Recent Insights into Hepatitis B Vaccine Design: A Review of Next-Generation Platforms and Adjuvants

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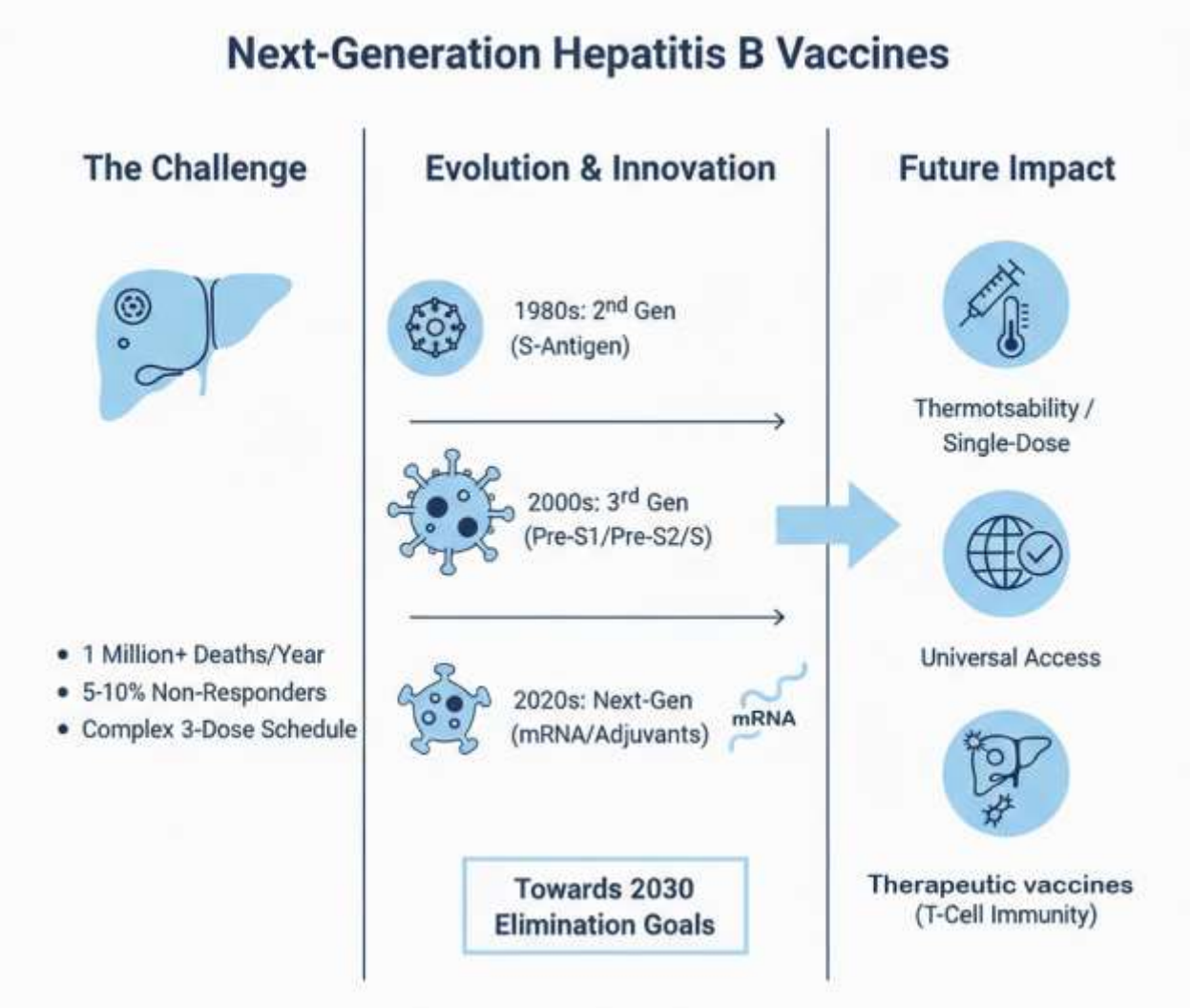
Abstract

Hepatitis B virus (HBV) remains a formidable global health challenge, contributing to over one million deaths annually through chronic complications such as liver cirrhosis and hepatocellular carcinoma. While traditional vaccination strategies have significantly reduced global prevalence since the 1980s, the path toward total elimination is hindered by specific limitations in current vaccine design. Standard second-generation recombinant vaccines, which utilize only the small (S) surface antigen produced in yeast, often require a complex three-dose regimen spanning six months. This schedule frequently leads to poor longitudinal compliance and fails to induce protective immunity in approximately 5% to 10% of the population, particularly among older adults, the obese, and the immunocompromised.

Recent insights into vaccine architecture have sparked a shift toward more sophisticated, next-generation platforms. This review explores the transition from single-antigen designs to "triple-antigen" third-generation vaccines that incorporate Pre-S1 and Pre-S2 components. These additions more closely mimic the natural viral envelope, providing a broader array of T- and B-cell epitopes that can bypass genetic non-responsiveness. Furthermore, the integration of potent new adjuvants, such as Toll-like receptor 9 (TLR9) agonists, has revolutionized the field by enabling two-dose schedules that achieve rapid seroconversion and higher antibody titers.

