Biology of Adenovirus and Its Use as a Vector for Gene Therapy

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INTRODUCTION

Adenoviruses were first discovered half a century ago by Rowe and colleagues, who were trying to culture adenoid tissue in the laboratory (Rowe et al., 1953). Since that time, nonhuman adenoviruses have been isolated from a number of species including chimpanzees, pig, mouse, and dog, as well as other mammalian and avian species (Shenk, 1996). Although human adenoviruses cause significant levels of respiratory, ocular, and gastrointestinal disease, they have been the object of intense study over the years mainly as a model system for basic eukaryotic cellular processes such as transcription, RNA processing, DNA replication, translation, and oncogenesis. Early on, it was shown that the virus could recombine during growth in culture (Lewis et al., 1966a,b; Pierce et al., 1968; Lewis and Rowe, 1970), ultimately setting the stage for the use of the virus as a vector for gene delivery into cells, animals, and humans. Many features of adenovirus make it well suited for gene delivery, including the ability to grow recombinant viruses to high titers, a relatively high capacity for transgene insertion, and efficient transduction of both quiescent and actively dividing cells, usually without incorporation of viral DNA into the host cell genome. These characteristics, as well as the development of many methods for manipulating the viral genome, have made adenovirus a popular choice as a gene delivery vehicle. This is evidenced by the fact that adenovirus is currently being used in roughly one-quarter of all gene therapy clinical trials, making it second only to the use of retroviral vectors (Journal of Genetic Medicine Website, www.wiley.co.uk/genmed/clinical). In this review, we summarize our current understanding of the life cycle of the human adenoviruses and then discuss how the structure and function of viral genes impact the use of the virus as a gene delivery vehicle. Readers desiring a more comprehensive coverage of the biology of the virus are directed to the volumes edited by Doerfler and Böhm (2003, 2004).

VIRUS STRUCTURE

The adenovirus virion is a nonenveloped icosahedral particle about 70–90 nm in size with an outer protein shell surrounding an inner nucleoprotein core (Fig. 1). The facets of the virus capsid are composed primarily of trimers of the hexon protein, as well as a number of other minor components including protein IIIa (pIIIa), pVI, pVIII, and pIX. The capsid vertices consist of the penton base, which acts to anchor the fiber protein, the moiety responsible for primary attachment of virions to the cell surface. Adenovirus cores contain the viral DNA as well as pV, mu, and the histone-like protein pVII. The genome itself is a linear, double-stranded DNA that is approximately 36 kb long. Each end of the genome has an inverted terminal repeat (ITR) of 100–140 bp to which the terminal protein is covalently linked. Genes are encoded on both strands of the DNA in a series of overlapping transcription units (Fig. 2). Virions also contain approximately 10 copies of the adenovirus protease, a cysteine endopeptidase that cleaves many of the structural preproteins into their mature form at the final stage of viral assembly.

THE VIRAL LIFE CYCLE

Binding and entry

The 51 distinct serotypes of human adenovirus have been classified into six groups (A–F) based on sequence homology and their ability to agglutinate red blood cells (Shenk, 1996). Most studies have been carried out on adenovirus serotype 2 (Ad2) and Ad5 and, unless otherwise stated, it should be assumed that the information below refers to work done on these serotypes. For all groups, except group B adenoviruses, initial attachment of virion particles to the cell surface occurs through binding of the fiber knob to the coxsackievirus B and adeno-
virus receptor (CAR). CAR is a type I transmembrane protein in the immunoglobulin superfamily and is present in many human tissues including heart, lung, liver, and brain (reviewed by Howitt et al., 2003). CAR normally functions as a cell-to-cell adhesion molecule on the basolateral surface of epithelial cells (Honda et al., 2000). Work has suggested that excess fiber produced during infection may act to disrupt epithelial barriers by blocking CAR function, thus promoting efficient spread of virus within and between tissues (Walters et al., 2002). The CD46 molecule, a complement-regulatory protein, has been identified as a cellular receptor for group B adenoviruses (Gaggar et al., 2003; Segerman et al., 2003; Sirena et al., 2004). Group B adenoviruses have therefore received considerable attention because of their ability to transduce cells, such as hematopoietic stem cells, dendritic cells, and malignant tumor cells, which can be resistant to infection by adenovirus groups that use CAR as the primary attachment receptor (Shayakhmetov et al., 2000; Rea et al., 2001; Havenga et al., 2002). Strategies that take advantage of the various cell tropisms between adenovirus groups have been developed, including the construction of chimeric viruses carrying fiber genes from alternative serotypes (Mastrangeli et al., 1996; Balamotis et al., 2004; Slager et al., 2004).

