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MINI REVIEW

The immune tolerant phase of chronic HBV infection: new perspectives on an old concept

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Chronic hepatitis B virus (HBV) infection progresses through distinct disease phases that are strongly associated with patient age. The so-called immune tolerant (IT) phase represents the classical early phase of infection; it is associated with high levels of HBV replication and lack of clinical signs of liver Inflammation. Whether this phase of HBV infection is also associated with immunological features of ''tolerance' has recently been challenged. Here, we review the data that dispute this concept of immune tolerance and then propose an alternative interpretation of the immunopathological events that take place during this early phase of CHB infection.
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INTRODUCTION

The development of chronic hepatitis B (CHB) is inextricably linked to the patient's age at the time of infection. Hepatitis B virus (HBV) is thought to exploit the immaturity of the neonatal immune system to establish a persistent infection, reflected in the 90% of neonates who develop chronicity following vertical or perinatal transmission, while children infected between the ages of 1 and 5 years are believed to have a 30% chance of developing chronic infection; conversely only 1%–5% of subjects infected in adulthood will develop CHB.[1,2] Historically, fetal development of tolerance to the virus *in utero*, through the transplacental transfer of viral proteins, was cited as a possible explanation for the high rates of chronic infection in infants.[3]

Patient age at the time of exposure does not only play a role in the outcome of infection, but also influences the clinical and virological features of the disease. Chronic HBV infection progresses through distinct disease phases that are strongly associated with patient age; the so called immune tolerant (IT) phase represents the classical early phase of infection, while the immune clearance and immune control phase are typically associated with adult patients (Figure 1a). These disease phases, which define the natural course of CHB, should mirror the different interaction between the host immune response and the virus.[2] However, the concept of immunological tolerance, a basic premise on which the disease is managed and treatment decisions are made, is increasingly being challenged.

A fundamental problem is that the IT phase, synonymous with HBV infection in young people, is a clinical categorisation used to define a disease state in an immune-mediated liver condition; broadly, it refers to hepatitis B e antigen positivity, high levels of HBV DNA and normal serum aminotransferases. The IT phase is usually asymptomatic and believed to be associated with minimal or no liver fibrosis on biopsy.[1,2] However, whether this clinical and virological phenotype is really linked with genuine features of immune tolerance and a lack of liver damage is questionable. Several data can be used to challenge this concept. The efficacy of Peg IFN-alpha and Peg IFN-alpha+nucleos(t)ide analogue therapy has been reported to be superior in 'immune tolerant' children than it is in adults.[4,5] The concept of immune tolerance present in HBV-infected young people contrasts with the epidemiological observation in malaria-HBV co-infected young patients in whom reduced parasitemia[6] and an increased incidence of cerebral malaria, a Th1-mediated malaria complication,[7] have been reported. In addition, a recent immunological characterization of IT HBV-infected adolescents did not reveal any

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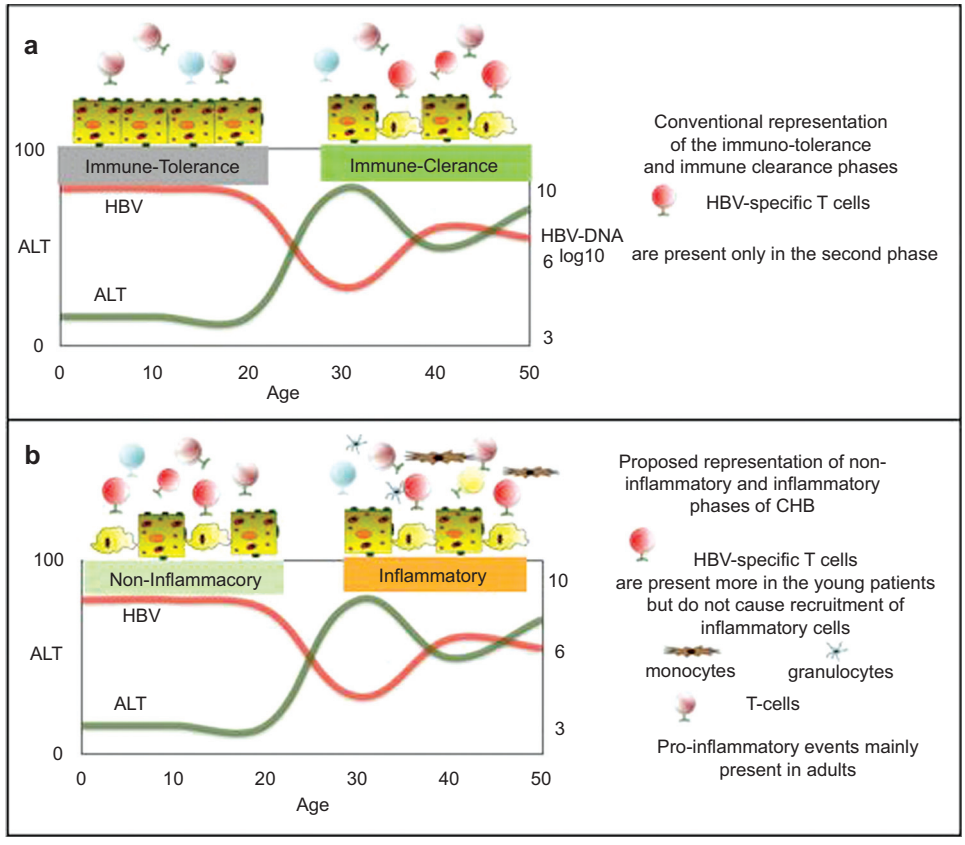