The review also evaluates the emergence of nucleic acid technologies, specifically mRNA-based delivery systems. By utilizing lipid nanoparticles to drive endogenous protein synthesis, these platforms trigger robust cellular immune responses that were previously difficult to achieve with protein-based subunits alone. As the scientific community targets the World Health Organization's 2030 elimination goals, these innovations in molecular design, delivery kinetics, and immunostimulatory enhancements represent the vanguard of preventative and potentially therapeutic intervention. This article synthesizes recent clinical and laboratory findings to provide a comprehensive overview of how these design breakthroughs are addressing the "non-responder" gap and simplifying global immunization logistics.



Graphical abstract

1. Introduction

The Hepatitis B virus (HBV) remains one of the most persistent and devastating global health challenges of the 21st century. Despite the availability of effective vaccines for over four decades, chronic HBV infection continues to be a leading cause of morbidity and mortality worldwide (Ondieki et al., 2015). According to recent epidemiological data, more than 296 million people are living with chronic hepatitis B, a condition that claims approximately 1.1 million lives annually due to its progression into liver cirrhosis and hepatocellular carcinoma (HCC) (Al-Busafi & Alwassief, 2024). The virus is highly infectious—estimated to be 50 to 100 times more infectious than HIV—and can be transmitted through percutaneous or mucosal exposure to infected blood and other body fluids, including vertical transmission from mother to child during childbirth.

The history of HBV vaccine design is a landmark in medical biotechnology. The first-generation vaccines, developed in the early 1980s, were derived from the plasma of chronic carriers. While

highly effective, these were limited by high production costs and public concerns regarding the safety of blood-derived products (Figure 1). This led to the development of second-generation recombinant vaccines in the mid-1980s, which utilized *Saccharomyces cerevisiae* (baker's yeast) to express the small (S) surface antigen (HBsAg). These vaccines became the cornerstone of global immunization programs, dramatically reducing the carrier rate in children in endemic regions from over 10% to less than 1% (Zhao et al., 2020).

However, as the global health community moves toward the World Health Organization's (WHO) ambitious goal of eliminating viral hepatitis as a public health threat by 2030, the limitations of these second-generation vaccines have become increasingly apparent. The primary hurdle is the "non-responder" phenomenon. Approximately 5% to 10% of healthy adults fail to develop a protective antibody titer (anti-HBs $\geq$ 10 mIU/mL) after the standard three-dose series (Mahmood et al., 2023). This lack of response is often linked to factors such as advanced age (over 40 years), tobacco use, obesity, and underlying medical conditions like diabetes or chronic kidney disease. Furthermore, the logistical complexity of the traditional 0, 1, and 6-month dosing schedule results in significant "drop-off" rates, where individuals fail to return for the final booster, leaving them with suboptimal long-term protection (Zhao et al., 2020).

To overcome these barriers, recent insights into vaccine design have shifted toward "precision vaccinology." This involves the integration of structural virology, immunology, and nanotechnology to create vaccines that are not only more potent but also more efficient in their delivery. Innovations such as the inclusion of the Pre-S1 and Pre-S2 domains—proteins that play a vital role in viral attachment to hepatocytes—offer a more comprehensive antigenic profile than the S-antigen alone (Shouval et al., 2015). Additionally, the development of novel adjuvants that target specific innate immune receptors has allowed for truncated dosing schedules, potentially increasing series completion rates in high-risk and mobile populations. This review will delve into these technological leaps, examining how modern design principles are being leveraged to close the gaps in global immunity and provide a definitive tool for HBV eradication.