After initial attachment to the cell surface, an exposed RGD motif on the penton base interacts with members of the αi integrin family, triggering virus internalization by clathrin-dependent, receptor-mediated endocytosis (Stewart et al., 1997; Meier et al., 2002). For Ad2 and Ad5, the acidic environment of the endosome induces escape of virions into the cytoplasm, although the mechanisms underlying this process are poorly understood. Once in the cytoplasm, dynein mediates trafficking of virions along microtubules toward the nucleus, where they subsequently dock with the nuclear pore complex (NPC) (Trotman et al., 2001; Kelkar et al., 2004). Disassembly of the cap-

![FIG. 1.](image1)  Schematic of the adenovirus particle, showing major components of the capsid and the core. Adapted from Shenk (1996).

![FIG. 2.](image2)  Map of the adenovirus genome and transcription units. The central, solid line represents the viral genome. Positions of the left and right ITRs, the packaging sequence (ϕ), the early transcription units (E1A, E1B, E2, E3, and E4), and the major late transcription unit (major late promoter [MLP], L1–L5) are shown. Arrows indicate the direction of transcription.
Early genes and DNA replication

The first viral transcription unit to be expressed is E1A. As with almost all adenovirus transcription units, E1A produces multiple mRNA and protein products by way of differential mRNA processing. Two E1A transcripts are produced during early infection: a 18S mRNA encoding the 289R (where R stands for amino acid residues) protein (in Ad5) and a 12S mRNA encoding the 243R protein. These proteins can immortalize primary cells in culture and, when expressed in conjunction with E1B proteins, cause tumors in rodents (reviewed by Ben-Israel and Kleinberger, 2002). Despite their transforming potential, the E1A and E1B genes, referred to collectively as the E1 region, are not associated with human cancers. During infection, the E1A proteins function to trans-activate the other adenovirus early transcription units (E1B, E2, E3, and E4) and to induce the cell to enter S phase in order to create an environment optimal for virus replication (Berk, 1986). The primary mechanism by which E1A forces quiescent cells to actively cycle is by interfering with proteins of the retinoblastoma (Rb) pathway (Harlow et al., 1986; Moran, 1993). Rb acts as a tumor suppressor by inhibiting cell cycle progression via binding to E2F, a transcriptional activator that promotes expression of genes necessary for driving the cell into S phase (Nevins, 1995). The 289R and 243R products are able to sequester Rb and release repression of E2F, allowing it to activate its target genes. E1A proteins have also been shown to modulate the activity of p107 and p130, two members of the Rb family that are also involved in regulating cell cycle progression (Parreno et al., 2001).

The E1A proteins have been shown to use a variety of mechanisms to subvert cell cycle checkpoints in addition to interfering with proteins of the Rb family. E1A can directly bind and inhibit components involved in cell cycle control such as the cyclin-dependent kinase inhibitor p21 (Chat-topadhyay et al., 2001). Furthermore, E1A can interact with a number of host factors involved in mediating chromatin structure including p400 (Fuchs et al., 2001) and the histone acetyltransferases (HATs) p300/CBP, pCAF, and TRRAP/GCN5 (Chakravarti et al., 1999; Hamamori et al., 1999; Lang and Hearing, 2003). Chromatin remodeling is thought to increase the accessibility of DNA to the transcriptional machinery, suggesting that interactions between E1A and HATs may act to promote the expression of genes necessary for induction of S phase. It has also been shown that the 289R protein can mediate expression of target genes by binding directly to cellular transcriptional machinery such as the transcription factor CBFI, a component of the Notch signaling pathway, and the mammalian mediator subunit, Sur2 (Ansieau et al., 2001; Stevens et al., 2002).

