Pathogenesis of liver involvement during dengue viral infections

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Summary
The dengue virus can infect many cell types and cause diverse clinical and pathological effects. We describe clinical and experimental observations that suggest that liver involvement occurs during dengue infections, and we outline the possible role played by host immune responses in this process.

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1. Introduction

Dengue is epidemic or endemic in virtually every tropical country. It is the most important viral haemorrhagic fever in the world. Infection may be clinically asymptomatic or give rise to undifferentiated fever, dengue fever (DF), dengue haemorrhagic fever (DHF) or dengue shock syndrome (DSS).

The dengue virus, an RNA virus, belongs to the Flaviviridae family, and consists of four serotypes (DEN1–4). The virus can infect many cell types and cause diverse clinical and pathological effects. Its main effects are on the vascular, muscular and haematological systems. However, both clinical and experimental observations suggest that there is liver involvement during dengue infection. This liver dysfunction could be a direct viral effect on liver cells or be an adverse consequence of dysregulated host immune responses against the virus.

In this mini-review we outline the clinical and experimental observations of liver involvement during dengue infections, and discuss the possible role played by host immune responses in this process.

2. Clinical observations

Clinical evidence of liver involvement in dengue infections includes the presence of hepatomegaly and increased serum liver enzymes. Hepatomegaly is frequent and is commoner in patients with DHF than in those with DF. Several studies document raised serum transaminase levels in dengue infection. Transaminase levels are also higher in DHF/DSS than in DF and tend to return to normal 14–21 d after infection.

Kuo et al. (1992) evaluated 270 dengue patients and found abnormal aspartate transaminase (AST) and alanine aminotransaminase (ALT) levels in 93.3 and 82.2%, respectively. Most had mild to moderate increases, while levels
Liver involvement in dengue infections (Chung et al., 1992). Although hepatitis B virus appears not to be influenced by concomitant hepatitis virus others do not see this (Suvatte et al., 1990). The course and anti-emetics) during the early phase of the illness, but potentiated by the intake of drugs (such as acetaminophen). Sequence of different dengue serotypes having varying tissue damage in dengue epidemics, greater degrees of liver damage are seen in most patients with symptomatic dengue infection (of predominantly DHF/DSS patients, severe liver dysfunction more rapidly than ALT levels. This is possibly because AST levels (Gholson et al., 1990), but it is similar to that seen with alcoholic hepatitis. The exact significance of this pattern seen in dengue is uncertain. It has been suggested that it may be due to excess release of AST from damaged myocytes during dengue infections (Chung et al., 1992). Although hepatitis B virus (HBV) is hyperendemic in parts of South America and in the Far East, no evidence exists that HBV infection acts as a co-factor for hepatic damage in dengue infections. In dengue infections, elevations in serum AST appear to be greater than ALT levels. This differs from the pattern in viral hepatitis, in which ALT levels are usually higher than or equal to AST levels (Gholson et al., 1990), but it is similar to that seen with alcoholic hepatitis. The exact significance of this pattern seen in dengue is uncertain. It has been suggested that it may be due to excess release of AST from damaged myocytes during dengue infections (Chung et al., 1992), but this has not been formally tested. Simultaneous measurement of muscle isoforms of lactate dehydrogenase and creatinine kinase may help further clarify this observation. The elevated AST levels tend to return to normal more rapidly than ALT levels. This is possibly because AST (12.5—22 h) has a shorter half-life than ALT (32—43 h) (Hawker, 1991).

Histological changes reported in the liver in dengue include: microvesicular steatosis, hepatocellular necrosis, Kupfer cell hyperplasia and destruction, Councilman bodies and cellular infiltrates at the portal tract (Bhamarapravati, 1989; Burke, 1968). Most reports are based on small numbers of samples obtained from fatal cases. The presence of thrombocytopenia and coagulative dysfunction makes it difficult to obtain samples from others. As such, one is unsure of the degree of changes present in those with milder disease. Steatosis occurs frequently in hepatitis of viral origin and no special significance can be attributed to this process in dengue infections. Hepatocellular necrosis in dengue generally affects the midzonal area and sometimes the centrilobular area. Reasons for this pattern may be that hepatocytes in this zone are more sensitive to anoxia or the products of an immune response (e.g. cytokines and chemokines) or that the dengue virus preferentially infects cells in this zone. In fact, dengue viral RNA and protein have been detected in midzonal hepatocytes, mostly around necrotic foci. Councilman (acidophilic) bodies correspond to hepatocytes showing the characteristic morphology of apoptosis. Inflammatory mononuclear cell infiltrates (of varying intensity) are seen in most specimens studied so far.