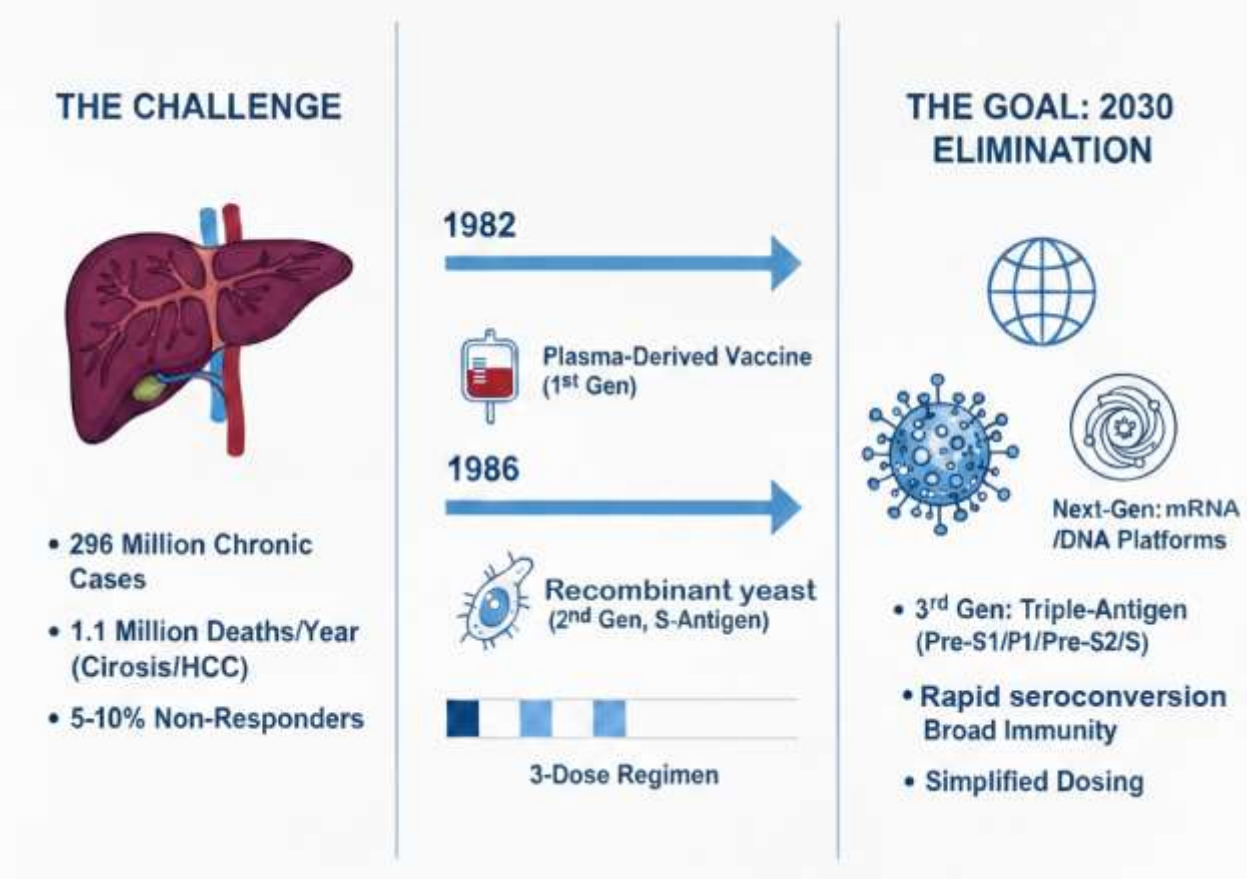


Figure 1: The evolution of HBV vaccine design

2. Evolution of HBV Vaccine Platforms

The technological trajectory of Hepatitis B vaccine design represents a masterclass in the evolution of modern vaccinology, moving from biological extraction to sophisticated genetic engineering. This evolution is generally categorized into three distinct generations, each defined by the complexity of the antigen and the host system used for production. Understanding this progression is essential to appreciating the current "next-generation" breakthroughs that aim to solve the persistent issue of vaccine non-responsiveness and complex dosing schedules.

The First Generation: Plasma-Derived Vaccines

In the early 1970s, the first Hepatitis B vaccines were developed by purifying the surface antigen (HBsAg) directly from the plasma of chronically infected individuals. This was a revolutionary concept at the time, as it used a viral byproduct—non-infectious subviral particles—to induce immunity. While these vaccines were highly immunogenic, they were fraught with logistical and safety concerns. The reliance on human donors meant a limited supply, and despite rigorous purification processes, there was a persistent public and clinical fear regarding the transmission of other blood-borne pathogens, such as HIV or Hepatitis C, which had not yet been fully characterized (Shouval et al., 2015).

The Second Generation: Recombinant Yeast Platforms

The mid-1980s marked a paradigm shift with the advent of recombinant DNA technology. Scientists successfully inserted the gene for the small (S) surface protein into *Saccharomyces cerevisiae* (baker's yeast). This allowed for the mass production of HBsAg in a controlled, sterile environment, eliminating the need for human plasma. These second-generation vaccines (e.g., Engerix-B and Recombivax HB) became the global standard. They proved to be exceptionally safe and effective for the majority of the population. However, their design is relatively simple; they contain only the 226-amino acid S-protein (Figure 2). Because the S-protein lacks the Pre-S1 and Pre-S2 domains found on the surface of the actual virus, the immune response is strictly focused on a limited set of epitopes. This simplicity is a primary reason why certain "non-responders" with specific HLA genotypes fail to recognize the antigen, and why a three-dose regimen over six months is required to achieve lasting memory (Zhao et al., 2020).

The Third Generation: Mammalian Cell Expression

To address the limitations of yeast-derived vaccines, third-generation vaccines were developed using mammalian cell lines (such as Chinese Hamster Ovary or CHO cells). Unlike yeast, mammalian cells can perform complex post-translational modifications, such as glycosylation, which more accurately mimic the viral particles produced during a natural human infection. Crucially, these vaccines (e.g., Sci-B-Vac or PreHevbrio) incorporate all three surface proteins: S, Pre-S2, and Pre-S1. The Pre-S1 domain is particularly significant because it contains the hepatocyte-binding site (the sodium taurocholate cotransporting polypeptide or NTCP binding site). By including these additional proteins, third-generation vaccines provide a much broader array of T-cell and B-cell epitopes. Clinical data indicates that this "triple-antigen" approach induces more rapid seroconversion and significantly higher antibody titers, particularly in older adults and those who previously failed to respond to second-generation vaccines (Shouval et al., 2015).

