Introduction to Viral Vectors

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Chapter 1

Introduction to Viral Vectors

James N. Warnock, Claire Daigre, and Mohamed Al-Rubeai

Abstract

Viral vector is the most effective means of gene transfer to modify specific cell type or tissue and can be manipulated to express therapeutic genes. Several virus types are currently being investigated for use to deliver genes to cells to provide either transient or permanent transgene expression. These include adenoviruses (Ads), retroviruses (γ-retroviruses and lentiviruses), poxviruses, adeno-associated viruses, baculoviruses, and herpes simplex viruses. The choice of virus for routine clinical use will depend on the efficiency of transgene expression, ease of production, safety, toxicity, and stability. This chapter provides an introductory overview of the general characteristics of viral vectors commonly used in gene transfer and their advantages and disadvantages for gene therapy use.

Key words: Adenovirus, Adeno-associated virus, Lentivirus, Retrovirus, Baculovirus, Poxvirus, Herpes virus, Virus infection, Virus structure

1. Introduction

The success of gene therapy relies on the ability to safely and effectively deliver genetic information to target cells, through either an ex vivo or an in vivo route. The former requires target cells to be extracted from the patient, transfected with the therapeutic gene, and returned to the patient once the gene transfer is complete. The in vivo route requires the vector to be introduced into the host, where it transduces target cells within the whole organism. Gene transfer has traditionally been achieved by the use of either viral or nonviral vectors. While nonviral methods are generally considered to be safer than viral transduction (1, 2), the production yield for plasmid DNA needs to be increased, and costs need to be decreased to make this a commercially viable gene-delivery method (3, 4); in addition, the gene transfer efficiency has to be improved. Consequently, only 17.9% of gene

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therapy clinical trials employ naked or plasmid DNA, whereas 45% of trials use either retroviral or adenoviral vectors (http://www.wiley.co.uk/genmed/clinical/).

Viruses have complex and precise structural features, which have adjusted through natural evolution for efficient transfection of specific host cells or tissues (5). A number of virus types are currently being investigated for use as gene-delivery vectors. These include adenoviruses (Ads), retroviruses (γ-retroviruses and lentiviruses), poxviruses, adeno-associated viruses (AAV), and herpes simplex viruses (HSV) (6). It is unlikely that any one of these vectors will emerge as a suitable vector for all applications. Instead, a range of vectors will be necessary to fulfill the objectives of each treatment (7).

2. Adenoviruses

Adenovirus (Ad) was first discovered in 1953 in human adipose tissue (8). This virus has since been classified into six species (A–F) that infect humans, and these species are subdivided into over 50 infective serotypes (9). From the variety of known Ads, researchers have concluded that viruses Ad2 and Ad5 of species C are the most effective for creating viral vectors for use in gene therapy (10). Ad vectors, now one of the most widely studied vector forms, are prominently used in worldwide clinical trials. As of March 2011, 402 of the total 1,703 gene-therapy clinical trials included studies with Ad vectors (http://www.wiley.co.uk/genetherapy/clinical).

2.1. Structure

2.1.1. The Capsid

The Ad capsid is a nonenveloped, icosahedral protein shell (70–100 nm in diameter) that surrounds the inner DNA-containing core. The capsid comprises 12 identical copies of the trimeric hexon protein (9). A pentameric penton base protein is located at each vertex of the capsid, and from it extends a trimeric fiber protein that terminates in a globular knob domain, as seen in Fig. 1 (11).

2.1.2. The Genome

The genome of the Ad is a linear, double-stranded DNA (dsDNA) ranging from 26 to 40 kb in length (12). This linear form is organized into a compact, nucleosome-like structure within the viral capsid and is known to have inverted terminal repeat (ITR) sequences (103 base pairs in length) on each end of the strand (11). The viral genome comprises two major transcription regions, termed the early region and the late region (13, 14). The early region of the genome contains four important transcription units (E1, E2, E3, and E4). Table 1 outlines the functions of each unit of the early region.

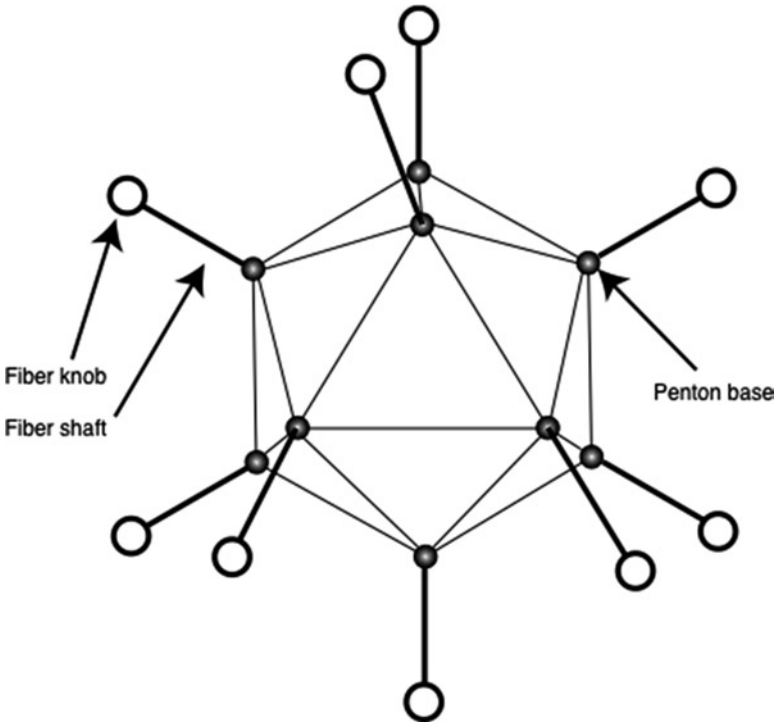

Fig. 1. Schematic diagram of an Ad capsid. The major structural protein of the capsid is the hexon. Penton capsomers, formed by association of the penton base and fiber, are localized at each of the 12 vertices of the Ad capsid.

