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Murine adenovirus infection of SCID mice induces hepatic lesions that resemble human Reye syndrome

(severe combined immunodeficiency/*scid* mutation)

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ABSTRACT **Murine adenovirus type 1 (MAV-1) infection of CB-17 SCID mice (which are homozygous for the severe combined immunodeficiency mutation) induces hepatic histopathologic and ultrastructural features that are strikingly similar to human Reye syndrome. Gross pathologic examination of MAV-1-infected mice revealed only pale yellow liver tissue. Histopathologic studies of tissue from MAV-1-infected mice revealed diffuse hepatic injury manifested by microvesicular fatty degenerative changes of hepatocytes and electron microscopic evidence of focal mitochondrial swelling with disruption of cristae and depletion of glycogen. Serum aminotransferase activities increased markedly in the infected animals; however, plasma ammonia levels were not elevated at the times assayed. Although all mice infected with MAV-1 died, neutralizing anti-MAV-1 monoclonal antibodies provided a dose-dependent delay in the appearance of clinical disease and hepatic histopathologic findings. Other findings included rare viral inclusions with only minimal inflammation in spleen, adrenal, and liver of infected mice. Our findings indicate that MAV-1 infection of SCID mice may provide important insights into the pathogenesis of the hepatic lesions of Reye syndrome.**

The etiology and pathogenesis of Reye syndrome (RS) remain elusive. A disease largely of children, RS is characterized clinically by a potentially reversible noninflammatory, nonicteric hepatitis with encephalopathy and histopathologically by microvesicular fatty degeneration of hepatocytes with mitochondrial injury (1–6). Although the incidence of RS has declined since its apparent peak in the late 1970s, mortality remains high (1–3). Prodromal viral illnesses have been noted in many cases, and epidemiologic evidence has led to proposals that RS is a postviral manifestation of influenza types A and B and varicella infection (1, 7–9). However, the typical histopathologic and biochemical abnormalities in RS suggest a toxin-mediated disease (1, 7, 8). Aspirin has been identified as one potential mediator of hepatic mitochondrial insult in RS (1, 10–14). Warnings that children with viral syndromes should not receive aspirin have been associated with an apparent decline in cases, but the relationship between pediatric aspirin use and the development of RS remains a matter of debate (1, 10–14). Other viral agents, toxins, endotoxin, and cytokines (15–22) have also been implicated in the pathogenesis of RS. None of these associations has been conclusively confirmed. Progress in understanding the etiology of the hepatic defects in RS has been hampered by the lack of a generally accepted animal model.

In the course of studying the pathogenesis of murine adenovirus type 1 (MAV-1) infection, we infected CB-17 mice that carry the severe combined immunodeficiency (SCID) mutation. The parental CB-17 strain is a congenic BALB/c mouse with only a single locus of the C57BL/6Ka mouse (23). Immunocompetent adult BALB/c and C57BL/6 mice do not experience lethal MAV-1 infection (refs. 24 and 25; unpublished observation). We have discovered that MAV-1 infection is lethal in adult SCID mice. Histopathological and ultrastructural studies of MAV-1-infected SCID mice have revealed hepatic lesions with striking similarities to those in human RS (3). This communication describes these findings and suggests that murine MAV-1 infection of SCID mice may provide a model to study the pathogenesis of the hepatic lesion of RS.

MATERIALS AND METHODS

Mice. CB-17 SCID mice were obtained from D. Myers, Cornell University. Mice were fed autoclaved food and water and maintained in autoclaved cages with fitted bonnets. Animals received a Septra (Burroughs Wellcome) solution for 2–3 days per week. No murine pathogens have been detected in our SCID colony.

Virus. MAV-1 obtained from S. Larsen, Indiana University School of Medicine, was passaged in tissue culture in murine L cells. Serial titrations of virus were performed on L cells to determine the median tissue culture infectious dose ($TCID_{50}$). Similarly processed uninfected cell "lysates" served as controls. Mice received i.p. inoculations of various amounts of MAV-1 diluted from a stock solution at 10^6 $TCID_{50}$ per milliliter in 100 μl of sterile 0.02 M phosphate-buffered saline (PBS), pH 7.2.

Monoclonal Antibodies (mAbs). 1A1, the IgG2a(κ) anti-MAV-1 neutralizing mAb used in mouse protection experiments, was one of many mAbs generated from a MAV-1-infected BALB/c mouse by previously described methods (26). These mAbs will be described elsewhere. The IgG2a concentration of 1A1 in ascites was determined by ELISA, after passage through a sterile 0.22-μm filter, by comparing its dilution curve to a standard curve derived with an IgG2a(κ) standard antibody (Organon Technika).