The dengue virus has been isolated from the liver of fatal cases (Burke, 1968; Rosen et al., 1989; Sumarmo et al., 1983). Some find it the main organ from which virus could be isolated (Bhamarapravati, 1997; Huerre et al., 2001; Rosen et al., 1989). For instance, using mosquito inoculation techniques, DEN-2 or DEN-3 viruses were recovered from the livers of 5 of 17 fatal cases, but rarely from other tissues (Rosen et al., 1989).

Using dengue-specific RT-PCR on liver samples from fatal cases, dengue RNA was detected in 11 of 15 (Rosen et al., 1999). Dengue RT-PCR has been done on paraffin-embedded samples from autopsies of 10 children with a clinical diagnosis of DHF/DSS 17 years after their death. In 44, 80 and 43% of cases, DEN-2 RNA was detected in the liver, spleen and lymph nodes (Saroli et al., 1999). Dengue viral RNA has also been detected in midzonal hepatocytes of archived paraffin-embedded autopsy tissues using an in-situ PCR method (Kangwanpong et al., 1995).

Immunohistochemistry and in-situ hybridization have been used to localize dengue antigens in naturally infected human tissues (Jessie et al., 2004), with immunoperoxidase methods suggested to be reliable and specific in diagnosing dengue or yellow fever infection from human archive samples (Hall et al., 1991).

Clinical manifestations in severe dengue disease and yellow fever are similar. Viruses causing them are closely related (both are flaviviruses, transmitted by the same group of vectors). Overall liver pathology in dengue appears to be similar to that observed during the early stages of yellow fever (Bhamarapravati, 1997). However, in yellow fever, liver cell necrosis tends to be more severe and extensive. In addition, immunofluorescence patterns in infected HepG2 cells differ. While dengue viral antigens are found as large perinuclear inclusions and small cytoplasmic foci, yellow fever antigens are homogeneously distributed throughout the cytoplasm (Marinneau et al., 1998). DEN-2 infection of HepG2 cells leads to the release of only a small amount of infectious particles and a slight increase in the number of viral antigen-containing cells over time. By contrast, yellow fever viruses replicated to high titres and infected all exposed cells (Marinneau et al., 1998). Furthermore, while dengue virus-infected cells died rapidly by apoptosis, the
highly productive yellow fever virus-infected cells did not show early cytopathic changes (Marianneau et al., 1998). It is possible that early apoptosis of infected hepatocytes followed by their rapid clearance by surrounding phagocytic cells may help to limit the spread of the dengue virus. By contrast, the delayed appearance of apoptotic liver cell death in yellow fever may occur too late. At this stage most cells may have already been infected, causing severe liver destruction.

The dengue virus is able to replicate in both hepatocytes and Kupffer cells (Huerte et al., 2001). Although dengue viruses can enter human Kupffer cells efficiently, replicative infection of these cells appears not to be very efficient (Marianneau et al., 1999). This may be because most viral particles enter such cells by phagocytosis, a process known to lead to viral degradation. In the smaller number of Kupffer cells in which virus replication can occur, the virus probably enters by receptor-mediated endocytosis and fusion. Two phases of Kupffer cell activation have been described. The first phase occurs shortly after infection with nitric oxide and IFN-γ production, with a second a few hours later, involving IL-6 and TNF-α synthesis (Marianneau et al., 1999).

Infection of hepatic cell lines HepG2 and THLE-3 with ChemiVax™-DEN1–4 and their parent viruses, wild-type DEN1–4 and YF17D, showed significant differences in growth kinetics (Brandli et al., 2005). The YF17D virus produced higher titres and caused extensive cytopathic effects earlier than ChemiVax™-DEN1–4 or wild-type DEN1–4 viruses. The lack of growth of chimeric viruses in human hepatic cells suggests that these viruses may be less hepatotrophic than YF17D virus vaccine in humans.

