNAi) inhibition of gene expression (Bass, 2000; Dave and Pomerantz, 2003; Stevenson, 2004). A frequently asked question concerns whether RNAi has significant advantages over antisense. However, it has been described that if a sufficient amount of antisense DNA can be delivered to the appropriate subcellular compartment, antisense inhibition of gene expression can be as potent as RNAi (McCaffrey *et al*., 2003; Morgan *et al*., 2005; Mourich and Marshall, 2005). RNAi is thought to catalytically trigger degradation of target mRNAs before translation (Cordeiro *et al*., 2003). However, this degradation is not complete because residual translation is observed. Antisense DNA acts at a different step in gene expression, by blocking translational initiation. It is therefore possible that antisense and RNAi could act synergistically to specifically result in more efficient gene silencing. We have successfully constructed a small hairpin RNA for *mfgl2* and preliminary data have shown its promising inhibitory effect on *mfgl2* expression in a cell culture system. Studies are underway to investigate its biological effects *in vivo* and its synergistic action when used together with *mfgl2* antisense plasmid.

Here we have demonstrated that a novel *mfgl2* antisense plasmid can be successfully used to block the expression of *mfgl2* in a cell culture system and in an *in vivo* animal model. We further showed that the administration of *mfgl2* antisense plasmid ameliorates liver pathology including inflammatory infiltration, fibrin deposition, and hepatocyte necrosis. Furthermore, it prolonged the survival period and elevated the survival rate of MHV-3-infected BALB/cJ mice, which normally died of a fatal disease, fulminant hepatitis. This study may provide an effective way to interfere with the potential therapeutic target

mfgl2 gene for microcirculation disorders including fulminant hepatitis, severe or marked chronic viral hepatitis (acute or chronic viral hepatitis), and acute rejection of allograft transplantation, in which *mfgl2* plays an important role (Ning *et al*., 2005; Zhu *et al*., 2005).

ACKNOWLEDGMENTS

The authors thank Professor Gary Levy for supplying the *mfgl2* cDNA clone, and Ms. Jingshang Hu for secretarial assistance during the preparation of this manuscript. This work was supported by the National Science Fund for Distinguished Young Investigators (no. 30225040 for Q.N., no. 30123019 for X.P.L.), from the National Science Foundation of China (NSFC), NSFC operating fund 30100171, 30571643, National Key Basic Research Program of China (2005CB522901), National High Technology Program (2002AA302202, 2003DF000034, 2003CB514112), and the National 10th Five-Year Plan Key Project (2004BA720A01).

REFERENCES

BASS, B.L. (2000). Double-stranded RNA as a template for gene silencing. Cell **101,** 235–238.

BESTOR, T.H. (2000). Gene silencing as threat to the success of gene therapy. J. Clin. Invest. **105,** 409–411.

CAPLEN, N.J., PARRISH, S., IMANI, F., FIRE, A., and MORGAN, R.A. (2001). Specific inhibition of gene expression by small double-stranded RNAs in invertebrate and vertebrate systems. Proc. Natl. Acad. Sci. U.S.A. **98,** 9742–9747.

CHAN, C.W., CHAN, M.W., LIU, M., FUNG, L., COLE, E.H., LEIBOWITZ, J.L., MARSDEN, P.A., CLARK, D.A., and LEVY, G.A. (2002). Kinetic analysis of a unique direct prothrombinase, fgl2, and identification of a serine residue critical for the prothrombinase activity. J. Immunol. **168,** 5170–5177.

CHENG, V.C., LO, C.M., and LAU, G.K. (2003). Current issues and treatment of fulminant hepatic failure including transplantation in Hong Kong and the Far East. Semin. Liver Dis. **23,** 239–250.

CHRIST, G.J., DAY, N., SANTIZO, C., SATO, Y., ZHAO, W., SCLAFANI, T., BAKAL, R., SALMAN, M., DAVIES, K., and MELMAN, A. (2004). Intracorporal injection of hSlo cDNA restores erectile capacity in STZ-diabetic F-344 rats *in vivo*. Am. J. Physiol. Heart Circ. Physiol. **287,** H1544–H1553.

COLEMAN, W.B. (2003). Mechanisms of human hepatocarcinogenesis. Curr. Mol. Med. **3,** 573–588.

CORDEIRO, M.F., MEAD, A., ALI, R.R., ALEXANDER, R.A., MURRAY, S., CHEN, C., YORK-DEFALCO, C., DEAN, N.M., SCHULTZ, G.S., and KHAW, P.T. (2003). Novel antisense oligonucleotides targeting TGF-β inhibit *in vivo* scarring and improve surgical outcome. Gene Ther. **10,** 59–71.

