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Recent advances in the molecular biology of hepatitis B virus: mutant virus and the host response

Recent advances in molecular biology have revolutionised the study of the hepatitis B virus. The polymerase chain reaction technique has allowed viral DNA from patients to be amplified and sequenced.[1] Such studies have shown that the genome of hepatitis B virus is highly variable and it is likely that no two viral isolates are genetically identical. Because hepatitis B virus gives rise to a number of distinct diseases it is tempting to suppose that different viral genotypes cause different diseases. Recipients of contaminated blood from patients with hepatitis B virus, however, do not necessarily develop the same disease,[2] indicating that different hepatitis B virus infections are caused by the interaction between a particular viral genotype and their host. In this review we shall discuss the association between various hepatitis B virus genomes and particular diseases and discuss the way in which variant viruses develop and give rise to disparate diseases.

Spectrum of hepatitis B virus hepatitis

Adult infection with hepatitis B virus causes a number of different diseases. The majority of infected adults develop an acute infection characterised by a hepatitis and a cellular immune response directed against the hepatitis B virus nucleocapsid protein, core.[3] In a minority of patients hepatitis B virus infection causes a severe, fulminant, hepatitis. A small number of infected adults do not develop an overt hepatitis and the normal immunological response to hepatitis B virus is impaired.[4] These patients do not eliminate the virus but develop a chronic infection which persists for many years and leads, ultimately, to either cirrhosis or hepatocellular carcinoma. Interferon is the treatment of choice for patients with chronic hepatitis B virus infection and a significant proportion respond and appear to eliminate the virus.[5] Many patients, however, do not respond to interferon. Adult infection with hepatitis B virus thus gives rise to a spectrum of diseases ranging from the relatively minor acute hepatitis through chronic interferon sensitive and interferon resistant hepatitis to the very severe fulminant hepatitis. There are two possible explanations for this disease spectrum: different viruses may differ in their virulence or the host response may vary.

Specific viral mutations associated with specific diseases

In 1989 Carman *et al*[1] identified a variant strain of hepatitis B virus that was associated with a specific disease. Although the majority of patients chronically infected with hepatitis B virus secrete hepatitis B virus e antigen (HBeAg) a minority do not. Carman *et al* studied patients with HBeAg-negative chronic hepatitis B virus infections. HBeAg is a protein encoded by the hepatitis B virus core gene. This gene contains two start codons (genetic sequences that initiate translation and protein production). When the first start codon is used for protein translation a large protein (precore) is formed. Precore contains a 'signal sequence' peptide that causes it to enter the endoplasmic reticulum, where it is degraded to form HBeAg. Translation from the second start codon of the core gene produces a smaller protein, core, that does not contain the signal sequence necessary for entry into the endoplasmic reticulum. Core protein cannot be degraded to HBeAg and its sole function is to form the viral nucleocapsid. When DNA from patients with HBeAg negative infections was sequenced a point mutation was identified between the two start codons. This mutation causes a stop codon to form which prevents the formation of the precore protein (the precursor for HBeAg) but does allow the nucleocapsid protein to be produced, by translation initiation at the second start codon. Hence viral particles are produced but HBeAg is not formed. The discovery of a mutant virus that was associated with a particular form of hepatitis B virus infection led to speculation that other mutations might be associated with specific diseases and a number of studies have addressed this issue.

One mutation – two diseases

In the early 1990s a number of groups studied the genotype of viruses that were associated with fulminant hepatitis. A common viral variant was identified in the majority of cases.[2 6] This mutation, however, was the same as that recognised in 1989 as the cause of HBeAg negative chronic hepatitis. Hence the same viral genotype can cause two distinct diseases, and clearly factors other than viral mutations must be involved in the pathogenesis of HBV infections.

Host response to hepatitis B virus

The observation that viral mutations alone cannot explain the variability of hepatitis B virus infections has led to a reevaluation of the patient's response to the virus. Two factors contribute to the host response to hepatitis B virus –

interferon production and the immunological response to hepatitis B virus antigens.

(a) INTERFERON PRODUCTION AND ITS EFFECTS

In an acute hepatitis B virus infection interferon is produced in large amounts.[7] Interferon increases the expression of hepatocyte HLA antigens[4] and induces the production of a group of enzymes (known as the RING genes)[8 9] that process viral proteins and allow them to associate with HLA antigens. In patients who are able to respond to hepatitis B virus proteins the HLA antigen processed viral antigen complex stimulates an immune response which results in the generation of cytotoxic T cells that lyse infected cells. Interferon thus interacts with the immune system and assists in the resolution of acute hepatitis B virus infections by presenting viral proteins to responsive immunocytes.

