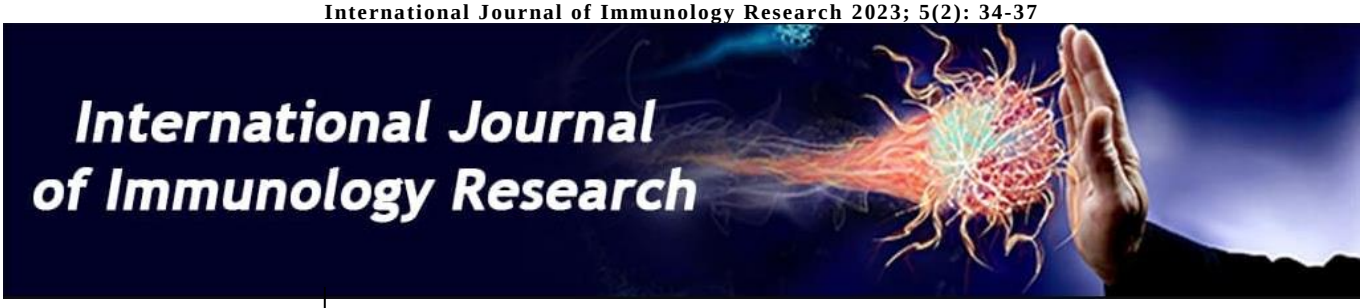

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Hepatitis B: Experimental models and therapeutic strategies

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Abstract
Hepatitis B Virus (HBV) infection remains a significant global health concern, with millions of people worldwide affected by chronic hepatitis B, leading to severe liver diseases such as cirrhosis and hepatocellular carcinoma. This review provides a comprehensive overview of the experimental models used to study HBV and the therapeutic strategies currently employed and under development. The review highlights the strengths and limitations of various in vitro and in vivo models, discusses the molecular mechanisms underlying HBV infection and pathogenesis, and explores the advances in antiviral therapies, including nucleos (t) ide analogs, immune modulators, and emerging gene-editing technologies.

Keywords: Hepatitis B, HBV, experimental models, therapeutic strategies, antiviral therapy, gene editing

Introduction
Hepatitis B virus (HBV) is a major cause of chronic liver disease, with over 250 million people living with chronic hepatitis B worldwide. Despite the availability of a highly effective vaccine, HBV infection continues to be a leading cause of liver cirrhosis and hepatocellular carcinoma (HCC). Chronic HBV infection is characterized by the persistence of viral covalently closed circular DNA (cccDNA) in the liver, which serves as a reservoir for viral replication and complicates efforts to achieve a complete cure. The development of effective therapeutic strategies requires a deep understanding of HBV biology, pathogenesis, and the host immune response. Experimental models are crucial for studying these aspects of HBV infection and for testing the efficacy of novel therapeutic approaches.

Main Objective
The main objective of this study is to provide a comprehensive overview of the experimental models used to study Hepatitis B virus (HBV) and to critically evaluate the therapeutic strategies currently employed and under development for managing chronic HBV infection.

Experimental Models for Hepatitis B
The study of Hepatitis B virus (HBV) and its interaction with the host immune system has been significantly advanced by the development and use of various experimental models. These models are crucial for understanding HBV biology, pathogenesis, and for evaluating the efficacy of potential therapeutic strategies. Due to the complexity of HBV infection and the limitations of human studies, in vitro and in vivo models have become indispensable tools in HBV research. This section discusses these models in detail, highlighting their applications, strengths, and limitations based on relevant studies.
In vitro models have been pivotal in dissecting the molecular mechanisms of HBV replication, gene expression, and host-virus interactions. Among the most commonly used in vitro systems are hepatoma cell lines such as HepG2, Huh7, and HepaRG. These cell lines are derived from human liver cancer cells and have been genetically modified to support HBV replication. For instance, HepG2.2.15 cells, a derivative of HepG2, stably express HBV DNA and produce infectious virions, making them a valuable model for studying viral replication and screening antiviral compounds.

Huh7 cells, although not naturally permissive to HBV, can be transfected with HBV DNA or infected with HBV particles when supplemented with sodium taurocholate cotransporting polypeptide (NTCP), the receptor required for HBV entry. HepaRG cells, a bipotent hepatic progenitor cell line, can differentiate into hepatocyte-like cells that support the entire HBV life cycle, providing a more physiologically relevant model than traditional hepatoma cell lines.

Despite their utility, these cell lines have limitations. One major drawback is that they do not fully recapitulate the natural infection process or the complex liver microenvironment. Additionally, they lack certain host factors and immune components that are critical for studying HBV-induced immune responses and liver pathology. To overcome these limitations, primary human hepatocytes (PHHs) have been employed as an alternative in vitro model. PHHs are isolated from liver tissue and can support the complete HBV replication cycle, including the formation of covalently closed circular DNA (cccDNA), which is a key feature of chronic HBV infection. Studies using PHHs have provided valuable insights into HBV-host interactions, particularly the mechanisms by which HBV evades the immune system and establishes chronic infection. However, the use of PHHs is hampered by their limited availability, variability between donors, short lifespan in culture, and susceptibility to dedifferentiation.