CROOKE, S.T. (2001). Basic principles of antisense technology. In: Crooke, S.T., ed. *Antisense Drug Technology: Principles, Strategies and Applications*. (Marcel Dekker, New York) pp. 1–28.

DAVE, R.S., and POMERANTZ, R.J. (2003). RNA interference: On the road to an alternate therapeutic strategy! Rev. Med. Virol. **13,** 373–385

DING, J.W., NING, Q., LIU, M.F., LAI, A., LEIBOWITZ, J., PELTEKIAN, K.M., COLE, E.H., FUNG, L.S., HOLLOWAY, C., MARSDEN, P.A., YEGER, H., PHILLIPS, M.J., and LEVY, G.A. (1997). Fulminant hepatic failure in murine hepatitis virus strain 3 infection: Tissue-specific expression of a novel fgl2 prothrombinase. J. Virol. **71,** 9223–9230.

DING, J.W., NING, Q., LIU, M.F., LAI, A., PELTEKIAN, K., FUNG, L., HOLLOWAY, C., YEGER, H., PHILLIPS, M.J., and LEVY, G.A. (1998). Expression of the *fgl2* and its protein product (prothrombinase) in tissues during murine hepatitis virus strain-3 (MHV-3) infection. Adv. Exp. Med. Biol. **440,** 609–618.

FUJIWARA, K., OGATA, I., OHTA, Y., HIRATA, K., OKA, Y., YAMADA, S., SATO, Y., MASAKI, N., and OKA, H. (1988). Intravascular coagulation in acute liver failure in rats and its treatment with antithrombin III. Gut **29,** 1103–1108.

FUNG, L.S., NEIL, G., LEIBOWITZ, J., COLE, E.H., CHUNG, S., CROW, A., and LEVY, G.A. (1991). Monoclonal antibody analysis of a unique macrophage procoagulant activity induced by murine hepatitis virus strain 3 infection. J. Biol. Chem. **266,** 1789–1795.

FURLING, D., DOUCET, G., LANGLOIS, M.A., TIMCHENKO, L., BELANGER, E., COSSETTE, L., and PUYMIRAT, J. (2003). Viral vector producing antisense RNA restores myotonic dystrophy myoblast functions. Gene Ther. **10,** 795–802.

GUIDOTTI, L.G., and CHISARI, F.V. (2001). Noncytolytic control of viral infections by the innate and adaptive immune response. Annu. Rev. Immunol. **19,** 65–91.

HIGUCHI, H., and GORES, G.J. (2003). Mechanisms of liver injury: An overview. Curr. Mol. Med. **3,** 483–490.

HONG, F., JARUGA, B., KIM, W.H., RADAEVA, S., EL-ASSAL, O.N., TIAN, Z., NGUYEN, V.A., and GAO, B. (2002). Opposing roles of STAT1 and STAT3 in T cell-mediated hepatitis: Regulation by SOCS. J. Clin. Invest. **110,** 1503–1513.

HOOFNAGLE, J.H., CARITHERS, R.L., JR., SHAPIRO, C., and ASCHER, N. (1995). Fulminant hepatic failure: Summary of a workshop. Hepatology **21,** 240–252.

KAKIMI, K., LANE, T.E., WIELAND, S., ASENSIO, V.C., CAMPBELL, I.L., CHISARI, F.V., and GUIDOTTI, L.G. (2001). Blocking chemokine responsive to γ-2/interferon (IFN)-γ inducible protein and monokine induced by IFN-γ activity *in vivo* reduces the pathogenetic but not the antiviral potential of hepatitis B virus-specific cytotoxic T lymphocytes. J. Exp. Med. **194,** 1755–1766.

LEIFELD, L., CHENG, S., RAMAKERS, J., DUMOULIN, F.L., TRAUTWEIN, C., SAUERBRUCH, T., and SPENGLER, U. (2002). Imbalanced intrahepatic expression of interleukin 12, interferon γ, and interleukin 10 in fulminant hepatitis B. Hepatology **36,** 1001–1008.

LIU, M., LEIBOWITZ, J.L., CLARK, D.A., MENDICINO, M., NING, Q., DING, J.W., D'ABREO, C., FUNG, L., MARSDEN, P.A., and LEVY, G.A. (2003). Gene transcription of fgl2 in endothelial cells is controlled by Ets-1 and Oct-1 and requires the presence of both Sp1 and Sp3. Eur. J. Biochem. **270,** 2274–2286.

LIU, M.F., CHAN, W.Y., McGILVRAY, I.D., NING, Q., and LEVY, G.A. (2001). Fulminant viral hepatitis: Molecular and cellular basis, and clinical implications. Expert Rev. Mol. Med. **3,** 1–19.

