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Hepatitis B viral infection of hepatic progenitor cells. Resolving unresolved questions?

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ABSTRACT

Accumulated data to date do not entirely explain the; propensity of the hepatitis B virus (HBV) to cause chronic infections in newborns; failure of antiviral agents to resolve infections or precise mechanism whereby HBV causes hepatocellular carcinoma (HCC). Based on the increased numbers of hepatic stem/progenitor cells (HPCs) present within the neonatal liver, the refractoriness of these cells to the effects of interferons and xenobiotics and their ability to undergo malignant transformation, we hypothesize that HBV infection of HPCs could explain these and perhaps other clinical features of chronic HBV.

1. Introduction

While much has been learned about the hepatitis B virus (HBV) since the Australia Antigen was first discovered by Blumberg et al. in the early 1960s, a number of key features of HBV infection remain unexplained. Included amongst these are why does exposure to HBV so often result in chronic infections of newborns; why do HBV infections promptly relapse following withdrawal of antiviral therapy and what is the precise mechanism whereby HBV induces hepatocellular carcinoma (HCC)? The purpose of this paper is to introduce the possibility that HBV infection of hepatic progenitor cells (HPCs) could help to explain these and other unresolved questions regarding HBV infection.

2. Chronicity of HBV infections in newborns

2.1. Dogma

Neonatal exposure to HBV results in chronic HBV infections because the newborn's immune system is too immature to achieve HBV clearance [1].

2.2. Supportive data

(1) Newborns and infants have functional differences in their immune system relative to adults [2,3]. (2) HBV and HBeAg in particular, has the capacity to induce "immune tolerance" and therefore, the offspring of mothers with high viral loads at the time of birth (HBeAg positive) are likely to acquire HBV tolerance and a chronic carrier state [4]. (3) HBV-transgenic mice have impaired T- and B-cell priming that could contribute to HBV persistence [5].

2.3. Conflicting data

While it is acknowledged that functional differences between the immune system of a newborn and adult exist, differences do not necessarily equate with inadequate responses. Indeed there are a number of findings to suggest the immune system of newborns is sufficiently responsive to protect against acute HBV infections becoming chronic. First, the immune response of newborns to HBV vaccination is both prompt and robust [6]. Second, young subjects with malaria and HBV co-infections, particularly those with high HBV-DNA levels, have reduced parasitemia compared to those with malaria alone and an increased incidence of cerebral malaria, manifestations of robust T-helper (Th)1 activity [7,8]. Third, adolescents with chronic HBV resulting from exposure as newborns or infants do not demonstrate tolerogenic T-cell features [9]. Finally, recent data indicate that fetal exposure to HBV *in utero*

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results in innate immune cell maturation and enhanced Th1activity as manifest by high IL-12, p40 and IFN-α2 but low IL-10 expression [10].

Together, the above reports suggest that HBV-infected newborns and infants present a "fully normal Th1 T-cell profile and do not show any increased defects in HBV-specific T-cell repertoire compared with HBV-infected adults" [10].

3. Hypothesis #1

HBV infection of hepatic progenitor cells (HPCs) within the newborn liver is responsible for the chronic HBV that occurs in this age group.

3.1. Rationale

Whereas HPCs constitute only a small percent (<1%) of the adult liver cell population, in newborns their prevalence is as high as 10–15% [11,12]. Moreover, essentially all of the HPCs are sufficiently differentiated (i.e. at the "hepatoblast" stage) to express the sodium taurocholate receptor required for HBV entry into cells. Thus, HBV infection of HPCs is more likely to occur in newborns than adults.

A unique feature of HPCs is that they represent an "immune privileged" cell type [13,14]. That property relates to their lack of or limited human leukocyte antigen-DR expression, ability to suppress the proliferation and activity of CD_4, CD_8 and NK lymphocytes, M_1 macrophages and inhibit the production of pro-inflammatory cytokines. In addition, it has been demonstrated that pluripotent human cells, human embryonic stem cells and human induced pluripotent stem cells have weaker responses to cytoplasmic double-stranded RNA, produce little interferon-β (IFN-β) and have attenuated responses to IFN-β (due to SOC_2 upregulation) [15]. Together, these features would favor HBV persistence within HPCs rather than the immune-mediated clearance that tends to occur with infection of mature hepatocytes.

