Original Article

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Progress of research on the immune tolerance of chronic HBV infection

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Abstract: Immune tolerance is a specific lack or negative response of T and B lymphocytes to antigen. According to different formation periods, immune tolerance can be divided into central and peripheral tolerances. The immune tolerance of the body to hepatitis B virus (HBV) after infection is the main cause of chronic HBV infection. In this paper, the functional defects of hepatitis B virus e antigen and dendritic cells, hyporesponsiveness of cytotoxic T lymphocyte, variation of helper T lymphocytes and cytokines, HBV genotype and genome, and the role of host gene polymorphism in the formation of immune tolerance in chronic HBV infection and its related research progress are introduced briefly.

Keywords: hepatitis B virus, hepatic DNA virus infection, immune tolerance

The history of human survival and development considerably involves fighting against pathogenic microorganisms. Pathogenic microorganisms cause a series of reactions after infecting the body through various routes. The human body can completely return to normal after most pathogens have been affected by the immune clearance of the body or active treatment. However, a small number of pathogens cause recessive infections, carrier states, or latent infections attributed to weak immune response or immune tolerance, as well as decreased pathogens and weak pathogenicity. Chronic infection caused by hepatitis B virus (HBV) is typically due to immune tolerance.

This paper reviews the understanding and research progress of scholars on immune response after HBV infection in the past 50 years.

Clinical outcomes after HBV infection are different. In adults infected with HBV, most patients exhibit acute hepatitis B, and almost all viruses can be removed from the body without treatment. Approximately 1% to 5% of patients develop chronic infections [1]. Children aged 1–5 years with HBV develop approximately 30% to 50% of chronic infections [1]. The risk of chronic infection of neonates infected with HBV during the perinatal period is as high as 80%–90% [1]. In this regard, some scholars believe that different performances are due to different host immune status after HBV infection in different age groups.

The causes of chronic HBV infection are believed to be multifaceted, including multiple factors, and are mainly related to the immune tolerance of the body to HBV, high HBV DNA load, HBV mutation, untimely or irregular treatment, and so on. The immune tolerance of the body to HBV is the most important cause of chronic infection.

1 HBV infection and central tolerance

The immune system undergoes a series of selections during the process of development, and the immature T and B lymphocytes that recognize their own antigens exhibit negative selection, leading to elimination of clones and formation of immune tolerance to their own tissues. Under normal physiological conditions, the

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selection process ensures that the immune system is stable without causing autoimmune diseases. However, in some pathological situations, the selection process can be used by pathogenic microorganisms to escape the immune clearance of the host for pathogens to effectively continue to infect the human body.

Tian *et al.* [2] have demonstrated that HBV can use this naturally occurring immune tolerance pathway to maintain its persistent infection in humans. Through a case–control study, Singh *et al.* [3] found that positive hepatitis B virus e antigen (HBeAg) indicates that HBV is in the active replication state, and small molecular weight of HBeAg plays an important role in the formation of intrauterine immune tolerance through the placental barrier, which considerably explains why children infected with HBV during perinatal and infantile stages are prone to develop chronic infections. The maternal immune system in pregnancy undergoes subtle changes to produce immune tolerance to the antigenic substance encoded by the heterologous gene of the father in the fetus without causing immunological damage to the fetus. Similarly, fetuses are also exposed to a large number of non-hereditary antigens produced by the mother, which can cause a series of changes to the fetal immune system, thus producing immune tolerance. Such mutual tolerance to allogeneic antigens between mother and fetus during pregnancy can effectively maintain intimate contact between maternal and fetal heterotypic genes. However, immunological changes to ensure tolerance to harmless exogenous antigens have become loopholes in the fetal and neonatal defense systems. Small molecule HBeAg in HBeAg-positive mothers can pass through the placental barrier and enter the fetus during pregnancy. However, the fetal immune system is immature, and this non-self-antigen is mistaken as a self-antigen, causing clonal elimination of immature T and B lymphocytes that can recognize HBV antigens, thereby forming immune tolerance to HBV. After birth of the fetus, T and B lymphocytes still develop, and the elimination of clones applied to immature T and B lymphocytes in response to the self-antigen still continues. Thus, once an infant is infected with HBV, the immune system exhibits difficulty in completely removing the virus, resulting in persistent infection. This feature is also a good explanation for a common clinical phenomenon: without artificial intervention, HBsAg-positive offspring of HBeAg-positive chronic hepatitis B (CHB) mothers carrying wild-type HBV become chronic HBV carriers, and HBsAg-positive offspring of HBeAg-negative CHB mothers usually develop acute self-limiting HBV infection [4].