3. Experimental observations

To infect cells, dengue viruses need first to attach to host cell surfaces. This attachment process is considered a major determinant of the viral host range and tissue tropism. The dengue viral envelope protein has been implicated as the viral attachment protein (Chen et al., 1996). Following penetration, internalization occurs by either endocytosis or direct fusion. Evidence exists for both receptor-mediated and non-receptor-mediated entry pathways.

There is considerable interest in determining the nature of cellular proteins used by different viruses to enter cells. Identification of such proteins may allow us to understand this process better. Heparin is able to inhibit DEN-2 virus invasion of the different liver cell lines (Thepparit et al., 2004). However, the degree of internalization varied between serotypes.

At present, exact mechanisms of interaction (including the nature of molecules that facilitate entry) between the dengue virus and liver cells are poorly defined. Glucose regulated protein 78 (GRP78) was reported to be used by DEN-2 to gain entry into HepG2 cells (a human hepatoblastoma cell line) (Jindadamrongwech et al., 2004). Thepparit and Smith (2004) suggested that the DEN-1 virus (but not the other dengue serotypes) used the 37/67 high-affinity laminin receptor to enter liver cells. This is a non-integrin cell surface molecule expressed on normal human liver cells, with upregulated levels on liver carcinoma cells. Laminin receptor binding appeared to occur both directly or via HS-dependent interactions. Interestingly, a similar mechanism has been previously proposed for entry of prion proteins (Gauczynski et al., 2001) and Sindbis viruses (Wang et al., 1992) into cells. Hilgard and Stockert (2000), studying DEN-1 binding protein expression using the Huh7 liver cell line, found the virus able to bind two membrane proteins (approximately 33 and 37 kDa in size). It is possible that the 37-kDa protein described in this report is similar to the molecule described by Thepparit and Smith (2004).

The permissiveness of a cell to infection by a virus is considered to be due to two factors: the ability of the virus to enter the cell, and the factors within the cell that enable the virus to replicate successfully. Cellular permissiveness to dengue virus infection appears to be modulated by viral serotype, strain and cell type. HepG2 cells show a marked influence of cell physiology, with cells in the G2 phase of the cell cycle having a higher susceptibility to infection and a higher rate of virus production per cell (Phoolcharoen and Smith, 2004). The link between the cell cycle and the permissiveness of liver cells to infection with the dengue virus may point the way to understanding the greater susceptibility of children towards the more severe forms of dengue infection (Phoolcharoen and Smith, 2004). Under experimental conditions, HepG2 cell binding of DEN-1 and DEN-2 was found to be non-saturable (Suksapatan and Smith, 2003), in keeping with results using Huh7 cells (Hilgard and Stockert, 2000). It is suggested there is a degree of cooperation in dengue virus binding onto liver cells. The binding of one virus to a liver cell serves to facilitate binding of further virus particles, thus making it easier for successive particles to bind. This is due to inducing conformational changes in cell surface binding molecules.

Dengue viruses have varying effects on liver cell lines in vitro. DEN-2 were able to attach equally well to five liver cell lines, but rates of replication and levels of virion production were higher in the differentiated cell lines (Huh7, PLC, M3B and Chang) than in a de-differentiated cell line (HAA227) (Lin et al., 2000). It is possible that specific cell differentiation factors may play important roles in viral replication within hepatocytes. Studies aimed at identifying such factors may allow us to understand this process better. Heparin is able to inhibit DEN-2 virus invasion of the different liver cell lines (Lin et al., 2002). Dengue viruses have differential susceptibility to heparin inhibition. This property may be used for screening and selecting mutant viruses for use in different animal models.
Liver involvement in dengue infections

of viraemia were noted. Liver involvement was noted in all individuals. Dengue viral antigens were found in the liver and serum but not the brain. Paes et al. found in vitro (Lin et al., 2000a) that dengue infection of liver cells upregulates RANTES mRNA expression by oxidant-dependent and independent pathways.

Following dengue infection, cellular apoptosis in the liver has been seen both in vivo and in vitro (Couvelard et al., 1999; Marianneau et al., 1996, 1997). The Councilman bodies are believed to be the remains of cells undergoing apoptosis (Huerre et al., 2001). Dengue virus infection of primary cultures of human Kupffer cells and a hepatoma cell line induces apoptosis, as evidenced by DNA laddering (Marianneau et al., 1997, 1999). Activation of the transcription factor NF-κB has been implicated in the induction of apoptosis (Marianneau et al., 1997). NF-κB decoys were able to inhibit HepG2 apoptosis, further pointing to the important role played by the NF-κB pathway in this process. Modulating this activity may have future therapeutic potential in patients with severe dengue-induced liver dysfunction.

