

# Hexadecyloxypropyl-cidofovir, CMX001, prevents adenovirus-induced mortality in a permissive, immunosuppressed animal model

Karoly Toth\*, Jacqueline F. Spencer\*, Debanjan Dhar\*, John E. Sagartz†, R. Mark L. Buller\*, George R. Painter‡, and William S. M. Wold\*<sup>§</sup>

\*Department of Molecular Microbiology and Immunology, Saint Louis University School of Medicine, 1100 South Grand Boulevard, St. Louis, MO 63104;

†Department of Comparative Medicine, Saint Louis University School of Medicine, 1402 South Grand Boulevard, St. Louis, MO 63104; and ‡Chimerix, Inc., 5007 Southpark Drive, Suite 200, Durham, NC 27713

Edited by Thomas E. Shenk, Princeton University, Princeton, NJ, and approved March 27, 2008 (received for review January 8, 2008)

**Adenoviruses (Ads) cause a wide array of end-organ and disseminated diseases in severely immunosuppressed patients. For example, ≈20% of pediatric allogeneic hematopoietic stem cell transplant recipients develop disseminated Ad infection, and the disease proves fatal in as many as 50–80% of these patients. Ad infections are a serious problem for solid-organ transplant recipients and AIDS patients as well. Unfortunately, there are no antiviral drugs approved specifically to treat these infections. A suitable animal model that is permissive for Ad replication would help in the discovery process. Here we identify an animal model to study Ad pathogenesis and the efficacy of antiviral compounds. We show that human serotype 5 Ad (Ad5) causes severe systemic disease in immunosuppressed Syrian hamsters that is similar to that seen in immunocompromised patients. We also demonstrate that hexadecyloxypropyl-cidofovir (CMX001) rescues the hamsters from a lethal challenge with Ad5. The antiviral drug provided protection both prophylactically and when given up to 2 days after i.v. exposure to Ad5. CMX001 acts by reducing Ad replication in key target organs. Thus, the immunosuppressed Syrian hamster is a powerful model to evaluate anti-Ad drugs, and its use can facilitate the entry of drugs such as CMX001 into clinical trials.**

antivirals | hamster

Adenoviruses (Ads) are widespread in the human population, with 52 known human serotypes (Ad1–Ad52) that are classified into seven species (A–G). In general, Ad1, Ad2, and Ad5 (species C), Ad3 and Ad7 (species B), and Ad4 (species E) cause respiratory illnesses, Ad40 and Ad41 (species F) cause enteric infections, and Ad8 and Ad37 (species D) cause eye infections (1). The infections are self-limiting in most people and result in lifelong immunity to the virus (1). Recently, Ad14, a species B Ad, emerged as a pathogen causing severe respiratory illness (2). Ads cause serious, often life-threatening illnesses in immunocompromised people (1, 3–6). The most severely affected group is pediatric hematopoietic stem cell transplant recipient patients. The most frequent posttransplantation complications in these children are pneumonia, associated with species A, B, and C Ads, and urinary infections and hepatitis, caused by species B and C Ads, respectively. Both species B and C Ads can cause disseminated disease in these patients that results in a 50–80% mortality rate.

To date there is no drug approved by the Food and Drug Administration (FDA) specifically to treat Ad infections. A key issue in identifying potential new drugs is the lack of a suitable animal model of systemic disease. Ads are fairly species-specific; thus, human Ads do not replicate in commonly used laboratory animals to a significant effect. Animal models exist to investigate topical Ad infection (7–10), but no systematic study has been performed in animals or in clinical trials to assess the effects of antiviral drug treatment on Ad replication and the pathology resulting from disseminated Ad infection. Most data on the

efficacy of antiviral compounds against Ad come from tissue culture experiments, individual case reports (3, 11–13), or small-scale prospective clinical studies (14). A promising anti-Ad drug in humans appears to be cidofovir, an acyclic nucleotide phosphonate cytosine analogue that acts as a chain terminator during DNA replication (15). Cidofovir is approved by the FDA for the treatment of CMV retinitis in AIDS patients. The drug is also effective against poxviruses (16), papillomaviruses (17), and the polyomavirus BK virus (18). However, cidofovir is not orally bioavailable, and its use can be associated with significant nephrotoxicity and hematological toxicity (19). Also, a substantial serotype-dependent variance in *in vitro* anti-Ad efficacy was reported with cidofovir (20).

A series of lipid-ester derivatives of cidofovir has been developed that decrease toxicity by reducing kidney exposure to the drug and increase oral bioavailability (21). CMX001 is one of these new derivatives. Its bioavailability and cellular uptake are significantly enhanced because of the lipid moiety, which is cleaved inside the cell by phospholipase to liberate cidofovir. This increased cellular uptake of CMX001 results in enhanced activity compared with cidofovir against multiple double-strand DNA viruses, including poxvirus (22–24) and CMV (25, 26) infections. Furthermore, the drug is 5- to 200-fold more potent *in vitro* than cidofovir against species A (Ad31), B (Ad3, Ad7), C (Ad5), and D (Ad8) Ads (27).

