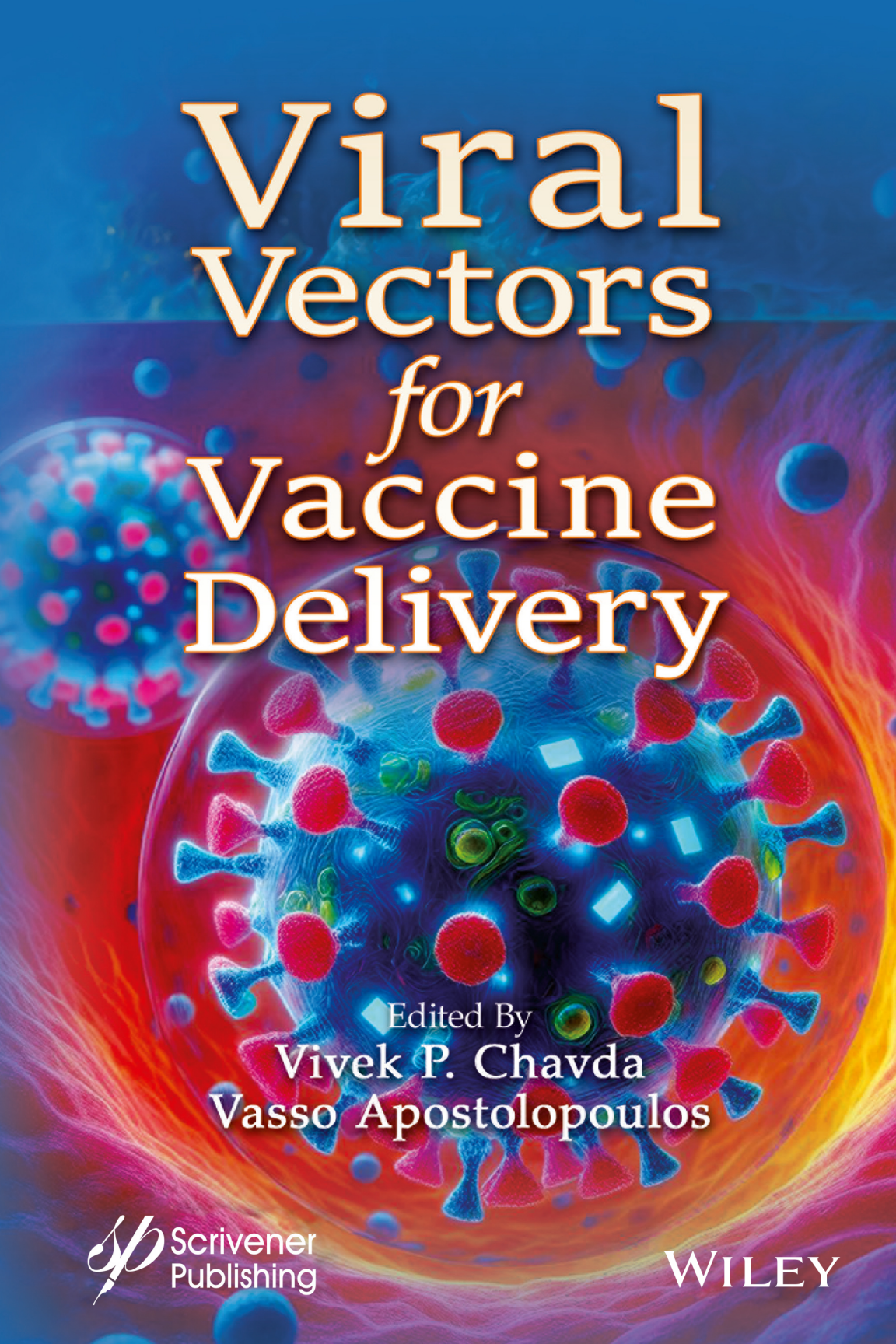
Viral
Vectors
for
Vaccine
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Edited By
Vivek P. Chavda
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Scrivener
Publishing
WILEY

Viral Vectors for Vaccine Delivery

Scrivener Publishing
100 Cummings Center, Suite 541J
Beverly, MA 01915-6106

Publishers at Scrivener
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Viral Vectors for Vaccine Delivery