Several mechanisms may be involved in dengue-induced liver cell apoptosis. These include direct cytopathic effects of the virus, mitochondrial dysfunction due to low flow hypoxia and the influence of cellular and humoral immune factors in the liver (as is observed in other types of viral hepatitis). The apoptotic process appears to be independent of p53 (Thongtan et al., 2004). Increased levels of endoplasmic reticulum stress have been speculated to induce apoptosis in dengue, as has been previously proposed with Japanese encephalitis, another member of the Flavivirus genus (Su et al., 2002). Recently, dengue virus-induced TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) expression has been suggested to be partly responsible for causing apoptosis (Matsuda et al., 2005). DEN-2 infection was shown to cause TRAIL promoter activation, whereas a proteasome inhibitor and TRAIL antibody were able to inhibit DEN-2-induced apoptosis.

An animal model for dengue was established by transplanting a human HepG2 cell line into severe combined immunodeficient (SCID) mice, followed by intraperitoneal infection with DEN-2 virus 8 weeks later (An et al., 1999). During the early post-infection stages, high viral titres were found in the liver and serum but not the brain. Paes et al. (2005) have used BALB/c mice to study liver injury following inoculation with a DEN-2 virus isolated from a human patient. Although the mice did not present with clinical signs and survived the infection, hepatic injury was seen in all individuals. Dengue viral antigens were found in the liver. Elevated transaminase levels correlating with a peak of viraemia were noted.

4. Immunopathogenic mechanisms

An excellent review on current knowledge of dengue pathogenesis has been recently published (Stephenson, 2005). Therefore, in this article, we will only describe salient features of the immune response during dengue infections and attempt to outline its relationship to liver involvement in dengue.

Both innate and adaptive host immune responses play important roles in determining the natural history of viral infections. Innate immune responses are induced rapidly and act as first-line defences until specific adaptive immune responses come into play. Differences in antibody, T-cell and cytokine responses are seen among patients with uncomplicated DF or DHF/DSS.

The immune enhancement and viral virulence hypotheses have been put forward to explain the causation of severe dengue disease. Antibody-dependent enhancement (ADE) attempts to explain the observation that individuals experiencing a secondary infection with a heterologous dengue viral serotype have a significantly higher risk of developing DHF/DSS. During secondary dengue infections, preexisting non-neutralizing antibodies may form complexes with the virus and enhance its uptake and replication in macrophages (Halstead and O’Rourke, 1977). The viral virulence hypothesis is based on the observation that some dengue viruses have greater epidemic potential than others. These virus-infected strains replicate faster and to higher concentrations and hence cause higher levels of viraemia.

Effective CD4+ and CD8+ T-cell responses have been suggested to play an important role in clearance of acute dengue viraemia. Following primary dengue, both serotype-specific and serotype-cross-reactive memory T cells are formed. On secondary exposure to a different viral serotype, most serotype-cross-reactive CD4+ and CD8+ T cells are able to augment infection by producing various cytokines (Kurane et al., 1990).

During dengue infection, monocytes, B cells, T cells and mast cells produce large amounts of cytokines. Serum concentrations of TNF-α, IL-2, IL-4 and IFN-γ are highest in the first three days of illness while IL-10, IL-5 and IL-4 tend to appear later (Chaturvedi et al., 1999). It has been suggested that predominant Th2 responses (IL-4, IL-5) occur in DHF/DSS, whereas Th1 (IFN-γ) responses seem to protect against severe infections.

Using MHC class I tetrameric complexes loaded with a peptide from the NS3 protein, expansion of CD8+ T cells with relatively low affinity for the currently infecting virus and higher affinity for serotypes presumed to have been encountered in the past were found (Mongkolpapaya et al., 2003). This was thought to result from the detrimental effects of original antigenic sin. It was argued that these ‘inappropriate’ T cells contribute to immunopathology while doing little to clear the virus. Another explanation suggested for these findings was that high antigenic load associated with a second dengue infection (due to immune enhancement), may preferentially drive high-affinity T cells into apoptosis, which would in turn increase the frequency of lower-affinity cells (Mongkolpapaya et al., 2003).