Cell cycle deregulation by E1A results in accumulation of the tumor suppressor p53. During the response to stressful stimuli, activation of the p53 pathway can result in apoptosis, preventing the survival of potentially damaged cells. In infected cells, the adenovirus E1B-55K protein acts to block p53-dependent apoptosis by directly binding p53 and inhibiting its ability to induce expression of proapoptotic genes (Sarnow et al., 1982; Ben-Israel and Kleinberger, 2002). Work has shown that the E4 region product E4orf6 cooperates with E1B-55K to carry out these functions (Querido et al., 2001a,b). Expression of E1A during infection also promotes apoptosis by sensitizing cells to the tumor necrosis factor α (TNF-α) and TRAIL (TNF-related apoptosis-inducing ligand)-mediated death receptor pathways (Shao et al., 1999; Routes et al., 2000). The second product of the E1B transcription unit, the 19K protein, is able to block downstream mediators of these pathways and inhibit programmed cell death (Perez and White, 2000; Tollefsen et al., 2001). In the case of TNF-α-mediated apoptosis, the E1B-19K protein can bind directly to the proapoptotic proteins Bak and Bax to prevent mitochondria-mediated apoptosis (Sundararajan et al., 2001). It is thought that these mechanisms for inhibiting apoptosis keep the cell alive as long as possible in order to maximize viral yields (Rao et al., 1992). In addition to its anti-apoptotic functions, the E1B-55K protein facilitates the transport of viral mRNAs to the cytoplasm during the late stages of infection (Pilder et al., 1986).

The E2 region encodes proteins necessary for replication of the viral genome: DNA polymerase, preterminal protein, and the 72-kDa single-stranded DNA-binding protein (reviewed by de Jong et al., 2003). Even though adenovirus replicates in the nucleus, it requires its own enzymatic machinery because of its chromosomal structure. It does not have telomeres, so the integrity of the DNA ends is ensured by the use of a viral protein, preterminal protein (pTP). The preterminal protein is covalently linked to the 5’ end of each genome strand and acts as a primer for the viral DNA polymerase. Initiation of polymerization begins by covalently attaching the first nucleotide of the growing chain to the pTP. Genome replication occurs via a strand displacement method mediated by the 72-kDa protein and the DNA polymerase.

Products of the viral E3 region function to subvert the host immune response and allow persistence of infected cells. The immune system has evolved a number of mechanisms for destroying virus-infected cells, including cell lysis by cytotoxic T lymphocytes and activation of receptor-mediated apoptotic pathways by chemokines. The E3-gp19K protein acts in two ways to prevent the presentation of viral antigens by the MHC class I pathway and subsequent cell lysis by cytotoxic T cells. E3-gp19K was first found to prevent translocation of MHC class I molecules to the cell surface by sequestering them in the endoplasmic reticulum (ER; Burgert et al., 1987). More recently, it has been shown that E3-gp19K can bind to TAP (transporter associated with antigen processing), an ER protein responsible for transporting cytosolic antigens into the lumen, suggesting that the E3-gp19K protein may directly interfere with the loading of peptides onto MHC class I molecules (Bennett et al., 1999). The E3-10.4K, 14.5K, and 14.7K proteins have all been shown to inhibit the induction of apoptosis by the chemokines TNF-α, Fas ligand (FasL), and TRAIL (Gooding et al., 1991; Shisler et al., 1997; Chen et al., 1998; Li et al., 1998; Benedict et al., 2001). In the case of E3-10.4K and 14.5K proteins, this inhibition occurs by inducing the clearance of chemokine receptors from the cell surface and targeting them to the lysosome for degradation, hence their designation as the receptor internalization and degradation (RID) complex. The E3-10.4K and 14.5K proteins have also been shown to block
TNF-α-induced secretion of the inflammatory mediator arachidonic acid, possibly via their induction of phospholipase A2 translocation to the cell membrane (Krajcso et al., 1996; Dimitrov et al., 1997).

