Involvement of the liver in dengue infections

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Abstract

Dengue virus, the causative agent of dengue fever (DF) and more severe forms of the disease – dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS) – can infect a number of different types of cells. While monocytes and macrophages are considered to be primary target cells driving the pathology of the disease, numerous studies have implicated the liver as a site of dengue virus replication and both clinical observations and experimental data support a role of the liver in dengue disease. This review aims to provide a brief overview of the data supporting a role of the liver in the pathology of dengue disease.

Keywords: Liver; dengue virus; dengue fever; dengue haemorrhagic fever; dengue shock syndrome.

Introduction

With an estimated 75–500 million infections per year and 3.6 billion people living at the risk of infection,[1] dengue is one of the most important public health problems in most tropical and subtropical countries. The causative agent of dengue, the dengue viruses (DEN-V), are transmitted to humans by the bite of infected female mosquitoes belonging to the Aedes family, most commonly Aedes aegypti and Aedes albopictus.[2]

Infection with DENV can be asymptomatic or can result in a wide spectrum of disease, varying from a mild, non-specific viral illness to dengue fever (DF) to the more severe forms of the disease – dengue haemorrhagic fever (DHF) and dengue shock syndrome (DSS). DF usually starts with a high fever and is often accompanied by a rash, headache and abdominal pains lasting 2–7 days. Frank haemorrhage is uncommon during DF but gastrointestinal bleeding, gingival bleeding and petechiae have been reported in some patients.[3] DHF can occur in primary infections but is more common in secondary infections and also starts with a high fever that is not significantly different from that observed in DF patients. Haemorrhagic phenomenon may be minimal, but around the time of defervescence, patients develop the hallmark of DHF, namely, significant plasma leakage, which in DSS is further characterized by
tachycardia and hypotension.\[4\] If the plasma leakage is severe and remains untreated, patients may develop shock, which can be fatal.\[4,5\]

While the main manifestations of severe dengue disease are primarily haemorrhagic in nature, a significant body of work has been accumulated which implicates the liver as a critical part of the disease pathology.

**Hepatomegaly**

Perhaps the most obvious sign of the involvement of the liver in dengue infections is the high proportion of dengue cases with liver enlargement. In one of the earliest large-scale series of clinical investigations into dengue, Halstead and colleagues observed that the frequency of liver enlargement was similar in both primary and secondary dengue infections, and the authors proposed that a moderate liver enlargement may be a part of the “normal” pathological response to dengue infection.\[6\] More recent studies have been somewhat divided, with some reports suggesting that hepatomegaly is present at between 50–100% of cases\[7-14\] while others document a significantly lower rate of hepatomegaly.\[15-17\] In addition, some studies support a higher rate of hepatomegaly in DHF/ DSS cases as opposed to DF cases\[13,14\] although this may not reach statistical significance.\[14\] On balance, the studies tend to support a high level of hepatomegaly in dengue cases, with perhaps a slightly higher rate in the more severe cases, although this may depend somewhat upon the exact case definitions used.

**Liver enzymes**

Upon injury to the liver, the enzymes, aspartate aminotransferase (AST) and alanine aminotransferase (ALT), are released into the bloodstream, and as a consequence these enzymes are believed to be sensitive indicators of liver damage. Perhaps, unsurprisingly, these enzymes are frequently elevated in dengue patients, as has been shown in numerous studies.\[7,9,12,14,16,18-22\] In one large series of patients examined for both AST and ALT levels, Kuo and colleagues evaluated 240 dengue patients from the 1987–1988 outbreak in Taiwan.\[20\] Elevated levels of AST and ALT were found in 93.3% and 82.2% of cases respectively. While the majority of patients had only mildly or moderately elevated levels of these transaminases, some 10% (11% and 7% for AST and ALT respectively) of patients had levels elevated by 10-fold or greater. Somewhat lower levels of liver enzyme disorder were noted by de Souza and colleagues in their study of 1585 dengue patients, and they observed alterations of AST and ALT levels in 63% and 45% of patients respectively.\[22\] Interestingly, however, the authors noted that the average levels of AST and ALT were significantly higher in DHF patients than in DF patients, an observation supported by other studies.\[9,14\] Several authors have noted that the levels of serum AST are greater than serum ALT,\[18,20,21\] which is in contrast to the normal finding with viral hepatitis.\[23\] Some evidence has suggested that there is a greater degree of involvement of the liver in infections with DENV-3 and DENV-4.\[24\] Overall, the studies are consistent with elevated levels of liver enzymes being a common characteristic of dengue disease, and as such possibly represent a discriminating factor in differentiating dengue from other febrile diseases,\[19\] but is of less use in differentiating DF from DHF.

