ALTERATION OF CLINICAL OUTCOME AND HISTOPATHOLOGY OF YELLOW FEVER VIRUS INFECTION IN A HAMSTER MODEL BY PREVIOUS INFECTION WITH HETEROLOGOUS FLAVIVIRUSES

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Abstract. Using a recently described hamster model of severe yellow fever (YF), we examined the hypothesis that prior infection with heterologous flaviviruses protects against severe or fatal YF. Hamsters were singly or sequentially infected with Japanese encephalitis virus, West Nile virus, and/or dengue-1 virus, and then challenged with a virulent strain of yellow fever virus (YFV). In contrast to control (naive) hamsters, many of which appeared clinically ill or died after YFV infection, the flavivirus-immune animals remained asymptomatic. The flavivirus-immune hamsters also had a reduced viremia and lower serum levels of alanine aminotransferase and total bilirubin, compared with naive hamsters, following YFV infection. Histologically, livers of animals in the flavivirus-immune and control groups showed comparable levels of multifocal necrapoptosis. However, steatosis was not observed in the flavivirus-immune animals, whereas naive hamsters developed extensive microvesicular steatosis in the liver following YFV infection. These findings suggest that hepatic steatosis is an adverse microscopic feature associated with severe disease in YFV infection. Our experimental results support earlier anecdotal reports that prior exposure of humans to heterologous flaviviruses reduces subsequent risk of fatal YFV infection.

INTRODUCTION

Monath and others have suggested that immunologic cross-protection by heterologous flaviviruses may provide partial protection against yellow fever (YF). In the 19th century, it was noted that indentured laborers from India and British troops who had served in India were less likely to contract severe YF during epidemics of the disease in Africa. Presumably, many of these people had been previously infected with dengue (DEN) viruses, and possibly West Nile (WN) or Japanese encephalitis (JE) viruses, during their residence in India. A retrospective investigation of a YF epidemic in Gambia in 1978 revealed that the inapparent-to-apparent infection ratio for YF was significantly higher in individuals with prior exposure to heterologous flaviviruses. In addition, there is limited experimental evidence from studies in monkeys supporting the concept that immunity to DEN and other flaviviruses may diminish the viremia and severity of yellow fever virus (YFV) infection.

To test the hypothesis that prior infection with heterologous flaviviruses protects against severe or fatal YF, a series of laboratory experiments were done using a recently described hamster model of the disease. The present paper reports our results that support the earlier observations and hypothesis that heterologous flavivirus antibodies are protective against severe YF. In addition to reduced levels of viremia and less severe alternation in liver function, the flavivirus-immune hamsters did not develop microvesicular steatosis, one of the classic pathologic features of severe YF infection. The significance of this lack of steatosis in the course of YFV infection is discussed.

MATERIALS AND METHODS

Viruses and animals. Five flaviviruses were used in this study; the Jimenez hamster-virulent (10th passage) strain of YFV; the Mochizuki high mouse passage (178x) strain of dengue-1 virus (DENV); the live attenuated SA14-2-8 vaccine strain of Japanese encephalitis virus (JEV); the New York 385-99 strain of West Nile virus (WNV); and the BeAr 23379 strain of St. Louis encephalitis virus (SLEV).

The hamsters used were juvenile and adult female Syrian golden hamsters (Mesocricetus auratus) obtained from Harlan Sprague Dawley (Indianapolis, IN). Animals were cared for in accordance with the guidelines of the Committee on Care and Use of Laboratory Animals (Institute of Laboratory Animal Resources, National Research Council) under an animal use protocol approved by the University of Texas Medical Branch. All work with infected animals was carried out in biosafety level-3 facilities.

Virus assay. Virus levels in blood of hamsters infected with YFV were titrated in cultures of the C6/36 clone of Aedes albopictus cells, as described previously. The presence or absence of viral antigen by immunofluorescence was used as the end-point. The YFV titers were calculated as the tissue culture infectious dose50 (TCID50) per milliliter of specimen by the method of Reed and Muench.