The E4 transcription unit encodes a number of proteins that have been known to play a role in cell cycle control and transformation; however, the mechanisms underlying these functions have remained unclear until recently. Early studies on the E4orf1 protein of Ad9 demonstrated that it is able to induce estrogen-dependent mammary tumors in mice (Javier et al., 1991). Interestingly, Ad9 E4orf1 can bind the candidate tumor suppressor ZO (zonula occludens) protein whereas the non-oncogenic Ad5 E4orf1 cannot (Glaunsinger et al., 2001). Furthermore, the transforming ability of Ad9 E4orf1 is dependent on its ability to stimulate membrane-associated phosphatidylinositol 3-kinase (Frese et al., 2003). These functions suggest that transformation by E4orf1 of Ad9 occurs via mechanisms distinct from those employed by E1 region proteins. In Ad2 and Ad5, E4orf3 and E4orf6 encode gene products with a number of diverse functions. Both proteins have been shown to increase the ability of E1 genes to transform primary rodent cells, increase the expression of viral late genes, and inhibit genome concatemerization by cellular DNA repair enzymes (reviewed by Tauber and Dobner, 2001; Stracker et al., 2002). In the case of E4orf6, enhanced transformation is thought to occur via its ability to block p53-mediated trans-activation by inhibiting the binding of p53 to cellular transcription factors (Dobner et al., 1996). E4orf6 has also been shown to cooperate with the E1B-55k protein in targeting p53 for degradation by cullin-containing ubiquitin ligases (Querido et al., 2001a). In addition to the functions listed, the E4orf3 protein also mediates the organization of nuclear structures termed PML oncogenic domains (PODs; Carvalho et al., 1995). Although the function of these domains is not clear, they have been shown to play a role in transformation, transcription, and apoptosis in infected cells (reviewed by Maul, 1998). Interestingly, an E4orf3 point mutant unable to facilitate the rearrangement of PODs was severely defective in viral DNA replication (Evans and Hearing, 2003). The E4orf6/7 transcript is produced by fusion of orf7 and a 5′ portion of orf6. The functions of this gene product appear to complement those of the E1A proteins in activating E2F-dependent promoters. Whereas E1A sequesters Rb, releasing repression of E2F, the E4orf6/7 protein binds directly to E2F and promotes its activation of viral and cellular promoters (Hardy et al., 1989; Huang and Hearing, 1989; Schaley et al., 2000). Most products of the E4 region have antipapoptotic effects; however, the E4orf4 protein interacts with protein phosphatase 2A to stimulate p53-independent apoptosis (Shtrichman et al., 1999). It is speculated that induction of programmed cell death pathways by E4orf4 may facilitate the release of progeny virions during the late stages of infection.

Late gene expression and viral assembly

Most adenovirus late genes are expressed from five regions, L1–L5, and are transcribed from one promoter, the major late promoter (MLP). The major late transcription unit (MLTU) encodes approximately 15 to 20 different mRNAs, all of which are derived from a single pre-mRNA by differential splicing and polyadenylation. These transcripts primarily encode structural proteins of the virus and other proteins involved in virion assembly. In addition, the 100K protein, a product of the L4 region, carries out a number of functions during the late phase of infection including acting as a chaperone for hexon trimerization and mediating recruitment of ribosomes to viral mRNAs (Cepko and Sharp, 1982; Xi et al., 2004). After the onset of DNA replication, transcription from the MLP is induced to high levels, ensuring the production of adequate amounts of structural proteins for the assembly of progeny virions. Manipulation of late genes encoding the structural components of the capsid has been explored as a strategy for changing the tropism of gene therapy vectors. Alterations of hexon, penton, fiber, and pIX have been shown to successfully mediate retargeting (Wickham et al., 1995; Dmitriev et al., 1998; Vigne et al., 1999; Vellinga et al., 2004).

