STUDIES ON THE PATHOGENESIS OF HEPATITIS DELTA VIRUS

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The primary aim of this study was to investigate the molecular basis of the cytopathic effect of hepatitis delta virus (HDV). HDV is a defective RNA virus that requires a helper function provided by hepatitis B virus (HBV) for its expression and replication. Natural infection with HDV occurs either as a coinfection with HBV, or superinfection of a HBV carrier and may result in fulminant, acute or chronic infection. Acute delta hepatitis results commonly in degenerative changes to the hepatocytes that include shrunken eosinophilic cytoplasm and pyknotic nuclei. The parenchyma is predominantly free of inflammatory cells, even in areas with significant histologic change, consistent with a direct cytopathic effect. In contrast, the histological features associated with chronic HDV infection are suggestive of a necro-inflammatory disease.

The virus-specific antigen, hepatitis delta antigen (HDAg), is expressed as two polypeptides (HDAg-p24 and HDAg-p27) that differ by the addition of 19 amino acids at the carboxy terminus of HDAg-p24 to create HDAg-p27. HDAg-p24 is expressed at high levels in hepatocytes during acute HDV infection, with only small amounts of HDAg-p27 being produced. As the HDV infection progresses to chronicity, the ratio of HDAg-p24:p27 in the liver is decreased. Furthermore, HDAg-p24 expression is necessary for HDV RNA replication, while HDV RNA replication is inhibited by the expression of HDAg-p27.

Thus, a major goal of this study was to investigate the proposal that HDAg-p24 is directly cytotoxic to hepatocytes during the acute phase of HDV infection, and to investigate the role of HDAg-p27 expression in the promotion of persistent infection. An in vitro cell culture model was developed to examine the direct effect of HDAg-p24
expression in stably-transfected eukaryotic cell lines. HDAg-p24 was shown to be
directly cytotoxic when expressed under the control of the inducible human
metallothionein (MTIIA) promoter in HepG2 and HeLa cells. In these cell lines, HDAg-
p24 expression was associated initially with a significantly reduced rate of RNA
synthesis followed by reduced rates of DNA synthesis, by a vast increase in cell
doubling times, and degenerative cytological changes which resembled those seen in
acute-HDV related hepatitis.

Site-directed mutagenesis was used to create HDV cDNA with the capacity to express
the large HDAG polypeptide, HDAg-p27. The HDAg-p27 gene was also placed under
the control of the MTIIA promoter and expressed in stably-transfected HeLa cells. In
contrast to the effects of HDAg-p24 expression, HDAg-p27 expression resulted in a
minimal effect on cellular RNA and DNA synthesis, no significant increase in cell
doubling times, and normal cytology. Thus, the non-cytotoxic nature of HDAg-p27 and
its ability to inhibit HDV RNA replication, are consistent with the proposal that HDAg-
p27 expression may promote persistence.

These results were confirmed in a transient transfection system using the Cos7 cell
line. In this system, transcription of a mutant form of HDAG mRNA in Cos7 cells that
contained a two nucleotide deletion to create a novel stop codon and thus a truncated
form of HDAG, was non-cytotoxic. This confirmed that HDAg-p24-related cytotoxicity is
due to the direct action of HDAg-p24 per se, and is not a result of events leading to
the expression of HDAg-p24. Furthermore, it was shown that HDAg-p27 could
modulate HDAg-p24 related cytotoxicity when coexpressed in the same cell.
It is possible that the transient interference of HBV replication following superinfection of a HBV carrier is a result of HDAg-p24-induced cytotoxicity during the acute phase of infection. The expression of high levels of HDAg-p24, but not HDAg-p27, was shown to inhibit the multiplication of both poliovirus and adenovirus. Thus, the mechanism of transient interference is not HBV-specific. During acute HDV infection, the high levels of HDAg-p24 expression may destroy HBV-producing cells, and this is reflected in a reduction of replicative markers of HBV in the serum and liver of infected patients.

Since HDAg-p27 is non-cytotoxic and may modulate cytotoxicity due to HDAg-p24, then continued expression of HDAg in chronic HDV infection may induce an immune response which is the cause of the continuing hepatitis. Consequently, the nature of the immune infiltrate in acute- and chronic- phase HDV-infected liver samples was examined by immunofluorescent staining. A dramatic decrease in the ratio of CD4+:CD8+ lymphocytes was observed in the persistently-infected liver samples compared with an acute phase sample. A high proportion of the CD8+ lymphocytes can be expected to represent cytotoxic T-lymphocytes (CTL). In addition, hepatic expression of HLA Class I antigens was detected in the majority of the persistently-infected liver samples. These results provide circumstantial evidence to suggest that HDAg may represent a target for CTL in persistent infection, although this finding will need to be confirmed.

On the basis of this study, a model is proposed for HDV pathogenesis. During the acute phase, HDAg-p24 represents the vast bulk of expressed HDAg, and this has a direct cytotoxic effect. The effect of HDAg-p24 is dose-dependent, so that it is likely that hepatocytes which express low levels of HDAg-p24 survive the cytotoxic action
of HDAg-p24. In addition, HBV replication is transiently suppressed as a result of the
cytotoxic action of HDAg-p24 on HBV-producing cells. Thereafter, the ratio of HDAg-
p24:p27 in the liver decreases leading to a reduced level of HDV RNA replication, and
in turn, persistent infection. During persistent infection HDAg may constitute a target
for CTL, and the action of these cells will determine the severity of ongoing hepatitis.