Antibody determinations. Serum antibodies to YFV and the other four flaviviruses were measured by a hemagglutination-inhibition (HI) test. Antigens for the HI test were prepared from brains of newborn mice inoculated intracerebrally with each of the viruses; the infected brains were processed by the sucrose-acetone extraction method. Hamster sera were tested by HI at serial two-fold dilutions from 1:20 to 1:5,120 at pH 6.6 (WNV, JEV, DENV, and SLEV) or 6.4 (YFV) with four units of antigen and a 1:200 dilution of goose erythrocytes, following established protocols.

Serum levels of alanine transaminase and bilirubin. Determinations of total bilirubin (TB) and of alanine aminotransferase (ALT) levels were done on fresh serum from clotted blood, using commercial kits (Total Bilirubin® and Infinity ALT®, Sigma Diagnostics, St. Louis, MO), according to the manufacturer’s instructions.

Experimental infection of animals. Four separate experiments were done. The viruses used and the sequence in which they were administered to the hamster were as follows: Exp-
identification of the specimens. Stained sections, the observer was blinded as to the group morphologically recognizable necrotic cells or foci were described in more detail in the Results. In each experiment, YFV was the final (challenge) virus administered. Following inoculation of YFV, the hamsters were bled daily for six consecutive days to monitor the level of viremia. On the sixth day after infection with YFV, the hamsters were exsanguinated and killed. Sera were collected for biochemical studies, including TB and ALT, and samples of liver, spleen, and for some animals, pancreas, were removed for histopathologic examination. Day 6 was selected as the time to evaluate the degree of organ pathology following challenge with YFV, since previous studies demonstrated that maximum tissue damage occurs on the sixth post-infection day in the hamster model of yellow fever.

**Histologic evaluation.** Samples of fresh liver, spleen, and pancreas were fixed in 10% neutral-buffered formalin for 24 hours and then in 70% alcohol for 1–2 days, before being processed for routine paraffin embedding. Four to five micron-thick sections were made and stained by the hematoxylin and eosin method. Some sections were also stained using the reticulin and Masson’s trichrome methods. Tissue sections were examined with an Olympus (Melville, NY) BX51 microscope equipped with UPlan/Apo lenses. For semi-quantitative analysis, a scheme described in a previous report was used, with modification. In analyzing histopathology of the liver, the degree of inflammatory infiltration, hepatocytic necroptosis, and steatosis were evaluated; the severity of each parameter was graded on a subjective scale of 0 to 4. For the spleen, a subjective impression of none, minimal, mild, moderate, and severe, was graded as 0, 1, 2, 3, and 4, respectively. The parameters assessed were lymphoid hyperplasia, lymphoid (white pulp) depletion, and macrophage proliferation (activation), as previously described. Pancreas was also examined in some of the animals; in this organ only the presence or absence of parenchymal necrosis was recorded.

**In situ TUNEL assay.** The terminal deoxynucleotidyl transferase-mediated dUTP nick-end labeling (TUNEL) technique was used to assay for apoptosis in sections of hamster liver tissue. The ApopTag peroxidase kit (Intergen Company, Purchase, NY) was used, following the manufacturer’s instructions, as previously described. For quantification, 10 random high power fields were examined, using a 40x objective, and the ApopTag-positive nuclei were counted. Due to occasional non-specificity of the in situ TUNEL assay, morphologically recognizable necrotic cells or foci were excluded from counting. While conducting the microscopic examinations of the hematoxylin and eosin–stained sections and the TUNEL-stained sections, the observer was blinded as to the group identification of the specimens.