Early events in Ad pathology have been studied extensively in mice, primarily in the context of gene therapy (28, 29); however, these studies could not address pathology resulting from virus replication because mice support human Ad replication very poorly. Several animal models have been proposed that are to some extent permissive for human Ad replication, including cotton rats (30, 31), pigs (32), dogs (33), and Syrian hamsters (34–36). However, these studies did not address replication of Ad under conditions of immunosuppression, as is observed in transplant patients. Here we report a Syrian hamster model to evaluate the efficacy of anti-Ad drugs under conditions of immunosuppression and disseminated Ad infection. We show that CMX001 is highly effective against Ad5 in this model.

## Results

**Immunosuppression Enhances the Replication of Ad5 in the Livers of Syrian Hamsters.** In an effort to model patients with a compromised immune system, we immunosuppressed adult Syrian ham-

Author contributions: K.T., R.M.L.B., and W.S.M.W. designed research; K.T., J.F.S., and D.D. performed research; G.R.P. contributed new reagents/analytic tools; K.T. and J.E.S. analyzed data; and K.T. and W.S.M.W. wrote the paper.

Conflict of interest statement: G.R.P. is an official and shareholder in Chimerix, Inc., a company planning to commercialize CMX001.

This article is a PNAS Direct Submission.

<sup>§</sup>To whom correspondence should be addressed. E-mail: woldws@slu.edu.

This article contains supporting information online at [www.pnas.org/cgi/content/full/0800200105/DCSupplemental](http://www.pnas.org/cgi/content/full/0800200105/DCSupplemental).

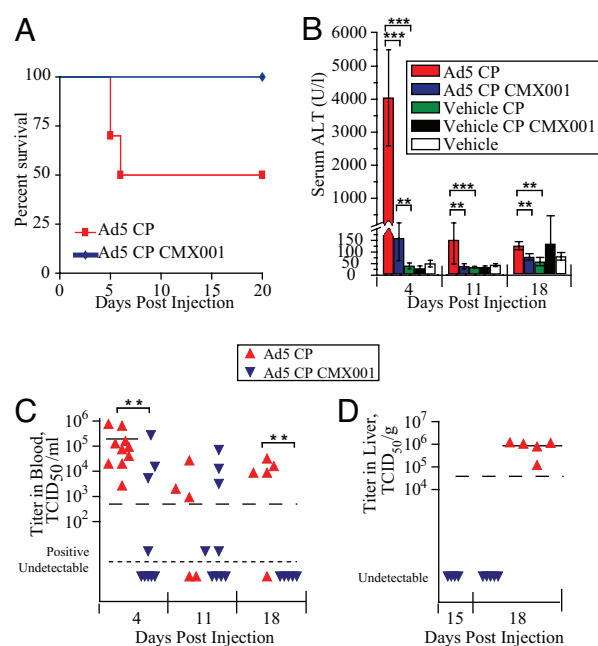
© 2008 by The National Academy of Sciences of the USA

sters with the alkylating drug cyclophosphamide (CP). On the day of dosing virus, total leukocyte counts usually dropped to an average of  $1,100 \pm 400$  per microliter with the treated animals compared with  $\approx 6,000 \pm 1,800$  per microliter with the immunocompetent ones and remained at low levels in the treated animals throughout the experiments. There was the same level of decrease in all leukocyte subsets [supporting information (SI) Table S1]. Erythrocyte and platelet counts did not change in response to CP treatment. Immunosuppressed animals immunized with Ad5 failed to raise anti-Ad neutralizing antibody (SI Methods and Fig. S1). With animals treated with CP, depletion of lymphoid tissue in the spleen and lymph nodes (data not shown) and the absence of infiltrating inflammatory cells in the liver were observed by histopathological examination, which further confirmed immunosuppression.

Immunosuppressed animals and immunocompetent controls were infected systemically with a single i.v. injection of Ad5 or AdEmpty, a replication-defective Ad vector. At day 7 after infection, most hamsters were moribund in the immunosuppressed, Ad5-injected group but not in other groups. In Syrian hamsters as in humans (3), the liver is one of the most severely affected organs during disseminated disease. The infectious viral titer was determined in the livers by a tissue culture 50% infectious dose (TCID<sub>50</sub>) assay. There was significantly more infectious Ad5 in the livers of immunosuppressed animals, especially at later time points (Fig. S2). AdEmpty was not detected in the liver of any hamster (Fig. S2), indicating that the Ad5 observed in the livers was the result of virus replication and not merely the input virus.