Figure 1 Conventional (**a**) and proposed new representations (**b**) of the immunological events occurring during the natural history of HBV infection. HBV, hepatitis B virus.

tolerogenic T-cell pattern.[8] Finally, histologically active disease with evidence of minimal chronic hepatitis or nonspecific reactive hepatitis has been reported in CHB children under the age of 10–12 years, considered IT.[9,10]

Here, we will review the data that dispute this concept of immune tolerance and then propose an alternative interpretation of the immunopathological events that take place during this phase of CHB infection.

DOES VERTICAL OR PERINATAL HBV INFECTION INDUCE IMMUNE TOLERANCE?

HBV is thought to capitalize on the immaturity of the newborn immune system and establish a persistent infection through the induction of a state of low or absent virus-specific response.[11] Acute hepatitis B infection in adults is almost invariably associated with control of HBV infection through the induction of an efficient HBV-specific T and B cell response.[12] On the contrary, HBV infection in infants or young children rarely causes acute hepatitis and results in the asymptomatic disease phase characterized by high levels of HBV replication and a low incidence of liver inflammation defined clinically as immune tolerance.[2] To explain this dichotomy, data from experimental animal models (i.e., HBV transgenic animals) have described the presence of immunological defects which impair HBV-specific T- and B-cell priming in neonatal animals;[3,13,14] thus predisposing to HBV chronicity.

However, the concept that HBV exposure at birth is only associated with viral persistence and an inability to trigger an antigen-specific adaptive immunity is not unequivocal. First, the concept that the neonatal immune system is in some way 'defective' appears more uncertain and there is growing evidence to suggest that neonatal immune responses defy such simple categorization. Both effector and regulatory immune responses are already in place during early fetal life.[15,16] Newborns have been shown to mount a virus-specific T-cell response.[17–19] The immune systems of newborns and infants are not 'defective' *per se*, but appear less prone to trigger a pro-inflammatory reaction which is likely an evolutionary adaptation to avoid dangerous immune reactions *in utero*.[20] Furthermore, a better analysis of data generated in natural HBV infection reveals that a proportion of neonates exposed to HBV at birth, mount a HBV-specific T-cell response. For example, two independent studies performed in HBsAg-negative children born to HBV-positive mothers[21,22] have demonstrated the presence of core and polymerase-specific T cells. Neonates of HBV^+ mothers have also been shown to have minimal or normal dendritic cell functions.[23–25]

Furthermore, the efficacy of HBV vaccination within the first year of life in HBV^+ children[26–27] raises considerable doubt that the state of HBV immune tolerance and defects in T–B cell interactions, detected in murine models,[28,29] rep-

resent the inevitable consequence of HBV exposure in neonates and children.

IMMUNOLOGICAL AND VIROLOGICAL PARAMETERS IN THE IMMUNE TOLERANT PHASE OF HBV INFECTION

Most of the evidence supporting the existence of an IT disease phase of HBV infection is based on clinical and virological parameters. HBV is not directly cytopathic and since HBV-specific CD8 T cells control HBV replication by recognition and killing of the HBV infected hepatocytes,[30] it is logical to interpret perturbations in alanine aminotransferase (ALT) level as a reflection of the presence or absence of HBV-specific T cells. Normal or minimal fluctuation in the liver enzymes has therefore been perceived as an indication of a lack of HBV-specific T-cell response. On the contrary, alterations in the liver enzymes and fluctuating levels of HBV DNA replication, which are observed more commonly in adulthood, are interpreted as an awakening of HBV-specific immunity.