Although virion assembly takes place in the nucleus, hexon trimerization begins in the cytoplasm, where the 100K protein associates with hexon soon after translation (Cepko and Sharp, 1983). Hexon trimers are then translocated to the nucleus in a process involving pVI (Wodrich et al., 2003). Once there, they associate with penton and minor capsid components to form the protein shell. Early pulse-chase experiments and the analysis of temperature-sensitive mutants suggested that genome encapsidation occurs via insertion of viral DNA into these preformed capsids (Edvardsson et al., 1976; D’Halluin et al., 1978a,b). Furthermore, characterization of viruses with mutations in the L1-52/55K gene indicates that this protein is required for the encapsidation process as infection with these mutants produces only empty or partially filled capsids (Hasson et al., 1989; Gustin and Imperiale, 1998). However, an alternative model has been suggested in which genome-containing cores nucleate assembly of the protein coat. Findings that the viral IVa2 protein is required for the formation of capsids and binds to the adenovirus packaging sequence, a region on the virus chromosome required for efficient genome packaging, support this model of assembly (Zhang and Imperiale, 2000, 2003; Zhang et al., 2001). Interestingly, the IVa2 protein was shown to bind the L1-52/55K protein in infected cells, although the relevance of this interaction remains unclear (Gustin et al., 1996). The L4-33K protein also appears to play a role in virus assembly as mutants carrying complete or partial deletions of this gene are defective in capsid formation (Fessler and Young, 1999; Finnen et al., 2001).

The packaging sequence itself is a series of seven repeats (A1–A7) at the left end of the genome (Hearing et al., 1987). Although each of the repeats fits a consensus motif, they are not functionally equivalent as A1, A2, A5, and A6 have been shown to be most important for genome encapsidation (Grable and Hearing, 1990). Once assembly and DNA encapsidation have occurred, the adenovirus protease cleaves a subset of the structural proteins into their mature form to produce fully infectious virions (reviewed by Mangel et al., 2003). Cell lysis and release of progeny virions occur approximately 30 hr postinfection in a process involving the E3-11.6K protein, also called the adenovirus death protein (ADP; Tollefson et al., 1996a). Unlike other products of the E3 region, ADP is produced only during the late phase of infection and is transcribed from the MLP rather than the E3 promoter (Tollefson et al., 1996b).
USE OF ADENOVIRUS VECTORS

For any gene therapy vector to attain utility in patients it must deliver genes to the intended target and provide expression for an appropriate length of time to achieve a therapeutic effect. Ideally, a vector should be administered systemically and infect only tissues in which gene delivery is desired, thus limiting toxicity to surrounding tissues and organs. Targeting adenovirus vectors to specific tissues has proven to be problematic, however, because of high-level expression of adenovirus receptors on many cell types. Many strategies have been developed that show promise in altering the tropism of adenovirus vectors (Curiel, 1999; Wickham, 2000). Much progress has also been made in achieving prolonged, robust expression of transgenes after vector administration. Improvements along these lines have been obtained mostly through deletion of multiple adenovirus genes, thereby reducing vector replication and the subsequent immune destruction of transduced cells. The construction and biology of these vectors are discussed below. A schematic showing the genome structure of commonly used vectors is presented in Fig. 3.

First-generation vectors

As described above, genes in the E1 region are necessary for activation of viral promoters and expression of both early and late genes. Thus, removal of the E1 coding sequence results in viruses that are severely impaired in their ability to replicate. Furthermore, the E1 region encodes the oncogenic transforming functions of the virus. For these reasons, replacement of the E1 region with transgenes was the initial strategy used in the construction of adenovirus vectors, giving rise to the so-called first-generation vectors. The ability to delete the E1 region alone allows approximately 5.1 kb for insertion of therapeutic genes because adenovirus can package up to 38 kb without affecting viral titer and growth rate (Bett et al., 1993).

Many of the first-generation vectors also contain a deletion in the E3 region, mainly for practical reasons. To optimize the yield of vectors in early experiments using overlap recombination, investigators used the Ad type 5 mutant dl309 or its derivatives, which contain in the E1 region two unique restriction sites due to partial deletion of E3 (Jones and Shenk, 1978). Thus, the likelihood of regeneration of the starting wild-type virus, which could arise as a result either of incomplete restriction digestion or religation of viral DNA in the cell, was minimized. Furthermore, E3 genes are entirely dispensable for virus growth in vitro and their removal, together with deletion of E1 genes, allows up to 8.2 kb for transgene insertion. Data have suggested that expression of E3 genes from vectors may be beneficial in vivo because of their ability to dampen many host immune processes. It has been reported that expression of the entire E3 region or the E3-gp19K product alone can increase persistence of transgene expression in some rodent models (Bruder et al., 1997; Ilan et al., 1997). However, conflicting data have shown that expression of the E3-gp19K protein has no effect on the length of transgene expression (Schowalter et al., 1997). These discrepancies may be due in part to differences in the nature of the transgene or the tissue type that was analyzed. Nevertheless, the inclusion of E3 genes in vectors remains an area of active investigation.

