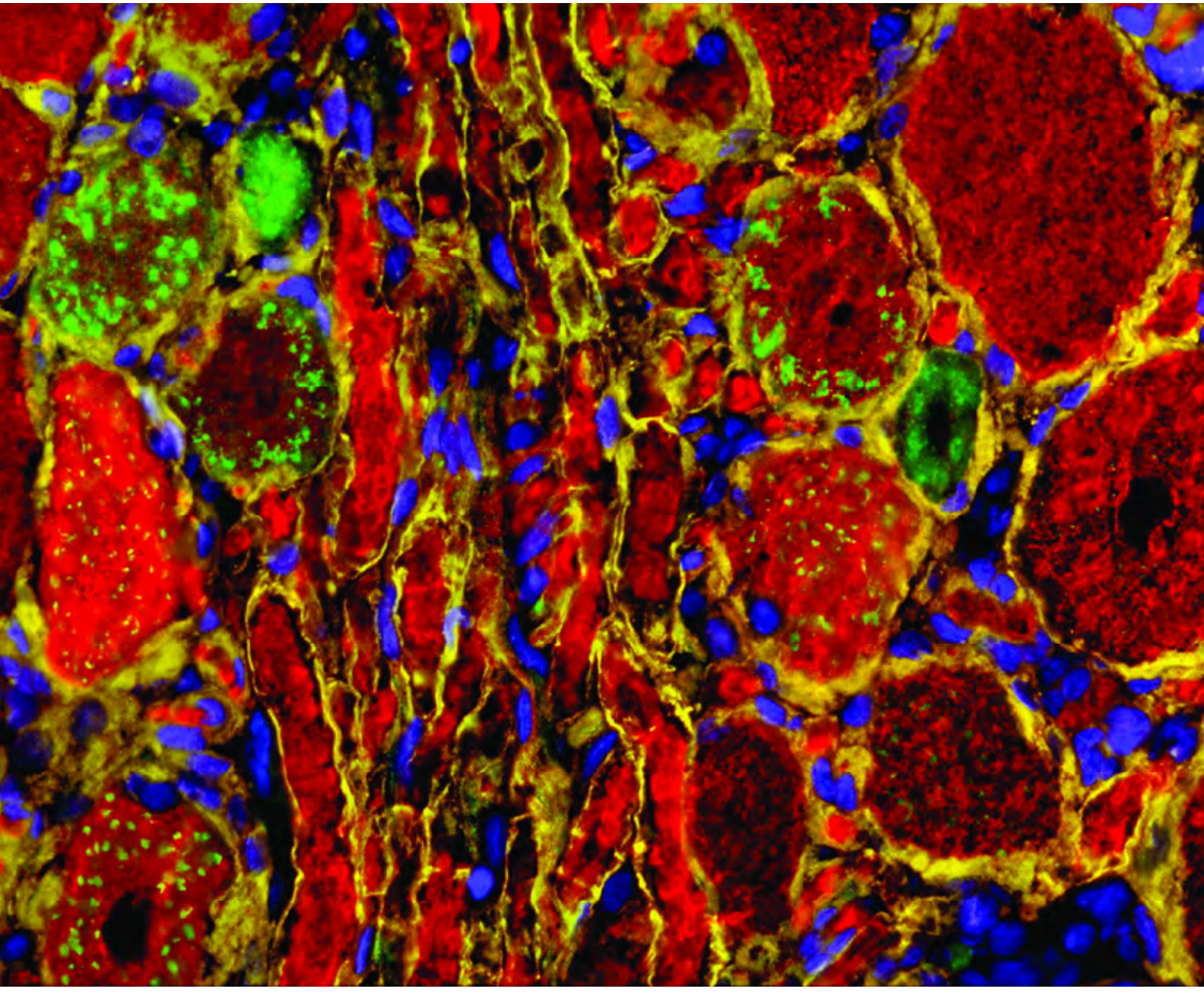
A publication of
The Williamsburg BioProcessing Foundation