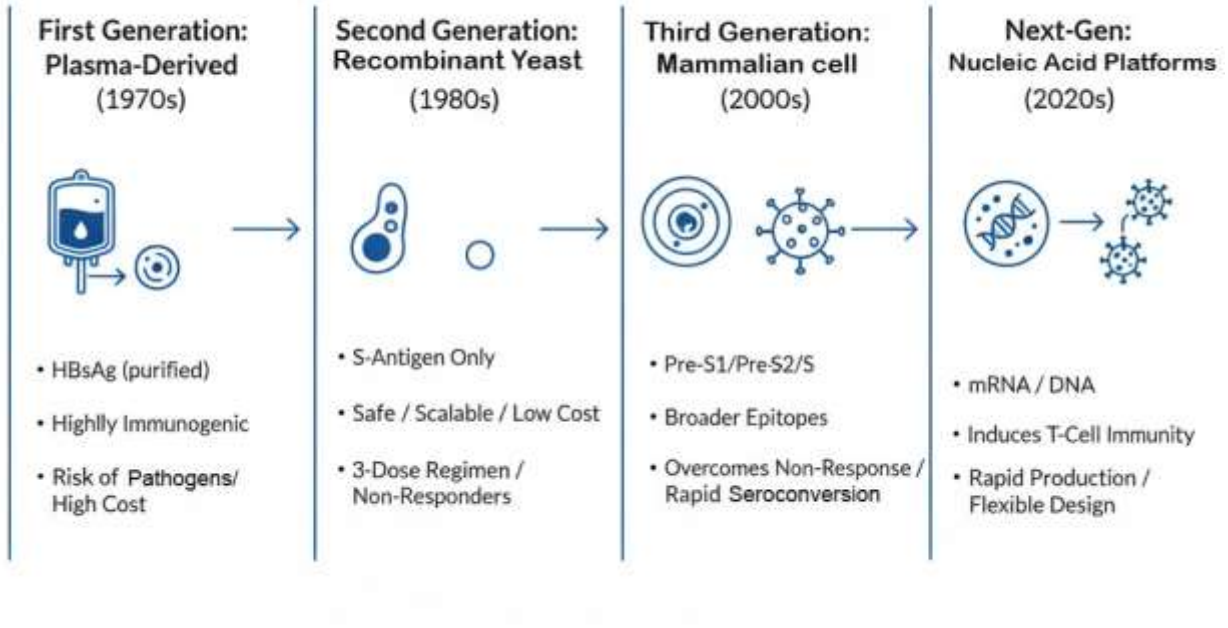


Figure 2: Evolution of HBV vaccine platforms

The Next Frontier: Nucleic Acids and Nanotechnology

Today, we are entering a "Next-Gen" era characterized by mRNA and DNA platforms. Inspired by the rapid deployment of COVID-19 vaccines, researchers are designing mRNA sequences that instruct the recipient's own muscle cells to produce HBV subviral particles. This method not only simplifies manufacturing but also stimulates the CD8+ T-cell response more effectively than protein-based vaccines, offering hope for both preventative and therapeutic applications (Mahmood et al., 2023).

Table 1: How the HBV vaccines have evolved in design over time

Generation	**Platform**	**Key Antigen(s)**	**Advantages**	**Limitations**
First	Plasma-derived	HBsAg (purified)	Highly immunogenic	Risk of blood-borne pathogens; high cost

Second	Recombinant Yeast	S-antigen (small)	Safe; scalable; low cost	Requires 3 doses; low response in 10% of adults
Third	Mammalian Cell	Pre-S1, Pre-S2, S	Rapid seroconversion; overcomes low-responsiveness	Higher manufacturing complexity
Next-Gen	mRNA / DNA	Synthetic HBV Genes	Rapid production; induces T-cell immunity	Requires advanced delivery (LNP)

3. Third-Generation Vaccines: The Triple-Antigen Approach

While second-generation yeast-derived vaccines have been the backbone of global immunization for decades, their reliance on a single, truncated protein—the small (S) antigen—presents a structural limitation that next-generation designs seek to rectify. The Hepatitis B virus (HBV) envelope is naturally composed of three surface proteins: the small (S), medium (M, which includes the Pre-S2 domain), and large (L, which includes both Pre-S1 and Pre-S2 domains) proteins. Third-generation vaccines, often produced in mammalian cell lines such as Chinese Hamster Ovary (CHO) cells, are designed to incorporate all three of these antigenic components, creating a "triple-antigen" profile that more faithfully mimics the native virion (Shouval et al., 2015).

The inclusion of the Pre-S1 and Pre-S2 domains is not merely a matter of structural mimicry; it provides a significant functional advantage in terms of immunogenicity (Figure 3). The Pre-S1 domain is particularly critical because it contains the specific amino acid sequence (residues 2–48) that mediates viral attachment to the host’s hepatocyte via the sodium taurocholate cotransporting polypeptide (NTCP) receptor (Al-Busafi & Alwassief, 2024). By including this domain in the vaccine, the immune system is trained to produce neutralizing antibodies that can directly block the virus's entry into liver cells. Furthermore, the Pre-S2 domain has been shown to enhance the immunogenicity of the S-antigen by providing additional T-cell help, which is vital for long-term immunological memory and robust B-cell activation (Vesikari et al., 2021).