**Prophylactic Use of CMX001 Prevents Ad5 Induced Mortality and Reduces Morbidity in Immunosuppressed Syrian Hamsters.** Hamsters immunosuppressed with CP were injected with vehicle or Ad5. Half of the animals in each group were treated with a daily dose of CMX001 for the duration of the experiment, starting on the day before virus injection. A nonimmunosuppressed, vehicle-treated group was included as well. By 6 days after infection, half (five of 10) of the Ad5-injected hamsters that were not treated with CMX001 died (Fig. 1A). All animals in this group lost weight, and the surviving animals gained weight at a very slow rate (Fig. S3A). At 4 days after injection, the levels of alanine aminotransferase (Fig. 1B) as well as aspartate aminotransferase (AST) (data not shown) were extremely high in these animals and did not return to normal up to day 18 after injection (the termination of the experiment), indicating significant hepatocellular damage. Infectious Ad5 could be detected in the blood (Fig. 1C) and livers (Fig. 1D) of most of these animals even at 18 days after infection. In contrast, no deaths occurred in the Ad5-injected, CMX001-treated group ( $n = 9$ ). These animals did not lose weight, although they exhibited a reduction in body weight gain compared with vehicle control animals (Fig. S3A). Their alanine aminotransferase levels were slightly elevated on day 4 but returned to normal by day 11 (Fig. 1B). Viremia was less severe with CMX001-treated hamsters, and all animals cleared the virus by day 18 (Fig. 1C). Infectious Ad5 was not detected in the liver of any animal in the drug-treated group at day 15 or at day 18 (Fig. 1D). We conclude from these data that CMX001 prevented disseminated Ad5 infection-induced death and greatly reduced viremia and hepatic pathology in immunosuppressed hamsters. Furthermore, by day 15, CMX001 reduced the viral load in the liver and blood to undetectable levels.

**CMX001 Reduces the Replication of Ad5 in Multiple Organs, Thus Decreasing Toxicity.** To investigate the effect of CMX001 in more detail, we conducted a short-term toxicology study in both immunosuppressed and immunocompetent hamsters, and we assayed the dissemination of Ad5 to several organs. Four groups of eight hamsters were infected i.v. with Ad5. Of these four

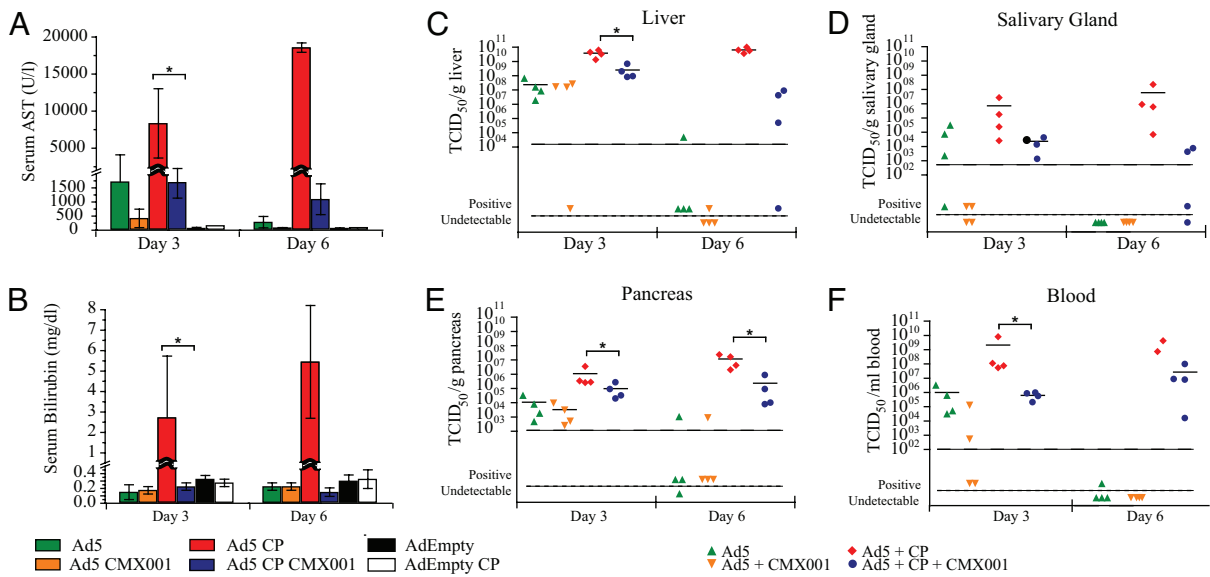


**Fig. 1.** CMX001 decreases Ad5-induced toxicity in Syrian hamsters. Immunosuppressed hamsters were infected i.v. with  $1.9 \times 10^{12}$  vp/kg Ad5. Half of the infected animals received a daily dose of 2.5 mg/kg CMX001 *per os*. Vehicle-injected animals were used as controls for all conditions. (A) CMX001 prevents Ad5-induced mortality. Animals were observed daily, and moribund hamsters were killed. No deaths occurred in any of the vehicle groups or in the CMX001-treated group. Ad5 CP vs. Ad5 CP CMX001,  $P = 0.0163$ . (B) CMX001 reduces Ad5-induced liver toxicity. At 4, 11, and 18 days after infection, serum was analyzed for alanine transaminase. The error bars signify standard deviation. (C) CMX001 reduces viremia. At days 4, 11, and 18, blood was collected from all animals that were alive at those time points and analyzed for the presence of infectious Ad5. The key applies to both C and D. (D) CMX001 eliminates Ad5 from the liver. Four hamsters of the Ad5 CP CMX001 group were killed on day 15; the remaining animals in this group and the Ad5 CP group were killed on day 18. Liver samples were collected from all these animals, and the infectious Ad5 titer was determined. In C and D in the scatter plots in subsequent figures, the symbols represent values from individual animals; the dashed line with the longer dashes signifies the limit of quantifiability, and symbols under the line with the shorter dashes represent samples in which no virus was detected. The horizontal bars correspond to means. \*,  $P < 0.05$ ; \*\*,  $P < 0.01$ ; \*\*\*,  $P < 0.001$ . Ad5 CP and Ad5 CP CMX001,  $n = 10$ ; vehicle CP and vehicle CP CMX001,  $n = 6$ ; vehicle,  $n = 5$ .