May/June 2003

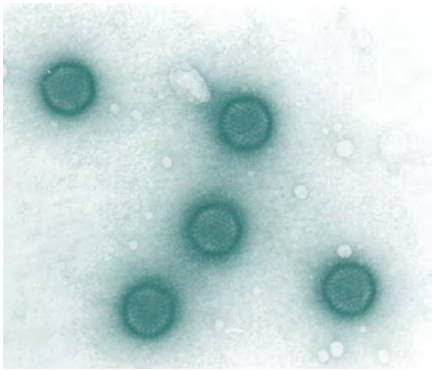
BioProcessing™ JOURNAL

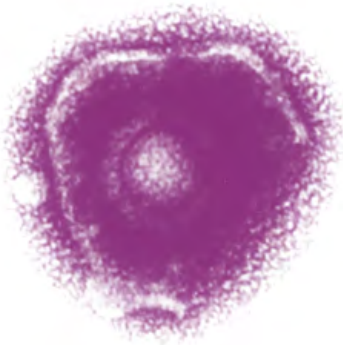
Advances & Trends In Biological Product Development

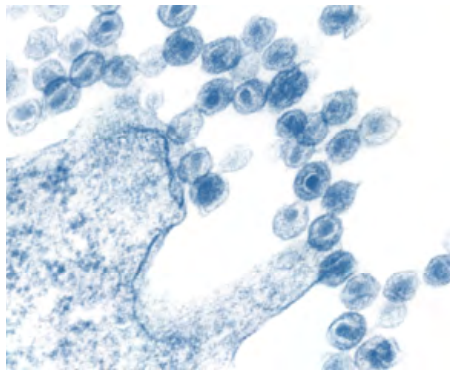


Vol. 2 No. 3

www.bioprocessingjournal.com







Photos courtesy E.W. Milne, Q-One Biotech Ltd.

VIRAL GENE VECTORS

BY SYBILLE L. SAUTER

Sybille Sauter (sybsau858@aol.com)
is a cofounder of
Virogenics Inc., San Diego, CA.

An astonishing range of viruses has provided building blocks for gene delivery systems, from the simple adeno-associated virus with a 5 kb genome to the complex poxviruses with 300 kb. This review focuses on non-replicating viral vectors that infect host cells just once, without producing infectious virus. This review focuses on nonreplicating viral vectors that infect host cells without producing infectious virus. Viral vectors are generally characterized by several criteria,including their ability to integrate into the host genome, coding capacity, titer, toxicity, immunogenicity, host range, duration of gene expression, and transient or stable production systems (Table 1). These are precisely the features that need to be carefully studied in the context of the application when deciding which vector to use.

Retroviral and lentiviral vectors. Retroviruses were discovered at the turn of the 20th century and are pathogens infecting vertebrates from fish to man. The hallmark of retroviruses is the reverse transcription of their RNA genome into DNA, which is then stably integrated into the host genome. The viral genome contains two 10 kb negative-strand RNA molecules coding for three components — *gag* encodes structural proteins, *pol* encodes enzymes,and *env* encodes the envelope protein. These genes are flanked by identical long terminal repeats (LTRs) on either end, providing regulatory functions (Fig. 1). The prototype retroviral vector is derived from the Moloney murine leukemia virus (MoMLV), and in 1990 was the first gene therapy vector to enter the clinic;it was used in an *ex vivo* mode to treat children suffering from the immune disease adenosine deaminase deficiency.[1] Since the 1980s, development of MoMLV-based vectors has guided the design and concepts of viral vectors in general. Basic principles, such as maximum deletion of viral sequence to accommodate heterologous genes, increasing safety by splitting up the viral genome, and the development of stable producer cell lines were established. MoMLV vectors have been employed in the majority of gene therapy trials to date and are,therefore, clinically well-characterized (Table 2). Other retroviruses used for vector construction include avian leucosis virus, Gibbon ape leukemia virus, spleen necrosis virus, and foamy viruses. Because retroviral vectors integrate into the host genome (potentially allowing lifelong expression) and are relatively non-immunogenic and non-toxic, they are well suited for the treatment of genetic disease. Drawbacks include the relatively low titer and the potential for insertional mutagenesis and germline transduction. Retroviral vectors also require actively proliferating cells at the time of infection, which eliminates many clinically relevant targets including neuronal, stem, and liver cells. Lentiviral vectors overcome this major shortcoming because they can transduce non-dividing cells; thus, they have received a lot of attention since their conception in the early 1990s. Lentiviruses belong to the family of Retroviridae, and otherwise display the same characteristics described for retroviruses — except for additional regulatory and accessory proteins that need not be included in the viral vector system. The prototype lentiviral vector is derived from HIV-1.[2] Several lentiviruses such as simian immunodeficiency virus and the non-primate lentiviruses such as feline immunodeficiency virus or equine infectious anemia virus were used for subsequent lentiviral vector development.[3–5]