Table 1
Early transcription units and their functions (Ad virus)

Transcription unit	Function
E1A	Activates early-phase transcription and induces the S phase of the host cell
E1B	Codes for E1B 19K and E1B 55K, which inhibit apoptosis and allow for viral replication
E2	Codes for DNA polymerase (pol), preterminal protein (pTP), and DNA-binding protein (DBP)
E3	Codes for proteins that block natural cellular responses to viral infection
E4	Codes for a variety of proteins that perform in DNA replication, mRNA transport, and splicing

2.2. Life Cycle

The early phase of adenoviral DNA invasion begins when the virus comes in contact with a host cell and ends at the onset of DNA replication. The globular knob domain of the viral capsid has a high affinity for the coxsackievirus and adenovirus receptor (CAR), which can be found on a variety of cells throughout the human body (15, 16). When the virus locates a host cell, the process of binding and internalization begins. The virus–host cell affinity between the fibrous knob and the CAR is heightened by the interaction of the penton base protein with secondary cellular receptors. The virus then travels through the cell membrane via receptor-mediated endocytosis, the virion is released, and the genome escapes the protein capsid and makes its way into the host cell nucleus, as depicted in Fig. 2.

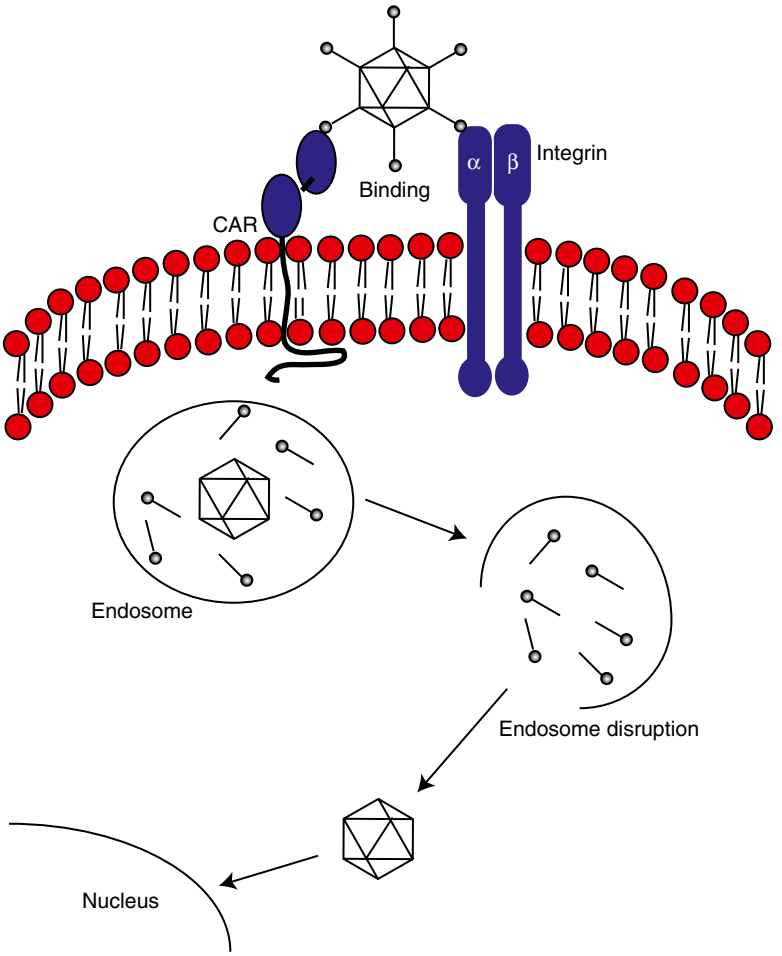

Fig. 2. Schematic representation of adenoviral infection. The Ad virion attaches to the host cell surface by CAR and integrin receptors. The virus enters the cell through clathrin-mediated endocytosis before viral DNA replication and transcription occur in the host nucleus.

Transcription of viral DNA begins when the genome enters the host cell nucleus. At this time, the E1A transcription unit of the early phase is transcribed, followed quickly by the E1B unit. Together, these two units help to prepare the genome for further transcription, shift the host cell into the S phase of replication, and inhibit apoptosis of the host cell. The E2 unit, the next to transcribe, encodes for DNA polymerase, a preterminal protein, and a DNA-binding protein, all of which are necessary for DNA replication. This process is followed by the transcription of the E3 unit, which inhibits the host cell from responding to the viral invasion. Finally, the E4 unit is transcribed to produce a variety of proteins required for DNA replication and movement into the late phase.

The late phase begins at the onset of viral DNA replication. This process begins at the origins of replication in the ITRs on either end of the viral genome, and the terminal protein at each end of the chromosome acts as the DNA primer. The products of late-phase transcription are expressed after a 20-kb section of the major late promoter has been transcribed. This section then undergoes multiple splicing cycles to return five encoding proteins of the late mRNA. These proteins are later used either to form the viral capsid or to assist in assembling the viral progeny. The host cell finally disintegrates and the virus is released.