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This edition first published 2025 by John Wiley & Sons, Inc., 111 River Street, Hoboken, NJ 07030, USA and Scrivener Publishing LLC, 100 Cummings Center, Suite 541J, Beverly, MA 01915, USA

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Library of Congress Cataloging-in-Publication Data

ISBN 978-1-394-27153-5

Front cover image courtesy of Adobe Firefly
Cover design by Russell Richardson

Set in size of 11pt and Minion Pro by Manila Typesetting Company, Makati, Philippines

Printed in the USA

10 9 8 7 6 5 4 3 2 1

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Preface

The development of vaccines has been a cornerstone of public health, preventing countless diseases and saving millions of lives. However, traditional vaccine approaches often face limitations, such as the need for multiple doses, the potential for adverse reactions, and the inability to induce long-lasting immunity against complex pathogens. In recent decades, viral vectors have emerged as a promising alternative, revolutionizing the field of vaccine development.

This book provides a comprehensive overview of viral vectors and their applications in vaccine delivery. Its chapters explore various aspects of viral vector technology, from the basic principles of viral vector construction to the latest advancements in gene editing and manufacturing.

To begin, the book introduces the concept of viral vectors and their advantages over traditional vaccine platforms. Subsequent chapters delve into the intricacies of viral vector construction, including the selection of appropriate viral backbones, the insertion of foreign genes, and the optimization of vector design for maximum immunogenicity. The role of adjuvants in enhancing the efficacy of viral vector vaccines is also discussed, highlighting their importance in boosting immune responses and improving vaccine potency.

The next section explores the different types of viral vectors used for vaccine delivery, including replication-competent and non-replicating vectors. Replication-competent vectors mimic natural infections, inducing a robust immune response, while non-replicating vectors are safer but may require multiple doses. Genetically modified viral vectors, such as those engineered for targeted delivery or enhanced antigen presentation, are also covered.

The book further delves into the specific applications of viral vectors in vaccine development, including their use in veterinary medicine and the development of vaccines against emerging infectious diseases. The

advantages and challenges associated with viral vector vaccines are discussed, along with the commercially available viral vector vaccines and the future potential of this technology.

This book serves as a valuable resource for researchers, scientists, and healthcare professionals working in the field of vaccine development. It provides a comprehensive understanding of viral vector technology and its potential to address the challenges of vaccine design and delivery. By exploring the latest advancements and future prospects, this book aims to contribute to the development of safer, more effective, and more accessible vaccines for a healthier global population. The editors are grateful to everyone who has supported their work and also wish to thank Martin Scrivener and Scrivener Publishing for their support and publication.

Editors
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1

Introduction to Viral Vectors

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Abstract

Viral vector manipulation is the most effective way to transfer genes to modify a specific cell type or tissue. Therapeutic genes can also be expressed through this technique. Many virus species are currently being studied for their ability to introduce genes into cells for transgenic expression, which can be either temporary or permanent. These comprise herpes simplex viruses, baculoviruses, adeno-associated viruses, poxviruses, γ-retroviruses, lentiviruses, and adenoviruses (Ads). The selection of a virus for regular clinical usage depends upon several factors, including transgenic expression effectiveness, production ease, safety, toxicity, and stability. An introduction to the general properties of viral vectors frequently used in gene transfer, as well as their benefits and drawbacks for gene therapy applications, is given in this chapter.

***Keywords*:** Viral vectors, gene transfer, transgene expression, adenoviruses, gene therapy

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Vivek P. Chavda and Vasso Apostolopoulos (eds.) Viral Vectors for Vaccine Delivery, (1–24) © 2025 Scrivener Publishing LLC

1.1 Introduction

For decades, traditional vaccination platforms such as live-attenuated or killed viral vaccines have been utilized effectively in inducing long-term immunity to various kinds of pathogenic human diseases. However, for many human infections, such vaccination platforms, especially live-attenuated vaccines, are unsuitable for human usage due to safety issues, low efficacy, or basic impracticality [1].