Adenoviral vectors. The pathogenic adenoviruses were discovered in the mid-1950s as the causative agent for acute respiratory disease in humans. Their double-strand DNA genome

codes for at least 12 structural and many non-structural proteins, flanked by inverted terminal repeats (ITRs), which are important in DNA replication. Adenoviruses do not integrate and the prototype adenoviral (Ad) vector is derived from the human Ad5 serotype virus.[6] More recently described Ad vectors include other human Ad serotypes and non-primate Ad vectors from chimpanzee Ad68 and canine Ad2. Ad vectors entered the clinic in 1993 to treat cystic fibrosis and have become the second most used gene therapy vector for the treatment of cancers and genetic disease (Table 2).[7] Advantages include their ease of production to very high titers of 1 x 10^{13} vp/ml, a large coding capacity of up to 36 kb in the "gutted" Ad system, non-integrating properties, and targeting potential. Disadvantages include short-term expression of the gene of interest,induction of inflammatory and immune responses, and possible toxicity at high systemic doses. The "gutted" Ad or helper-dependent Ad system has demonstrated long-term expression of the gene of interest.

Adeno-associated vectors. The nonpathogenic adeno-associated virus (AAV) was discovered in the mid-1960s as a contaminant of adenoviruses — hence its name. AAV has a simple single-strand DNA genome with two open reading frames (*rep* and *cap*) for the nonstructural and structural proteins flanked by ITRs, which are essential for replication and integration. AAV can be either lytic or latent,and in the presence of helper virus (Ad or herpes simplex, for instance) wild-type AAV lyses its host cell, whereas in the absence of helper virus it stably integrates into the genome or is maintained as an episome. Site-specific integration is driven by the *rep* genes. Most recombinant AAV vectors are deleted in *rep* and *cap* to increase vector packaging capacity and, therefore, do not integrate in a site-specific manner. Vector genomes are mainly maintained as stable episomes and, depending on the target cell and experimental system investigated, to a smaller degree by non-specifically integrated provectors. The prototype AAV vector is derived from human AAV2, and was first described in 1984; gene therapy trials to treat cystic fibrosis commenced in the mid-1990s.[8] Advantages include long-term expression and lack of pathogenicity or toxicity. Disadvantages include the small capacity of 4.5 kb and possible insertional mutagenesis by the integrated forms. Alternative AAV vectors derived from human serotypes 1,3, 4, and 5 have recently been shown to display different tropism.[9] Less-developed vector systems derived from autonomous parvoviruses, such as minute virus of mice (MVMp) and H-1, may be useful for cancer therapy due to their oncolytic properties and tropism for transformed tissues.

Herpes simplex vectors. Herpes simplex viruses were discovered more than 80 years ago and can cause a variety of