The first-generation vectors are the most commonly used viral vectors in gene-therapy trials (11). These vectors, based on Ads 2 and 5 of species C, have the E1 region of the genome deleted to allow more genomic space for foreign DNA (10, 17). The E3 region may also be deleted for viral DNA to be replicated in culture. These eliminations allow the insertion of approximately 7.5 kb of DNA into the vector. Another vector form used in gene therapy is known as the "gutted" vector, in which all adenoviral DNA is excised except for the ITRs and packing signals. These vectors allow up to 36 kb of foreign DNA to be accommodated within the viral vector.

2.3. Preclinical Gene Transfer and Clinical Trials

Adenoviral vectors have many benefits that account for their growing popularity in gene-therapy trials; however, they also have some limitations that must be overcome before they can be used for a wide range of treatment options. Some of these advantages and disadvantages are listed in Table 2.

The Ad vector is most commonly associated with studies of cancer treatment. In one study, these vectors successfully delivered tumor suppressor genes p53 and p16 to tumor growths. The Ad vector responsible for the delivery of the p53 gene was the first to be approved for gene-therapy treatment (18). Suicide gene therapy, or prodrug therapy, has also been studied as a cancer treatment option. Suicide therapy uses viral proteins to metabolize

Table 2
Advantages and disadvantages of adenoviral vectors

Advantages	Disadvantages
Ability to infect both dividing and quiescent cells	Long-term correction not allowed
Stability of recombinant vectors	Humoral and cellular immune response from high vector doses
Large insert capacity	
Nononcogenic	
Can be produced at high titers	

nontoxic drugs into a toxic form, resulting in cell death. Recently, a phase I/II suicide-gene-therapy clinical trial has been completed in prostate-cancer patients, using an E1/E3-deleted replication-deficient Ad (CTL102) encoding the bacterial nitroreductase enzyme in combination with prodrug CB1954 (19). A total of 19 patients received virus plus prodrug, and 14 of these had a repeat treatment. Minimal toxicity was observed in patients, including those that received repeated dosages. The greatest reduction in prostate-specific antigen (PSA) was 72%; however, less than 40% of patients showed a PSA reduction greater than 10% (20). Furthermore, an increased frequency of T cells recognizing PSA was detected in 3 out of 11 patients following therapy, suggesting that this direct cytotoxic strategy can also stimulate tumor-specific immunity (19).

Gene therapy using adenoviral vectors has also been employed in the study of various liver diseases because of the vector's ability to affect nondividing cells and its high concentration in the liver after administration (21, 22). A recent study has assessed the therapeutic effect of an Ad vector carrying PAI-1 small interfering RNA (siRNA) on hepatic fibrosis. Histological and immunohistochemical analysis showed a significant reduction of liver fibrosis in rats that received the vector. The vector was able to correct the levels of matrix metalloproteinases and their inhibitors and to stimulate hepatocyte proliferation while concurrently inhibiting apoptosis (23).

Other popular research done with Ad vectors includes studies of stem cell differentiation (24), AIDS (25), cardiovascular disease (26), and pulmonary tuberculosis (27, 28). Adenoviral vectors have been widely studied and are likely to be prominent in the future of gene therapy.

3. Adeno-Associated Virus

AAV originates from the *Dependovirus* genus of the *Parvovirus* family and was first discovered in 1965 as a coinfecting agent of the Ad (29). This small virus is naturally replication-defective and requires the assistance of either a helper virus, such as the Ad or the herpes virus, or some form of genotoxic stress to replicate within a host cell nucleus (30).

3.1. Structure

3.1.1. The Capsid

The AAV capsid is a nonenveloped, icosahedral protein shell, 22 nm in diameter (30). Each serotype of this virus has its own characteristic capsid with a special affinity for certain host cell receptors, allowing it to be used to target a variety of tissue types (31–33).

3.1.2. The Genome

The genome of AAV is composed of a linear, single-stranded DNA with two open reading frames flanked on each end by a 145-bp ITR sequence (30–32). The 5′ open reading frame contains nucleotides that code for four important replication proteins, Rep 78, Rep 68, Rep 52, and Rep 40. The 3′ open reading frame codes for three capsid proteins, VP1, VP2, and VP3. Table 3 outlines the functions of each of these proteins.

3.2. Life Cycle

AAV serotype 2 is the most commonly used AAV vector in gene-therapy clinical trials. The life cycle of this viral serotype begins with the binding of the viral capsid to the host cell via negatively charged heparan sulfate proteoglycans (HSPGs); this attachment is enhanced by coreceptor integrins and various growth factor receptors (29) that help to bind the viral vector to the host cell surface. The vector is taken up by the cell through clathrin-mediated endocytosis (30, 31). Internalization is quickly followed

Table 3
Functions of Rep and Cap proteins (AAV)

Protein	Function
Rep 40 Rep 52	Participate in the generation and accumulation of single-stranded viral genome from the double-stranded replicative intermediates
Rep 68 Rep 78	Interact with Rep-binding elements and ITR terminal resolution sites to assist in the DNA replication process
Cap (vp1, vp2, vp3)	All share the same V3 regions but have different N-termini – used to form the capsid structure in a ratio of 1:1:10

by acidification of the endosome and release of the viral genome. It is not fully understood how the viral genome is able to integrate with the host cell nucleus; however, researchers have found that a helper virus is required to penetrate the host nuclear membrane before the AAV genome can begin replication (29–31). Once inside the nucleus, the AAV DNA integrates with the S1 site of chromosome 19 (33) and replication commences, producing the four Rep proteins and the three Cap proteins outlined in Table 3.