groups, two were immunosuppressed with CP and two were not. One immunocompetent and one immunosuppressed group were treated with daily doses of CMX001 for the duration of the study, starting on the day before virus injection; four animals were killed on each of days 3 and 6. In addition, there were two groups infected with AdEmpty (at the same dose as Ad5); one group was immunosuppressed, and the other was not.

Animals in the immunosuppressed, Ad5-treated group were moribund by day 4 or 5, and two animals were found dead on day 5. At the time the animals were killed, their serum transaminase and bilirubin levels were extremely high (Fig. 2A and B). Serum transaminases were elevated, although to much a lesser extent, in the immunosuppressed Ad5-infected animals that were treated with CMX001 (Fig. 2A); however, the hamsters were not clinically ill. Bilirubin levels were normal in these hamsters (Fig. 2B), indicating less severe hepatocellular damage. Importantly, CMX001 markedly reduced the infectious viral titers in the livers, salivary glands, pancreas, and blood of treated hamsters (Fig. 2C–F).

With animals killed on day 6, microscopic examination of the livers of the immunosuppressed, Ad5-infected hamsters revealed



**Fig. 2.** CMX001 attenuates Ad5-induced toxicity in hamsters by reducing viral replication. Immunosuppressed and immunocompetent hamsters were infected with Ad5 or with AdEmpty, and one immunosuppressed and one immunocompetent Ad5-infected group were treated with CMX001 ( $n = 4$  for all groups). (A and B) Serum AST and bilirubin levels were analyzed at the indicated times. (C–F) Infectious virus titer was determined in liver, salivary gland, pancreas, and blood. The horizontal bars in the scatter plots represent means. \*,  $P < 0.05$ .

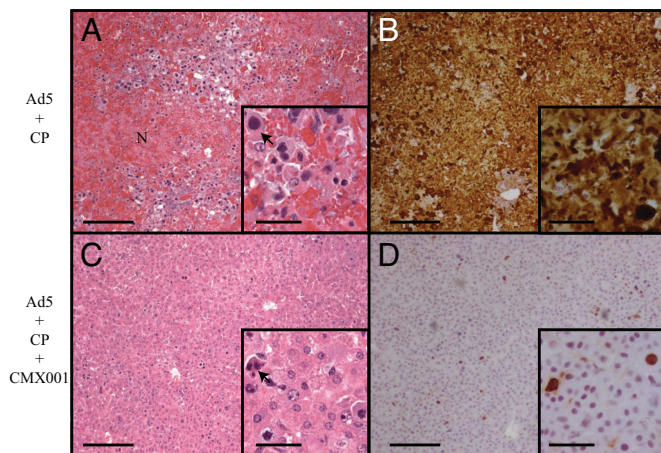
diffuse hepatocellular necrosis of massive severity, reaching throughout the liver with only scattered foci of histologically viable hepatocytes (Fig. 3A). In contrast, the livers of Ad5-infected, immunosuppressed hamsters that were treated with CMX001 were dramatically less severely affected than their nontreated counterparts (Fig. 3C). This difference was even more apparent when the liver samples were examined for Ad replication by immunohistochemical (IHC) staining for the Ad fiber protein. The overwhelming majority of hepatocytes were fiber-positive with the untreated animals (Fig. 3B), whereas only scattered cells stained with the treated animals (Fig. 3D). The staining was cytoplasmic and nuclear in both cases. Because Ad

virions uncoat and lose their fibers upon infection, nuclear staining for fiber cannot derive from the input virus. Furthermore, fiber is expressed in the late phase of infection, after the viral genome has replicated; therefore, the presence of fiber in the nucleus signifies Ad replication in that cell. With immunocompetent, Ad5-infected hamsters the beneficial effect of treatment with CMX001 was clearly apparent at day 3 based on a reduction in severity of hepatocellular changes (data not shown). By day 6, no significant differences in liver histopathology were seen in the immunocompetent hamsters with or without treatment with CMX001 (data not shown).

Cytopathologic changes (acute degeneration with intranuclear inclusion bodies) were observed in the adrenal cortex (Fig. S4A) and the pancreatic islets (Fig. S4C) of the Ad5-infected, CP-treated hamsters, and these findings were corroborated by IHC for Ad5 fiber (Fig. S4B and D). IHC suggested that the principal cell type infected in the pancreatic islets were the glucagon-producing  $\alpha$  cells, which was for the most part confirmed by immunofluorescent staining for glucagon (Fig. S4E) or insulin (Fig. S4F) together with the Ad5 fiber protein (SI Methods). CMX001 treatment largely prevented cytopathology in these endocrine tissues (data not shown). No such lesions were seen in Ad5-infected immunocompetent hamsters with or without CMX001 treatment (data not shown).