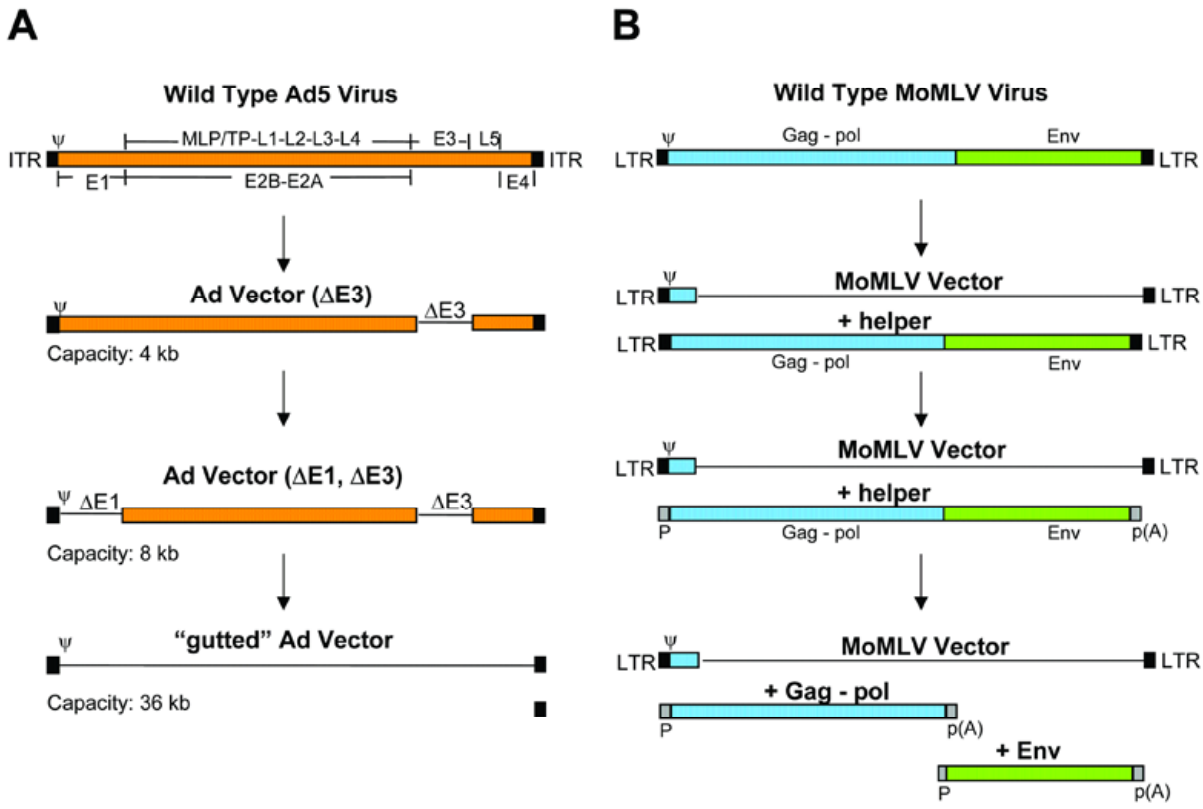


Figure 1. Schematic representation of several Ad vector generations evolving toward increased coding capacity, resulting in the "gutted" Ad vector with a maximum capacity of 36 kb. Helper functions are provided *in trans* and are not shown (A). Schematic representation of several generations of retroviral vector components in stable producer lines showing the trend towards a "split genome" while reducing viral sequences (B). ITR (inverted terminal repeat), LTR (long terminal repeat), P (promoter), p(A) (poly A signal), Ψ (packaging signal).

Table 1.

Viral Vector	Virus genome	Capacity	Titer[a]	Integrating	Long-term expression	Immuno-genicity	Infects non-dividing cells
Retrovector (MoMLV)	RNA 8.3 kb	8–9 kb	1×10^8 to 1×10^9	Yes	Yes	Low	No
Lentivector (HIV)	RNA 9.2 kb	8–9 kb	1×10^8 to 1×10^9	Yes	Yes	Low	Yes
Ad vector	dsDNA 36 kb	up to 36 kb	1×10^{11} to 1×10^{13}	No	No	High	Yes
AAV vector	ssDNA 5 kb	4.5 kb	1×10^{11} to 1×10^{13}	Yes[c]	Yes	Low	Yes
Herpes vector	dsDNA 150 kb	up to 152 kb	5×10^8 to 5×10^{9}[b]	No	Possible	Medium	Yes
Poxvirus vector	dsDNA 130–300 kb	>10kb	5×10^9 to 1×10^{10}	No	No	Medium	Yes
Alphavirus vector	RNA 12 kb	4.5 kb	1×10^9 to 1×10^{10}	No	No	Low	Yes

[a] Titers are defined as colony-forming or infectious units/ml for retro-, lenti-, and alphavirus vectors; viral particles/ml for Ad vectors;viral genomes/ml for AAV vectors, and plaque-forming units/ml for herpes and poxvirus vectors.
[b] Titers of replicating HSV vectors;HSV amplicon vectors have a lower titer.
[c]AAV vectors can either stably integrate into the genome or persist as episomal elements.

diseases. The hallmark of HSV is the ability to remain latent as an episomal element in their host for life and enter the lytic stage,causing lesions at or near the site of infection after spontaneous or stress-induced reactivation. The HSV viral vector prototype is derived from HSV-1 and was first described in 1982.[10] HSV-1 has a complex double-strand DNA genome of 152 kb with at least 80 known genes. Various degrees of viral gene removal result in either replication-competent or -incompetent (HSV amplicon) vector systems.[11] Amplicon vectors contain only the minimum *cis*-acting sequences required for replication and packaging in the presence of HSV-1 helper functions provided *in trans*. The first HSV vectors to enter the clinic in 1997 were conditionally replicating vectors for the treatment of brain tumors. The technical difficulties associated with producing pure vector preparations with sufficient titers currently hamper HSV amplicon use in the clinic. Advantages of HSV amplicons include a large coding capacity, little to no immunogenicity or toxicity, and a non-integrating episomal presence with the potential for long-term expression.