Although first-generation vectors have proven to be highly promising as vehicles for gene delivery, problems do exist. The first drawback associated with these vectors becomes apparent during vector production. Recombination between the E1 region sequences in the complementing cell line and the recombinant virus can give rise to viral progeny with functional E1 genes that are replication competent (Lochmuller et al., 1994). Thus, recombinant virus stocks must be assayed for the presence and expression of E1 genes before use. Moreover, E1-deleted packages can only be made in cell lines that provide these functions in trans. Although a number of cell lines that express E1 proteins have been developed, the most widely used is the 293 cell line, a human embryonic kidney-derived line that has been transformed by the adenovirus E1 region (Graham et al., 1977). Production of E1-deleted vectors was initially carried out by homologous recombination in mammalian cells between constructs carrying the left and right ends of the genome (Chinnadurai et al., 1979). However, this method proved to be inefficient and has prompted the development of techniques relying on standard cloning in bacteria and subsequent transfection of recombinant chromosomes into mammalian cells for virus production (reviewed by Danthine and Imperiale, 2000). Removal of the E1 region alone allows approximately 5.1 kb for insertion of therapeutic genes because adenovirus can package up to 38 kb without affecting viral titer and growth rate (Bett et al., 1993).

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ence of replication-competent viruses. Helper cell lines such as PER.C6 and 911, in which the overlap between E1 sequences in the cell and those commonly present on recombinant virus chromosomes is reduced, have been constructed in order to minimize this occurrence (Fallaux et al., 1996, 1998). The second and more troublesome problem associated with the use of first-generation vectors is their stimulation of a cellular immune response, resulting in the destruction of transduced cells that are expressing therapeutic transgenes. Indeed, a number of early studies showed that administration of E1-deleted vectors to immune-competent animals results in only transient transgene expression (Yang et al., 1994a, 1995; Dai et al., 1995). It is theorized that the immune response is stimulated by low levels of replication that can occur even in the absence of the E1 genes. This idea is supported by findings that genome replication and late gene expression can occur from E1-deleted vectors in vivo (Yang et al., 1994a,b). Although stimulation of a robust immune response may preclude the use of first-generation vectors in some settings, they still remain promising for applications requiring short-term gene expression such as cancer therapy and vaccination.

Second-generation vectors

To prevent the immune response generated by low-level replication of E1-deleted viruses, vectors deleted for multiple genes have been created to inhibit viral gene expression more effectively. These second-generation vectors have been constructed primarily by the removal of E2 and E4 coding sequences, also providing the benefit of a larger capacity for transgene insertion. The major drawback encountered during construction of these multiply deleted viruses is the need for isolation of cell lines expressing the missing functions in trans. Although this can be a time-consuming process, vectors propagated in these cells are less likely to undergo recombination to give replication-competent viruses. In the case of E2 genes, cell lines have been produced that stably express the single-stranded DNA-binding protein, preterminal protein, the viral DNA polymerase, or a combination of the three (Schaack et al., 1995; Amalfitano et al., 1996; Gorziglia et al., 1996; Amalfitano and Chamberlain, 1997). Vectors containing deletions in these genes are incapable of genome replication, and in the case of polymerase-deficient vectors, no replication occurs even in the presence of high levels of E1A (Amalfitano et al., 1998). Furthermore, experiments in immune-competent mice demonstrate that transgene expression from vectors lacking the DNA polymerase gene was sustained much longer than from vectors without the deletion (Hu et al., 1999). Results from experiments in which all or part of the E4 region has been deleted are less clear. As described above, the E4 region encodes products involved in many aspects of viral replication. It was thus theorized that removal of all or part of the E4 transcription unit would impair viral replication and gene expression such that an immune response would not be triggered. Rodent models have suggested that the deletion of some or all E4 proteins may affect the length and level of transgene expression; however, this regulation appears to be both tissue and promoter specific (Armentano et al., 1997; Brough et al., 1997; Dedieu et al., 1997; Wang et al., 1997; Lusky et al., 1998; Grave et al., 2000).