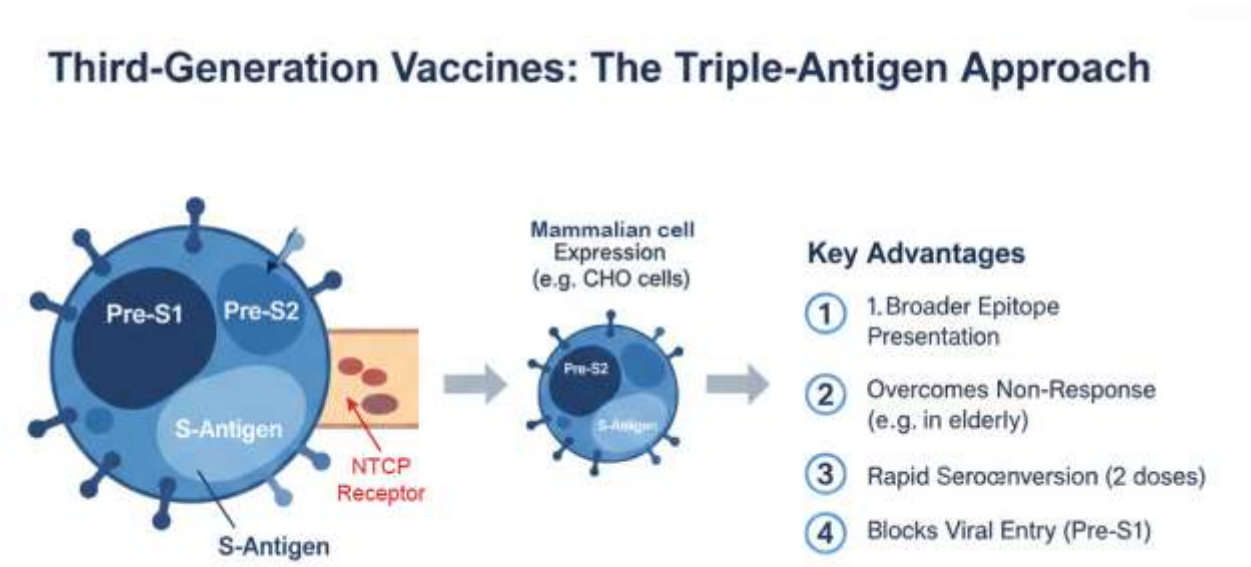


Figure 3: The triple-antigen approach in HBV vaccine design

One of the most compelling arguments for the triple-antigen design is its ability to overcome "non-responsiveness" in specific populations. Clinical studies have demonstrated that individuals who fail to respond to traditional S-only vaccines often possess specific Human Leukocyte Antigen (HLA) haplotypes that do not efficiently present S-antigen peptides to T-cells. Because third-generation vaccines like PreHevbrio (Sci-B-Vac) contain a much broader array of epitopes across the Pre-S1 and Pre-S2 regions, they provide the immune system with "more bites at the apple," increasing the likelihood that at least one peptide-MHC complex will trigger an effective response (Diaz-Mitoma et al., 2021). In a large-scale Phase III clinical trial, the triple-antigen vaccine achieved a seroprotection rate of 91.4% in adults aged 65 and older, compared to only 76.5% for the conventional second-generation vaccine (Vesikari et al., 2021).

Moreover, the manufacturing process in mammalian cells allows for complex post-translational modifications, such as glycosylation, which are absent or different in yeast-derived platforms. Proper glycosylation is essential for the correct folding of the HBsAg particles and the preservation of conformational epitopes that are the primary targets of neutralizing antibodies (Mahmood et al., 2023). Recent insights suggest that these mammalian-cell-derived particles are more stable and exhibit higher affinity for antigen-presenting cells (APCs), leading to more rapid seroconversion. Instead of the typical six-month window required for second-generation vaccines to reach peak titers, triple-antigen platforms often induce protective levels of antibodies after just two doses (Diaz-Mitoma et al., 2021). This shift toward multi-antigenic design represents a critical step in tailoring vaccination to high-risk groups, including the elderly, diabetic patients, and those with chronic kidney disease, ensuring that no population is left vulnerable to HBV infection.

4. mRNA and Nucleic Acid Technologies

The rapid acceleration of vaccine technology during the COVID-19 pandemic has provided a robust blueprint for the next generation of Hepatitis B virus (HBV) prevention. While protein-subunit vaccines have been the historical standard, the shift toward nucleic acid-based platforms—specifically messenger RNA (mRNA) and DNA—represents a fundamental change in how the immune system is primed to recognize HBV. Unlike traditional recombinant vaccines that deliver a pre-manufactured protein, mRNA vaccines deliver the genetic instructions (encoded in synthetic mRNA) for the host's own cells to produce the HBsAg (Mahmood et al., 2023).