**Liver specimen studies**

While both hepatomegaly and alterations in liver enzymes point to the involvement of the liver in the disease, they are unable to distinguish between the result of a bystander
or secondary effect. As such, a number of studies have sought to provide direct evidence for the involvement of the liver in the disease process. The earliest of these studies undertook direct histological investigations of specimens from the livers of fatal cases of dengue infection. The predominant findings in these studies were microvesicular steatosis and small foci of hepatocellular necrosis in addition to the presence of councilman bodies, Kupffer cell hyperplasia and mononuclear cell infiltrates at the portal tract. In this respect, the liver damage seen in fatal dengue cases is significantly less severe than that seen in fatal cases of yellow fever virus infection. While relatively uncommon, cases of fulminant hepatitis have also been documented.

Several studies have used an immunohistochemical approach to detect the presence of dengue antigens in liver specimens. These studies have predominantly used antibodies directed against dengue E protein, although one recent study used an antibody directed against dengue NS3 protein, which gives a greater degree of certainty that infected cells are undergoing viral replication and do not reflect the presence of endocytosed or phagocytosed virus particles without viral replication. The majority of the studies detect, to a greater or lesser extent, the presence of dengue antigen in hepatocytes, the major cell type composing the liver. Interestingly, while some studies have suggested that 80–90% of hepatocytes show immunoreactivity, other studies fail to detect dengue antigen in hepatocytes at all. Whether this extreme difference is due to methodological or sample-preparation differences, or truly reflects a different tissue tropism of some dengue virus lineages remains unclear at this point. While some studies have detected the presence of dengue antigen in Kupffer cells, the study by Balsitis and colleagues did not detect the presence of immunoreactive NS3 in these cells, suggesting thereby that Kupffer cells do not support replication of the dengue virus, and immunoreactivity to dengue structural proteins noted by others may reflect phagocytosed virus. This would be consistent with the studies by Marianneau and colleagues who showed that dengue was efficiently taken up by isolated primary human Kupffer cells, but that the infection was non-productive.

Dengue-specific RT-PCR and in situ hybridization has been employed in several studies to detect the presence of the dengue genome. As with the studies utilizing immunohistochemistry, while the majority of the studies detect the presence of the dengue genome in liver samples, and more specifically in hepatocytes and to a lesser extent in Kupffer cells, other studies do not detect the presence of the dengue genome in either hepatocytes or Kupffer cells. Again, this may well reflect either methodological differences or inherent differences in virus tropism.

Several studies have recovered infectious dengue virus from liver specimens and, in particular, Rosen and colleagues recovered infectious dengue serotype 2 or 3 from 5 out of 17 liver specimens from fatal cases of dengue infection through the mosquito inoculation technique. However, these studies do not use defined populations of cells from the liver and as such, no information as to the cell types involved in the disease process is available.

**Mouse model studies**

A number of studies have utilized various mouse model systems to understand the pathogenesis of dengue infections. Perhaps the simplest model is the intraperitoneal and intravenous injection of DENV-2 into BALB/c mice. Histopathological analysis of these
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Animals showed severe liver injury, including hepatocyte swelling and vacuolization, necrosis and steatosis. Liver damage was more severe, and occurred earlier in the intravenous injection system as compared to the intraperitoneal injection system. Ultrastructural studies showed the accumulation of cytoplasmic lipid droplets as well as mitochondrial swelling in hepatocytes, possibly related to the induction of apoptosis. Evaluation of liver enzymes showed a peak of both AST and ALT at seven days post-infection, which was correlated to the extent of liver injury. Dengue virus antigens were detected not only in hepatocytes but also in Kupffer cells.

While SCID mice are apparently resistant to dengue infection, SCID mice transplanted with human liver cell lines HepG2 or Huh-7 or with K562 (an erythroleukemia cell line) cells and intraperitoneally injected with DENV-2 or DENV-4 have all been used as model systems, with somewhat conflicting results. For example, while An and colleagues report high levels of the virus in the liver of SCID mice with transplanted HepG2 cells in the early stage post-infection, Lin and colleagues report little or no involvement of the liver in the same SCID mice engrafted with human K562 cells.