**RESULTS**

**Establishment of heterologous flavivirus infection(s) in hamsters, their antibody response, and subsequent challenge with YFV.** Experiment 1. Infection of naive hamsters with YFV (Group YF control). Sixteen adult hamsters (11–12 weeks old) were inoculated intraperitoneally with 10^6 TCID_{50} of YFV. The animals in this group showed clinical signs of severe YF (lethargy, anorexia, tremors) on the fifth and sixth days after infection, as described previously. Eight of the animals were bled daily for six consecutive days to monitor the level of viremia. On the sixth day after infection, these eight animals were exsanguinated to obtain serum for liver function studies, and tissue samples were taken at necropsy for histopathologic examination. The remaining eight hamsters were observed for 14 days; two of the animals died on sixth and seventh days, giving a mortality rate (25%) similar to that previously described.

**Experiment 2. Infection with JEV, followed by YFV (Group J/E/YF).** Ten subadult hamsters (5–6 weeks old) were inoculated subcutaneously with JEV strain SA14-2-8. Twenty-eight days later, the animals were bled to determine the HI antibody titers to JEV and YFV antigens, which are shown in Table 1. The hamsters in this experiment all developed a strong HI antibody response to JEV antigen (1:640–1:1,280), but they also had lower levels of cross-reacting antibodies to YFV antigen (1:80–1:160). Thirty-one days after JEV infection, the 10 hamsters were inoculated intraperitoneally with 10^5 TCID_{50} of YFV and were then bled for six consecutive days to determine the level of the resulting YFV viremia. Six days after infection with YFV, the hamsters were killed and necropsied to obtain tissue samples for histopathologic analysis. No sera were obtained for liver function tests from this group of animals. The animals in this group did not appear sick when killed on the sixth day of YFV infection.

**Experiment 3. Sequential infection with DENV, WN, and YFV (Group DEN/WN/YF).** Twenty-six juvenile hamsters (3–4 weeks old) were inoculated intracerebrally with the Mo-chizuki strain of DENV. This virus strain produces an asymptomatic infection in juvenile and adult hamsters. Twenty-seven days after DENV infection, 10 of the animals were bled to determine their immune status. At this time, all of the hamsters had HI antibodies to DENV, with titers ranging from 1:160 to 1:640. The 26 animals were then inoculated intraperitoneally with 10^4 TCID_{50} of WNV strain New York 385-99. Thirty-one days after WNV infection, 18 of the hamsters were inoculated intraperitoneally with 10^5 TCID_{50} of YFV and 10 were bled for six consecutive days to determine the level of viremia. Ten of the 18 YFV-infected animals were killed on the sixth day for histopathologic analysis; the remaining eight survived and did not appear ill. The other eight DENV/WNV-infected animals did not receive YFV and were used as YFV-negative controls.

**Experiment 4. Sequential infection with SLEV, WN, and YFV (Group SLE/WN/YF).** An additional 26 juvenile hamsters were inoculated subcutaneously with 10^6 TCID_{50} of SLEV strain BeAr 23379. Thirty days later, the animals were inoculated intraperitoneally with 10^4 TCID_{50} of WN strain New York 385-99. Forty-five days after WNV infection, nine of the hamsters in this group were bled to determine their flavivirus HI antibody titers. Eighteen of the original 26 hamsters were then given 10^6 TCID_{50} of YFV. These animals
Hemagglutination-inhibition (HI) antibody titers* in St. Louis encephalitis virus (JEV) and yellow fever virus (YFV) antigens 28 days after infection with JEV

<table>
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</table>

* Reciprocal of highest positive serum dilution.

were bled daily for six days, as described earlier, to determine the level and duration of the subsequent YFV viremia. On the sixth day after inoculation of YFV, 10 of the hamsters were exsanguinated and necropsied. The other eight YFV-infected hamsters survived infection and did not appear ill. The eight remaining hamsters from this group of 26 that had been previously infected with SLEV and WNV, but not YFV, were exsanguinated and necropsied to serve as YFV-negative controls.

The HI antibody titers in the eight hamsters from Experiment 4 immediately before and 6 days after infection with YFV are shown in Table 2. Following immunization with SLEV and WNV, these animals already had HI antibody titers (range = 1:160–1:640) against YFV antigen. Six days after infection with YFV, the animals had a further increase in their flavivirus HI antibody titers (Table 2), indicating an amnestic type of antibody response.