In reality, both experimental data in animal models and HBV infection in humans have shown that ALT measurement cannot be used as a surrogate of a virus-specific T-cell response. Overt T-cell immunity against hepatocytes performed in adenovirus infected mice[31] takes place without alteration in the serum ALT. Furthermore, adoptive transfer of HBV-specific T cells can cause substantial inhibition of HBV replication without increased levels of ALT, an observation that led to the understanding that efficient HBV clearance from infected hepatocytes is also mediated by a cytokine-mediated non-cythopathic effect.[32,33]

Besides, the quantification of circulating and intrahepatic HBV-specific T cells in CHB patients have shown that their quantity is not proportional to the level of biochemical activity reflected in the serum ALT.[28,29] The robust inflammatory events in the liver that are associated with significant ALT elevations, are actually associated with an intrahepatic recruitment of granulocytes, monocytes and non-antigen-specific T cells.[28,34–37]

A direct demonstration that HBV immune response is not completely absent during the IT phase of CHB disease was shown in our recent work. We studied the immune response in CHB-infected adolescents with ostensible IT disease, based on their clinical and virological profile; analysis of the circulatory T cells demonstrated that these patients did not display any tolerogenic T-cell features. Moreover, they could mount a perfectly normal Th1 T-cell response and even harbour HBV-specific T cells. Notably, these HBV-specific T cells, though weak and functionally impaired as one would expect to see in patients with chronic hepatitis B, were in fact quantitatively and functionally superior to those found in CHB-infected adults in the 'immune clearance' phase of disease.[8] In addition to these immunological data, analysis of HBV quasispecies in children with an IT clinical profile showed significant HBV diversity.[38] Despite this work focusing primarily on the analysis of HBV diversity in adult patients with CHB, the data collected in children with an IT clinical profile were compatible with the presence of a potential immune pressure against HBV during this initial phase of infection.

DOES THE IMMUNE TOLERANT PHASE OF HBV INFECTION STAND UP TO CLOSER SCRUTINY IN THE CLINIC?

We have discussed the limitations of serum ALT as a surrogate of HBV-specific T-cell activity and we summarized the data suggesting that the IT phase of disease is not associated with a complete absence of HBV-specific T-cell immunity. However, it is also important to understand whether normal liver enzymes and the potential presence of antiviral immunity can fully exclude ongoing disease activity within the liver.

Indeed, it has already been proposed by others that there is likely a low but persistent immune destruction of infected hepatocytes, by low-level cytotoxic T lymphocyte infiltration, leading over time to an adaptive response of the liver, with a possible clonal emergence of hepatocytes resistant to HBV infection; a scenario that could explain the progressive decrease of HBV replication levels over time.[39]

Studies in CHB-infected children under the age of 10–12 years, considered IT, were shown to have histologically active disease with evidence of minimal chronic hepatitis or non-specific reactive hepatitis, despite ALT levels being described as within the normal range[9,10] and clonal hepatocyte repopulation, an indirect measurement of targeted killing of HBV-infected hepatocytes, has been detected in patients in the IT disease phase (Kennedy and Mason, manuscript in preparation). Whether such low-level hepatocyte lysis can initiate the process for the development of liver cirrhosis and/or HCC later in adult life is at this juncture, difficult to predict.

AGE-DEPENDENT CHANGES IN IMMUNITY

We have reviewed clinical and experimental evidence showing that HBV-specific T-cell immunity and some degree of liver damage are not completely absent in the initial phase of chronic HBV infection. However, how can we explain the differences in the clinical and virological profiles associated with age?

A possible explanation can be related to the fact that immune responses are not identical during the life of an individual. TLR-mediated immune function has been shown to change in different periods of life;[40] the production of anti-inflammatory cytokines (e.g., IL-10) is high in preterm infants, then progressively declines over the first years of life, but is superior in children when compared to adults. Conversely, pro-inflammatory cytokines (e.g., IL-1beta, TNF-alpha) steadily increase during early life[40] until they reach the state of chronic low-grade systemic inflammation called 'inflammaging' that is characteristic of elderly subjects.[41] Similarly, T-cell response shifts from a Th2/Treg type response in newborns to a more Th1 response in children/adults [20] with a progressive increase of effector memory T-cell pools, which are more sensitive to cytokine-mediated activation.[42]

Can the different virological and inflammatory patterns observed during the natural history of HBV infection be caused by the age-related modulation in immune function?

The concept that pathological processes might be modulated by age is established in other infections. For example, during the 1918 influenza pandemic, children, despite experiencing

the same rate of clinical influenza, had a much lower mortality rate than young adults.[43] This phenomenon has also been observed in other bacterial and viral infections[44] and has been associated with the lower production of pro-inflammatory cytokines in children.