In a phase 1 clinical trial of a live-attenuated dengue virus vaccine, side effects produced by the vaccine virus strain's under-attenuation were observed [2].

Certain infections, including the Ebola and Marburg viruses belonging to the Filoviridae family, are so deadly that live-attenuated vaccines are not even considered because the risk of the vaccine strain becoming under-attenuated or reverting to a pathogenic state would be too great. The persistent need for developing novel, safer, and more effective vaccine platforms has led researchers to explore alternate approaches for vaccine production, including DNA vaccines, viral-vectored immunizations, and recombinant protein subunit vaccines. One of the most promising platforms for recombinant vaccine research is the viral vector. A viral vector is comparable to a small delivery device that can transport genetic material to the cell nucleus. The viral vector has the genetic material loaded and packaged into it. The purpose of using viral vectors for vaccination is to introduce the target pathogen's naturally existing antigens to the immune system without the infectious pathogen [1].

Viral vectors can be categorized into two main groups based on their genomic behavior within host cells: those that integrate into the host cellular chromatin, such as oncoretroviruses and lentiviruses, and those that primarily exist as extrachromosomal episomes within the cell nucleus, including adeno-associated viruses (AAV), adenoviruses (Ads), and herpesviruses. This classification is crucial for understanding their mechanisms of action and potential applications in gene therapy and vaccination. The selection of viral vectors for clinical use is influenced by several critical factors, including stability, toxicity, safety, ease of manufacturing, and the efficiency of transgene expression. These considerations ensure that the chosen vector is suitable for the intended therapeutic application while minimizing risks to the patient. Viral vectors encompass both RNA and DNA viruses, which can be further divided based on their genomic structure into single-stranded (ss) and double-stranded (ds) genomes. For instance, retroviruses typically possess an ssRNA genome that must be

reverse-transcribed into dsDNA before integration into the host genome. In contrast, Ads are characterized by their dsDNA genomes and are known for their ability to transiently express genes without integrating into the host genome.

The distinct properties of each viral vector type contribute to their effectiveness in various therapeutic contexts. For example, retroviral vectors are particularly effective for stable gene integration in dividing cells, while AAVs are favored for their low immunogenicity and ability to transduce both dividing and nondividing cells. Ads offer high transduction efficiency and large packaging capacity, making them suitable for delivering larger genetic payloads [3, 4].

1.2 Baculovirus Vectors

A safe, nontoxic, non-integrative vector with a high replication capability is the baculovirus. Since baculoviruses may infect both latent and growing cells, they are also a highly versatile, inexpensive vector with a wide tissue and host tropism. Additionally, they are more biosafe since they only reproduce in insect cells—not in mammalian cells. Baculoviruses are a desirable choice for gene transfer due to their advantageous characteristics. Regenerative medicine, anticancer treatments, and vaccine development have all benefited greatly from the substantial advancements made in using baculoviruses in gene therapy. Nowadays, the main applications of baculoviruses are in the manufacture of vaccines and recombinant proteins. New avenues for the production of vaccines of the next generation have been made possible by the stimulation of mucosal and systemic immune responses by baculoviruses through oral or intranasal delivery. This human-friendly virus will undoubtedly be promoted as a viable vector for clinical applications if further knowledge about the biology of baculoviruses and their interactions with non-native hosts is obtained [5, 6].

The term "baculovirus" refers to the unique rod-shaped viral particles produced in infected insect cells, known as occlusion bodies. Baculoviruses are frequently employed in insect cell culture systems as expression vectors. The baculovirus can be modified to include foreign genes in its genome, which allows the virus to infect insect cells. This makes it possible to produce significant quantities of recombinant proteins for scientific or commercial uses.

Baculoviruses have been assessed as potential carriers of antigens to elicit immunological responses, making them attractive candidates for the production of vaccines [5].