Reduced YFV viremia and alteration of liver function in hamsters previously infected with heterologous flaviviruses. As shown in Figure 1, YFV was present in the blood of the flavivirus-naive (control) hamsters within 24 hours after infection, peaked on day 3 (mean titer = 10^7.5 TCID<sub>50</sub>/mL), and had largely disappeared by day 6. The disappearance of infectious virus from peripheral blood corresponds with the development of HI and IgM antibodies in the hamster model of yellow fever. Six days after YFV infection, the serum ALT and TB levels in the naive animals were markedly elevated (Figure 2).

Despite the presence of pre-existing antibodies to heterologous flavivirus (Tables 1 and 2), the hamsters in Experiments 2, 3, and 4 (JE/YF, DEN/WN/YF, and SLE/WN/YF, respectively) still developed a significant YF viremia after challenge with YFV (Figure 1). The level and pattern of viremia among animals in these three experiments were similar; the peak virus titers were lower and virus replication was delayed, compared with that of naive hamsters after YFV challenge.

The mean serum ALT and TB levels in each experimental group on the sixth day after YFV infection are shown in Figure 2. Following challenge with YFV, hamsters previously infected with SLEV and WNV (Experiment 4) developed elevated levels of ALT and TB. However, the magnitude of ALT and TB alteration was less than that in naive hamsters infected with YFV (Figure 2). The mean ALT and TB values in the DEN/WN/YF group of animals (Experiment 3) were also lower than the values obtained in the YFV control hamsters and were comparable to liver function results obtained with the hamsters in Experiment 4 (SLE/WN/YF). In contrast, the ALT and TB levels in the eight control animals (SLE/WN only) were within normal limits for hamsters, indicating that the alteration of liver function was due to YFV infection and not to the prior SLEV and WNV infections.

Histopathologic changes in hamsters challenged with YFV after previous infection with heterologous flaviviruses. Hamsters infected only by DENV and WNV or by SLEV and WNV (YFV-negative controls in Experiments 3 and 4, respectively) showed no histologic abnormalities in the liver, spleen, kidneys, lymph nodes or heart (Figure 3A and B). In contrast, the YFV-infected animals in Experiments 3 and 4 showed mild-to-moderate lobular inflammatory cell infiltrations in the liver on the sixth post-infection day, with scattered but frequent hepatocytic necrapoptosis (Figure 3C–F). In most of these hamsters, the liver pathology was characterized by evenly distributed (scattered) foci of inflammation in the form of microgranulomas, which consisted of small clusters of Kupffer cells, lymphocytes, and a few eosinophils. Some of these microgranulomas contained an apoptotic hepatocyte, which appeared as condensed pink cytoplasm, with or without a pyknotic nucleus (Councilman body). In a few cases, the inflammation was less discrete in distribution but more evenly scattered in the sinusoidal spaces. Some portal tracts also had inflammatory cell infiltration, but the piecemeal necrosis or interface hepatitis was not noted. The YF control hamsters also exhibited diffuse microvesicular steatosis six days after challenge with YFV (Figure 3G and H), as previously described. In contrast, no significant steatosis was found in livers of hamsters in the DEN/WN/YF and SLE/WN/YF groups (Figures 3C–F and 4). A semi-quantitative histologic scoring showed no difference in severity of inflammation or necrapoptosis between the YF control hamsters and the YFV-infected hamsters of Experiments 3 and 4 (Figure 4). Histopathologic changes in the spleen were similar among animals in all four experiments, but with certain differences in severity. While the non-YFV-challenged hamsters in Experiments 3 and 4 showed no pathology in the spleen (Figure 5A), all of the animals challenged with YF virus showed hyperplasia of the lymphoid areas, as characterized by immunoblastic cell proliferation (Figure 5B) or expansion of the lymphoid

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**Table 1**

Hemagglutination inhibition (HI) antibody responses of hamsters to Japanese encephalitis virus (JEV) and yellow fever virus (YFV) antigens 28 days after infection with JEV

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* Antibody titers against corresponding antigens: JEV Ag = yellow fever viral antigen; WNV Ag = West Nile viral antigen; SLEV Ag = St. Louis encephalitis viral antigen. HI antibody titers are expressed as the reciprocal of highest positive serum dilution.