The process for creating vectors from AAVs begins with the deletion of genes coding for the Rep and Cap proteins. This deletion provides approximately 5 kb of packing space for foreign DNA. The new DNA is inserted into the "gutted" virus that contains only the ITRs. The ITRs contain all *cis*-acting elements necessary for replication and packaging in the presence of a helper virus. The Rep and Cap proteins and all necessary adenoviral helper genes are expressed on either one or two plasmids. The expression of Ad genes from a plasmid eliminates the need for coinfection with wild-type adenovirus. Production of AAV vectors requires cotransfection of human embryonic kidney cells (HEK293) with the gutless AAV and one or two helper plasmids (29, 34, 35).

3.3. Preclinical Gene Transfer and Clinical Trials

Adeno-associated viral vectors are most widely used in tissue engineering studies. For such applications, these vectors possess a wide range of advantages; however, some obstacles must still be overcome for these vectors to become commercially approved and be available for treatment. A list of the benefits and limitations of the AAV vectors may be found in Table 4.

Table 4
Advantages and disadvantages of AAV vectors (36)

Advantages	Disadvantages
Nonpathogenic	Smaller size limits the amount of foreign genes that can be inserted
Broad host and cell type tropism range	Slow onset of gene expression[a]
Transduce both dividing and nondividing cells	
Maintain high levels of gene expression over a long period of time (years) in vivo	

[a] *Note*: In the case that single-stranded AAV vectors are used; using self-complementary AAV vectors (double-stranded AAV vectors), the gene expression is more rapid, as the transduction is independent of DNA synthesis (37)

In various animal studies, AAV vectors have been used to treat skin burns (38), excision wounds (39), and incision wounds (40) and have shown great promise for the future. Researchers have also found AAV vectors to be stable in various tissues, including the brain (41), as well as in many different cell types, including muscle (42) and retina cells (43).

The wide range of tissues that can be affected by AAV vectors is due in large part to the unique capsid of each AAV serotype. For example, AAV2 (the most commonly used AAV serotype) has a high affinity for HSPGs (44) – receptors found in a variety of cell types – whereas AAV5 will bind to the platelet-derived growth factor receptor (PDGFR), commonly found on the cells of the brain, lung, and retina (45, 46). Other serotypes whose receptors have not been determined still show an obvious affinity for specific cell types. For example, AAV1, AAV6, AAV7, and AAV8 are attracted to muscle, lung, muscle and liver, and liver cells, respectively (47–49). Further studies have been done with the so-called "mosaic" serotypes, where researchers combined two different AAV vectors and discovered that these mosaics often maintained affinity for the receptors associated with both serotypes (50, 51). Once inside the host cell, rAAV vectors stay mostly episomal (in both human and nonhuman cells) (52). However, stable expression of the vector is possible for extended time periods, often in excess of 1 year, for several cell types including brain (41), muscle (42), and eye (43).

Unlike vectors used in other gene-therapy trials, the main focus of AAV trials has been on monogenetic diseases (53%), followed by cancer (23%) (36). Cystic fibrosis is the most frequently targeted disease. Repeated administration of aerosolized AAV vector containing the cystic fibrosis transmembrane regulator (AAV-CFTR) is well tolerated and safe (53, 54); however, in a phase 2B clinical trial, no statistically significant improvement was seen in patients receiving AAV-CFTR compared to placebo (55). AAV vectors have also been used to treat hemophilia B with some success. In a phase 1/2 dose-escalation clinical study, rAAV-2 vector expressing human F.IX was infused through the hepatic artery into seven subjects. There was no acute or long-lasting toxicity observed at the highest dose, which was able to produce a therapeutic effect. However, in contrast to previous work performed in dogs (56), the expression of therapeutic levels of F.IX only lasted 8 weeks as a result of immunogenic destruction of hepatocytes expressing the AAV antigen (57). Other diseases that have been treated with rAAV vectors are Canavan disease (58), infantile neuronal ceroid lipofuscinosis (59), Parkinson's disease (60), and α1-anti-trypsin deficiency (61).

4. Retroviruses

Retroviruses are known for their ability to reverse the transcription of their single-stranded RNA genome, thus creating dsDNA to replicate after infecting host cells. These viruses are most generally categorized as either simple (oncogenic retroviruses) or complex (lentiviruses and spumaviruses) (62). This section discusses the simple oncogenic retroviruses – most commonly the murine leukemia virus – before discussing the complex retroviruses in the lentivirus section. The oncogenic retroviruses are limited by their inability to infect non-dividing cells; however, they are considered extremely useful for tissue engineering studies, particularly those concerning bone repair.

4.1. Structure

4.1.1. The Capsid

The retroviral capsid is an enveloped protein shell that is 80–100 nm in diameter and contains the viral genome (52). The envelope structure surrounding the capsid is actually a lipid bilayer that originates from the host cell and contains both virus-encoded surface glycoproteins and transmembrane glycoproteins (63). The basic retroviral structure is similar to lentiviruses (HIV-1 – shown in Fig. 3).