Mouse Infection and Antibody Protection Studies. Four- to six-week-old CB-17 SCID mice were used for all experiments. The serum concentration of IgM in these mice was <1 μg/ml. Virus infection was performed as described above. One milligram, 100 μg, or 1 μg of mAb 1A1 diluted in 100 μl of sterile PBS was administered i.p. to groups of five female SCID mice 1 hr before MAV-1 infection. Anti-ricin IgG2a and anti-ricin IgG2b mAbs were used as controls.

Infection Experiments. The first infection experiment performed was a virus titration study. Thirty 4-week-old female SCID mice were divided into six groups: five groups received serial dilutions of MAV-1 (10^4, 10^3, 10^2, 10, and 1 $TCID_{50}$) and the sixth group received PBS. Data for histopathologic,

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Abbreviations: MAV-1, murine adenovirus type 1; RS, Reye syndrome; SCID, severe combined immunodeficiency; $TCID_{50}$, median tissue culture infectious dose; mAb, monoclonal antibody; TNF, tumor necrosis factor.

ultrastructural, and biochemical studies were obtained from a second infection experiment. Four groups, each consisting of five SCID mice, were studied: group I received 5×10^4 $TCID_{50}$ of MAV-1 only; groups II and III were controls receiving 100 μl of uninfected lysate and PBS, respectively; and group IV received 5×10^4 $TCID_{50}$ of MAV-1 one hour after receiving 50 μg of mAb 1A1. All mice were female except those in group II, which were male.

Histopathologic and Ultrastructural Studies. mAb-treated, control, and MAV-1-infected mice were killed by cervical dislocation when the MAV-1-treated mice were moribund. Under sterile conditions, organs were removed and samples of brain, lung, heart, spleen, liver, kidney, and adrenal were fixed in phosphate-buffered 3.7% formaldehyde for histologic examination. Paraffin-embedded tissues cut at 5 μm were stained with hematoxylin and eosin and oil red O. Liver tissue was fixed within 5 min in 3% glutaraldehyde, postfixed in osmium tetroxide, dehydrated in graded ethanol solutions, and embedded in epoxy resin (Epon 812) for electron microscopy. Sections (1 μm) were cut and stained with alkaline toluidine blue. Thin sections were placed on copper grids and stained with uranyl acetate and lead tetroxide. Grids were examined in a Zeiss 9 electron microscope at 80 kV. Histopathologic and ultrastructural examination of all tissues was performed by one of us (S.M.F.) without initial knowledge of the virus and/or antibody treatment status of the animals.

Chemistries. Mice from groups I–IV (second infection experiment) were bled by retroorbital sinus puncture 2 days prior to experimental manipulations and again at the time of sacrifice on day 8. Blood from the five mice in each of the four experimental groups was pooled. Serum aspartate aminotransferase, alanine aminotransferase, bilirubin, and glucose were measured on a Technicon Chem-1 analyzer. Plasma ammonia concentrations were determined on fresh specimens with the Sigma kit (Sigma, catalog no. 170-B).

RESULTS

The response of SCID mice to MAV-1 was examined in infection and mAb protection experiments. The virus titration experiment demonstrated that MAV-1 infection is lethal in SCID mice. The time to reach a lethal endpoint was dependent upon the amount of virus administered (Fig. 1). All mice receiving virus died; uninfected mice all survived. Death followed the onset of clinical illness within 18–24 hr. Disease manifestations were the same regardless of virus dose and were marked by the abrupt onset of lethargy, poor grooming and feeding, ruffled fur, hunched posture, and unsteady gait.

mAb protection experiments demonstrated that although mAb administered 1 hr prior to MAV-1 infection prolonged the survival of treated animals, all MAV-1-infected animals eventually died. One milligram of mAb 1A1 prolonged the median survival time (LT_{50}) of mice given 10^5 $TCID_{50}$ of MAV-1 from 7 days to 44 days. With 5×10^4 $TCID_{50}$ of MAV-1, 100 μg of 1A1 prolonged the LT_{50} from 8 days to 26 days and 1 μg prolonged the LT_{50} from 8 days to 14 days. MAV-1-infected mice receiving anti-ricin mAbs were not protected and died with the same time course as MAV-1-infected mice receiving no mAb.