Immunocompetent C57BL/6 mice injected with a high titre of DENV-2 showed elevated levels of AST and ALT on days 3, 5 and 7 post-infection, which was elevated further after a second inoculation. Dengue RNA was detected in the liver of infected mice and a strong correlation was found between T cell activation and hepatic cellular infiltration. Dengue infection of interferon receptor-deficient (AG129) mice is uniformly lethal, and the presence of immunoreactive NS3 was detected in hepatocytes, implying dengue virus replication in these cells being consistent with reports of dengue virus infection of human primary hepatocytes.

While some of these studies failed to detect the involvement of the liver, other studies have demonstrated a clear evidence of the involvement of the liver and of hepatocytes specifically. Where liver involvement is seen in the model systems, the changes observed reflect the pathology of human disease.

Isolated primary liver cells

To date, only two studies have investigated the susceptibility to dengue infection of primary (untransformed) human liver cells. The first study isolated Kupffer cells from liver sections obtained during partial hepatectomy for liver cancer and demonstrated that while dengue virus entered into Kupffer cells efficiently, no viral progeny were produced and that infected cells underwent cell death by apoptosis. The second study utilized commercially obtained hepatocytes purified from liver transplantation “cut-downs” and demonstrated productive infection with DENV-2 strain 16681 as assessed by plaque assay and cellular expression of both structural and non-structural proteins (E and NS1). A significant cytokine response was observed which included the up-regulation of TRAIL, MIP-1α, MIP-1β, IFN-β, IL-8 and RANTES. The profile of cytokine induction was similar, but not identical to the profile generated by DENV-2-infected HepG2 cells undertaken in parallel with the infection of the primary hepatocytes. Although not formally evaluated, evidence of the induction of apoptosis in response to DENV-2 infection was observed in the infected primary hepatocytes which showed extensive nuclear fragmentation. Infected primary hepatocytes additionally
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showed the up-regulation of TRAIL, a type-II transmembrane protein that has significant proapoptotic effect in liver cells, and that has been proposed to be the primary mediator of apoptosis induction in dengue-infected transformed hepatocytes.

Studies on transformed liver cells

Transformed liver cells are broadly permissive to dengue infection and a large number of studies have investigated cellular responses to dengue infection in transformed liver cells. These studies have investigated virus attachment, entry, replication and the consequent liver cell death. Heparin sulphate has been implicated in dengue virus attachment to liver cells, either as a direct virus receptor or, more probably, as either a low affinity-binding molecule or receptor co-factor. Two cell surface-expressed proteins have been implicated as liver cell-expressed dengue virus receptors, and it has been proposed that these proteins act in a serotype-specific manner.

The first protein implicated, GRP78 or BiP, was proposed to act as a receptor for DENV-2 while the 37/67kda high-affinity laminin receptor protein has been implicated as a DENV-1 receptor. A critical role for GRP78 as a chaperone protein in the replication of dengue has also been suggested. The 37/67kda high-affinity laminin receptor protein has additionally been implicated as a receptor for Sindbis virus, tick-borne encephalitis virus, and Venezuelan equine encephalitis virus, while GRP78 has been implicated as a co-receptor for coxsackie virus A9. The issue of serotype-specific entry of the dengue virus into liver cells is somewhat controversial, as other identified dengue virus receptors such as DC-SIGN and Hsp79/90 in different cell types do not show such a specificity, although in some cases, not all serotypes have been investigated. Dengue virus entry to liver cells has been suggested to occur predominantly, but not solely, by clathrin-coated pit-mediated endocytosis and it has been proposed that as much as 20% of virus entry to liver cells may occur through alternate pathways. While somewhat controversial, support for the concept of multiple entry pathways has also been presented in studies on dengue virus entry into Vero (monkey kidney) cells. One study has suggested that dengue virus entry into HepG2 cells is modulated at least in part by the cell cycle, as has been found with dengue entry into insect cells.

Two studies have investigated global gene expression changes in HepG2 cells by either microarray analysis or cDNA-AFLP. Both studies estimate that some 500 gene transcripts belonging to a number of different pathways are differentially regulated in response to dengue infection. The microarray analysis identified a number of genes involved in the innate immune response and, in particular, pattern recognition genes such as TLR3, TLR8, RIG-1 and MDA5 were identified as being up-regulated. Some genes identified such as IL-6, IL-8, RANTES and IFN- were consistent with data generated from analysis of dengue-infected primary hepatocytes. Perhaps significantly, the microarray analysis of dengue virus-infected HepG2 cells also detected the up-regulation of caspases 8 and 10 (see next section).