4.1.2. The Genome

The genome of the retrovirus is a linear, nonsegmented, single-stranded RNA, 7–12 kb in length (62). The simple class of retroviruses contains three major coding segments and one small coding domain. The major segments contain three genes – gag, pol, and env – which code for proteins important in viral integration,

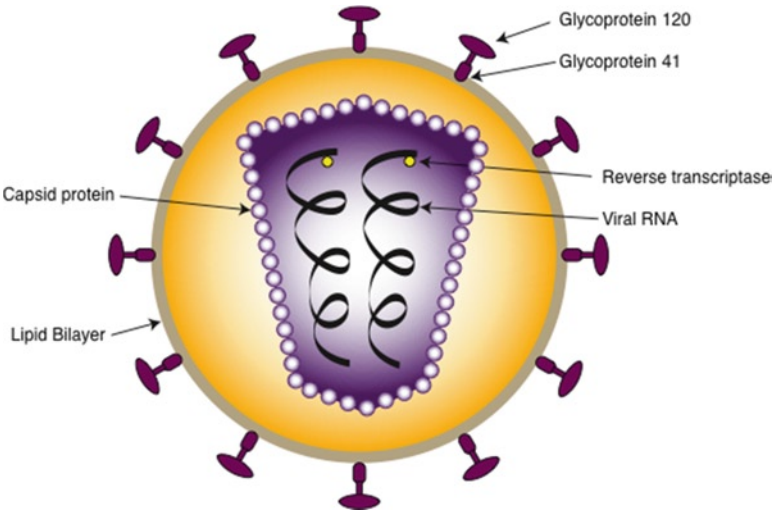

Fig. 3. Schematic diagram of the basic physical structure of a retrovirus (shown here is the structure of the HIV-1 lentivirus).

Table 5
Functions of retroviral genes

Protein	Function
gag	Codes for the viral core
pol	Codes for reverse transcriptase and integrase
env	Codes for surface and transmembrane components of the viral envelope proteins
pro (small coding domain)	Encodes a viral protease

replication, and encapsulation (52). The small coding domain contains the pro gene, which encodes for viral protease (63). A more detailed description of the coding segments and their protein products may be found in Table 5.

4.2. Life Cycle

The life cycle of the retrovirus begins when the glycoproteins of the viral envelope attach to specific host cell receptors. The viral envelope then fuses with the cellular membrane of the host, and the viral core is released into the host cell cytoplasm. Proteins coded for by the pol gene are then used to begin viral transformation. Viral reverse transcriptase is used to create a dsDNA genome from the original single-stranded RNA of the virus. As the viral dsDNA cannot pass through the nuclear membrane in nondividing cells, the integration of the virus is only possible when the cell is cycling, after the breakdown of the nuclear membrane. Subsequently, viral integrase helps the newly formed dsDNA integrate into the host cell genome, where it will remain a permanent part of the host cell, now known as a provirus. With respect to virus replication, RNA polymerase II transcribes the provirus to mRNA, which codes for viral proteins. After the virus reassembles in the cytoplasm, it escapes the cell by budding out from the cellular membrane, where the capsid receives its envelope (64).

Retroviral vectors must be replication defective. To achieve this, all of the *trans*-acting elements of the genome (gag, pol, and env genes) are removed, leaving only the attachment sites, the long terminal repeat, the packaging signals, and the sites important for viral gene expression. Removal of gag, pol, and env genes provide space for the gene of interest to be inserted into the viral genome. Vector replication can only occur in packaging cell lines. Packaging cells are transfected with plasmids containing the gag, pol, and env genes, which they consistently express allowing for retroviral proliferation (65, 66). To increase safety, the gag and pol genes are contained in one plasmid, while the env gene is contained in another. The vector gene is contained in a third plasmid

(split-genome packaging plasmid). This allows multiple regenerations of the vector to be produced without any risk of a replication-competent retrovirus being formed (66).

4.3. Preclinical Gene Transfer and Clinical Trials

Retroviral vectors are widely used in studies of tissue repair and engineering. Because these vectors can be used to infect dividing cells without producing any immunogenic viral proteins while also becoming a permanent part of the host cell genome, they have proven to be an extremely useful tool in gene-therapy research. These vectors are limited only by their relatively small carrying capacity and their inability to infect nondividing cells; however, these disadvantages have not kept them from being the most widely used vectors in the research of gene and cell therapy (67).

One of the most common types of study done with these viral vectors involves bone repair. Current methods of bone grafting are limited by the availability of source grafting material and the dangers of disease transfer. However, retroviral vectors have recently been used to deliver various growth factors and differentiation factors to both mature bone cells and stem cells that have been used in tissue scaffolding, and various animal studies have yielded promising results (68, 69). Retroviral vectors have also been used in the repair of damaged cartilage (70, 71) and in the formation of tissue-engineered blood vessels for the treatment of cardiovascular disease (72).

In addition, retroviral gene therapy has also been used in clinical trials, among others, to treat X-linked severe combined immunodeficiency (X-SCID) in infants and preadolescents (73, 74). Autologous CD34+ hematopoietic cells were transduced ex vivo with retroviral vectors containing the open reading frame of human *IL2RG* cDNA. Significant improvements in T-cell function have been observed, although one study reported leukemias in four patients secondary to retroviral insertional mutagenesis (75).