Advances in stem cell technology have led to the development of stem cell-derived hepatocyte-like cells as a promising alternative to PHHs. Induced pluripotent stem cells (iPSCs) and embryonic stem cells (ESCs) can be differentiated into hepatocyte-like cells that exhibit many characteristics of primary hepatocytes, including the ability to support HBV replication. These stem cell-derived models offer the advantage of being renewable and less variable compared to PHHs. Moreover, they provide a platform for studying patient-specific responses to HBV infection and evaluating personalized therapeutic approaches. However, these models are still being refined to fully mimic the functional properties of primary hepatocytes and to overcome challenges related to incomplete maturation and heterogeneous cell populations.

In vivo models are essential for studying the pathogenesis of HBV, immune responses, and for testing therapeutic interventions in a whole-organism context. Chimpanzees were historically the primary animal model for HBV research due to their susceptibility to natural HBV infection. Studies using chimpanzees have been instrumental in understanding the acute and chronic phases of HBV infection, as well as in the development of the HBV vaccine. However, ethical concerns, high costs, and the limited availability of chimpanzees have led to a decline in their use.

To address the limitations associated with chimpanzees, researchers have developed humanized mouse models, which have become increasingly important in HBV research. These models involve the transplantation of human hepatocytes into immunodeficient mice, such as uPA/SCID and FRG mice, creating a chimeric liver that supports HBV infection and replication. Humanized mouse models are valuable for studying HBV-host interactions, evaluating antiviral drugs, and investigating immune responses in a more human-relevant context. For example, studies using humanized mice have provided insights into the role of the immune system in controlling HBV infection and the mechanisms by which HBV establishes chronic infection. However, these models also have limitations, including incomplete humanization of the liver and the absence of a fully functional human immune system, which can restrict their ability to fully model HBV-induced liver disease and immune responses.

Transgenic mouse models have also been used to study HBV. These models are genetically engineered to express HBV proteins, allowing researchers to investigate the effects of HBV gene expression on liver pathology and immune responses. While transgenic mice have provided valuable insights into the mechanisms of HBV-induced liver damage and the role of viral proteins in immune evasion, they do not replicate the natural course of HBV infection and lack the cccDNA reservoir, which is crucial for chronic infection.

In conclusion, the development of in vitro and in vivo models has significantly advanced our understanding of HBV biology and pathogenesis. While each model has its strengths and limitations, they collectively provide a comprehensive toolkit for studying HBV and testing therapeutic strategies. The continued refinement of these models, along with the integration of emerging technologies, will be essential for overcoming the remaining challenges in HBV research and for developing more effective treatments for chronic hepatitis B.

Therapeutic Strategies for Hepatitis B

The treatment of Hepatitis B virus (HBV) infection has evolved significantly over the past decades, with multiple therapeutic strategies now available to manage the disease. However, curing chronic HBV infection remains a significant challenge due to the persistence of covalently closed circular DNA (cccDNA) in the liver. Current therapies primarily aim to suppress viral replication, reduce liver inflammation, and prevent progression to cirrhosis or hepatocellular carcinoma (HCC). This section explores various therapeutic strategies for HBV, including nucleos (t) ide analogs, immune modulators, therapeutic vaccines, and emerging technologies such as gene editing.

Nucleos (t) ide analogs (NAs) represent the cornerstone of HBV antiviral therapy. These drugs act by inhibiting the reverse transcriptase activity of the HBV polymerase, which is essential for viral replication. Among the most widely used NAs are tenofovir disoproxil fumarate (TDF), tenofovir alafenamide (TAF), and entecavir. These agents are highly effective at suppressing HBV replication, resulting in significant reductions in serum HBV DNA levels. Long-term studies have shown that NAs can prevent the progression of chronic hepatitis B to cirrhosis and reduce the risk of developing HCC. For example, a study by Chang *et al*. demonstrated that entecavir significantly lowered the incidence of liver complications in patients with chronic hepatitis B compared to untreated controls. However, while NAs effectively suppress viral replication, they do not directly target cccDNA, meaning that viral replication can resume if treatment is stopped. This necessitates long-term or even lifelong treatment for many patients, which can be associated with adherence issues, drug resistance, and side effects.

Immune modulation is another therapeutic strategy that has garnered interest due to the role of inadequate immune responses in chronic HBV infection. Historically, interferon-alpha (IFN-α) was one of the first immune modulators used to treat chronic hepatitis B. IFN-α has both antiviral and immunomodulatory effects, promoting the clearance of infected hepatocytes and enhancing the immune response against HBV. However, IFN-α therapy is associated with

significant side effects, including flu-like symptoms, depression, and hematological abnormalities, limiting its use to a subset of patients who can tolerate the treatment.