Poxviruses. Poxviruses are a diverse family of pathogenic viruses infecting insects and vertebrates. The most notorious member, variola virus, causes smallpox. Some poxviruses, such as avipox, have large DNA genomes of 130–300 kb that code for more than 200 open reading frames. Poxviruses replicate exclusively in the cytoplasm, resulting in high-level, transient gene expression. A prominent member is the vaccinia virus, which has successfully been used as a smallpox vaccine. Today, poxviruses are broadly used as gene delivery vehicles in preclinical and clinical studies, and recent engineering efforts have generated systems that produce replication-defective vectors. These efforts have been fostered by occasional severe complications during smallpox eradication using vaccinia virus. One example of a poxvirus with an improved safety profile is modified vaccinia Ankara (MVA) — a highly attenuated virus originally derived from vaccinia. Avipox vectors are another safe alternative to vaccinia vectors because they only replicate in avian cells. Advantages include a relatively high titer, large coding capacity, little to no toxicity, and established large-scale production methods. The disadvantages of avipox vectors are transient expression and high immunogenicity, which makes them more suited for vaccines and immunotherapy purposes.

Alphaviruses. Mosquito-borne alphavirus infections range from seroconversion to devastating diseases, although most human infections are subclinical. Alphaviruses have a 12 kb positive-strand RNA genome that functions directly as mRNA coding for four nonstructural and four structural proteins. Nonstructural proteins mediate replication into full-length minus strands,that produce new genomic RNAs and a subgenomic mRNA encoding structural proteins. Replication takes place in the cytoplasm and leads to the characteristic transient, high-level amplification of alphavirus genomes and proteins. Alphavirus vectors have been derived from Sindbis, Semliki Forest, or

Venezuelan equine encephalitis virus (VEE) and are currently being tested in preclinical studies.[12–15] Potential advantages include high-level protein expression, broad host range, and low immunogenicity. The small capacity of 4.5 kb may be limiting, but transient protein expression followed by destruction of target cells may render these vectors particularly useful for vaccines or cancer applications.

Other vectors. Vectors derived from polyomaviruses (SV40),hepadnaviruses (HBV), orthomyxoviruses (influenza), rhabdoviruses (VSV), poxviruses (vaccinia), and coronaviruses are less developed, produce infectious virus, or are more frequently used for vaccine applications. Chimeric vector development is challenging and still at an early stage. Hybrid vectors, including Ad-retro, Ad-AAV, alphavirus-retro, alphavirus-VSV, and HSV-AAV, have been described with the hope of generating a vector that combines more desired properties than currently available systems.[16]

TRENDS AND ASPECTS OF VIRAL VECTOR DEVELOPMENT

The evolution of viral vector systems is typically determined by characteristics of the specific virus. Nevertheless, there are general trends in viral vector development that are true for most systems, involving improvements in areas such as coding capacity, safety, potency, and production.

Coding capacity. Elimination of viral sequences to accommodate heterologous genetic information was an early step in vector development. Deleting non-essential sequences while providing helper functions *in trans* increases the virus' coding capacity. The resulting "gutted" vector genome is mainly — and in some cases exclusively — dedicated to carrying therapeutic genes (Fig. 1A). Although the average cDNA is only 2 kb, a larger coding capacity is needed to deliver exceptionally large genes such as dystrophin (14 kb), genomic DNA fragments, more than one gene, and regulatory elements (promoters, poly A signals, and elements for regulation of gene expression).

Safety. One safety issue for the clinical use of viral vectors is the potential for generating replication-competent virus (RCV) that spread to neighboring cells, possibly causing disease. This scenario is particularly disastrous for HIV-based vectors, and, therefore, stringent regulations are in place to screen clinical vector lots for RCV. RCVs are generated by recombination of viral components, and efforts to reduce this risk include splitting up the viral genome, avoiding sequence overlap between vector components (and generally reducing vector sequences), and avoiding murine cell lines for MoMLV-based vector production (Fig. 1B).[17] The choice of a cell line is important because approximately one percent of mammalian genomes encode endogenous retroviral sequences facilitating homologous recombination with murine MoMLV vectors.