T-cell responses in dengue infections need to be well regulated, to avoid specific downsides. For instance, in the process of viral elimination, damage to vital target organs may occur. As in the case of the other hepatitis viruses, dengue virus-specific CD4+ and CD8+ T cells may cause liver cell damage by direct cytolysis and/or cytokine-mediated effects. Cytokines produced during the immune response may also have ill effects, such as promoting the excessive deposition of extracellular matrix.
Bhamarapravati et al. (1967) examined the liver pathology of 100 fatal paediatric DHF cases (aged 5 months to 14 years) and reported that cellular infiltration was noted in 64 cases. Lymphocytic cells, megakaryocytes and rarely neutrophils were observed in the sinusoids. Cellular infiltration around the portal tract comprised lymphocytes, plasmacytoid cells and some histiocytes. In a recent study by Huére et al. (2001), in five fatal paediatric cases (aged 10 months to 6 years), little or no inflammatory infiltrate was found in four cases and a moderate periportal infiltrate in one. Chen et al. (2004) infected immunocompetent C57BL/6 mice with high titres of DEN-2 strain 16681 and studied lymphocyte activation and hepatic cellular infiltration. T cells in the infected mice were found to be activated and functionally active, as evidenced by the production of IFN-γ. Most of the activated cells were CD8+ T cells. Liver enzyme elevation and hepatic T-cell infiltration were found to coincide with the kinetics of T-cell activation. Hepatic cellular infiltrates consisted predominantly of T cells. While most CD4+ T cells clustered around the portal vein, most CD8+ T cells were scattered in the hepatic acinus. Flow cytometric analysis of liver infiltrating T cells showed that nearly two-thirds were CD8+ T cells.

Gagnon et al. (1999) have postulated that CD4+ cytotoxic T cells (CTLs) may mediate liver damage in dengue through a mechanism involving bystander lysis. CD4+ T-cell-mediated cytotoxicity is thought to occur via two main pathways: release of perforin and granzymes from the activated CTL or the interaction of Fas ligand on T cells with Fas on the target cell. The Fas/Fas-L pathway could contribute to the destruction of cells presenting viral antigens as well as non-viral antigen-presenting bystander cells that express Fas. Dengue viral capsid-protein-specific CD4+ T-cell clones were able to mediate bystander lysis of HepG2 and Jurkat cells. It has been suggested that dengue virus-specific CTL may be activated by dengue virus-infected Kupffer cells, and in turn lyse hepatocytes via a bystander mechanism. However, such bystander lysis has still not been demonstrated in vivo.

At present, the part played by the host immune response in liver damage is unclear. Differences in the intensity of mononuclear cell infiltrates in the liver have been reported in human and animal studies. Although it has been suggested that cellular infiltrates are lower following arboviral infections than following hepatitis B or C infection, reports in human and animal models of dengue suggest significant infiltrates may occur. It is known that activated viral-specific T cells are able to bind specific receptors on virus-infected cells and induce their apoptosis. It is possible that some T cells that enter the liver may cause damage and either undergo apoptosis or leave the liver. They may also produce a range of cytokines/chemokines, and cause damage to target organs (such as the liver) at a distance from the site of immune activation.

5. Conclusion

In summary, clinical and experimental observations suggest that liver involvement occurs during dengue infections. Clinical evidence includes hepatomegaly and increased serum liver enzymes, with liver involvement being more pronounced in the more severe forms of infection. Dengue viral antigens have been found within hepatocytes, and the virus appears to be able to replicate in both hepatocytes and Kupffer cells, and dysregulated host immune responses may play an important causative role in liver damage. Modulating these immune responses may have a therapeutic potential.

There are limitations in the investigation of liver involvement in dengue infection. Immunopathological lesions in the liver are difficult to study in patients with thrombocytopenia and coagulative dysfunction; present knowledge is based mainly on post-mortem specimens and animal models.

Conflicts of interest statement

The authors have no conflicts of interest concerning the work reported in this paper.

References


Daughday, C.C., Brandt, W.E., McCown, J.M., Russell, P.K., 1981. Evidence for two mechanisms of dengue virus infection of
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adherent human monocyes: troyin sensitive virus receptors and troyin resistant immune complex receptors. Infect. Immun. 32, 469–473.