None-the-less, an essential component of this hypothesis requires evidence that HBV can infect HPCs. Here, the data is somewhat conflicting but trends toward such infections occurring. For example, HBV has been reported not to infect human bone marrow mesenchymal stem cells and human adipose-derived mesenchymal stem cells, at least not in their de-differentiated or uninduced state [16,17]. However, other reports describe successful infection and replication of HBV within human bone marrow mesenchymal stem cells, differentiated umbilical cord matrix stem cells and induced pluripotent stem cell-derived hepatocellular systems [18–20]. Moreover, HBV markers have been identified in non-tumor associated HPCs adjacent to HCC in chronic HBV carriers [21]. Thus, HBV infection of HPCs is likely to occur and would be expected to result in immunity of the virus from host mediated immune responses.

4. HBV relapses following withdrawal of antiviral therapy

4.1. Dogma

HBV relapses following withdrawal of antiviral therapy stem from persistence of covalently closed circular-DNA (ccc-DNA) within adult hepatocytes [22].

4.2. Supporting data

ccc-DNA represents a potentially, fully functional HBV replicative intermediate [23]. Thus, the loss of ccc-DNA is generally considered a prerequisite to viral clearance. Moreover, presently available antiviral agents at best lower but do not eradicate ccc-DNA from infected hepatocytes [24,25].

4.3. Conflicting data

There is a conceptual flaw in the ccc-DNA theory for viral persistence despite long-term antiviral therapy in that the life-span of an adult hepatocyte is 200–300 days and at the time of death, autophagy and apoptosis occur resulting in the loss and removal of all cell constituents including ccc-DNA [26]. Moreover, even if ccc-DNA were to survive the apoptotic and clearance process, it still does not possess the necessary protein/lipid encapsulation required for hepatocyte uptake [27]. In addition, liver macrophages do not possess the enzymatic activity and other cellular components required to support HBV replication, packaging and export [27]. Thus, were antiviral therapy able to completely inhibit HBV replication leaving only ccc-DNA intact, and were that therapy to be administered for the duration of a hepatocyte life span, theoretically, HBV infections within the liver should clear.

5. Hypothesis #2

HBV infections relapse following withdrawal of antiviral therapy as a result of residual virus remaining in the HPC population of the liver.

5.1. Rationale

The rationale for proposing that relapses following withdrawal of antiviral therapy relate to HBV infection of HPCs, is based on the long-term survival of these cells which has been estimated to be decades and their resistance to antiviral therapy [15,28,29]. Specifically, as indicated above, HPCs have an attenuated response to interferons and therefore, interferon therapy would not be expected to successfully eradicate HBV from HPCs [15]. With respect to nucleos(t)ide analogue therapy, data are limited. Evidence of HBV-infected HPCs being relatively resistant to entecavir therapy can be derived from *in vitro* studies wherein entecavir had limited antiviral effects on HBV infected stem cells maintained in a micropatterning format and cocultured with fibroblasts until the cells differentiated into a more mature hepatocyte [19,30]. In addition, like other stem/progenitor cells, HPCs manifest decreased drug uptake, altered intracellular drug metabolism and enhanced drug export [31,32]. The latter resulting from upregulation of multidrug resistance protein transporters [33]. Thus, were HBV-infected HPCs resistant to antiviral therapy, cessation of such therapy would result in a prompt relapse. The same reservoir of residual virus might also help to explain recurrences of HBV in otherwise resolved HBV infections when immunosuppressive agents are introduced rather than implicating undetectable amounts of residual HBV within mature hepatocytes, subgenomic viral DNA integrations or yet unidentified extrahepatic sources of the virus.

6. HBV-induced hepatocellular carcinoma

6.1. Dogma

HBV induces HCC by directly or indirectly causing mutations to host hepatocyte DNA that results in malignant transformation of the infected hepatocyte [34].

6.2. Supportive data

HBV-DNA integrates into the host cell genome and in the process, mutates host DNA shortly following infection [35]. Moreover,