No treatment-related microscopic lesions were observed in the lungs, kidneys, and salivary glands in any of the hamsters. No significant microscopic lesions were detected in any of the organs examined with any of the AdEmpty-infected hamsters.

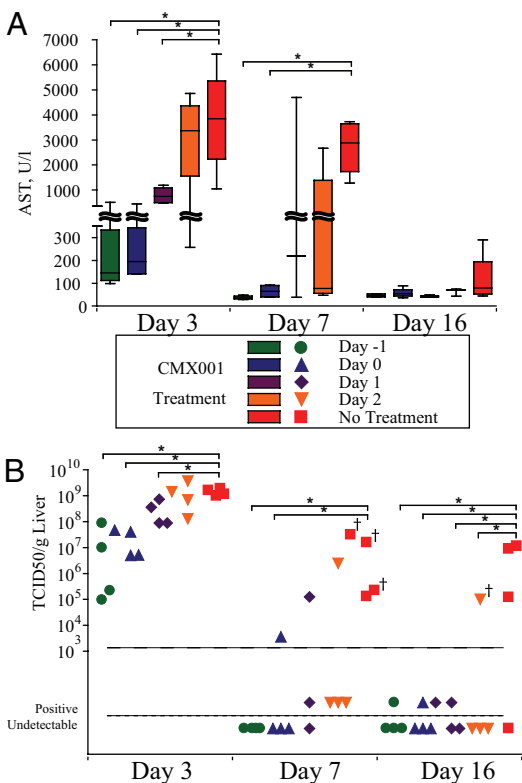
We conclude that, in accord with *in vitro* observations, CMX001 attenuates Ad-induced pathology by inhibiting virus replication in the target organs, most significantly in the liver.



**Fig. 3.** CMX001 decreases Ad5-induced lesions in the liver. Livers of hamsters killed at day 6 in the experiment described in Fig. 2 were subjected to histopathological and IHC evaluation. Animals infected with Ad5 and not treated with CMX001 exhibited extensive coagulation necrosis throughout the liver (A) and widespread replication of Ad5, demonstrated by staining for fiber (B). Treatment of Ad5-infected hamsters with CMX001 resulted in a significant reduction in hepatocellular injury (C) and greatly reduced IHC staining for fiber (D). The arrows indicate intranuclear inclusion bodies. (Scale bars: 200  $\mu\text{m}$  for the larger images and 50  $\mu\text{m}$  for Insets.) N, necrosis.

**CMX001 Is Effective in Reducing Ad5 Replication and Ad5-Caused Pathology When Administered After Exposure to the Virus.**

To examine whether CMX001 is effective when given after Ad infection, immunosuppressed hamsters were infected with Ad5 and treated with daily doses of CMX001, starting a day before, 6 h, 1 day, or 2 days after virus injection. One group was left untreated. Four animals were killed from each group at days 3, 7, and 16, and serum and livers were collected.



**Fig. 4.** CMX001 rescues hamsters from a lethal challenge of Ad5 when given 1 day after exposure and has a beneficial effect when given 2 days after exposure. Immunosuppressed hamsters were injected i.v. with Ad5 on day 0. The animals were treated daily with CMX001, starting a day before (day -1), 6 h after (day 0), 1 day after (day 1), or 2 days after (day 2) virus injection. One group of hamsters was left untreated (No Treatment). (A) Serum AST levels were examined on days 3, 7, and 16. The boxes depict the interquartile range, the whiskers are maxima and minima, and the horizontal bar in the boxes represents the median.  $n = 4$  for all groups except for the day-1 group at day 7 and the day-2 group at day 16, where  $n = 3$ . (B) The amount of infectious Ad5 in the liver was determined.  $n = 4$  for all groups at all time points. †, animal was moribund or was found dead. \*,  $P < 0.05$ .

On day 6, three of the remaining eight hamsters in the untreated group were moribund. Both serum AST levels (Fig. 4A) and infectious virus levels in the liver (Fig. 4B) were very high with all of the hamsters in the untreated group at days 3 and 7 and remained elevated at day 16. With the animals that received CMX001 2 days after Ad5, there was one hamster found dead at day 10, but the surviving animals had normal serum AST levels by day 16 (Fig. 4A). Animals in this group had lower virus burden by day 7, and no infectious Ad5 was detected in the livers of the surviving animals at day 16 (Fig. 4B). The antiviral effect was more pronounced with the animals receiving the drug starting from day 1, and the efficacy of the treatment starting 6 h after virus infection was virtually indistinguishable from that of prophylactic use of CMX001 (Fig. 4A and B).

## Discussion

**Animal Model for Disseminated Ad Infection in Immunosuppressed Patients.** We have developed an immunosuppressed, permissive animal model in which Ad5 replicates in multiple organs including the liver, salivary gland, and pancreas and causes an illness similar to that seen in immunosuppressed patients. Replication of Ad5 was greatly enhanced (342-fold by day 3) in the livers of immunosuppressed hamsters compared with the immunocompetent controls, and high levels of infectious virus were recovered at 18 days after infection. Hamsters with an intact immune

system cleared the virus by day 7. The increase in Ad replication in immunosuppressed hamsters occurred despite the fact that CP is known to suppress Ad DNA replication (37). Therefore, our results emphasize the importance of an early immune response against Ads, probably by the innate immune system.