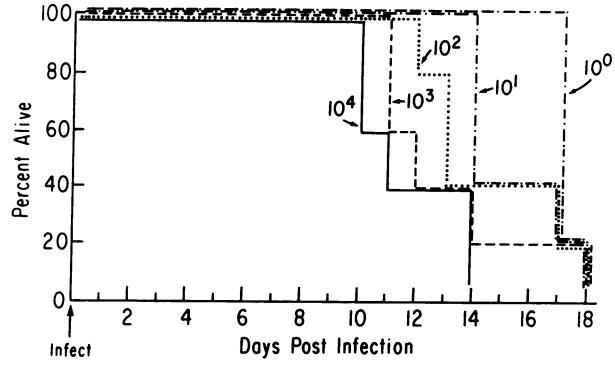

FIG. 1. Virus titration survival curves of MAV-1-infected SCID mice. Each group consisted of five 4-week-old female mice. The amounts of MAV-1 (1 to 10^4 $TCID_{50}$, administered i.p.) are shown. There were no deaths of uninfected (PBS-injected) mice.

Histopathologic and ultrastructural examinations were performed on SCID mice in groups I–IV. Four MAV-1-infected (group I) mice were moribund on day 8 when they were sacrificed and dissected; the fifth animal died on day 7. Three lysate and PBS control (groups II and III) mice, and two mAb-treated (group IV) mice, all without clinical illness, were sacrificed and dissected with the infected animals. The three cage mates of the mAb-treated mice were observed until death on days 11 (1) and 27 (2) postinfection. The two cage mates of the PBS and lysate control mice (groups II and III) remained well.

The livers of virus-infected (group I) animals were pale and yellow at autopsy. This was not observed in the control (groups II and III) or antibody-treated (group IV) animals. Light microscopy showed histopathologic changes in liver, adrenal, and spleen of MAV-1-infected (group I) animals only. Liver was remarkable for multifocal, diffuse microvesicular intrahepatic fat accumulation in virtually all lobules, confirmed by staining with oil red O. Three of eight mAb-treated (group IV) and control (groups II and III) animals demonstrated only rare hepatic microvesicular fat restricted to isolated lobules. The other five mice demonstrated essentially no fatty changes at all. In marked contrast to the control and mAb-treated animals, liver sections of all of the MAV-1-infected (group I) mice were notable for intense and extensive staining with oil red O (Fig. 2). Hepatocyte microvesicular fatty degeneration also occurred in mAb-protected animals when disease spontaneously occurred. This was observed in a mouse that was dissected 56 days after the administration of MAV-1 and mAb 1A1. Occasional large intranuclear eosinophilic viral inclusions characteristic of MAV-1 were noted in liver (Fig. 3), spleen, and adrenal. In MAV-1-infected mice, only rare foci of hepatocyte necrosis were observed in association with small collections of polymorphonuclear cells. Examination of splenic tissue revealed marked histiocytic and stromal activation with giant cells. Viral inclusions in spleen and liver were confirmed by immunoperoxidase staining with polyclonal BALB/c anti-MAV-1 antiserum (data not shown).

Electron microscopic examination of liver sections revealed abnormalities in MAV-1-infected (group I) mice that were not seen in the control or mAb-treated animals (Fig. 4). Mitochondrial swelling, loss of matrix density, disruption and loss of cristae, and glycogen depletion with numerous cytoplasmic fat globules were noted. No viral particles were seen by electron microscopy in hepatocyte nuclei of MAV-1-infected mice; however, viral particles were confirmed in one spleen.

Only MAV-1-infected (group I) mice developed biochemical abnormalities. On day 8 after infection, when the mice were in a moribund state, aspartate aminotransferase was 13 times higher than the baseline value (810 units vs. 61 units) and alanine aminotransferase was 10 times higher (298 units vs. 31 units), whereas serum bilirubin concentrations remained normal. In mAb-protected (group IV) animals, the aspartate aminotransferase value was 93 units before infection and 135 units on day 8 and the alanine aminotransferase value was 43 units before infection and 41 units on day 8. Significant fluctuations from preinfection values did not occur in groups II and III, although the lysate (group II) animals had unexplained elevations at the beginning of the