In contrast to the data which suggests that some 500 genes have altered transcriptional regulation, proteomic analysis of dengue-infected HepG2 cells have only identified some 17 proteins as being differentially expressed, and these proteins were primarily...
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involved in the regulation of transcription and translation. \[82\] Interestingly, a secretome analysis of dengue-infected HepG2 cells identified significantly more proteins as being differentially regulated by dengue virus infection, with 35 proteins being down-regulated and 24 proteins being up-regulated in infected HepG2 cells. \[83\] Several proteins, including \(\alpha\)-enolase, superoxide dismutase (SOD), peptidyl-prolyl isomerase A and B (cyclophilins A and B), tissue inhibitor of metalloproteinases 1 and 2 (TIMP-1 and 2) and macrophage migration inhibitory factor (MIF) identified in the secretome of dengue virus-infected HepG2 cells (as opposed to the secretome of uninfected HepG2 cells), have been previously identified in other virus infections or inflammation situations. \[83\]

Two groups have shown that autophagy, the lysosomal degradation pathway, is activated in response to dengue virus infection of liver cells \[84-86\] and it has been suggested that in liver cells, autophagic vesicles act as sites for dengue virus replication. \[86\] Again, as with dengue virus receptor usage, \[66,67,87\] it has been proposed that interactions between the dengue virus and the autophagy pathway are serotype-specific. \[84,86\] Given that the endocytosis pathway interacts with the autophagic pathway, a model has been proposed to link dengue virus entry and replication in liver cells in terms of a continuing interaction with membranes of an endosomal-autophagosomal lineage. \[88\]

Apoptosis

Cellular apoptosis in the liver upon dengue virus infection has been reported both in vivo and in vitro. \[35,40,63,89,90\] Histological examination of the livers of fatal cases of dengue virus infection note the presence of councilman bodies which are believed to be the remains of cells undergoing apoptosis. \[38\] Dengue virus infection of primary cultures of human Kupffer cells and hepatoma cell lines induces apoptosis as evidenced by DNA laddering, \[63,89-91\] and infection of primary human hepatocytes produces morphological changes characteristic of apoptosis. \[59\] The mechanism by which the dengue virus induces apoptosis remains to be clearly elucidated. While some authors have proposed that the apoptosis of liver cells occurs by a p53-independent mechanism, based in part on the high level of apoptosis in the p53-null cell line Hep3B, \[63\] other authors have proposed that apoptosis occurs by a p53-dependent mechanism. \[92\] In 2005, Matsuda and colleagues \[61\] presented evidence that Apo2L or TRAIL (tumour necrosis factor-related apoptosis-inducing ligand) is induced by dengue infection and that this interacts with the Apo2L/TRAIL receptor DR5/TRAIL-R2 expressed on the surface of liver cells \[61\] inducing apoptosis. This would suggest that apoptosis was induced primarily through an extrinsic apoptosis pathway. In contrast Nasirudeen and Liu, who have suggested apoptosis primarily results through an intrinsic, mitochondrially-mediated pathway. \[92\] However, more recently, Nasirudeen and Liu also suggested that apoptosis in liver cells may be mediated through caspase 1. \[93\] Other authors have proposed that the critical event in triggering apoptosis in dengue-infected hepatocytes is the interaction between the dengue virus capsid protein and the human death domain-associated protein Daxx, \[94\] and that nuclear localization of the dengue capsid protein is essential for the interaction with Daxx and subsequent apoptosis. \[95\] Further research is required to integrate the various suggested models of induction of apoptosis in liver cells as a consequence of dengue virus infection.
Alternate mechanisms

A number of alternatives to direct infection of liver cells have been suggested to account for the involvement of the liver in dengue disease. These mechanisms include damage induced by the infiltration of activated lymphocytes, especially CD8+ T cells, bystander lysis mediated by CD4+ cytotoxic T cells after activation by dengue-infected Kupffer cells, as well as the action of antibodies against dengue proteins cross-reacting with host cell proteins. Overall, the theories of an alternate mechanism of liver damage in dengue infection are somewhat less well supported than models proposing direct infection of cells. However, there is no evidence ruling out either model, and it is possible that both mechanisms, direct and indirect liver involvement, may occur together in dengue infections.

Conclusions

A large body of clinical and experimental evidence points to the involvement of the liver in the pathobiology of dengue virus infections of humans. The balance of evidence suggests that hepatocytes are directly involved in the infection as sites of dengue replication, possibly adding to the total viral burden. It remains to be seen whether the increasing body of information on the involvement of the liver can be translated into hepato-protective treatments, and whether this would reduce either the severity of the disease or the likelihood of patients developing more severe forms of the disease.

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References


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