Morbidity was not seen immediately after administration of input virus, but rather occurred after virus replication. Therefore, we conclude that the pathology was caused by virus replication in the organs, especially the liver.

Ad-induced lesions were detected by histopathological analysis in the adrenal gland and the pancreas of CP-treated animals, and this was corroborated by IHC staining for the Ad virion protein fiber. Interestingly, Ad5 replication in the pancreas was mostly limited to pancreatic islets, primarily to the  $\alpha$  cells, whereas in the adrenal gland Ad5 infected the cortical cells. Infection of these two endocrine tissues by Ad5 was not observed in immunocompetent hamsters. We are not aware of reports on whether Ad5 has a preference for infection of endocrine tissues in humans; our hamster data suggest that this should be examined in patients with Ad-mediated mortality.

**CMX001 Represses Replication of Ad5 in Multiple Organs, and It Suppresses Ad5-Induced Morbidity and Mortality.** We have shown that the antiviral drug CMX001 prevented Ad5-induced mortality and significantly reduced morbidity in the treated animals, as judged by the absence of rapid weight loss and the lack of greatly elevated serum transaminase levels that would indicate liver damage. Viremia was reduced in both intensity and duration in the hamsters that received the drug, and Ad5 was cleared from their livers by day 15.

CMX001 significantly repressed replication of Ad5 in the liver, salivary gland, and pancreas of immunosuppressed hamsters, as demonstrated by decreased recovery of infectious Ad5 from these organs. Histopathological analysis showed that CMX001 could prevent extensive damage to the liver, one of the major target organs for Ad5. Importantly, viremia largely mirrored viral replication in all three organs examined; thus, viremia can be used as a surrogate marker for virus replication. The effect of the drug was less significant in immunocompetent animals, possibly because the immune system cleared the virus.

CMX001 was effective when administered 1 day before infection with the LD<sub>50</sub> of Ad5 and then daily throughout the experiment. Given its potency, it is not surprising that CMX001 was also beneficial even when given as late as at 2 days after infection, a time when large amounts of infectious progeny virus can be recovered from the livers. With patients, viremia can be detected a median 3 weeks before disease develops, which may provide time for treatment (3). Thus, CMX001 may be useful both prophylactically and therapeutically.

We propose that the Syrian hamster can be used as an animal model to investigate Ad pathogenesis in immunocompromised patients. Furthermore, our data establish the immunosuppressed Syrian hamster as a powerful model to evaluate the efficacy of anti-Ad drugs. One such compound, CMX001, reduced Ad5 cytopathology and abolished mortality caused by disseminated Ad infection. Therefore, this promising drug warrants clinical development for the treatment of Ad infections.

## Materials and Methods

**Immunosuppression of Syrian Hamsters.** In all experiments female hamsters (5–6 weeks old) weighing  $\approx 100$  g were used. At 4–7 days before administering Ad5, the hamsters were injected i.p. with 140 mg/kg CP followed by a twice-weekly regime of i.p. injection of 100 mg/kg CP for the duration of the experiment.

**Infection of Syrian Hamsters with Ad.** Human Ad5 was obtained from American Type Culture Collection. The replication-deficient Ad vector AdEmpty has no transgene; this vector was obtained from Introgen Therapeutics. The viruses

were purified either by column chromatography or by three consecutive CsCl isopycnic gradient centrifugations. The virus was extensively dialyzed against 10 mM Tris (pH 8.2) and 10% glycerol and stored at  $-80^{\circ}\text{C}$  in single-use aliquots. Virus particle (vp) titers were established by HPLC; infectious titers were determined by TCID<sub>50</sub> assays. For infections, the hamsters were anesthetized with ketamine–xylazine mix and injected i.v. (into the jugular vein) with  $1.9 \times 10^{12}$  vp/kg ( $1.6 \times 10^{11}$  TCID<sub>50</sub>/kg) Ad5 or  $1.9 \times 10^{12}$  vp/kg ( $2.5 \times 10^{11}$  TCID<sub>50</sub>/kg) AdCMVpA in a single bolus of 200  $\mu\text{l}$  diluted in 10 mM Tris (pH 8.2) and 10% glycerol. This dose of Ad5 causes  $\approx 50\%$  mortality in immunosuppressed Syrian hamsters.

**Treatment with CMX001.** CMX001 was obtained from Chimerix. The drug was formulated in PBS and was administered by oral gavage at 2.5 mg/kg. Hamsters were treated daily for the duration of the 3-week survival experiment and for 6 consecutive days, starting on the day before virus injection, for the acute toxicology/biodistribution experiment. This dosing schedule models a possible prophylactic treatment for transplant patients. For the postexposure experiment, treatment started at the indicated times and lasted throughout the experiment.