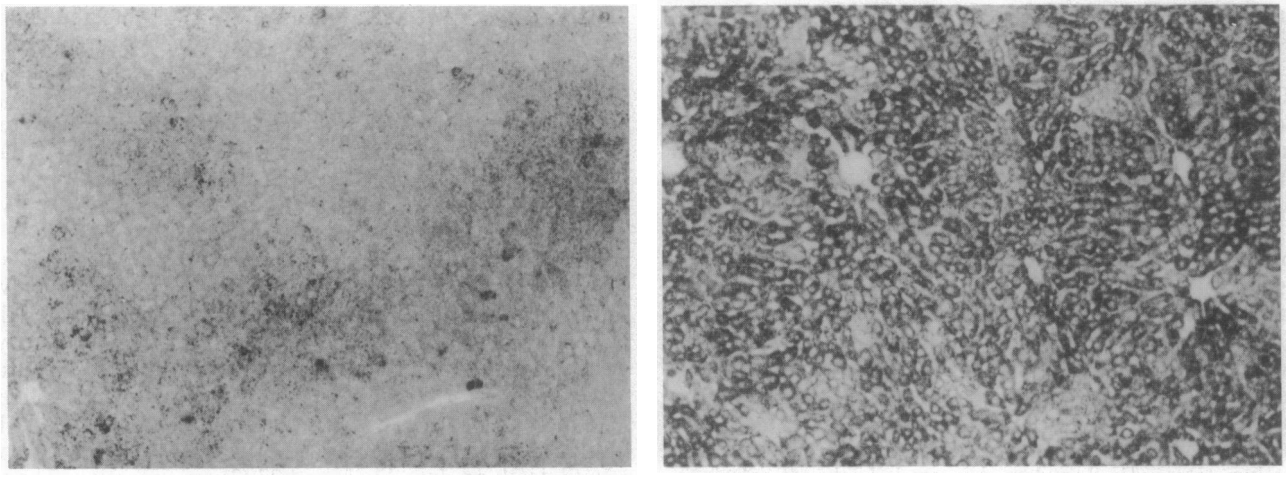

FIG. 2. (*Left*) Cryostat section of lysate control (group II) liver stained with oil red O for intracellular lipid. There is minimal staining (dark droplets) in focal areas of hepatic lobules. Cellular architecture, not demonstrated with this stain, is normal. (×80.) (*Right*) Cryostat section of MAV-1-infected (group I) liver stained with oil red O. There is intensely stained, diffuse microvesicular lipid (dark droplets) involving all lobules. Cellular architecture is seen in Fig. 3. (×80.)

experiment. However, no hepatic pathology was detected in these animals. Plasma ammonia concentrations did not rise from preinfection values in any of the groups (data not shown). The mean serum glucose of MAV-1-infected (group I) mice was 405 mg/dl (four mice), compared with a mean of 165 mg/dl (15 mice) for groups II–IV. The histopathologic, electron microscopic, and biochemical findings reported here have been confirmed in additional animals.

DISCUSSION

Several human adenovirus serotypes have been reported in association with clinical and histopathologic evidence suggestive of RS in children, including hepatic microvesicular fatty degeneration without mitochondrial ultrastructural changes or hyperammonemia (15, 21, 22). Although adenovirus dissemination to liver is uncommon (22), it has been described in immunocompromised children (27). Recently, disseminated adenovirus infection including hepatitis has been reported in patients with SCID and with human immunodeficiency virus infection (28). In contrast to both human RS and MAV-1 infection of SCID mice, these patients had severe hepatic necrosis resembling adenoviral disease described in liver transplant patients (29).

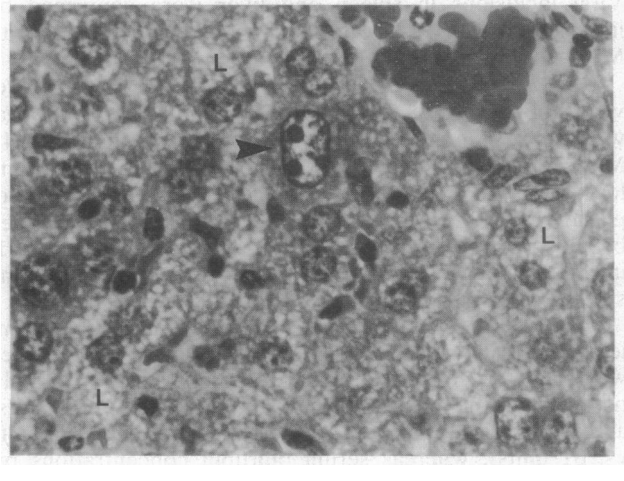

FIG. 3. Light micrograph of MAV-1-infected (group I) liver. Microvesicular lipid (L), extracted during tissue processing, is seen as small clear spaces in virtually all hepatocytes within this field. A large, swollen hepatocyte nucleus (arrowhead) has two intranuclear viral inclusions and clearing of the chromatin. There is no necrosis or inflammatory reaction associated with this virus-infected cell. (Hematoxylin and eosin; ×600.)