HBV vaccine is immunologically effective in HBV-negative infants born to HBV-positive mothers. This phenomenon contradicts the traditional immune tolerance view of intrauterine HBV exposure-induced immune tolerance [5]. Moreover, epidemiological, clinical, and experimental evidence have also questioned the concept of immune tolerance in HBV infection [6]. Hong *et al.* [7] found that HBV, a virus that is believed to use the immune system of the fetus and newborn to establish a chronic infection virus, can unexpectedly train the fetal and neonatal immune systems, promoting the development of innate immune cells and the helper T lymphocytes (Th) 1. By contrast, this process enhances the antibacterial ability of immune cells in fetal cord blood *in vitro*. These training effects are associated with changes in the cytokine environment characterized by low interleukin (IL)-10 and the commonly high IL-12p40 and interferon alpha-2 (IFNα2). This process not only reveals the potential symbiotic relationship between HBV and its natural host but also demonstrates the plasticity of the fetal immune system after intrauterine virus exposure. Possibly, this virus–host symbiosis and interaction can explain why HBV is so tenacious in most people. However, the impact of HBV on the immune system of infants and young children with vertical transmission remains to be further explored.

2 HBV infection and peripheral tolerance

2.1 Dendritic cells (DCs) and immune tolerance

DCs are currently the most powerful specific antigen-presenting cells, which can significantly stimulate the initial T lymphocyte proliferation and directly activate the initial T lymphocyte response to secrete various cytokines and chemokines to regulate the function of other immune cells. DC function is impaired in patients with chronic HBV infections [8]. Upregulation of programmed death molecule 1 ligand on myeloid DC in CHB patients significantly inhibits immune function of T lymphocytes, and chronic inflammation of the

liver promotes programmed death molecule 1 ligand expression on myeloid DC, resulting in HBV-specific T lymphocyte function defects and sustained replication of the virus [9]. Lan *et al.* [10] found that HBeAg may inhibit DC maturation. Chen *et al.* [11] found that HBeAg can upregulate the expression of Toll-like receptor 3 on DC and reduce the secretion of IFNγ, which may be one of the molecular mechanisms involved in HBV immune tolerance. Shi *et al.* [12] found that HBV can inhibit the interaction between natural killers and plasma cell-derived DC, thus affecting plasma cell-derived DC-induced natural killers secreting IFNγ and leading to chronic HBV infection.