**Establishing the Infectious Titer of Ad in Hamster Organs.** At the time that the animals were killed, the caudal half of the right lateral lobe of the liver, one of the submandibular salivary glands, the splenic branch of the pancreas, and whole blood collected by cardiac puncture were snap-frozen in liquid nitrogen and stored at  $-80^{\circ}\text{C}$ . The organs were processed and the extracts were titered as described previously (38). The solid tissues were homogenized by using a bead-beater apparatus (TissueLyser; Qiagen) with tungsten-carbide beads. All samples were freeze-thawed three times and sonicated for 7 min, then clarified by centrifugation in a microcentrifuge. The samples were serially diluted in a 96-well plate, and HEK-293 cells were infected with the dilutions. This cell line is permissive for the replication of both Ad5 and AdEmpty. Some wells were spiked with a known amount of Ad5 as a positive control to test for inhibition of infection by the extract. The infected plates were incubated for 14 days; then the assay was read, and TCID<sub>50</sub> titers were

calculated according to the Reed–Muench method. Samples under the limit of quantifiability were marked “positive” if they resulted in at least one positive well or “undetectable” if no viral cytopathic effect was detected in any of the wells. For clarity, data collected from moribund animals or ones that were found dead are shown together with data collected at the next scheduled killing.

**Histopathological Evaluation.** Immunocompetent or immunosuppressed hamsters were infected i.v. with Ad5 or AdEmpty, and half of the Ad5-infected animals were treated with six daily oral doses of 2.5 mg/kg CMX001. Liver, kidney, lung, spleen, mesenteric lymph node, pancreas, adrenal gland, and salivary gland were collected at the time the animals were killed (day 3 and day 6; four hamsters per group per time point) and immersion-fixed in 10% neutral buffered formalin. The organs were trimmed, embedded in paraffin, sectioned, and stained with H&E. Serial sections from each paraffin block were immunohistochemically stained for Ad fiber. Briefly, antigen retrieval using DIVA Decloaker (Biocare Medical) was conducted before incubation with an anti-Ad-fiber mouse monoclonal antibody (4D2; NeoMarkers). Secondary antibody incubation and chromogen staining were done by using the Dako-Cytomation EnVision+ System-HRP (Dako North America) for mouse primaries kit. The slides were counterstained with hematoxylin.

**Statistical Analysis.** Because the distribution of data did not satisfy the requirements for parametric analysis, nonparametric tests were used to assess statistical significance. The Kruskal–Wallis test was used to detect the overall treatment effect, and the Mann–Whitney *U* test was used to perform pairwise comparisons. Differences with  $P \leq 0.05$  were considered significant. Means were not calculated and statistical analysis was not performed on groups in which samples were missing. With groups in which infectious virus titers for some samples were not quantifiable, the limit of quantification was used instead to calculate statistical significance.

**ACKNOWLEDGMENTS.** This research was supported by National Institutes of Health Grants R01 CA118022 and R41 AI075744 (to W.S.M.W.).