Interestingly, a detailed, albeit small, longitudinal study has shown that more dramatic changes in serum ALT levels occur in CHB patients around the 20- to 25-year-old age bracket,[38] which is exactly the period where more pronounced pro-inflammatory events are observed in humans.

IMMUNOLOGICAL EVENTS IN HBV INFECTION: AN ALTERNATIVE HYPOTHESIS

Based on the epidemiological, clinical and experimental data summarized above, we propose an alternative interpretation of the immune events occurring during the different phases of chronic HBV infection.

The state of high HBV replication and low ALT levels present in children and young CHB patients might be caused by the presence of an anti-viral 'non-inflammatory' immune response (Figure 1b). As we have summarized above, data obtained from young patients with CHB (aged between 15 and 30 years) show that, at least in this period, the absence of serological markers of liver inflammation is not associated with a complete absence of HBV-specific T-cell response.[8] Furthermore, signs of limited killing of hepatocytes[9,10] and of virological immune escape[38] can be detected in patients in the IT disease phase. Thus, in the initial phase of HBV infection, the few and functionally impaired HBV-specific T cells might, similar to adults, try to contain the HBV infection by cytokine-mediated control and by killing HBV-infected hepatocytes. However, as a consequence of the reduced pro-inflammatory cytokine milieu and of the limited pool of T cells responding to by-stander cytokine- mediated activation, this HBV-specific T-cell response does not trigger nonspecific inflammatory events. In contrast the so called 'immune clearance phase' of chronic hepatitis B, characterized by abnormal serum ALT levels, fluctuations in the levels of HBV replication and clear histological signs of liver inflammation; may differ not by the number or function of HBV-specific T cells (that are more profoundly exhausted in CHB infected adult patients),[8] but the higher propensity to trigger 'inflammatory' events (Figure 1b). Liver inflammation can still be triggered by HBV-specific CD8 T cells, but it is unlikely a direct effect of a quantitative or functional recovery, which is in fact theoretical and has never actually been demonstrated in adult CHB patients. In these subjects, HBV-specific T-cell response has never been shown to be proportional to liver inflammation.[28] Furthermore, episodes of heightened liver inflammation, like those observed during hepatic flares, occur without any detectable increase in circulating HBV-specific T cells,[45] while they are associated with fluctuations in chemokines[45] and natural killer cell activation.[46] Alternatively, liver inflammatory events in adults may derive directly from pro-inflammatory reactions triggered, for example, by bacterial products as shown in a model of hepatic steatosis.[47] It is notable that increased and persistent production of pro-inflammatory cytokines like IL-1beta and TNF-alpha can directly inhibit HBV replication[48,49] and thus, explain the reduced HBV-DNA levels observed in adults patients.

CONCLUSIONS

CHB is a dynamic disease with an unpredictable course. The assumption that those infected at birth or in early childhood enter a phase of protracted immune tolerance with minimal or no disease progression over two to three decades appears increasingly implausible. Furthermore, as we discussed here, this clinical categorization lacks meaningful immunological data to support the concept of tolerance; while clinically the concept of a generic IT disease phase appears increasingly unsound.

Experimental and epidemiological data from HBV infection raise doubts that the natural progression of chronic HBV infection can be dictated by a simple quantitative difference in adaptive immunity against the virus. The oversimplistic view that vertical or perinatal transmission of HBV results in a phase of HBV-specific immune tolerance which is then lost in adulthood is not supported by our increased understanding of the effect of age on the immune response, nor is it supported by our better insight into the immune events triggered by HBV infection. The arguments presented here challenge the concept of a generic IT disease phase in young people and thus importantly, raise questions about the premise on which treatment decisions are made.

Additional data need to be gathered from HBV-exposed newborns and CHB-infected children, in whom limited studies have been performed to date. The potential role and function of many other components of the immune response, such as natural killer or natural killer T cells that are highly abundant in the liver, should also be analyzed in the context of age.[50] Should more data support the proposed thinking, some practical consequences might arise. Firstly, if young CHB patients exhibit a more conserved HBV-specific immune response, than that observed in adult patients, but do not trigger a full blown pro-inflammatory reaction; therapy designed to boost HBV-specific immunity (vaccine therapy, check points inhibitors) is more likely to be successful and indeed less damaging in young patients. Secondly, we should start to consider and evaluate CHB in adults as an inflammatory rather than a viral induced disease. The efficacy of therapy based on suppressing platelet function in blocking the development of HCC in HBV transgenic mice represents the first step towards this analysis.[51,52]

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