2.2 Cytotoxic T lymphocytes (CTLs) and immune tolerance effect

CTLs are proliferated and differentiated from peripheral lymphoid tissues by $CD8^+$ T lymphocytes and then leave the lymphoid tissue to accumulate at the site of the infection under the action of chemokines. This process specifically kills the host cells of intracellular parasites and other pathogens without damaging normal tissue. Th1 and HBV-specific CTL immune responses play a key role in HBV clearance, but the CTL response ability of CHB patients is diminished [13]. HBcAg can activate Toll-like receptor 2 on Kupffer cells, causing Kupffer cells to secrete additional IL-10 and inhibit the immune function of HBV-specific $CD8^+$ T lymphocytes [14]. This process mediates the depletion of anti-HBV $CD8^+$ T lymphocytes and induces liver immune tolerance to HBV. Programmed death molecule 1 (PD-1)/B7 homolog 1 (B7-H1) signaling pathway can negatively regulate the immune response of T and B lymphocytes, is closely related to the functional depletion of HBV-specific $CD8^+$ T lymphocytes, and participates in the formation of immune tolerance after HBV infection. The expression of PD-1 and B7-H1 is upregulated in HBV infection [15]. Blocking the B7-H1/PD-1 signaling pathway can enhance DC-mediated T lymphocyte immune response and antiviral capacity [16]. Thus, HBV-specific CTL functional defects are one of the important reasons for the chronicity of HBV infection. Interestingly, Schurich *et al.* [17] found that the antiviral efficacy of HBV-specific T lymphocytes is related to their energy metabolism state. Functionally depleted HBV-specific T lymphocytes limit the plasticity of their energy metabolism caused by functional defects in the intracellular mitochondria and can thus rely only on glycolysis for energy supply. In young CHB patients during the stage of immune tolerance, the immune response to HBV has been initiated *in vivo* [18]. However, the T lymphocytes do not exhibit immunological tolerance characteristics, even stronger than the ability of T lymphocytes in healthy humans to produce Th1-type cytokines [19]. Although biochemical and serological evidence suggests that the host is immune tolerant to HBV, the host initiates an anti-HBV immune response during the immune tolerance phase. HBV is potentially dangerous in patients with CHB who are considered to be latent in immune tolerance [20].

2.3 Th and cytokines and immune tolerance

Initial $CD4^+$ T lymphocytes can be differentiated into different Th subpopulations after antigen stimulation, including subgroups, such as Th1, Th2, Th3, and Th17, which can regulate humoral and cellular immune functions by secreting different cytokines. Th1 mainly secretes cytokines, such as IFNγ and TNF, to induce cellular immune responses against infection by pathogens, such as HBV. Th2 mainly secretes IL-like cytokines, such as IL-4, IL-5, IL-10, IL-13, and so on, to induce and promote B lymphocyte-mediated humoral immune responses. In physiological conditions, Th1 and Th2 are mutually restricted in equilibrium to maintain normal immune function and play an important role in the pathogenesis of body infections. After HBV infection in humans, changes in liver microenvironment and certain components of HBV, such as HBeAg, can promote the transformation of Th1 cell population into Th2 cell population. This phenomenon causes an imbalance of Th1/Th2, reduction of antiviral substance secreted by Th1, and attenuation of anti-HBV-specific T lymphocyte immune response, which cause chronic HBV infection. IL-35 is an immunosuppressive cytokine secreted by regulatory T lymphocytes. *In vitro* experiments have shown that IL-35 can also inhibit the proliferation of HBV-specific CTLs, weaken the immune responses of anti-HBV cells, and play an important

role in the formation of immune tolerance in HBV infection [21]. Therefore, IL-35 is considered to be closely related to the chronicity of HBV infection.

2.4 HBV genotype and genomic variation and immune tolerance

The current study found that HBV contains eight major genotypes (A–H) and two newly discovered genotypes (I and J). The genotypes in China are mainly types C and B. Compared with patients infected with C genotype, patients with B genotype exhibit early HBeAg seroconversion and less progress to chronic hepatitis, cirrhosis, and hepatocellular carcinoma [22]. The A_2 subtype mainly causes chronic HBV infection in adults, and co-infection of B and C is the main cause of chronic mother-to-child transmission of HBV infection in East Asia [23]. The HBV genome is unique and precise in structure but is susceptible to mutation, and mutation at certain specific sites may be associated with chronic HBV infection. The preS deletion mutation is the most frequently reported mutation. A large in-frame deletion mutation has been detected in the preS region of the gene encoding the HBV envelope protein, and the mutation sites are often clustered at the 3′ end of the preS1 region and the 5′ end of the preS2 region [24]. Mutation in this region not only causes downregulation of HBV envelope protein expression but also induces the mutation of identifiable epitopes of T and B lymphocytes, which escape the combination with neutralizing antibodies, such as preS and anti-S antibodies, resulting in immune tolerance of T lymphocytes to the target antigen [23]. Such deletion mutation is mainly found in the HBV genes A_1, B, and C, which may be associated with prolonged viral replication in perinatal infection. The basic core promoter region of the preS and preC regions is another site susceptible to mutation, and A1762T/G1764A double mutation is the most common basic core promoter mutation. The most common mutation in the preC region is the mutation of G1896A site codon TGG to the stop codon TAG. This mutant genotype D is more common than genotype A, and mutation in these two sites causes the reduction and disappearance of HBeAg, respectively [25]. Given that HBeAg can be expressed on the hepatocyte membrane and is the target of host immune attack, the lack of HBeAg may cause immune escape of HBV and promote persistent infection of HBV [26], causing damage to liver cells, which considerably explains why HBeAg-negative patients sometimes exhibit severe liver damage.