MARSDEN, P.A., NING, Q., FUNG, L.S., LUO, X., CHEN, Y., MENDICINO, M., GHANEKAR, A., SCOTT, J.A., MILLER, T., CHAN, C.W., CHAN, M.W., HE, W., GORCZYNSKI, R.M., GRANT, D.R., CLARK, D.A., PHILLIPS, M.J., and LEVY, G.A. (2003). The Fgl2/fibroleukin prothrombinase contributes to immunologically-mediated thrombosis in experimental and human viral hepatitis. J. Clin. Invest. **112,** 58–66.

McCAFFREY, A.P., MEUSE, L., KARIMI, M., CONTAG, C.H., and KAY, M.A. (2003). A potent and specific morpholino antisense inhibitor of hepatitis C translation in mice. Hepatology **38,** 503–508.

MOCHIDA, S., OGATA, I., HIRATA, K., OHTA, Y., YAMADA, S., and FUJIWARA, K. (1990). Provocation of massive hepatic necrosis by endotoxin after partial hepatectomy in rats. Gastroenterology **99,** 771–777.

MONDELLI, M., and EDDLESTON, A.L. (1984). Mechanisms of liver cell injury in acute and chronic hepatitis B. Semin. Liver Dis. **4,** 47–58.

MORGAN, R.A., WALKER, R., CARTER, C.S., NATARAJAN, V.,

TAVEL, J.A., BECHTEL, C., HERPIN, B., MUUL, L., ZHENG, Z., JAGANNATHA, S., BUNNELL, B.A., FELLOWES, V., METCALF, J.A., STEVENS, R., BASELER, M., LEITMAN, S.F., READ, E.J., BLAESE, R.M., and LANE, H.C. (2005). Preferential survival of $CD4^+$ T lymphocytes engineered with anti-human immunodeficiency virus (HIV) genes in HIV-infected individuals. Hum. Gene Ther. **16,** 1065–1074.

MOURICH, D.V., and MARSHALL, N.B. (2005). Antisense approaches to immune modulation for transplant and autoimmune diseases. Curr. Opin. Pharmacol. **5,** 508–512.

NAKAMOTO, Y., and KANEKO, S. (2003). Mechanisms of viral hepatitis induced liver injury. Curr. Mol. Med. **3,** 537–544.

NING, Q., BROWN, D., PARODO, J., CATTRAL, M., GORCZYNSKI, R., COLE, E., FUNG, L., DING, J.W., LIU, M.F., ROTSTEIN, O., PHILLIPS, M.J., and LEVY, G.A. (1998). Ribavirin inhibits viral-induced macrophage production of TNF, IL-1, the procoagulant fgl2 prothrombinase and preserves Th1 cytokine production but inhibits Th2 cytokine response. J. Immunol. **160,** 3487–3493.

NING, Q., LIU, M., KONGKHAM, P., LAI, M.M., MARSDEN, P.A., TSENG, J., PEREIRA, B., BELYAVSKYI, M., LEIBOWITZ, J., PHILLIPS, M.J., and LEVY, G. (1999). The nucleocapsid protein of murine hepatitis virus type 3 induces transcription of the novel fgl2 prothrombinase gene. J. Biol. Chem. **274,** 9930–9936.

NING, Q., LAKATOO, S., LIU, M., YANG, W., WANG, Z., PHILLIPS, M.J., and LEVY, G.A. (2003). Induction of prothrombinase fgl2 by the nucleocapsid protein of virulent mouse hepatitis virus is dependent on host hepatic nuclear factor-4α. J. Biol. Chem. **278,** 15541–15549.

NING, Q., SUN, Y., HAN, M., ZHANG, L., ZHU, C., ZHANG, W., GUO, H., LI, J., YAN, W., GONG, F., CHEN, Z., HE, W., KOSCIK, C., SMITH, R., GORCZYNSKI, R., LEVY, G., and LUO, X. (2005). Role of fibrinogen-like protein 2 prothrombinase/fibroleukin in experimental and human allograft rejection. J. Immunol. **174,** 7403–7411.

O'GRADY, J.G., and WILLIAMS, R. (1986). Management of acute liver failure. Schweiz Med. Wochenschr. **116,** 541–544.

PAWLOTSKY, J.M. (2002). Molecular diagnosis of viral hepatitis. Gastroenterology **122,** 1554–1568.

RAKE, M.O., FLUTE, P.T., PANNELL, G., and WILLIAMS, R. (1970). Intravascular coagulation in acute hepatic necrosis. Lancet **14,** 533–537.