Recent advances in immune therapy for HBV have focused on developing novel agents that can modulate the immune system without the severe side effects of IFN-α. These include immune checkpoint inhibitors, therapeutic vaccines, and adoptive T cell therapies. Immune checkpoint inhibitors, such as those targeting the PD-1/PD-L1 pathway, have shown promise in reactivating exhausted HBV-specific T cells, potentially allowing the immune system to control or clear the virus. For example, a study by Zhang *et al*. demonstrated that blockade of the PD-1/PD-L1 pathway in chronic HBV patients could partially restore T cell function and reduce viral loads. However, immune checkpoint inhibitors can lead to immune-related adverse events, and their long-term safety and efficacy in HBV treatment are still under investigation.

Therapeutic vaccines are another strategy aimed at boosting the immune response to HBV. Unlike prophylactic vaccines, which prevent HBV infection, therapeutic vaccines are designed to stimulate an immune response in individuals already infected with the virus. Several therapeutic vaccines are currently in development, including those based on DNA, proteins, and viral vectors. These vaccines aim to elicit robust T cell responses that can target and eliminate HBV-infected cells. A recent clinical trial of the therapeutic vaccine GS-4774 showed that it could induce HBV-specific T cell responses, although its ability to achieve sustained viral control in combination with NAs remains to be determined.

Gene editing is an emerging technology that holds the potential to cure HBV by directly targeting and eliminating cccDNA, the key reservoir of the virus in the liver. CRISPR-Cas9, a widely used gene-editing tool, has shown promise in preclinical models for cleaving HBV DNA and preventing viral replication. For instance, Seeger and Sohn demonstrated that CRISPR-Cas9 could specifically target and degrade HBV cccDNA in infected cells, effectively reducing viral replication. However, challenges remain regarding the delivery of CRISPR-Cas9 to hepatocytes in vivo, as well as concerns about off-target effects and the potential for unintended genomic alterations. Despite these challenges, the potential of gene editing to eliminate cccDNA makes it a highly attractive approach for achieving a functional cure for HBV.

Combination therapy is also being explored as a strategy to enhance treatment outcomes for chronic HBV infection. Combining NAs with immune modulators, such as IFN-α or checkpoint inhibitors, could potentially increase the likelihood of viral clearance by simultaneously suppressing viral replication and boosting the immune response. Additionally, the combination of antiviral agents with gene-editing technologies may offer a synergistic approach to targeting both viral replication and cccDNA reservoirs. Clinical trials are currently underway to evaluate the safety and efficacy of various combination therapies for chronic HBV.

In conclusion, while significant progress has been made in the treatment of chronic HBV infection, challenges remain in achieving a complete cure. Current therapies, including nucleos (t) ide analogs and immune modulators, are effective at suppressing viral replication and preventing disease progression but do not eliminate cccDNA, requiring long-term treatment. Novel therapeutic strategies, including therapeutic vaccines and gene editing, hold promise for targeting HBV more effectively and potentially achieving a functional cure. Future research should continue to focus on optimizing these approaches and evaluating combination therapies to overcome the limitations of existing treatments and improve patient outcomes.

Conclusion

Hepatitis B remains a significant global health challenge, with millions of people affected by chronic infection that can lead to severe liver diseases such as cirrhosis and hepatocellular carcinoma. Despite advancements in therapeutic strategies, achieving a complete cure for chronic hepatitis B remains elusive, primarily due to the persistence of covalently closed circular DNA (cccDNA) in the liver. The current therapeutic approaches, including nucleos(t)ide analogs and immune modulators, have proven effective in suppressing viral replication and preventing disease progression, yet they fall short of eradicating the virus, necessitating long-term treatment and management. Experimental models have been indispensable in advancing our understanding of HBV biology, pathogenesis, and in evaluating the efficacy of therapeutic strategies. Both in vitro models, such as hepatoma cell lines and primary human hepatocytes, and in vivo models, including humanized mouse models and transgenic mice, have provided valuable insights into HBV-host interactions and the mechanisms underlying chronic infection. However, these models also have limitations that must be addressed to fully replicate the complexity of human HBV infection and its associated immune responses. Emerging therapeutic strategies, particularly gene-editing technologies like CRISPR-Cas9, offer new hope for directly targeting and eliminating cccDNA, potentially leading to a functional cure. Additionally, immune-based therapies, including therapeutic vaccines and immune checkpoint inhibitors, are being actively explored to enhance the host immune response against HBV. The combination of these novel approaches with existing antiviral therapies holds promise for more effective management of chronic hepatitis B and may pave the way toward achieving a cure. Future research should continue to refine experimental models to better mimic human HBV infection, allowing for more accurate evaluation of new therapies. Moreover, the development of combination therapies that target both viral replication and immune modulation could offer synergistic effects, improving treatment outcomes for patients. As we move forward, the integration of innovative technologies and a deeper understanding of HBV pathogenesis will be crucial in overcoming the challenges associated with chronic HBV infection and ultimately improving the lives of those affected by this persistent virus.

Conflict of Interest

Not available

Financial Support

Not available

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