1.3 Adenovirus Vectors

Adenoviral vectors (AdVs) have emerged as the most widely used vehicle for gene therapy in cancer treatment. These vectors are also employed in vaccination strategies to deliver foreign antigens and in various gene therapy applications. In many cases, AdVs are engineered to be replication-defective; this involves the deletion of essential viral genes, which are then replaced with a genetic cassette that expresses a therapeutic gene. Such modifications allow for targeted gene delivery while minimizing the risk of viral replication in healthy tissues. In the context of cancer therapy, replication-competent AdVs, known as oncolytic vectors, have been developed. These vectors are specifically designed to replicate within cancer cells, utilizing the natural lytic cycle of the virus to induce cell death selectively. By exploiting the unique vulnerabilities of tumor cells, oncolytic Ads can effectively target and destroy malignant tissues while sparing normal cells.

Numerous clinical trials have demonstrated the safety and therapeutic efficacy of both replication-defective and replication-competent AdVs. For instance, studies have shown that these vectors can elicit robust immune responses against tumors, enhancing their potential as therapeutic agents. The ability of AdVs to infect a broad range of cell types and their capacity to induce strong cellular and humoral immune responses further support their utility in cancer treatment. Moreover, AdVs can be engineered to express immune-modulatory molecules or tumor-specific antigens, which can enhance antitumor immunity. This versatility makes them suitable not only for direct cancer therapies but also for combination strategies with existing treatments such as immune checkpoint inhibitors [7].

Although Ads have been used as gene delivery vehicles since the invention of gene therapy, Ad vaccines, like mRNA vaccines, are a more recent approach [8, 9]. The viral replication genes E1 and/or E3 are removed and substituted for the desired transgene, like an antigen, to form a vector. This prevents the virus from expressing the desired antigen and stops it from replicating its genome after infection. In comparison to mRNA vaccines, Ads have a number of advantages, such as the previously mentioned low cost and thermostability [10].

Ad vector vaccinations generally elicit robust transgenic antigen-specific cellular (specifically, $CD8^+$ T cells) and/or humoral immune responses, making them immunogenic vaccines [11].

The potential of AdVs to elicit a potent and well-balanced immune response makes them ideal for use in the COVID-19 pandemic. These vectors have been studied as vaccine agents for a variety of infectious diseases

[12, 13]. Early AdV systems faced biological challenges, but the distinct molecular characteristics of these vectors facilitated the rapid development of vaccines with complex designs [10, 14].

AdVs have the benefit of high infection effectiveness and a significant cargo limit. AdVs have a significant disadvantage in that they are highly cytotoxic; nevertheless, this characteristic can be useful when the vectors are employed as oncolytic viruses. Although there are drawbacks of transient gene expression, safety benefits outweigh them as gene expression on unintentional targets would be transient [14].

AdVs are categorized into first, second, and third generations based on their genetic makeup. First-generation AdVs can only be produced using a packaging cell line that expresses the E1 protein; they are not capable of self-replication. The most widely utilized packaging cells are human embryonic kidney 293 cells.

Most human cells produce E1A-like proteins; therefore, even first-generation AdVs with deleted E1 sections can cause robust host immune responses and persistent cytotoxicity in transduced host cells. Another strategy was to develop a second-generation Ad without E2 and E4 deletions in order to lessen the host cell's immune response to the vector. The E2 section encodes genes related to Ad replication, while the E4 region encodes regulatory proteins related to DNA transcription.

Second-generation Ads continue to elicit host immune responses and reduce transgenic expression in target cells by expressing adenoviral proteins through the remaining genes. The production of third-generation AdVs, often referred to as "helper-dependent vectors," involves co-introducing a "helper adenovirus"—a virus that carries the genes required for replication—into the packed cells. Third-generation Ads can be contaminated by helper Ads, and self-propagating Ads can result from homologous recombination between helper viruses and packing cells [15].

Gendicine, a recombinant human p53 adenovirus, was approved by the China Food and Drug Administration (CFDA) in 2003 as a first-in-class gene therapy product for head and neck cancer treatment. Gendicine is a biological medication that can be given in three different ways: intravascular infusion, intracavity, or minimally invasive intratumoral injection. The wild-type (wt) p53 protein produced by Gendicine-transduced cells has a tumor-suppressive role in response to cellular stress. It induces apoptosis, senescence, and/or autophagy, depending on the conditions of the cellular stress. It also promotes cell-cycle arrest and DNA repair. Gentacine has demonstrated notably higher response rates when combined with radiation and chemotherapy than when used with traditional therapies alone. Additionally, its safety record is really good. Apart from head and neck

cancer, other cancer types and illness stages have also been effectively treated with metronidazole. No major side effects have been noted, with the exception of 50 to 60% of patients experiencing vector-associated transient fever, which persisted for a few hours [16].

