

## Dengue Hemorrhagic Fever: Pathology and Pathogenesis

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Dengue Hemorrhagic Fever is the most severe manifestation of human infection by the mosquito-borne flavivirus Dengue. Dengue virus is an enveloped virus, with a single stranded, positive sense RNA genome that encodes three structural genes (E, PrM, C) and seven nonstructural genes. There are four antigenically distinct serotypes of Dengue virus (DEN1-4). Geographic expansion of the range of dengue serotypes and the *Aedes aegypti* vector has been accompanied by dramatically increasing numbers of Dengue fever and DHF cases. DHF is distinguished from classic Dengue Fever (DF) by the presence of vascular leak, manifesting as hemoconcentration, hypoproteinemia, serous effusions, and in the most severe cases, shock. DHF has been classified into four grades based on clinical indicators, with grades III and IV representing Dengue shock syndrome (DSS). DHF occurs most commonly in children and is associated with secondary infection by a heterologous Dengue serotype. DHF is generally associated with higher viremia titers than DF. Thrombocytopenia is a constant feature of dengue infections, but the mechanism of this is not clear. DIC is seen in only a few instances of grades III-IV DHF. Plasma leak coincides with defervescence and clearance of viremia, suggesting immunopathological mechanism of endothelial injury as opposed to direct effects of virus.

The pathology of fatal DHF has been well described in large autopsy series. Hemorrhages of the pleura, epicardium, gastrointestinal mucosa and skin are present, and serous effusions and edema of retroperitoneal soft tissues are prominent. Histopathologic manifestations are dominated by the liver lesion, which consists of variable degrees of hepatocellular necrosis, primarily midzonal. Other features of the associated hepatitis, such as presence of Councilman bodies and Torres bodies are reminiscent of Yellow Fever. Spleens show atrophy of the white pulp, both T and B cell areas, along with increased numbers of reactive lymphocytes in the red pulp, correlating with the presence of atypical lymphocytes in the peripheral blood. Capillaries and arterioles in several organs show endothelial swelling, minimal perivascular inflammation and edema, and rare apoptotic endothelial cells. In general, histopathologic changes do not explain the profound microvascular insufficiency characteristic of this disease.

Dendritic cells and cells of the mononuclear phagocyte system are important early targets of infection. Immature Langerhans cells are permissive for infection and are likely the earliest target after infection by the bite of an infected mosquito. Antibody dependent enhancement of monocyte infection has been demonstrated in primary unfractionated cultures of human peripheral blood leukocytes and splenocytes infected with various DEN isolates. In tissues obtained at autopsy or biopsy, immunohistochemistry demonstrates viral antigen in hepatocytes, Kupffer cells, splenic macrophages, and, focally, in endothelial cells.

DHF is believed to be immunologically driven. The Halstead hypothesis states that secondary infection by a different Dengue strain results in antibody dependent enhancement of mononuclear phagocyte infection. Secondary dengue infections are also associated with generation of cross-reactive T cell responses originating from T memory cells. Severe disease is associated with immunological activation markers, such as sIL-2R, IL-2, and activated immunophenotype of peripheral blood monocytes. The degree of liver injury correlates not with viremia, but with markers of immune activation. Mechanisms of endothelial injury are likely multiple and include direct viral effects and indirect effects of cytokines and other mediators. Overproduction of inflammatory cytokines, such as  $IFN\gamma$ ,  $TNF\alpha$ , MCP-1, and IL-8 has been documented in serum of DHF patients. Monocyte/macrophages and activated T cells are among the probable sources of these mediators. Antibodies generated against the viral NS1 protein cross-react with microvascular endothelial cells and may initiate endothelial injury. Infected endothelial cells show altered expression of VEGF receptors and matrix metalloproteinases, which participate in the regulation of endothelial permeability. The viral protein NS1 interacts with the complement inhibitory protein clusterin, suggesting alterations in complement regulation. The identification of cross-reactive anti-E antibodies that bind plasmin peptides suggest possible interference with fibrinolysis/coagulation systems. Any explanation of vascular leak syndrome in DHF must take into account the relatively sparse infection of microvascular endothelial cells and paucity of frank endothelial damage in fatal human cases. Because animal models that recapitulate the natural history and pathology of DHF are not available, significant gaps remain in understanding the kinetics and sites of viral replication and their relationship to plasma leakage syndromes.

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## Bullet Points and keywords

- Dengue hemorrhagic fever is an acute febrile syndrome with vascular leak, caused by the widely distributed, mosquito born flavivirus Dengue. DHF is most commonly seen in children experiencing a secondary infection with a heterologous serotype of dengue virus. Seroepidemiologic and immunologic studies suggest that pre-existing, non-neutralizing antibody enhances infection of target mononuclear phagocytes and increases virus replication.
- The pathology of fatal DHF has been well described in human autopsy series, but information on the kinetics and location of viral replication in tissues during natural infection is sparse. Animal models that faithfully recapitulate the all aspects of the natural history and pathology of DHF are not available.
- Major pathologic findings are hemorrhages, edema, and midzonal, paucicellular necrosis in the liver. Large reactive lymphocytes are seen in peripheral blood and lymphoid tissues. There are small foci of mild perivascular inflammation, edema, and endothelial swelling in microvasculature of many organs. Important target cells include dendritic cells, mononuclear phagocytes, hepatocytes, and focally, endothelial cells.
- Suggested mechanisms of vascular leak include overproduction of pro-inflammatory cytokines by activated T cells and monocyte/macrophages, direct viral effects on regulation of endothelial permeability, alterations in regulation of complement and fibrinolytic systems, and antiviral antibody that cross-reacts with endothelial cells.

Keywords: Dengue virus, hemorrhagic fever, cytokines, endothelium, immunopathology, hepatitis