5. Lentiviruses

Lentiviruses, a subcategory of the retrovirus family, are known as complex retroviruses based on the details of the viral genome. The most common example of a lentivirus is the human immunodeficiency virus type 1 (HIV-1).

5.1. Structure

The lentiviral capsid is the same as that of the simple retroviruses described in Subheading 4. The lentiviral genome, like that of other retroviruses, contains a single-stranded RNA, 7–12 kb in length (62). However, while the genome contains the same genes

Table 6
Genes expressed in HIV-1 lentivirus in addition to the simple retrovirus genes described in Table 1

Protein	Function
rev	An RNA-binding protein that acts to induce the transition from the early to the late phase of HIV gene expression
tat	An RNA-binding protein that enhances transcription 1,000-fold
nef	Disturbs T-cell activation and stimulates HIV infectivity
vpr	Mediates HIV to infect nondividing cells
vpu	Enhances the release of HIV-1 from the cell surface to the cytoplasm
Vif	A polypeptide necessary for the replication of HIV-1

These genes are nonessential and absent in lentiviral vectors. The *rev* gene along with the simple genes *gag*, *pol*, and *env* are expressed on plasmids that are present in packaging cells

as the simple retroviruses (gag, pol, and env, see Table 5), it also comprises six other genes – two regulatory genes and four accessory genes – that code for proteins important for viral replication, binding, infection, and release. Table 6 outlines each of these six genes and the functions of their expressed proteins. The most common lentiviral vector is made from HIV-1. In these vectors, the original genes present in the simple virus, all four of the additional accessory genes, and one of the regulatory genes are deleted to create space for the insertion of foreign genes (76, 77). In contrast to the simple retroviruses, LV vectors are generally produced by transfection of HEK293 or 293T cells. The first of two necessary helper plasmids contains the gag, pol, and rev genes; the other plasmid contains the env gene (78). A further plasmid brings in the recombinant LV vector sequence.

5.2. Life Cycle

The life cycle of the lentivirus is representative of the retrovirus family, in that the glycoproteins of the viral envelope are attracted to specific cellular receptors; the envelope then fuses with the host cell membrane, and the core is released into the cytoplasm. Soon after this internalization, the single-stranded RNA is transcribed in reverse with the help of viral proteins to form a double-stranded genome that is incorporated into the host genome. However, some important differences do take place in the life cycle of the lentivirus (64). First of all, gene expression occurs in two separate phases, known as the early and late phases, which are separated by the binding of the rev protein (79). Second, the lentivirus is capable of infecting nondividing cells via proteins expressed from the vpr gene (80).

Finally, the tat gene, found only in complex retroviruses, is essential for the replication of HIV-1 (81).

The self-inactivating expression vector (SIN) is another vector form of the lentivirus, in which the U3 promoter is deleted, causing transcriptional inactivation of the provirus. This vector form limits both genome mobility and possibilities of recombination in the host cell (78, 82).

Oftentimes, the vectors used in gene-therapy trials are given an envelope surrounding the capsid structure that is composed of very specific glycoproteins, namely, the vesicular stomatitis virus glycoprotein (VSV-G), which allows the vector a high tropism and the ability to infect a wide variety of cell types (83).

5.3. Clinical Trials

Lentiviral vectors possess many advantages over other simple retroviral vectors. For example, lentiviral vectors can infect mouse and rat embryos to generate transgenic animals with high tissue-specific expression of transgene (84). Since these vectors also have a relatively large carrying capacity for foreign genomic material (52), it is suggested that they can be used to produce other transgenic animal species.

Lentiviral vectors have traditionally been used in studies dealing with nondividing host cells, such as those of the nervous and cardiac systems (85). The first clinical trial using a LV vector was approved in 2002. Since then, eight other protocols have received approval, and 11 others have been submitted or are under review (86). The first trial to be approved was for VRX496™ (lexgenleucel-T) anti-HIV RNA therapy (87). The vector has been shown to be safe and offers short-term efficacy and is currently in phase I/II trials. Other diseases to be targeted with LV vectors are adrenoleukodystrophy (ALD) (88), a progressive neurodegenerative disease that causes diffuse demyelination and primarily affects the CNS, Parkinson's disease (89), sickle cell anemia and β-thalassemia (90), HIV (91), and cancer immunotherapy (92). A comprehensive review of forthcoming clinical trials can be found in D'Costa et al. (86).

6. Baculoviruses

The most commonly studied baculovirus is known as the *Autographa californica multiple nucleopolyhedrovirus* (AcMNPV). It was originally thought that this virus was incapable of infecting mammalian cells; however, in 1983 several studies showed that baculovirus could be internalized by mammalian cells (93, 94), and they were used for the expression of human interferon β (95). Subsequently, AcMNPV has been successfully internalized in a number of human cells, with some of the viral genome reaching

the host cell nucleus (96, 97). Though the baculovirus is not the most widely studied virus in gene therapy, it is nonpathogenic to human cell lines and is unable to replicate in mammalian cells. These are considerable safety advantages and may be a distinct advantage over other viral vectors.

6.1. Structure

The baculoviral capsid is a rod-shaped protein shell (40–50 nm in diameter and 200–400 nm in length) that is naturally protected by a polyhedron coat. While this coat does provide viral protection, the virus does not need it to exist. Some of the most studied of these viruses are actually in the "budded" form, which consists of an envelope that surrounds the capsid and that contains glycoproteins essential for viral binding to host cells (98). The genome of the baculovirus is a complex circular, dsDNA containing the genes necessary for viral infection of host cells. An in-depth description of AcMNPV genes can be found in Cohen et al. (99).