1.4 Poxvirus Vectors

Poxviruses are a large, complex virus belonging to the Poxviridae family that can affect both vertebrates and invertebrates. Poxviruses are comparatively large and oval-shaped viruses. Poxviridae is subdivided into two subfamilies: Chordopoxvirinae (vertebrate poxviruses) and Entomopoxvirinae (insect poxviruses). The subfamily Chordopoxvirinae has been further divided into nine genera, four of which contain viruses that cause diseases in humans (Orthopoxvirus, Parapoxvirus, Molluscipoxvirus, and Yatapoxvirus). Smallpox and molluscum contagiosum are diseases that affect humans, whereas the other two are zoonotic infections. The virion is enclosed, and the genome is protected in a protein sheath. Among all DNA viruses, poxviruses are distinct in that they can only reproduce outside of the nucleus, in the cytoplasm of the host cell. To encode the various enzymes and proteins involved in viral DNA replication and gene transcription, a large genome is therefore required [17].

Poxvirus infections can cause lesions, skin nodules, or a severe rash. Variola virus, the causative agent of smallpox, caused enormous morbidity and mortality in human communities before its effective eradication. The smallpox vaccine, which was essential in the eradication of smallpox, is based on the vaccinia virus (VV), a poxvirus closely related to the variola virus. The vaccination protected against smallpox without developing it. Though smallpox is no longer around, other poxviruses can infect humans. Monkeypox and VVs are two examples. Monkeypox is related to smallpox but produces a less severe sickness in humans. The smallpox vaccine is based on the VV.

Poxviruses have been utilized as vectors for foreign gene expression in mammalian cells. Modified poxviruses, such as modified vaccinia Ankara (MVA), are used as vaccine vectors in the treatment of a variety of infectious diseases. Poxviruses are open to genetic modification because of their enormous genomes. This has been used for various applications, including gene delivery vector application in research and the development of recombinant vaccines [18].

The ability of poxviruses to produce cellular and humoral immunity, their huge genome size with several immunomodulatory genes, and their

tolerance for significant heterologous gene insertions are the major characteristics that make them good antigen delivery platforms and vaccine vectors. Initially, the VV was designed to express heterologous genes. Later, with promising results, the potential of using swinepox, parapoxvirus, and avipoxvirus as vectors was additionally examined. In order to mitigate the safety risks associated with wt poxviruses, a variety of severely attenuated strains with replication defects have been created, primarily by repetitive cycles in cell culture [19].

The thymidine kinase (TK) gene has been specifically targeted for insertional inactivation in the majority of recombinant poxviruses produced too far. This involves introducing a heterologous gene into the TK locus within the poxvirus genome. Poxviridae family recombinant VV vectors proliferate and transcribe their genome in infected cells' cytosol. Thus, it is important that viral DNA is incorporated into the host's genome. VV is the preferred vector for transient gene expression since it infects almost all types of mammalian cells. Its massive, adaptable genome makes it possible to insert large DNA segments up to 25 kb in size. This virus has three distinct stages in its infectious cycle. Genes in the early phase code for enzyme proteins, while genes in the intermediate phase control the expression of genes in the late phase, which codes for structural proteins. Promoting the expression of the inserted gene of interest is possible by means of the 7.5-kDa protein that encodes the promoter gene, which is active during both the early and late phases of infection. The wt VV is cytolytic, while less virulent poxvirus vectors like MVA or fowl pox virus are commonly used for cell transduction.

To create safer and more versatile poxvirus-vectored vaccine candidates, other immunomodulatory genes have also been used recently. In heterologous prime-boost vaccination regimens, in which poxvirus vectors are combined with other killed or DNA vaccine formulations, it has been demonstrated that poxvirus vectors are highly effective. The number of vaccinations based on the poxvirus has been approved for use against various animal infections, such as the canine distemper virus (CDV), rabies virus (RabV), avian influenza virus (AIV), and West Nile virus (WNV) [19].