A critical design insight in modern mRNA HBV vaccines is the use of lipid nanoparticles (LNPs). These LNPs serve a dual purpose: they protect the fragile mRNA from enzymatic degradation and facilitate its entry into host cells, typically myocytes or resident dendritic cells at the injection site. Once inside the cytoplasm, the mRNA is translated by the host's ribosomes into HBsAg (Figure 4). Because the antigen is synthesized endogenously, it is processed via the Major Histocompatibility Complex (MHC) Class I pathway, in addition to the MHC Class II pathway (Li et al., 2024). This is a significant departure from protein-based vaccines, which primarily stimulate MHC Class II and B-cell responses. By activating the MHC Class I pathway, mRNA vaccines are uniquely capable of inducing robust CD8+ cytotoxic T-lymphocyte (CTL) responses, which are essential for identifying and eliminating virus-infected hepatocytes (Li et al., 2024).

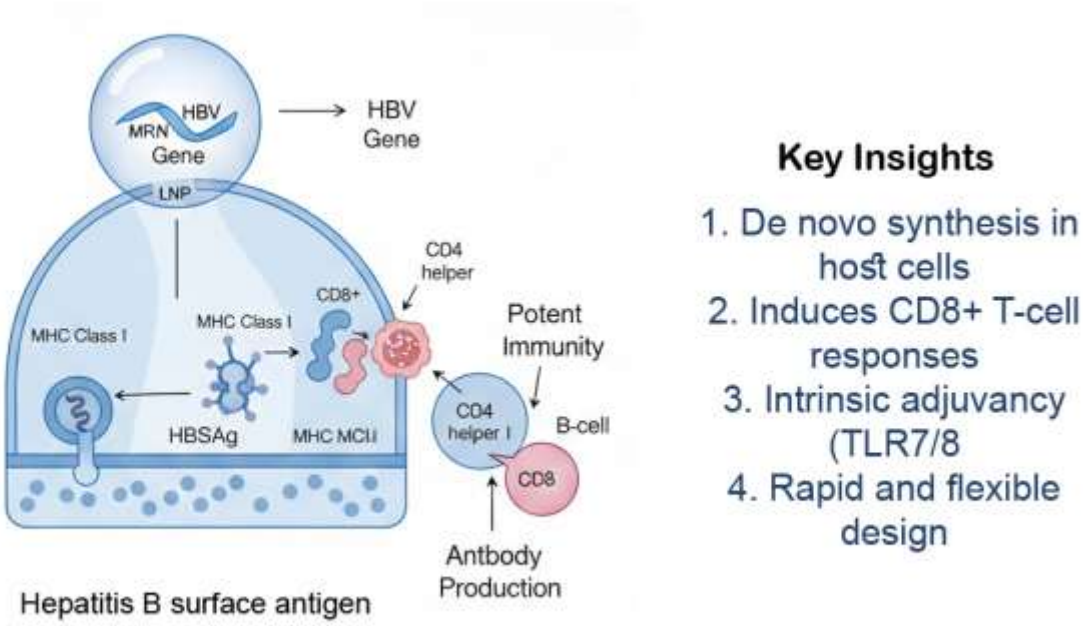


Figure 4: mRNA and nucleic acid technologies in HBV vaccine design

Recent research has also highlighted the "intrinsic adjuvancy" of mRNA molecules. The single-stranded RNA and the LNP components are recognized by innate immune sensors, such as Toll-like receptors (TLR7 and TLR8) and RIG-I-like receptors. This recognition triggers the production of Type I interferons and pro-inflammatory cytokines, creating an immunostimulatory environment that enhances the subsequent adaptive immune response without the need for traditional aluminum-based adjuvants (Mahmood et al., 2023). In preclinical models, mRNA-LNP vaccines encoding the large surface protein (containing Pre-S1, Pre-S2, and S) have demonstrated the ability to induce higher neutralizing antibody titers and more potent T-cell responses than conventional recombinant proteins (Al-Busafi & Alwassief, 2024).

Furthermore, the programmable nature of mRNA allows for "plug-and-play" design modifications. Researchers can now easily engineer chimeric mRNA sequences that incorporate multiple HBV genotypes or specific mutations to address regional viral variations. This flexibility is particularly vital for developing therapeutic vaccines intended for patients with chronic HBV. In these patients, the immune system is often in a state of "T-cell exhaustion" due to prolonged exposure to high levels of viral antigen. Next-generation mRNA designs are being optimized to include co-stimulatory molecules or check-point inhibitors encoded within the same platform to "re-awaken" the exhausted T-cell population (Li et al., 2024). While many of these nucleic acid platforms are still in various phases of clinical trials, the ability to induce a multi-dimensional immune response—combining high-titer antibodies with potent cellular immunity—positions mRNA as a leading candidate for the next era of HBV intervention (Mahmood et al., 2023).

5. Breakthroughs in Adjuvant Systems

The evolution of Hepatitis B vaccine design has recently centered on the transition from traditional "depot-forming" adjuvants to sophisticated "immunostimulatory" molecules. For decades, aluminum salts (alum) were the primary adjuvants used in HBV vaccines. While safe, alum primarily induces a Th2-biased immune response, which is effective for generating antibodies but relatively weak at stimulating the cellular (Th1) immunity necessary for long-term viral clearance and memory (Mahmood et al., 2023). Recent insights into the innate immune system’s role in shaping adaptive responses have led to the design of novel adjuvant systems that target specific pattern recognition receptors (PRRs).