RHOADS, J. (2003). Natural history and epidemiology of hepatitis C. J. Assoc. Nurses AIDS Care **14**(5 Suppl.)**,** 18S–25S.

STEPHENSON, M.L., and ZAMECNIK, P.C. (1978). Inhibition of Rous sarcoma viral RNA translation by a specific oligodeoxyribonucleotide. Proc. Natl. Acad. Sci. U.S.A. **75,** 285–288.

STEVENSON, M. (2004). Therapeutic potential of RNA interference. N. Engl. J. Med. **351,** 1772–1777.

TAMM, I., DORKEN, B., and HARTMANN, G. (2001). Antisense therapy in oncology: New hope for an old idea? Lancet **358,** 489–497.

WEISS, B., DAVIDKOVA, G., and ZHOU, L.W. (1999). Antisense RNA gene therapy for studying and modeling biological processes. Cell Mol. Life Sci. **55,** 334–358.

YACYSHYN, B.R., BOWEN-YACYSHYN, M.B., JEWELL, L., TAMI, J.A., BENNETT, C.F., KISNER, D.L., and SHANAHAN, W.R., Jr. (1998). A placebo-controlled trial of ICAM-1 antisense oligonucleotide in the treatment of Crohn's disease. Gastroenterology **114,** 1133–1142.

YIN, X., YUTANI, C., IKEDA, Y., ENJYOJI, K., ISHIBASHI-UEDA, H., YASUDA, S., TSUKAMOTO, Y., NONOGI, H., KANEDA, Y., and KATO, H. (2002). Tissue factor pathway inhibitor gene delivery using HVJ-AVE liposomes markedly reduces restenosis in atherosclerotic arteries. Cardiovasc. Res. **56,** 454–463.

ZHU, C.L., YAN, W.M., ZHU, F., ZHU, Y.F., XI, D., TIAN, D.Y., LEVY, G.A., LUO, X.P., and NING, Q. (2005). Fibrinogen like protein2/fibroleukin expression and its correlation with disease progression in murine hepatitis virus type 3-induced fulminant hepatitis and in patients with severe viral hepatitis B. World J. Gastroenterol. **11,** 6936–6940.

Address reprint requests to:
Dr. Qin Ning
Laboratory of Infectious Immunology
Department of Infectious Disease
Tongji Hospital
1095 JieFang Avenue
Wuhan 430030, China

E-mail: qning@tjh.tjmu.edu.cn

Received for publication September 26, 2005; accepted after revision February 21, 2006.

Published online: May 23, 2006.

This article has been cited by:

1. Sui Gao , Ming Wang , Huali Ye , Jianwen Guo , Dong Xi , Zhimo Wang , Chuanglong Zhu , Weiming Yan , Xiaoping Luo , Qin Ning . 2010. Dual Interference with Novel Genes mfgl2 and mTNFR1 Ameliorates Murine Hepatitis Virus Type 3-Induced Fulminant Hepatitis in BALB/cJ MiceDual Interference with Novel Genes mfgl2 and mTNFR1 Ameliorates Murine Hepatitis Virus Type 3-Induced Fulminant Hepatitis in BALB/cJ Mice. *Human Gene Therapy* **21**:8, 969-977. [Abstract] [Full Text] [PDF] [PDF Plus]
2. Zeguang Wu, Meifang Han, Tao Chen, Weiming Yan, Qin Ning. 2010. Acute liver failure: mechanisms of immune-mediated liver injury. *Liver International* **30**:6, 782-794. [CrossRef]
3. Z. G. Wu, W. M. Yan, W. Guo, T. Chen, Y. Zou, H. W. Wang, X. J. Wang, X. J. Yang, Y. L. Lu, X. P. Luo, Q. Ning. 2010. Telbivudine preserves T-helper 1 cytokine production and downregulates programmed death ligand 1 in a mouse model of viral hepatitis. *Journal of Viral Hepatitis* **17**, 24-33. [CrossRef]
4. Sui Gao, Ming Wang, Jian-wen Guo, Dong Xi, Xiao-ping Luo, Qin Ning. 2010. Construction of mTNFR1shRNA plasmid and its biological effects on MHV-3 induced fulminant hepatitis in BALB/cJ mice. *Virologica Sinica* **25**:1, 52-58. [CrossRef]
5. Dong Xi, Zhi-Mo Wang, Sui Gao, Chuan-Long Zhu, Jian-Wen Guo, Xiao-Ping Luo, Qin Ning. 2007. Construction of shRNA of fulminant hepatitis related gene mfgl2 and investigation of its biological effects in vitro. *Virologica Sinica* **22**:5, 366-373. [CrossRef]