Another approach aimed at improving the safety of retro- and lentiviral vectors employs self-inactivating (SIN) vectors. SIN vectors are engineered with a deletion in the 3' LTR that eliminates the enhancer and promoter function. After infection and reverse transcription of the viral genome, these modifications are incorporated into both the 3' and 5' LTRs, resulting in transcriptionally inactive LTRs at both ends of the integrated vector. The integrated vector can only initiate mRNA production from the internal promoter driving the gene of interest but not from the LTRs. SIN vectors may, therefore, reduce the chance of insertional activation of potentially harmful genes such as proto-oncogenes as well as RCV production.

Another safety concern is viral vector toxicity, caused by the vector component itself and/or various contaminants of vector preparations. Viral vector toxicity increases with dose, although most vectors show few (if any) toxic effects at clinically relevant doses; however, high-dose Ad vector via systemic administration can result in serious toxicity.[18] Vectors with increased efficacy are necessary to avoid high vector doses, since low- to mid-level Ad doses are not plagued by these problems.

Efficacy. Route of administration, pseudotyping, cell-specific targeting, titer, regulated gene expression, and pre-existing anti-vector antibodies all influence *in vivo* efficacy of viral vectors. Delivering vectors to specific tissues should increase efficacy by increasing the vector concentration at the target site. The most straightforward approach is direct injection of vector in or near the affected target organ. Depending on the application, this strategy can work well; for example, intracoronary delivery of Ad-FGF4 to treat ischemic heart disease. Cell-specific homing is more complex and requires knowledge of the virus' receptor. Initially, the host range of retroviruses was redesigned through the use of heterologous viral envelopes with different tropism — a process called "pseudotyping." More sophisticated targeting approaches involve modifying the viral envelope itself. This strategy has proven difficult for retro- and lentiviral vectors, but the envelope proteins of Ad vectors are more amenable to reengineering.[19] Although promising, targeted Ad vectors still need further development and evaluation in preclinical studies before becoming a reality in the clinic. Successful targeting *in vitro* has not always translated into targeting *in vivo*. Improving the amount of vector delivered is a very important component for in vivo efficacy and increasing production titers (number of viral particles per ml) has received much attention. Significant steps were taken to improve titers of stable retroviral producer lines from the originally reported 1 x 10^3 to 1 x 10^8 cfu/ml.[17]

Life-long expression of the therapeutic gene is the goal for genetic disease treatments, although promoter silencing, particularly of retroviral vectors, has made this difficult. Recent advances in the understanding of promoter silencing may help researchers avoid repeat administration and thus increase vector potency. Also, fine-tuning of expression levels by regulatable systems may not only contribute to efficacy, but

may be necessary for diseases where only a narrow range of therapeutic protein levels can be tolerated. Last, the efficiency of viral vector therapy can be diminished by preexisting anti-vector antibodies. This has been thought to be a particular problem for the highly immunogenic Ad vectors, because more than half of the U.S. population already has anti-Ad antibodies from natural infections. However, some studies have shown that the neutralizing capacity of the population is much less frequent. Generating Ad vectors from different serotypes may circumvent that this issue for initial dosing. The problem remains for repeat administration circumstances and may be dependent on route of administration.

Production. Manufacturing large amounts of clinical-grade vector material has been facilitated by developing stable vector producer lines — a good example of this is in the retroviral vector field, but most other vector systems have seen published work in this area. Evaluating producer clones (often more than 100) to select the top candidate is tedious but worthwhile. The main criteria are high titer, stability during extended cultures, and good scale-up characteristics.[20] Vector production from stable producers ensures batch-to-batch consistency and much has been learned about the importance of optimal seeding density, harvesting times, and temperature for optimal production for a number of vector systems. A variety of techniques are available to propagate attachment-dependent producer lines such as the CellCube™ bioreactor, Cell Factories™, tissue culture flasks, roller bottles, and the more sophisticated perfusion-based systems. Purification of vector particles from either vector-containing supernatants or vector-containing cells is specific to each vector system. Once purified, the vector is stored in a well-buffered formulation that may contain sugars or other supplements to stabilize vector particles. Storage conditions, vial size, and material all affect vector potency.