5.1. TLR9 Agonists and the CpG Revolution

The most significant breakthrough in recent years is the integration of CpG oligonucleotides, specifically CpG 1018, into HBV vaccine formulations. CpG 1018 is a synthetic DNA sequence that mimics bacterial and viral DNA, acting as a potent agonist for Toll-like receptor 9 (TLR9). Unlike alum, which primarily sequesters the antigen at the injection site, CpG 1018 directly stimulates B cells and plasmacytoid dendritic cells (pDCs). This stimulation triggers the production of pro-inflammatory cytokines such as Interleukin-12 (IL-12) and Interferon-alpha (IFN-alpha), which are critical for driving a Th1-biased immune response (Al-Busafi & Alwassief, 2024).

The clinical impact of this design is best exemplified by Heplisav-B. By utilizing CpG 1018, this vaccine achieved a seroprotection rate of 95.4% compared to 81.3% for traditional alum-adjuvanted vaccines (Vesikari et al., 2021). Most notably, the potency of this adjuvant allows for a **two-dose regimen** administered over just one month (0 and 4 weeks). This is a monumental shift from the traditional six-month, three-dose schedule, addressing the chronic issue of patient non-compliance and "drop-off" during the long intervals required by second-generation designs (Zhao et al., 2020).

5.2. Combinatorial and Nano-Adjuvants

Beyond single TLR agonists, current research is exploring combinatorial adjuvant systems (AS) that target multiple immune pathways simultaneously. For instance, the AS04 adjuvant system (used in Fendrix) combines aluminum hydroxide with monophosphoryl lipid A (MPLA), a TLR4 agonist. This combination has proven particularly effective in "difficult-to-vaccinate" populations, such as patients with chronic kidney disease or those undergoing hemodialysis, by providing a more intense and sustained activation of antigen-presenting cells (Shouval et al., 2015).

Furthermore, nanotechnology is being leveraged to create "smart" adjuvants. Recent designs involve encapsulating HBsAg within polymeric nanoparticles or using saponin-based molecules like QS-21, which can enhance both the breadth and the magnitude of the immune response (Mahmood et al., 2023). These advanced delivery systems ensure that the antigen and the adjuvant are delivered to the same dendritic cell at the same time, maximizing the efficiency of immune priming. Experimental designs using calcium phosphate nanoparticles combined with CpG have also shown promise in inducing high levels of IgG2a antibodies and Interleukin-6 (IL-6), which correlate with superior protection in preclinical models (Mahmood et al., 2023). These adjuvant breakthroughs are not merely incremental improvements; they represent a fundamental redesign of the vaccine's interaction with the host's innate immune system, ensuring higher protection rates across diverse and aging populations.

6. Challenges and Future Directions

Despite the profound technological leaps in Hepatitis B vaccine design, the transition from laboratory innovation to global clinical impact remains fraught with structural and biological hurdles (Figure 6). As the scientific community aligns its efforts with the World Health Organization’s (WHO) 2030 goal of eliminating viral hepatitis as a public health threat, several critical challenges must be addressed. Chief among these is the "last mile" of immunization: the birth dose. While next-generation vaccines offer superior immunogenicity, their impact is nullified if they cannot reach newborns in resource-limited settings within the first 24 hours of life. Currently, global birth dose coverage hovers around 45%, leaving millions of infants vulnerable to vertical transmission and a 90% risk of developing chronic infection (Al-Busafi & Alwassief, 2024).