2.5 Host gene polymorphism and immune tolerance

The human leukocyte antigen (HLA) gene complex is the major histocompatibility complex of humans, which is the most important genetic factor determining host immunity, and is also one of the most important host factors associated with clinical outcomes of HBV infection. Full-genomic association analysis has shown that single nucleotide polymorphisms near HLA-DP, HLA-DQ, and HLA-DR are closely related to the clinical outcome of HBV infection. The relationship between HLA gene polymorphism and chronic HBV infection has become a hot topic in recent years. Alleles HLADRB1*11/*12 and DQB1*0301 are globally associated with chronic HBV infection [27]. Zhu *et al.* [28] found four loci that can independently cause chronic HBV infection, that is, HLA-DPβ1 site 84–87, HLA-DRβ1*13 site 71 and rs400488, and HLA-C site 15. Chang *et al.* [29] found that rs9277535 (HLA-DPB1), rs9276370 (HLA-DQA2), rs7756516 and rs7453920 (HLA-DQB2), and rs9366816 near HLA-DPA3 are significantly associated with chronic HBV infection. DQB1*0301 and DQB1*0303 are related to persistent HBV infection in the Xinjiang Uyghur Autonomous Region [30]. In Saudi Arabian HBV-infected patients, the three single-nucleotide gene polymorphisms rs2856718, rs7453920, and rs9275572 in the HLA-DQ region are increasingly sensitive to chronic HBV infection [31]. Interestingly, genetic polymorphisms at some HLA loci can protect the body from persistent HBV infection. In the Asian population, HLA-DPA1 and HLA-DPB1 genes show significant protective effects to resist chronic HBV infection [32].

Through two independent case–control studies, Wang *et al.* [33] found that the HLA-DPA alleles rs3077 and rs9277535 in the Han population significantly reduce the risk of CHB, and HLA-DP rs9277535 is associated with the reduced CHB risk in the Zhuang population.

3 Problems and prospects

Seeking to break HBV immune tolerance has always been a hotspot and a difficult point of research. People want to solve this problem by various ways, such as combining or sequentially using nucleoside and nucleotide drugs with peginterferon α to increase the negative conversion rate of HBsAg, research on therapeutic vaccines, use of various immunopotentiators, and developing small molecules of antiviral drugs.

However, no significant substantive progress has been made. In recent years, with some advances in research related to HBV immune tolerance, some questions that need to be explored in depth have also emerged. Given that the anti-HBV immune response of the host can be observed after HBV infection in the so-called immune tolerance period, should the traditional concept of immune tolerance be redefined or the natural process of HBV infection in humans be re-divided? Is it possible to consider the difference in the immune tolerance period in the choice of timing of antiviral therapy? Given that the immune system of infants and young children exhibits plasticity, is it the best time to treat HBV when HBV exists in the body for a short time and the immune system of the infant is not fully mature? Other new drugs and methods and other issues need to be further studied and revealed in the treatment of breaking immune tolerance of HBV infection.

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