Previously unrecognized tissue specificity for liver has been noted in other SCID mouse models of viral infection (30, 31). However, unlike the severe viral hepatitis observed in these models, MAV-1 infection of CB-17 SCID mice results in liver histopathology with a striking resemblance to the hepatic lesions reported in human RS (1–5, 9). SCID mice develop microvesicular fatty hepatocyte degeneration without significant inflammation, and ultrastructural changes including mitochondrial pleomorphism, matrix disruption, cristalysis, and glycogen depletion. These findings are elicited by the administration of MAV-1 alone. The delay in the appearance of hepatic lesions in MAV-1-infected SCID mice that occurs with neutralizing anti-MAV-1 mAb treatment confirms that MAV-1 infection is responsible for the lesions observed. Further similarities between MAV-1-infected SCID mice and human RS include elevated aminotransferase activities with normal bilirubin values. MAV-1-infected SCID mice have not manifested hyperammonemia. Their rapid demise may preclude its development, or increases in plasma ammonia may not have been detected due to transient elevations that were not sustained. Although often noted, the role of hyperammonemia in the pathogenesis of human RS is unresolved (1).

Existing murine models of RS (32–40) have addressed the roles of numerous viral agents, aspirin, and toxins. These models have various discrepancies with the human disease including, in some, the absence of abnormal hepatic histopathologic and ultrastructural findings (32). One spontaneous outbreak of a RS-like illness with characteristic hepatic lesions including mitochondrial abnormalities was reported in young BALB/cByJ mice (34). These animals developed infection in their normal habitat. Some, but not all, affected mice were shown to be infected with murine intestinal coronaviruses. However, these findings were not reproducible with subsequent coronavirus infection of the same strain (1, 34). In SCID mice, the amount of MAV-1 or neutralizing mAb administered determines the timing and appearance of clinical illness and detectable hepatic lesions.

In immunocompetent mice, MAV-1 infection via multiple routes is lethal only in very young (suckling) animals (24, 25). Histologically, viral inclusions associated with necrosis in spleen, adrenal, brain, heart, and kidney have been described (25). Detectable viral inclusions also occur in liver, adrenal, and spleen of MAV-1-infected SCID mice; however, this is