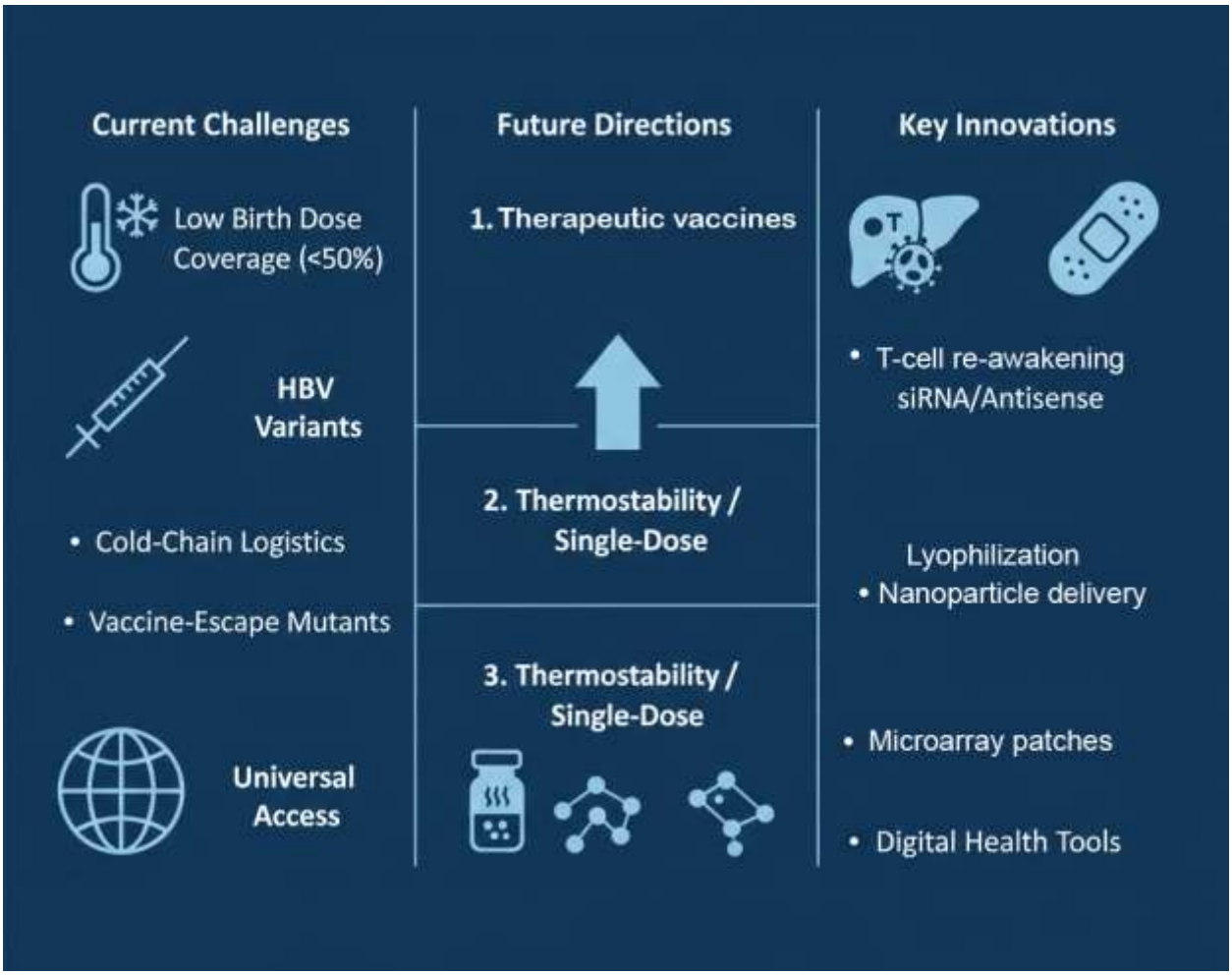


Figure 6: Challenges and future directions in HBV vaccine design

A significant area of active research is the development of **therapeutic vaccines**. Unlike preventative vaccines, which aim to induce neutralizing antibodies (humoral immunity), therapeutic vaccines are designed to break immune tolerance in patients already living with chronic HBV (CHB). The primary challenge here is the exhaustion of HBV-specific T-cells and the presence of hepatitis B surface antigen (HBsAg) "decoy" particles that saturate the immune system (Li et al., 2024). Recent insights suggest that a "functional cure"—defined as the sustained loss of HBsAg—will likely require a combinatorial approach. This includes using mRNA or DNA platforms to jump-start CD8+ T-cell responses while simultaneously using siRNA (small interfering RNA) or antisense oligonucleotides to lower viral protein loads (Mahmood et al., 2023). This "reduction-then-restoration" strategy represents the current vanguard of HBV cure research.

Logistical barriers also dictate the direction of future vaccine design. The requirement for a "cold chain"—continuous refrigeration from manufacture to administration—is a major impediment in tropical and developing regions. Recent breakthroughs in **thermostability** are addressing this by exploring the use of spray-drying or lyophilization (freeze-drying) to create vaccines that remain stable at room temperature for months. Additionally, nanotechnology is being utilized to develop

single-dose, controlled-release vaccines. By encapsulating HBsAg in biodegradable PLGA [poly(lactic-co-glycolic acid)] microspheres, researchers aim to create a "pulsatile release" system that mimics the traditional three-dose schedule with a single injection, significantly reducing the burden on healthcare infrastructure (Zhao et al., 2020).

Finally, the emergence of **HBV variants** poses a potential threat to vaccine efficacy. While the "a" determinant of the HBsAg is relatively conserved, "vaccine-escape mutants" (such as the G145R mutation) have been documented in infants born to HBsAg-positive mothers (Al-Busafi & Alwassief, 2024). Next-generation designs must therefore be "variant-proof." The flexibility of mRNA platforms is particularly advantageous here, as they can be rapidly updated to include multiple genotypes or mutated sequences. Furthermore, the inclusion of the highly conserved Pre-S1 domain in third-generation vaccines provides a broader protective shield, as mutations in this region are often detrimental to the virus's ability to infect liver cells (Shouval et al., 2015). Moving forward, the integration of digital health tools for tracking vaccination and the development of needle-free delivery systems, such as microarray patches, will be essential to achieving universal coverage and finally ending the HBV epidemic.

7. Conclusion

The landscape of HBV vaccine design is transitioning from simple recombinant proteins to sophisticated multi-antigenic and nucleic acid-based systems. By leveraging the Pre-S1/Pre-S2 domains and novel TLR agonists, next-generation vaccines are poised to eliminate the "non-responder" gap and simplify immunization schedules, bringing the goal of global HBV elimination by 2030 within reach.

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