VIRAL VECTORS FOR GENE THERAPY AND VACCINES

Access to a variety of viral vectors, along with advanced vector technology, has not yet created the "ideal gene therapy vector."[22] An ideal vector might combine high titer, easy production, large coding capacity, regulatable long-term expression, site-specific integration, targeting, lack of immunogenicity, and the ability to infect dividing and non-dividing cells. However, due to the limitations of currently available systems, there is no single preferred gene therapy vector; rather, researchers must choose carefully from the available systems to ensure success in the clinic. Additionally, what is ideal for one indication and route of administration may not be ideal for another.

Since the advent of human gene therapy in 1990, more than 550 clinical trials have been conducted worldwide and our growing understanding of vector biology, and *in vivo* efficacy has advanced the field of human gene ther-

Table 2.

Viral Vectors	Gene Therapy trial started	Number gene therapy trials	Number of patients	Applications
Retrovector	Yes	> 200	> 1,750	Cancer > monogenic and infectious disease
Lentivector	No[a]	-	-	-
Ad vector	Yes	> 160	> 640	Cancer > monogenic and cardiovascular disease
AAV vector	Yes	> 12	> 35	Monogenic disease > cancer
Herpes vector	Yes[b]	> 3	> 20	Cancer
Poxvirus vector	Yes	> 30	> 80	Cancer > infectious disease
Alphavirus vector	No[a]	-	-	-

Source: Journal of Gene Therapy website www.wiley.co.uk/genmed

[a] Start of clinical trial planned for 2003.
[b] Replication-competent HSV vectors.

apy. Most clinical trials have addressed cancers and genetic disorders (Table 2); more recently, gene therapies have been extended to the treatment of infectious, cardiovascular, and neurodegenerative diseases. To date, approximately 2,500 patients have been treated, mostly with retroviral vectors (Table 2). Predominant use of retroviral vectors during the early years shifted to Ad vectors — in particular for cancer treatment. More recently, trials with AAV vectors have been initiated for genetic disorders due to their lack of pathogenicity and toxicity.

Recent well-publicized events drew much attention to gene therapy trials, starting with Jesse Gelsinger's death in 2000 — the first patient for whom death could be directly attributed to the gene therapy treatment, an Ad vector. Then, a report on the first gene therapy success caused reason for optimism when several children were successfully treated for a fatal form of severe combined immunodeficiency syndrome using retroviral vectors. Later, when several of these patients had developed leukemia (due to insertional mutagenesis of the vector), it was not only a disappointment but caused concerns about the safety of integrating viral vectors. Presently, it is unclear to what extent gene therapeutic interventions will be effective in patients, and the future will tell whether viral-based gene therapy will indeed become a reality.

As for vaccines, the "ideal vaccine vector"should have most of the properties listed for gene therapy vectors;however, there is no need for integration, long-term expression, or targeting. This shortlist suggests that several currently available vector systems are already well suited for vaccines. Historically, some of the first viral vaccine vectors were derived from viruses that were used whole to vaccinate against related viruses. Examples include the immunization of military recruits with wildtype Ad4 and Ad7 to prevent adenoviral-induced respiratory disease and vaccination with vaccinia to prevent smallpox. In fact, poxviruses are widely used for vaccine vector development, having the advantage of a clinically proven safety profile. One consideration for preclinical and clinical research is preexisting immunity, for example to vaccinia because of smallpox vaccination. Also, preexisting or induced antivector antibodies could potentially reduce efficacy of subsequent vaccinations through immediate clearance, although recent data suggest that may depend on the specific vector and route of administration. More recently, the focus has shifted to nonmultiplying gene delivery systems, to avoid safety concerns that may arise from vectors producing infectious progeny.

Strategies such as immunizing with proteins, inactivated virus, or attenuated virus have been successful for a range of infectious diseases such as smallpox, measles,and rabies, but these approaches have not proven feasible for an HIV vaccine because of safety concerns or lack of efficacy. Subsequently, alternative modalities have been developed, and viral vectors in particular have shown excellent efficacy in inducing both protective and therapeutic immunity in animal models. Potential advantages of virus-derived vectors over other vaccine technology include high-level production of protein antigens directly within host cells, potential adjuvant effects from the vector component, and high transduction efficiency of host cells. Currently, several viral vector-based HIV vaccines derived from alphaviruses, Ad, VSV, rabies virus, poliovirus, poxviruses, and AAV are in preclinical and clinical studies.[21]

Overall, viral vectors are very promising tools for vaccine development,and may help generate vaccines to those diseases that are difficult to immunize against. Perhaps they are not the "magic bullet" we once thought, but they still remain one of the most promising weapons in the research arsenal.