VV is a member of the poxvirus family. An icosahedral papovavirus is called Simian virus-40(SV40). Recently, alterations have been implemented in Simian virus-40 (SV40) to enable it to serve as a vector for gene delivery. Gene transfer vectors that exhibit certain distinctive characteristics include recombinant SV40 (rSV40) vectors: SV40 is a widely recognized virus, and it is simple to create nonreplicative vectors at titers of 1012 IU/ml. Additionally, these successfully transduce both dormant and proliferating

cells, are nonimmunogenic, and provide a broad variety of cell types with sustained transgene expression. The limited ability to clone viruses and the potential risks associated with the random integration of the viral genome into the host genome are currently the drawbacks of using rSV40 vectors for gene therapy [20].

1.5 Herpes Virus Vectors

Herpesviruses are well known for being able to cause latent infections. Some cells may experience the virus going dormant after an initial active infection, and subsequent reactivations can result in recurring infections. Many individuals carry herpes viruses without showing any symptoms, and these kinds of viruses are very widespread. Some people, particularly those with compromised immune systems, may experience more severe herpesvirus infections and consequent complications [21].

Alphaherpesvirinae, Betaherpesvirinae, and Gammaherpesvirinae are the three subfamilies of the nine herpes viruses that are known to infect humans (Figure 1.1).

Herpesviruses with reduced virulence are engineered to carry heterologous immunogens that specifically target a number of hazardous and important infections. These compounds are remarkable for their ability to elicit humoral and cell-mediated immune responses, as well as to accept large amounts of foreign DNA.

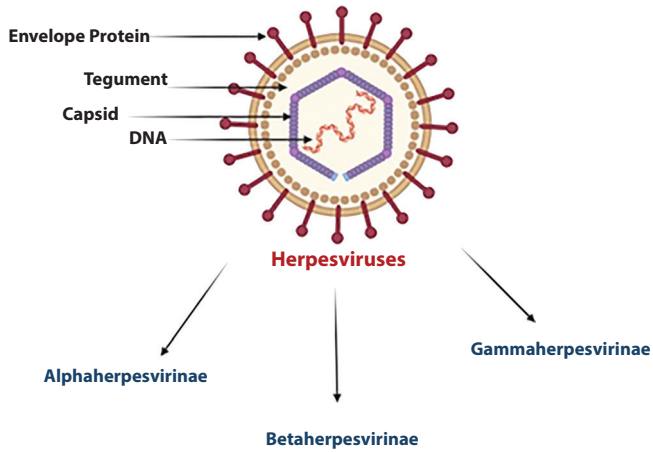

Figure 1.1 Structure and types of herpesviruses.

A strong basis for the advancement of herpesvirus-based vectors is established by a better understanding of the interaction between the vector and the host. Currently, a variety of molecular techniques, including codon optimization, homologous and two-step en passant mutagenesis, BAC technology, and the CRISPR/Cas9 system, are used to facilitate the generation of herpesvirus-based recombinant vaccine vectors [22].

Herpes simplex virus (HSV) vectors are appropriate for transferring and expressing several large therapeutic genes in brain neurons over an extended period of time, even in the total absence of viral gene expression. The advanced vector technologies are safe, long-lasting, and non-inflammatory within the nucleus of nerve cells. HSV may eventually be utilized to treat a number of neurodegenerative disorders with an identified inherited cause [21].