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**Table 2**

Hemagglutination-inhibition (HI) antibody titers* in St. Louis encephalitis virus- and West Nile virus-immune hamsters of experiment 4, immediately before and 6 days after yellow fever virus (YFV) challenge

<table>
<thead>
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</table>

* Reciprocal of highest positive serum dilution.
follicles. At the same time, there was splenic macrophage hyperplasia (Figure 5D). Among hamsters in the YF control group (Experiment 1), white pulp depletion was more prominent (Figure 5C). As summarized in the semi-quantitative analysis in Figure 4, the flavivirus immune groups (DEN/WN/YF and SLE/WN/YF) exhibited more lymphoid hyperplasia, less severe lymphoid depletion, and similar splenic macrophage activation compared with the YF control group in Experiment 1. The YFV-infected hamsters in Experiment 3 showed mild-to-moderate lymphoid hyperplasia in the spleen, but no significant lymphoid depletion or splenic macrophage hyperplasia.

Pancreatic tissue was also examined in some of the hamsters. As previously reported,8 moderate pancreatic necrosis was found in naive hamsters challenged with YFV (Experiment 1), but no necrosis was observed in the pancreas of the flavivirus-immune animals (Experiments 2-4) after YFV challenge.

In hamsters previously infected with JEV and then challenged with YFV, the liver showed mild-to-severe inflammatory cell infiltration of the portal tracts, as well as in the lobular parenchyma, predominantly by activated lymphocytes and plasma cells. Occasional eosinophils were also noted. The portal inflammation exhibited a perivascular pattern centered mainly around the portal veins. The bile ducts showed no evidence of injury. There was no interface hepatitis (piecemeal necrosis) in these lesions. The lobular inflammation was characterized by scattered to frequent hepatocytic necroptosis, accompanied by the accumulation of lymphocytes and plasma cells in the nearby sinusoidal spaces (Figure 6A). However, no bridging necrosis or large areas of confluent necrosis were noted as observed in the YF control group. Occasionally, prominent peri-central venule inflammation was seen. Again, no significant steatosis was observed in these hamsters. The spleen exhibited marked macrophage proliferation (Figure 6B), as well as lymphoid hyperplasia.

**In situ TUNEL analysis.** An in situ TUNEL stain was performed on the liver sections from each group of hamsters, and the apoptotic indices were scored individually, as previously described.8 The mean indices of the groups are shown in Figure 7. No statistically significant difference was found among any of the groups challenged with YFV (Experiments 1–4). No apoptosis was observed in livers of YFV-negative control hamsters from Experiments 3 and 4 (Figure 7).
FIGURE 3.  **A**, Normal histology of liver from a hamster previously infected with Saint Louis encephalitis (SLE) and West Nile (WN) viruses.  **B**, Normal histology of liver from a hamster previously infected with Dengue (DEN) and WN viruses.  **C** and **E**, Liver from a hamster previously exposed to SLE and WN viruses six days after challenge with yellow fever (YF) virus. Moderate inflammatory cell infiltration and scattered Councilman bodies (necropoptotic bodies) can be seen.  **D** and **F**, Liver from a hamster previously infected with DEN and WN viruses six days after challenge with YF virus. Moderate inflammatory cell infiltration and scattered Councilman bodies (necropoptotic bodies) can be seen.  **G** and **H**, Liver from a YF virus-infected control (flavivirus naive) hamster. There is diffuse microvesicular steatosis, and scattered Councilman bodies can be seen. (Magnifications: ×50 in **A, B, C, D,** and **G;** ×100 in **E, F,** and **H**.)
FIGURE 4. Histologic scoring system used to quantitate liver and spleen pathology. SLE = St. Louis encephalitis; WN = West Nile; YF = yellow fever; D1 = dengue-1; Infl = inflammation; Necrapop = necrapoptosis; Steat = steatosis; Lymph Hyp = lymphoid hyperplasia; WP Depl = white pulp depletion; Mac Prolif = macrophage proliferation. The values for the SLE/WN groups were zero and thus are not visible on the graph.