ACKNOWLEDGMENTS

I would like to thank Drs. Mehdi Gasmi (Ceregene), Silvia Perri (Chiron), and Richard Rigg (MediGene) for their in-depth knowledge and discussions of viral vector systems.

REFERENCES

1. Mulligan RC. The basic science of gene therapy. Science 1993:260;926–932.
2.Naldini L, Verma IM.Lentiviral vectors. Adv Virus Res 2000:55;599–609.
3. Johnston JC et al. Minimum requirements for efficient transduction of dividing and nondividing cells by feline immunodeficiency virus vectors. J Virol 1999:73;4991–5000.
4. Poeschla EM, Wong-Staal F, Looney DJ. Efficient transduction of nondividing human cells by feline immunodeficiency virus lentiviral vectors. Nat Med 1998:4;354–356.
5.Olsen JC. EIAV, CAEV and other lentivirus vector systems. Somat Cell Mol Genet 2001:26;131–145.
6.Graham FL, Hitt MM, Parks RJ. Structure and genetic organization of adenovirus vectors. In: The Development of Human Gene Therapy. Cold Spring Harbor, NY; Cold Spring Harbor Laboratory Press:1999. p 61–86.
7. Zabner J et al. Adenovirus-mediated gene transfer transiently corrects the chloride transport defect in nasal epithelia of patients with cystic fibrosis. Cell 1993:75;207–16.
8.Hermonat PL,Muzyczka N. Use of adeno-associated virus as a mammalian DNA cloning vector:transduction of neomycin resistance into mammalian tissue culture cells. Proc Nat Acad Sci 1984:81;6466–6470.
9. Davidson BL et al. Recombinant adeno-associated virus type 2,4, and 5 vectors: transduction of variant cell types and regions in the mammalian central nervous system. Proc Nat Acad Sci 2000:97;3428–3432.
10. Spaete RR, Frenkel N. The herpes simplex virus amplicon: a new eucaryotic defective-virus cloning-amplifying vector. Cell 1982:30;295–304.
11. Burton EA et al. Use of the herpes simplex viral genome to construct gene therapy vectors. Methods Mol Med 2003:76;1–31.
12. Dubensky TW Jr et al. Sindbis virus DNA-based expression vectors:utility for in vitro and in vivo gene transfer. J Virol 1996:70;508–619.
13.Schlesinger S. Alphavirus vectors:development and potential therapeutic applications. Expert Opin Biol Ther 2001:1;177–191.
14.Liljestrom P, and Garoff H.A new generation of animal cell expression vectors based on the Semliki Forest virus replicon. Biotechnology 1991:9;1356–1361.
15. Caley IJ et al. Humoral,mucosal, and cellular immunity in response to a human immunodeficiency virus type 1 immunogen expressed by a Venezuelan equine encephalitis virus vaccine vector. J Virol 1997:71;3031–3038.
16. Breakefield XO, Lam PY. Hybrid vector designs to control the delivery, fate and expression of transgenes. J Gene Med 2000:2;395–408.
17.Sheridan PL et al.Generation of retroviral packaging and producer cell lines for large-scale vector production and clinical application: improved safety and high titer. Mol Ther 2000:2;262–275.
18.Lozier JN et al. Toxicity of a first-generation adenoviral vector in rhesus macaques. Hum Gene Ther 2002:13;113–124.
19.Curiel DT. Strategies to adapt adenoviral vectors for targeted delivery. Ann NY Acad Sci 1999:886; 158–171.
20. Fong TC et al. The use and development of retroviral vectors to deliver cytokine genes for cancer therapy. Crit Rev Ther Drug Carr Sys 2000:17;1–60.
21. Polo JM, Dubensky TW Jr. Virus-based vectors for human vaccine applications. Drug Discov Today 2002:7;719–727.
22. <1046> Cell and Gene Therapy Products, U.S. Pharmacopoeia.