Noncytolytic Mechanisms Involved in Hepatitis B Virus Clearance

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Persistence versus clearance

Central to our understanding of hepatitis B virus pathogenesis from infancy throughout adulthood is our understanding of the factors mediating viral persistence versus clearance. HBV infection itself appears noncytopathic, as evidence by the normal growth and appearance of infected cells in culture and the apparently normal liver in many infected newborns. Thus, because the disease spectrum associated with HBV is extraordinarily variable, it is widely believed that the host response plays a critical role in the pathogenesis of the associated liver disease. Infected newborns usually develop immune tolerance and proceed to becoming chronic carriers, whereas the majority of infected adults clear the viral infection without long-term damage to the liver. The HLA-class I restricted T cell responses in adults with active infection who clear the virus are generally polyclonal, avid, and multispecific, whereas neonates and adults progressing to chronic disease exhibit diminished and limited CTL repertoires. These findings led to initial conclusions that the quality and strength of the CTL response was primarily and selectively responsible for HBV clearance.

Considerations in CTL-mediated killing

Does the CTL in humans and animals who clear virus act by killing all of the HBV-infected cells ? The liver in humans with HBV (and in animals such as woodchucks with WHV, and in ducklings with DHBV) is saturated with virus ; all of the hepatocytes contain virus upon immunohistochemical analysis within the first month following infection. Potentially, if all of infected hepatocytes were killed simultaneously, fulminant liver failure would result. Patients and animals clearing infection generally demonstrate complete clearance from hepatocytes within one month. *In vivo* bromodeoxyuridine staining of liver, to determine rate and extent of hepatocyte turnover, demonstrates only 2-3% of the cells turning over at any one time. Contemporaneous measurement of hepatocyte apoptosis, an end result of CTL response, demonstrates 1-10% of cells undergoing involution. The question thus becomes whether infected individual cells are cured of infection, or are they killed and replaced by protected hepatocyte precursors ?

Steps required for intracellular clearance

If indeed virus is cleared from individual hepatocytes, many mechanisms are likely involved in curing cells of virus and viral precursors. The covalently closed circular DNA of the virus in the nucleus must be eliminated, as this serves as the amplicon for replication and serves as the template for pregenomic RNA synthesis (which is required as a template for later reverse transcription/replication). Capsids and pregenomic RNA must be eliminated from the cytoplasm, and circulating virions must be eliminated from the circulation and extrahepatic reservoirs to prevent reinfection of cured hepatocytes. Likely, the humoral antibody response is important in the last regard, but little is understood regarding virus specific mediators providing intracellular clearance of viral products and precursors.

The transgenic mouse model for studying immunopathogenesis

To address the relative roles of CTL-induced killing versus CTL-directed curing in the clearance of HBV from liver, an elegant transgenic mouse model was developed by Chisari and colleagues. Particular transgenic strains containing overlength HBV transgenes produce intact and infectious HBV particles. The replication pattern of HBV within liver is similar to that found in normal infections in permissive animals, although the template for replication in these mice is the transgene and not ccc DNA. Transgenic animals are tolerant to infection since they have been exposed to viral antigens throughout embryogenesis. Genetically identical animals absent the transgene can be immunized with specific viral antigens to generate specific CTL. These lymphocytes are transferable to transgenic animals in quantitative doses. Particular responses can then be studied in a controlled cohort accounting for age, gender, and viral titer. Using this model, Guidotti *et al.* established that adoptively transferred CTL completely abolished viral transcription and capsid formation 5 days after CTL infusion. At the dose transferred,

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it was estimated that less than 10% of the hepatocytes underwent cytolysis, which was confirmed by immunohistochemical analyses, and cells undergoing mitosis were rare. Cell curing dominated over cell killing by at least a ten-to-one ratio.

Two separate mechanisms were inferred to be operational based on kinetic data. Although viral capsids and viral RNAs were both completely abolished in the background of minimal necroinflammatory CTL activity, the viral capsids were eliminated 1-2 days prior to reductions in viral RNA. Whether another mechanism is operational for removal of ccc DNA could not be assessed, since the transgenic mice do not contain this viral species.

Guidotti *et al.* next assessed whether CTL-mediated killing was required for the paracrine phenomenon of CTL-mediated curing. Similar to the experiments just described, CTLs against specific HBV core epitopes were transferred into mice containing the HBV transgenes who were also knocked out for genes essential for CTL-mediated apoptosis. These HBV-positive, perforin-negative mice cleared viral transcripts and replication intermediates to the same extent and with the same kinetics. Necroinflammatory activity, as demonstrated by serum ALT and histochemical staining, was notably absent. This infers that antigen-specific CTL is required to activate and regulate antigen-nonspecific cytokine cascades. Notably, tumor necrosis factor-alpha and interferon-gamma were implicated as key humoral elements induced during CTL migration required for cure. This was demonstrated by pretreating CTL-transfused animals with antibodies to both of these two cytokines, completely blocking the CTL-induced inhibition of viral capsids and RNA. Whether these cytokines are wholly responsible for CTL-induced curing, and the mechanism(s) by which they act in curing are issues for current investigation.

Role of cytokines in transcriptional regulation

Other studies have investigated the means by which inflammatory cytokines modulate viral gene expression *in vitro*. Romero and Lavine prepared reporter constructs in which luciferase gene expression was driven by various HBV promoter elements. These constructs were transfected into immortalized human hepatoma cell lines treated with various concentrations and combinations of recombinant cytokines. Both tumor necrosis factor-alpha and interferon-gamma significantly inhibited reporter gene expression in a dose-dependent and additive fashion. Nuclear run-on assays demonstrated that expression was diminished due to inhibition of transcription from the viral promoter.

Conclusion

The spectrum of outcomes in individuals with HBV infection likely depends on the quality of the CTL response. A vigorous qualitative response likely initiates a quantitiatively sufficient cytokine response in individuals going on to clear infection. An insufficient or partial response may result in perpetual necroinflammatory activity without adequate cytokine release for intracellular cure. This promotes long term complications of fibrosis, cirrhosis and hepatocellular carcinoma. Further understanding of the means by which cytokines cure cells will help in the design of rational and natural therapeutic options for individuals with chronic HBV infection.

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