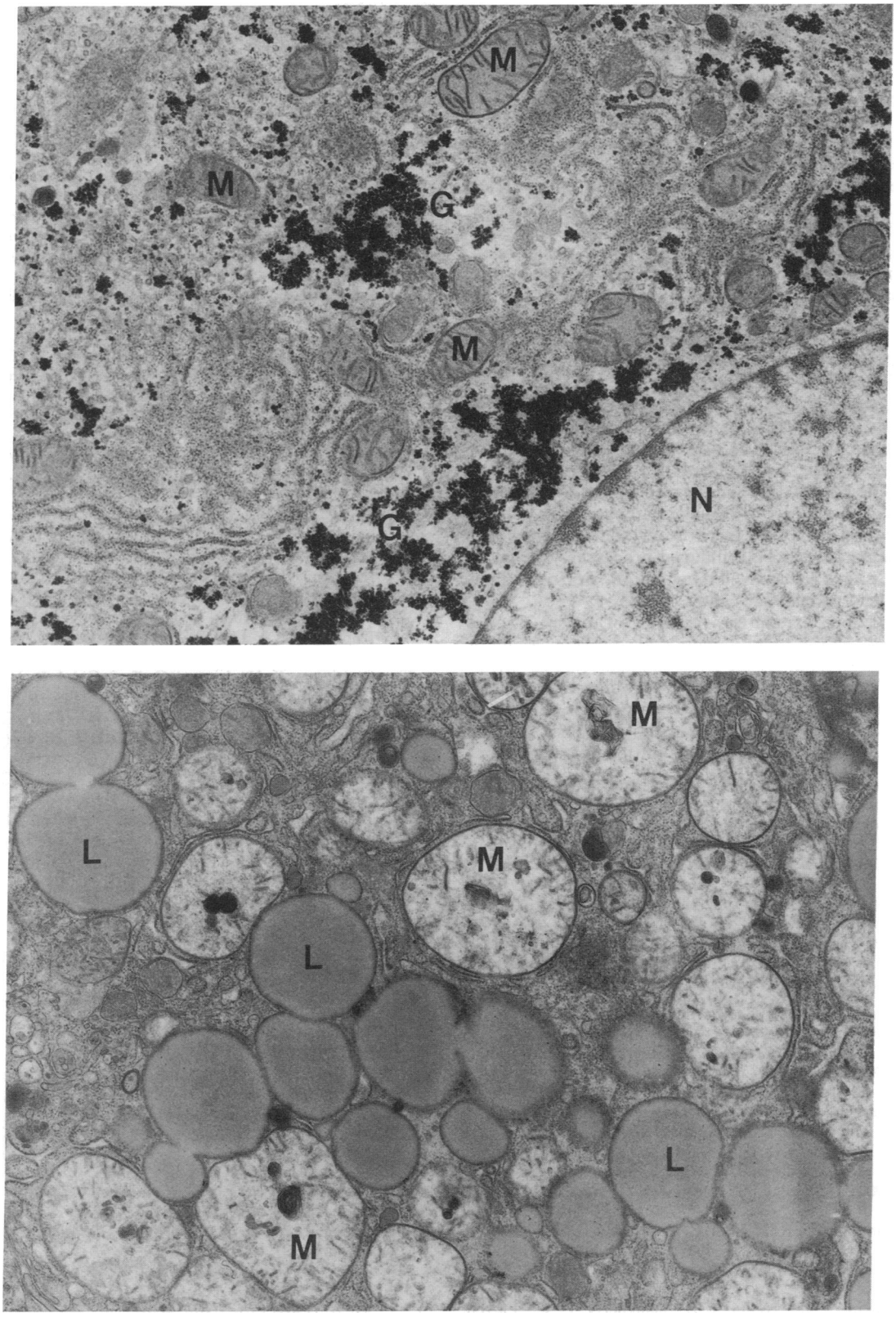

FIG. 4. Electron micrographs. (*Upper*). Lysate (group II) control liver. Mitochondria (M) are small and regular in shape with intact cristae and matrix. Abundant electron-dense glycogen (G) is present throughout the cell. A portion of the hepatocyte nucleus (N) is seen. There are no lipid droplets in the cytoplasm. (×16,400.) (*Lower*) Portion of hepatocyte cytoplasm from a MAV-1-infected animal. Note that the magnification is approximately the same as in *Upper*. The mitochondria (M) are markedly swollen, with clearing of matrix, crystalysis, and the presence of electron-dense myelin-like material. Multiple lipid droplets (L) are present throughout the cytoplasm. Glycogen is absent. (×17,100.)

only rarely associated with cellular necrosis and inflammation. The relative absence of hepatic inflammation and necrosis is a hallmark of human RS (1), although periportal hepatic necrosis has been reported (6). MAV-1-infected SCID mice may succumb to toxic processes before the onset of hepatic necrosis. The latter finding has been reported in

organ transplantation, human SCID, and human immunodeficiency virus infection (28, 29). In this regard, toxic properties have been attributed to the adenovirus penton (41). It has been suggested that the aminotransferase elevations, hyperglycemia, and encephalopathy observed in some human adenovirus infections could be due to this protein (22).

SCID mice may be able to adequately clear viral particles and thus circumvent widespread virus dissemination, as cytokine-mediated processes related to natural killer (NK) cell and macrophage function are unimpaired (42, 43). Low-dose endotoxin, compromised interferon responses, and augmented release of tumor necrosis factor (TNF) have been implicated in the toxic pathogenesis of RS (17–20). In this regard, human adenovirus-infected cells are killed *in vitro* by TNF and γ-interferon (44). Expression of the human adenovirus early region (E3) genes may modulate host responses to infection, including the abrogation of TNF responses (45–48). This anti-TNF effect has been mapped to the 14.7-kDa E3 gene product (48). Sequences homologous to the human adenovirus E3 genes have not been demonstrated in the MAV-1 genome (49). The absence of these genes may have important pathogenic implications for MAV-1 infection of immunodeficient (SCID) mice, especially with respect to a potential role for TNF in disease pathogenesis. Indeed, MAV-1 infection of SCID mice bears some clinical and histologic similarities to TNF-induced processes. The latter may include shock, glucose dysregulation, deranged lipid metabolism, and splenic activation (50).

Our observations extend the spectrum of murine disease attributable to MAV-1 to an immunodeficient host. Many of the clinical and histopathologic features manifested by MAV-1-infected SCID mice are compatible with human RS. Further insights into the pathogenesis of RS, and perhaps answers to many unresolved issues in adenovirus pathogenesis, may be gained as we proceed to develop this intriguing model.

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