In patients who develop chronic hepatitis B virus infections interferon production is impaired[10] and an appropriate immune response does not develop. It is not yet clear why some patients do not produce interferon. Hepatitis B virus contains two proteins which affect the production of interferon – the X protein can stimulate the production of β-IFN[11] while the core protein can inhibit its production.[12] Presumably the amount of interferon produced in any HBV infection depends upon the relative activities of these two proteins. It should be noted that studies of interferon induction to date have examined the effects of hepatitis B virus on the induction of β-IFN. Hepatocytes produce α-IFN and not β-IFN however (Foster, Thomas, MacNair, and Thurz – unpublished data) and hence the interactions between hepatitis B virus and interferon production have not yet been adequately studied.

Interferon deficiency cannot be the sole determinant of chronic hepatitis B virus infection – if it was, then all patients with chronic hepatitis B virus infections should respond to interferon therapy. Recent work has shown that hepatitis B virus contains a protein (terminal protein) that can inhibit the cellular response to interferon.[13] In patients with chronic hepatitis B virus infections the amount of terminal protein in biopsy specimens varies and patients who express large amounts of this interferon inhibitor usually fail to respond to interferon therapy (Foster, Goldin, Stark, and Thomas – submitted for publication). The outcome of any hepatitis B virus infection may therefore depend upon the induction of and the cellular response to interferon.

(b) IMMUNOLOGICAL RESPONSE TO HEPATITIS B VIRUS

In an acute infection, or a chronic infection that resolves with interferon therapy, viral eradication is the result of elimination of infected hepatocytes by cytotoxic T cells.[4] Cytotoxic T cells only recognise viral antigens which have been processed by the RING proteins (see above) and then presented on the cell surface by HLA class I antigens. Only a small number of viral proteins are processed in this way and within each target protein there are antigenic epitopes that combine specifically with certain HLA antigens.[3] The epitope that combines with an HLA antigen depends on the nature of the HLA protein. Hence the viral target for cytotoxic T cell mediated lysis varies in different patients and depends upon the patient's HLA type. For hepatitis B virus it is now clear that the core protein is the main target for cytotoxic T cells and the antigenic epitopes within the core protein that associate with the HLA antigen A2 have now been identified.[3] The epitopes that associate with other HLA antigens have not yet been found.

CHANGING VIRUSES AND THE HOST RESPONSE

Sequential studies from patients with chronic hepatitis B virus infections have shown that viral mutations develop during the course of an infection and that the dominant viral strain changes.[14 15] We believe that 'new' viruses arise spontaneously during a chronic infection and that immunological pressure then selects mutant viruses that have a survival advantage. Some viruses – for example, human immunodeficiency virus – HIV – are known to escape from the immune system by generating novel cytotoxic T cell epitopes[16] that cannot be recognised by the host and it seems likely that variant hepatitis B viruses develop in the same way. The HBeAg negative mutation (described above) often develops during a chronic hepatitis B virus infection and its development is associated with an exacerbation of the hepatitis.[15] Presumably the host immune system develops a response to HBeAg (either humoral or cell mediated) and is able to eliminate hepatocytes that express HBeAg. The virus responds to this host selection pressure by eliminating the immunological target – HBeAg negative mutant viruses will have a significant survival advantage and hence HBeAg negative infection develops.

The production and effects of interferon may profoundly influence the course of an hepatitis B virus infection and both core and terminal protein are known to alter the interferon system (see above). These proteins are encoded by a closely related region of hepatitis B virus DNA (the two genes overlap, and the proteins are produced from different open reading frames). We now know that in chronic infections the DNA that encodes these two proteins often mutates,[14 15] presumably because of immunological selection pressure and the 'new', mutated viruses may differ in their interferon response characteristics. In a host in which HBV has mutated such that the immune system cannot recognise viral antigens the effects of IFN may be of little consequence. If the same virus infects another host whose immune system can respond to the presented viral antigens, then the production and actions of interferon may play a vital role in either the elimination of the virus or its persistence. Hence a virus that has adapted to one host may cause a quite separate disease in a new one.

Conclusion

Recent advances in molecular biology and immunology have greatly increased our understanding of the pathology of hepatitis B virus. The different diseases caused by this variable virus are caused by a complex interaction between the host's immune system, the interferon response and the viral genotype. It seems likely that in chronically infected patients viral variants develop that are able to avoid the host's antiviral mechanisms and hence the virus is able to persist for many years. A virus that has adapted to one host may, however, be poorly adapted to another and when transferred to a new patient the same virus may be rapidly eliminated or give rise to an overwhelming hepatitis. A complete understanding of how this virus evolves to escape the immune system is now within sight and such an understanding may lead to improved therapy for the millions of patients infected with this ubiquitous pathogen.

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