FIGURE 5. A, Normal histology of spleen from a hamster previously infected with St. Louis encephalitis (SLE) and West Nile (WN) viruses. Note the distinct border of the lymphoid follicle surrounding a small arteriole (white pulp). B, Spleen from a hamster previously infected with SLE and WN viruses six days after challenge with yellow fever (YF) virus. Note the lymphoid hyperplasia characterized by the proliferation of immunoblastic cells, and macrophages (arrowheads). C, White pulp depletion of spleen from a flavivirus-naive (control) hamster six days after infection with YF virus. D, Splenic macrophage hyperplasia, from a hamster previously infected with dengue-1 and WN viruses six days after challenge with YF virus. Arrowheads show tangible-body macrophages. (Magnifications: ×25 in A; ×50 in B, C, and D.)
Figures 6A and 6B are not described in the text.

**DISCUSSION**

The results of this study support previous observations and speculation that immunity to heterologous flavivirus reduces the severity of YFV infection. In the present series of experiments, prior infection of hamsters with JEV, SLEV, WNV and DENV protected the animals from fatal disease after challenge with YFV. The protective effect of prior heterologous flavivirus infection was demonstrated by lower levels of viremia, less severe alteration of liver function (as measured by levels of TB and ALT), and an absence of hepatic steatosis in the flavivirus immune groups (Experiments 2–4) compared with the flavivirus-naive (YF control) group. A similar protective effect by prior heterologous flavivirus infection has been reported in a hamster model of WN encephalitis.

Our studies showed that hamsters previously exposed to other flaviviruses developed inflammation and hepatic necroptosis similar in extent to that observed in naive hamsters after YFV challenge. However, in the flavivirus-immune animals, no steatosis was found, in contrast to the extensive microvesicular steatosis observed in the flavivirus-naive hamsters following infection with YFV. The lack of steatosis was correlated with a clinically milder form of YFV infection as shown by lower viremia, less alteration of liver function, and generally asymptomatic infection. Histologic changes in the spleen were also different in the flavivirus-immune hamsters compared with the flavivirus-naive group in that there was more prominent lymphoid hyperplasia and much milder white pulp depletion in the former animals. Another difference noted between the immune hamsters and the naive group was the lack of pancreatic necrosis in the former. In summary, the flavivirus-immune hamsters demonstrated splenic lymphoid hyperplasia, a lack of microvesicular steatosis in the liver, and an absence of pancreatic necrosis compared with the pathology observed in naive animals infected by YFV.

In human cases of YF, although abnormalities are noted in multiple organs, the most consistent findings are seen in the liver. Microvesicular steatosis is a prominent microscopic feature of YFV infection in liver of susceptible vertebrate hosts, including humans, monkeys, or hamsters. In the hamster YF model, small numbers of minute fatty vesicles start to appear in some of the hepatocytes by the third or fourth day after YFV infection; by the fifth or sixth day, the fatty vesicles have spread to involve more cells in larger areas, and they usually occupy the entire cytoplasm (Figure 3G and H).

We have observed the association of liver steatosis with an adverse outcome in other experiments with YFV in the hamster model. For example, in studies comparing the virulence (mortality rate) of different YFV strains in hamsters, splenic lymphoid hyperplasia and a lack of liver steatosis were consistent findings in hamsters infected by less virulent strains of YFV (Fisher AF and others, unpublished data). These findings suggest that extensive steatosis is related to more severe clinical disease in YFV infection. However, steatosis may not be the cause for the adverse outcome of YFV infection in these hamsters; instead it may be simply a marker for severe liver dysfunction.