6.2. Life Cycle

The life cycle of the baculovirus is still not fully understood; however, researchers have come to some conclusions about its binding, internalization, and nuclear uptake. Though the method of virus–host cell interaction is not clear, researchers do agree that the glycoprotein Gp64 is necessary for this interaction to occur (98). Researchers also agree that the virus is then taken into the cell via some form of endocytosis, possibly clathrin-mediated endocytosis, though some other internalization methods may coexist (97, 100–102). Once inside the cytoplasm, the virus frees itself through acidification of the endosome (96, 103). Scientists agree that the transfer of the nucleocapsid is somehow blocked in the cytoplasm of the host cell, and some say that this is due to the various microtubules throughout the cytoplasm. This conclusion is supported by the fact that transport time decreases when these microtubules have been chemically disintegrated (104). Once the viral genome finally reaches the host cell nucleus, it is ready to be taken up into the cell; however, it appears that this uptake process may vary depending on the type of host cell. Some cells take up the genome directly through nuclear pores, while others seem to transport the viral genome to different subcellular compartments. Still others appear to degrade the viral genome prior to nuclear uptake (105). Though researchers have not yet completely understood the life cycle of the baculovirus, this virus and its capabilities are being continually studied.

6.3. Preclinical Gene Transfer and Clinical Applications

Though baculoviruses are not yet a widely studied vector form, they do possess a number of benefits that have awakened the curiosity of many researchers. First, they do not replicate inside mammalian host cells and are not toxic (106). Second, baculoviral DNA has been known to automatically degrade inside host cells

over time (107, 108). Also, because the baculovirus only infects insects and invertebrates, humans do not appear to have preexisting antibodies or T-cells specifically against baculovirus (109). Finally, these viruses may be constructed into vectors with a DNA carrying capacity of up to 38 kb, allowing the delivery of a large amount of foreign genomic material to the host cells (110). The main drawback associated with baculovirus is a rapid, complement-mediated inactivation. To overcome this, researchers have successfully coated virus particles with polyethylenimine, protecting them against complement inactivation (111, 112).

Not only does this virus have a variety of promising characteristics, but it has also been proven to be practical in a number of gene-therapy trials. Baculoviruses have been used in animal studies to deliver genes to a wide range of cell types, including carotid artery (113), liver (114, 115), brain (116, 117), and skeletal muscle (118). This relatively new vector form has already caught the interest of many scientists and will likely play a large role in the future of gene therapy.

7. Herpes Simplex Virus

Actually, many different varieties of the HSV have been discovered. The most common of these, known as HSV-1, is well known by the average person as the viral cause for cold sores. One of the most intriguing aspects of this virus is its ability to infect a host and then remain latent for a period before reappearing again (119). Research on this virus continues in hopes that its unique characteristics will lead to a breakthrough in gene therapy.

7.1. Structure

7.1.1. The Capsid

The HSV has an icosohedral protein shell that is covered by a viral envelope. Embedded within the envelope are a variety of glycoproteins important for the viral attachment to host cellular receptors. Tegument is a layer of proteins and enzymes coded for by the viral genome that lies between the capsid core and the viral envelope (119). Figure 4 illustrates this capsid structure.

7.1.2. The Genome

The HSV genome consists of a dsDNA (152 kb in length) that codes for up to 90 different proteins important for viral attachment and replication (120). This genome is further organized into what are known as unique long and unique short segments, and these segments are capped on each end by inverted repeat sequences (52).

7.2. Life Cycle

One of the most interesting characteristics of the HSV is its ability to remain latent in host cells after the initial infection and then to reappear spontaneously (119). The life cycle of this virus begins

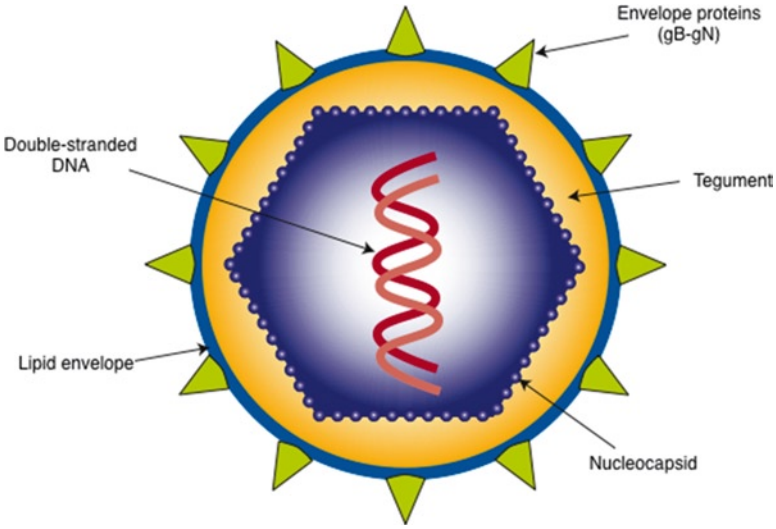

Fig. 4. Schematic diagram showing the structure of the herpes simplex virus.