- Wold WSM, Horwitz MS (2007) Adenoviruses. *Field's Virology*, eds Knipe DM, Howley PM (Lippincott, Williams, & Wilkins, Philadelphia), 5th Ed, pp 2395–2436.
- Centers for Disease Control and Prevention (2007) Acute respiratory disease associated with adenovirus serotype 14—four states, 2006–2007. *Morbidity Mortality Weekly Report* 56:1181–1184.
- Ison MG (2006) Adenovirus infections in transplant recipients. *Clin Infect Dis* 43:331–339.
- Kojaoglanian T, Flomenberg P, Horwitz MS (2003) The impact of adenovirus infection on the immunocompromised host. *Rev Med Virol* 13:155–171.
- Chakrabarti S, et al. (2002) Adenovirus infections following allogeneic stem cell transplantation: Incidence and outcome in relation to graft manipulation, immunosuppression, and immune recovery. *Blood* 100:1619–1627.
- Feuchtinger T, Lang P, Handgretinger R (2007) Adenovirus infection after allogeneic stem cell transplantation. *Leuk Lymphoma* 48:244–255.
- Trousdale MD, et al. (1995) Studies of adenovirus-induced eye disease in the rabbit model. *Invest Ophthalmol Vis Sci* 36:2740–2748.
- Romanowski EG, Yates KA, Gordon YJ (2001) Antiviral prophylaxis with twice daily topical cidofovir protects against challenge in the adenovirus type 5/New Zealand rabbit ocular model. *Antiviral Res* 52:275–280.
- Gordon YJ, Romanowski E, Araullo-Cruz T, De Clercq E (1992) Pretreatment with topical 0.1% (S)-1-(3-hydroxy-2-phosphonylmethoxypropyl)cytosine inhibits adenovirus type 5 replication in the New Zealand rabbit ocular model. *Cornea* 11:529–533.
- Kaneko H, et al. (2004) The cotton rat model for adenovirus ocular infection: Antiviral activity of cidofovir. *Antiviral Res* 61:63–66.
- Muller WJ, et al. (2005) Clinical and in vitro evaluation of cidofovir for treatment of adenovirus infection in pediatric hematopoietic stem cell transplant recipients. *Clin Infect Dis* 41:1812–1816.
- Lankster AC, et al. (2007) Disseminated adenovirus infection in children after allogeneic stem cell transplantation: Control by cidofovir and immune recovery. *Bone Marrow Transplant* 39:530.
- Abe S, et al. (2003) Oral ribavirin for severe adenovirus infection after allogeneic marrow transplantation. *Bone Marrow Transplant* 32:1107–1108.
- Hoffman JA, Shah AJ, Ross LA, Kapoor N (2001) Adenoviral infections and a prospective trial of cidofovir in pediatric hematopoietic stem cell transplantation. *Biol Blood Marrow Transplant* 7:388–394.
- De Clercq E, Holy A (2005) Acyclic nucleoside phosphonates: A key class of antiviral drugs. *Nat Rev Drug Discov* 4:928–940.
- De Clercq E, Neyts J (2004) Therapeutic potential of nucleoside/nucleotide analogues against poxvirus infections. *Rev Med Virol* 4:289–300.
- Calista D (2000) Resolution of recalcitrant human papillomavirus gingival infection with topical cidofovir. *Oral Surg Oral Med Oral Pathol Oral Radiol Endod* 90:713–715.
- Rinaldo CH, Hirsch HH (2007) Antivirals for the treatment of polyomavirus BK replication. *Expert Rev Antiinfective Ther* 5:105–115.
- Plosker GL, Noble S (1999) Cidofovir—A review of its use in cytomegalovirus retinitis in patients with AIDS. *Drugs* 58:325–345.
- Gordon YJ, et al. (1991) Inhibitory effect of (S)-HPMPC, (S)-HPMPA, and 2'-nor-cyclic GMP on clinical ocular adenoviral isolates is serotype-dependent in vitro. *Antiviral Res* 16:11–16.
- Ciesla SL, et al. (2003) Esterification of cidofovir with alkoxyalkanols increases oral bioavailability and diminishes drug accumulation in kidney. *Antiviral Res* 59:163–171.
- Buller RM, et al. (2004) Efficacy of oral active ether lipid analogs of cidofovir in a lethal mousepox model. *Virology* 318:474–481.
- Quenelle DC, et al. (2004) Oral treatment of cowpox and vaccinia virus infections in mice with ether lipid esters of cidofovir. *Antimicrob Agents Chemother* 48:404–412, and erratum (2004) 48:1919.
- Smee DF, et al. (2004) Effects of four antiviral substances on lethal vaccinia virus (IHD strain) respiratory infections in mice. *Int J Antimicrob Agents* 23:430–437.
- Bidanset DJ, Beadle JR, Wan WB, Hostetler KY, Kern ER (2004) Oral activity of ether lipid ester prodrugs of cidofovir against experimental human cytomegalovirus infection. *J Infect Dis* 190:499–503.
- Kern ER, et al. (2004) Oral treatment of murine cytomegalovirus infections with ether lipid esters of cidofovir. *Antimicrob Agents Chemother* 48:3516–3522.
- Hartline CB, et al. (2005) Ether lipid-ester prodrugs of acyclic nucleoside phosphonates: Activity against adenovirus replication in vitro. *J Infect Dis* 191:396–399.
- Liu Q, Muruve DA (2003) Molecular basis of the inflammatory response to adenovirus vectors. *Gene Ther* 10:935–940.
- Lieber A, et al. (1997) The role of Kupffer cell activation and viral gene expression in early liver toxicity after infusion of recombinant adenovirus vectors. *J Virol* 71:8798–8807.
- Prince GA, et al. (1993) Pathogenesis of adenovirus type 5 pneumonia in cotton rats (*Sigmodon hispidus*). *J Virol* 67:101–111.
- Toth K, et al. (2005) Cotton rat tumor model for the evaluation of oncolytic adenoviruses. *Hum Gene Ther* 16:139–146.
- Jogler C, et al. (2006) Replication properties of human adenovirus in vivo and in cultures of primary cells from different animal species. *J Virol* 80:3549–3558.
- Ternovoi VV, et al. (2005) Productive replication of human adenovirus type 5 in canine cells. *J Virol* 79:1308–1311.
- Hjorth RN, et al. (1988) A new hamster model for adenoviral vaccination. *Arch Virol* 100:279–283.
- Thomas MA, et al. (2006) Syrian hamster as a permissive immunocompetent animal model for the study of oncolytic adenovirus vectors. *Cancer Res* 66:1270–1276.
- Zarubae VV, et al. (2007) Effect of 6-azacytidine on the course of experimental adenoviral infection in newborn Syrian hamsters. *J Chemother* 19:44–51.
- Parsons PG, et al. (1990) Relationships between resistance to cross-linking agents and glutathione metabolism, aldehyde dehydrogenase isozymes and adenovirus replication in human tumour cell lines. *Biochem Pharmacol* 40:2641–2649.
- Toth K, Spencer JF, Wold WS (2007) Immunocompetent, semi-permissive cotton rat tumor model for the evaluation of oncolytic adenoviruses. *Adenovirus Methods and Protocols*, eds Tollefson AE, Wold WSM (Humana, Totowa, NJ), pp 157–168.