1.6 Epstein-Barr Virus Vectors

The Epstein-Barr virus (EBV) vector is characterized by its ability to persist in the host cell nucleus as an episome, rather than integrating into the host genome. This property renders it a highly accessible and efficient vector for use in human cell lines. The EBV vector comprises two key DNA components: the nuclear antigen-1 (EBNA1) gene, which is expressed by the virus, and the OriP sequence, which serves as the origin of plasmid replication. The EBNA1 protein plays a crucial role in maintaining the stability of the episome by binding to the OriP sequence. This interaction facilitates the anchoring of OriP-containing plasmids to nuclear proteins within the host cell, allowing them to remain stable episomes during cell division. As a result, these plasmids can replicate alongside the host's cellular machinery without disrupting genomic integrity. The unique characteristics of EBV vectors make them particularly suitable for various applications in gene therapy and vaccine development. Their episomal nature allows for sustained expression of therapeutic genes without the risks associated with genomic integration, such as insertional mutagenesis. This aspect is especially important in therapeutic contexts where long-term gene expression is desired. Furthermore, EBV vectors can be engineered to enhance their functionality. For instance, modifications can be made to improve their replication efficiency or to include additional regulatory elements that enhance transgene expression. This adaptability makes EBV vectors a valuable tool in both research and clinical settings [23].

Research on alpha herpes virus has significantly improved several genetic techniques, and EBV replication is comparable to that of other

herpes viruses. However, EBV is distinct from alpha herpes viruses in that it induces latency in human B cells and modifies their growth [24].

EBV vectors with OriP-containing plasmid replicate once throughout the cell cycle in parallel with the host chromosomes. The high-affinity matrix attachment area containing oriP is responsible for anchoring EBV vectors, which have a chromatin-like structure, to the nuclear matrix in latently infected cells.

The two noncontiguous portions that make up OriP are the dyad symmetry (DS) element and the family of repetitions (FR). A 30-bp repeat sequence is present in 20 tandem flawed copies in the FR and four similar copies in the DS, the other region. The majority of sequences for EBNA1 binding are present in these 30-bp repeats.

With the use of EBV-based vectors, deficient human cell lines can be effectively corrected by cDNA or genomic DNA transfections leading to complementation and cloning of the correcting gene. It is possible to target tumor cells that express EBNA-1 specifically with vectors containing only oriP in EBV-associated neoplasms such as nasopharyngeal carcinoma and Burkitt's lymphoma [25].

1.7 Retrovirus Vectors

Gene therapy commonly uses viruses, specifically retroviruses, as vectors. The genetic material found in retroviruses is RNA. Reverse transcription is the process by which the retrovirus converts RNA into DNA once it enters the host cell. The provirus, which is the viral DNA that has been created, is integrated into the host cell's DNA. Proviruses often provide no risk to users. There is a significant risk, though, as some retroviruses have the ability to turn healthy cells malignant.

Retroviruses must render them harmless prior to using them as a vector. For instance, by deliberately deleting a gene that codes for the viral envelope, the retrovirus can be made inert. A retrovirus cannot enter the host cell if it does not have the envelope. A single envelope-defective retrovirus can multiply into many viral particles with the help of helper viruses. Helper viruses possess the usual genes that produce envelopes. Because the vector virus has a malfunctioning envelope gene, it can multiply together with the helper virus when it infects host cells (Figure 1.2). The vector and helper viruses multiply billions of times by repeatedly replicating in the host cells. It is possible to isolate and purify the vector viruses from the helper viruses. It is crucial to isolate vector viruses and ensure they are

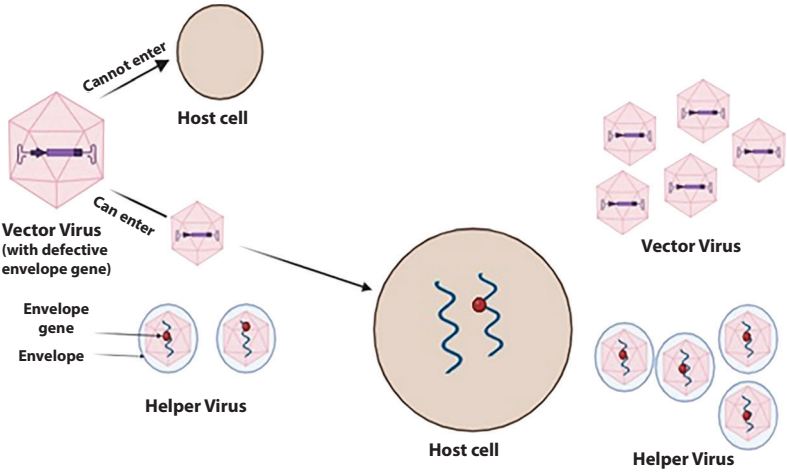

Figure 1.2 Large-scale production of vector viruses using helper viruses.

completely free of helper viruses. The health of patients receiving gene therapy is seriously threatened by helper virus contamination.