Table 7
Functions of α, β, and γ genes of HSV-1

Protein class	Function
α	Major transcriptional regulatory proteins – necessary for the synthesis of β and γ proteins
β	Include DNA polymerase and transcriptional factors involved in viral replication
γ	Primarily serve as structural proteins

when it binds to host cell surface receptors via glycoproteins of the viral envelope. The virus is then taken into the cell, where it is delivered to the nucleus. Once the virus reaches the nucleus, the viral capsid binds to the nuclear membrane and releases the viral DNA into the host cell nucleus. Transcription of the viral DNA is a complex process involving multiple steps with the help of a variety of proteins. These proteins are classified into three groups, termed α, β, and γ proteins. The α proteins are also known as the immediate early proteins, and the β proteins are called the early proteins. The γ proteins are referred to as the late proteins, and DNA replication begins following their transcription (63). Table 7 provides a more detailed explanation of these gene products and their functions.

The HSV-1 has been used to develop two different types of viral vectors. The replication-defective vectors work in much the same way as the adenoviral and retroviral vectors. The α genes

involved in viral DNA replication are deleted, and the foreign gene of interest is inserted into the viral genome. Helper cells are then used to take the place of the deleted genes, and the vector is ready for infection. The other type of HSV-1 vector is known as the amplicon vector. In this vector form, plasmids that contain the HSV-1 origin of replication, all necessary packaging signals, and the gene of interest are cotransfected with a helper virus and inserted into a cell line that supports the growth of the helper virus. Replication of this vector is prevented by either deletion of the α genes or temperature influence.

7.3. Preclinical Gene Transfer and Clinical Applications

The main advantage of the HSV is its ability to remain latent within host cells after infection. This distinctive feature, along with the fact that the virus is naturally neurotropic, allows it to infect neural cells and, therefore, to assist in treating neural diseases. Most of the animal studies performed with HSV have involved either the treatment of brain tumors or Parkinson's disease. In both cases, gene therapy using the herpes simplex viral vector has shown promising results (121).

8. Poxviruses

The poxvirus is most widely known for its use as a vaccine against smallpox. Recombinant gene expression of this virus was first performed in 1982 (122, 123), and it was one of the first animal viruses to be used as a gene-transfer vector (124). The most commonly studied strains of this virus include the MVA and NYVAC viruses, as they are naturally replication-defective in most human tissues and they lack the ability to produce infectious virus in human host cells (125).

8.1. Structure

The poxvirus capsid is acquired in the host cell cytoplasm after DNA replication. Some of the protein products of viral genome replication form the capsid during viral reassembly. These mature virions are wrapped by an envelope structure that originates from the *trans*-Golgi to form intracellular enveloped viruses. These viruses later fuse with the inner cell membrane, are released from the Golgi envelope, and are reenveloped by the host cell membrane upon escape (126).

The MVA and NYVAC poxviruses have unusually large dsDNA genomes (178 and 192 kb, respectively) (127). Approximately 100 genes are specifically conserved in poxviruses, while the existence of other genes helps to define the different viral strands. All of the genes present in the viral genome are valuable, and few introns, if any, exist in many of the viral strands (128).

8.2. Life Cycle

The life cycle of the poxvirus begins when the glycoproteins of the viral envelope attach to host cell surface receptors. Once the virus enters the cytoplasm, it is thought to form a type of replication center enclosed by the rough endoplasmic reticulum. Unique to the poxvirus, replication of the viral genome actually takes place in the host cell cytoplasm, as opposed to the host cell nucleus (129). Viral gene expression is an extremely complex cascade mechanism that leads to the production of early, intermediate, and late transcription factors, along with structural proteins and various enzymes (130). Immature virions are formed followed by the production of mature virions that are enveloped in a double membrane structure by the *trans*-Golgi and that are released at the host cell membrane and reenveloped by the lipid bilayer that makes up the host cell membrane (126).

8.3. Preclinical Gene Transfer and Clinical Applications

The most widely used poxviral vectors originate from the MVA and NYVAC viral strains. These strains are often chosen because they are naturally replication-defective and unable to produce infective viruses in human tissues (125).

These viruses have long been used as vaccines against diseases such as smallpox but are now being studied as viral vectors to be used against other viral, parasitic, and bacterial diseases, including HIV, West Nile fever, and tuberculosis (131, 132). These viral vectors are used to elicit an immune response against foreign diseases that have become resistant to the drugs once used to kill them (133). However, they are not dangerous because they are naturally replication-defective in human cells.

Poxviral vectors are also being used in immunomodulation gene therapy, in which they can safely deliver tumor-associated antigens to tumor cells, causing an immune response against those tumor cells (124). While not the most commonly studied vector forms, poxviral vectors have proven applicable to the treatment of many diseases for the treatment of various forms of cancer, and researchers are highly interested in their impact on treatment options in the future.

9. Summary

The ability of viruses to deliver foreign DNA to cells for therapeutic purposes has been exploited in numerous different contexts. The diverse nature of different vectors and the variability of different diseases mean that there will almost certainly be no "one size fits all" vector. Clinical trials have shown that certain vectors have great potential for specific diseases. For example, retroviral vectors have had great success in treating X-SCID, whereas lentiviral vectors have been used to target various neurological diseases, including

Parkinson's and ALD, and other clinical trials have employed AAV vectors to treat monogenic disorders, such as Duchenne muscular dystrophy and hemophilia B. Although no viral vector has yet received clinical approval in Europe or the USA, the encouraging results from clinical trials, coupled with continual improvements in vector design and safety, shows that this technology has immense potential.

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