Massive hepatic steatosis (particularly the microvesicular type) occurs in other acute liver diseases, such as Reye’s syndrome. Its presence and rapid onset may indicate severe functional “shut-down” of the liver, and may explain the high mortality seen in diseases such as YF and Reye’s syndrome. This acutely developing phenomenon suggests dysfunction of mitochondria, since at the early stages, overt cellular degeneration is rarely observed. Mitochondrial changes also probably play an important role in the activation of apoptosis or necroptosis. The latter is a major component in YF liver pathology.

The hepatic necroptosis that occurs in YF ranges from scattered, individual Councilman bodies to foci of multiple hepatocytes forming coagula, and diffuse mid-zonal necrosis. In advanced or severe YF cases, the mid-zonal pattern can be obscured by the involvement of zones 1 and 3 as well. The pathologic features of necroptosis observed in the hamster model of YF suggest that a spectrum of changes occurs during the process. This hypothesis is supported by in situ TUNEL staining, which revealed nuclear DNA fragmentation in the liver before discernable cell lysis occurred. In addition, typical apoptotic bodies of different stages were seen in livers of YFV-infected hamsters, using electron microscopy (Xiao SY, Popov VL, Tesh RB, unpublished data). We suspect that liver cell necrosis in human cases of YF also begins...
as apoptosis, directly induced by virus infection, and not by necroinflammatory injuries. This view is based on the lack of consistent inflammatory cell infiltration in the disease, and the fact that the Councilman body, the morphologic evidence of apoptosis, was first described in YF livers. When viewed in the later stages of the disease, such as at autopsy, the necrosis is so extensive and advanced, and may be complicated by other secondary changes, that the process can no longer be simply attributed to apoptosis.

We suspect that YFV causes morphologic and functional compromise to the mitochondria of the hepatocytes, which contributes to cell apoptosis, and at the same time causes abnormal lipid peroxidation and microvesicular fatty changes. The pathogenesis of YFV-induced hepatic necrosis is largely unknown; but our future research will focus on how YFV triggers the cellular apoptosis pathways, as well as steatosis.

Although no studies have been reported in examining the relationship between mitochondrial dysfunction and YFV infection, studies with another flavivirus, hepatitis C virus, indicate that it induces steatosis in human patients and transgenic mice by the binding of hepatitis C virus core protein with host cell mitochondrial protein. More recently, using immunogold electron microscopy, we have found YF viral antigen to be associated with hepatocyte mitochondria, in addition to rough endoplasmic reticulum (Xiao SY, Popov VL, Tesh RB, unpublished data). The functional compromise of the hepatocytes may also affect the synthetic functions of the liver, thus contributing to the bleeding diathesis observed in patients with YF.

Much has been written during the past three decades on the phenomenon of immune enhancement with sequential flavivirus infection. Most of epidemiologic and laboratory studies of this phenomenon have focused on DENV infection and an attempt to explain why some individuals develop dengue hemorrhagic fever (DHF) and dengue shock syndrome (DSS). One dominant theory is that individuals with pre-existing antibody to one DENV serotype are predisposed to the more severe DHF/DSS when subsequently infected by a different DENV serotype. However, our experimental studies with hamster models of YF and WN encephalitis suggest that pre-existing flavivirus antibodies are protective and that they do not increase the severity of these two diseases. Thus, immune enhancement does not appear to be a general phenomenon with all flavivirus infections.

Received December 19, 2002. Accepted for publication March 13, 2003.

Financial support: This study was supported by the National Institutes of Health (grants AI-10984 and AI-50175, NOI-AI-25489) and the Centers for Disease Control and Prevention (grant U50/CCU 620541).

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