A retroviral vector carrying a maximum 8-kb size therapeutic DNA is useful for transforming the cells. However, the integration and delivery efficiency of therapeutic DNA is poor. To achieve high efficiency of integration, packaged retroviral RNA particles are used.

1.8 Lentivirus Vectors

Lentiviruses are among the most popular and useful viral vectors in the laboratory. Lentiviruses have two advantages: they have a large genetic capacity and can transduce both proliferating and nondividing cells. At the beginning of the 1990s, investigators developed viral vector systems based on retroviruses, such as the Moloney murine leukemia virus (MMLV).

Only actively dividing cells were susceptible to infection by the vectors, but they also had the ability to integrate into the genome and support transgenic expression for a long time. While nondividing cells might be infected by a different type of adenovirus-based vector, transgenic expression would not be produced over time. The packaging, envelope, and transfer plasmids constituted the original lentiviral vector system. The HIV-1 provirus mutant included in the packaging plasmid was unable to package itself because it lacked a few necessary proteins. The cell types that the vector could infect were determined by the viral envelope present on

the envelope plasmid. Finally, the necessary transgene and HIV-1 long-terminal repeats (LTRs) were included in the transfer plasmid, helping to facilitate the integration of the virus into the host genome. After these plasmids were co-transfected, 293T cells released transgene-containing lentiviral particles into the medium, which could be collected for use in research. Lentiviral vectors are still widely used for tracing and targeting brain cells because they can carry a significantly larger genetic cargo (8 kb versus 4.5 kb) than adeno-associated viral vectors, even though the latter can also target nondividing cells (Figure 1.3) [26, 27].

Lentiviruses have been studied for decades by researchers. Because it naturally inserts genetic material into cells, especially stem cells, HIV is the most well-known lentivirus. The lentiviral vector is built using an HIV virus blueprint. The HIV virus is made up of nine genes. Researchers take three or four different genes from the HIV virus's blueprint to create the lentiviral vector, which increases the vector's ability to transfer genetic material. Now, more genes will be added in order to produce the desired therapeutic effect. Since only a small portion of the nine genes from the original viral blueprint are used, HIV infection is impossible due to incomplete genomes.

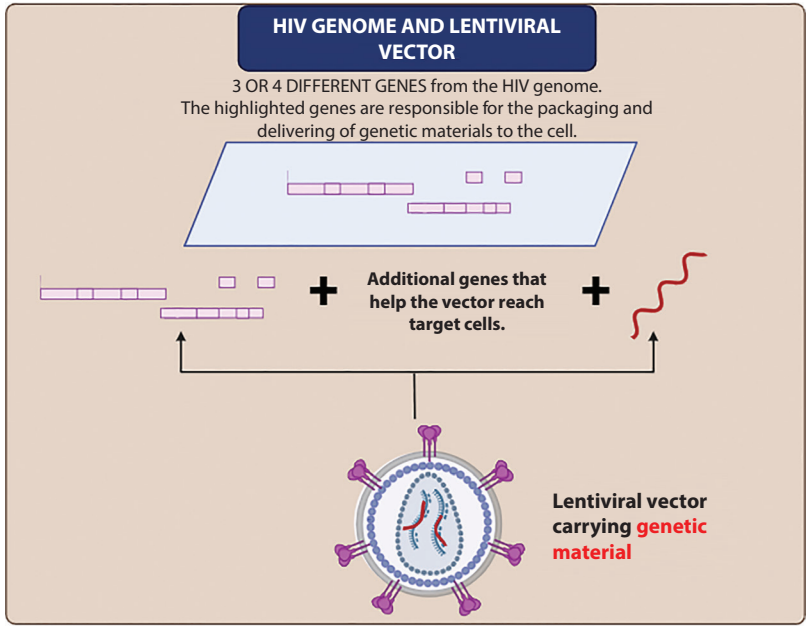

Figure 1.3 HIV genome and lentiviral vector.