Helper-dependent vectors

The approach that holds perhaps the most promise for long-term gene expression in the absence of complicating effects due to the presence of viral genes is that of gutted, or helper-dependent, adenovirus vectors (Clemens et al., 1996; Chen et al., 1997). In this strategy, all of the viral structural genes are deleted from the viral chromosome, leaving just the two ITRs and the packaging signal. Such a chromosome can accommodate up to 37 kb of transgene sequences. To propagate the helper-dependent genome, the presence of a helper virus that provides the functions required for replication and assembly is required, as production of a complementing cell line has not been possible because of the need for high levels of some virion components and the toxicity of some of these proteins to the cell. The main problem to date is the inability to completely separate virions containing the helper-dependent chromosome from those containing the helper virus genome (Steinwaelder et al., 1999; Sandig et al., 2000). Early strategies that were pursued to reduce helper virus contamination included the use of a helper virus carrying a mutated packaging signal, and minimizing the size of the helper-dependent chromosome compared with that of the helper virus with the hope that the two types of virions could be separated on the basis of their different densities. Even using these techniques, however, helper-dependent virus preparations contained significant levels of contaminating helper virus. More recently, helper viruses in which the packaging sequence is flanked by loxP or frt sites have been constructed (Parks et al., 1996; Umana et al., 2001). When these viruses are used to propagate helper-dependent vectors in cells expressing Cre and Flp, respectively, the packaging sequence on the helper virus is excised, resulting in a significantly lower percentage of contaminating helper virus. Indeed, by deriving improved helper cell lines and culture conditions, helper virus levels can be reduced to below 0.01% (Palmer and Ng, 2003). However, recombination between helper-dependent and helper chromosomes, leading to helper chromosomes that can be packaged, is still encountered during virus propagation. A novel method using baculovirus to provide helper functions was reported to allow for production of helper-dependent vectors without contamination by helper virions, although attempts to use this process for large-scale preparations also resulted in the formation of replication-competent viruses (Cheshenko et al., 2001). Nevertheless, in vivo studies using helper-dependent vectors have produced promising results (DelloRusso et al., 2002; Ehrhardt and Kay, 2002; Mian et al., 2004; Muruve et al., 2004; Pastore et al., 2004).

Oncolytic vectors

Gene correction strategies require that vectors deliver therapeutic genes to target tissues and allow for the persistence of transduced cells. Adenovirus vectors that aim to selectively kill target tissues, termed oncolytic or conditionally replicating adenoviruses (CRAds), have been developed for the treatment of cancer. Malignant cells often have mutations in tumor suppressor genes required for regulating cell cycle progression, such as the p53 and Rb1 genes. Selective replication of oncolytic adenoviruses lies in their ability to replicate only in cells in which these cell cycle checkpoints have been disrupted. As
described above, the E1B-55K protein normally inhibits the ability of p53 to induce apoptosis in infected cells. Thus, it was posited that a vector deleted for the E1B-55K gene should be able to replicate in and lyse only a cell in which p53 function has been lost (Bischoff et al., 1996). Indeed, vectors deleted for the E1B-55K gene have been tested in animal models and clinical trials and show promise in selectively destroying malignant tissues (Heise et al., 1997; McCormick, 2003). Interestingly, the dependence of these viruses on mutations in p53 to replicate is unclear as they seem able to replicate in tumor cells regardless of p53 status, all the while sparing normal cells from lysis (Goodrum and Ornelles, 1998; Hall et al., 1998; Rothmann et al., 1998; Harada and Berk, 1999). Cancer cells lacking Rb function have been targeted in a similar way, using viruses in which the Rb-binding domains, but not the domains necessary for promoter activation, have been deleted from the E1A proteins (Fueyo et al., 2000). An alternative approach for achieving conditional replication is to place the E1A and/or E2 and E4 transcription units under the control of a promoter active only in malignant tissues. One example of this strategy is placement of E1A under the control of the human telomerase reverse transcriptase (hTERT) promoter, a promoter active in many cancer cells but not in normal tissues (Zou et al., 2004). Most recently, oncolytic adenoviruses carrying “suicide genes” have been used, thereby increasing the toxicity of vectors to target tissues (Hawkins and Hermiston, 2001; Hawkins et al., 2001).

THE IMMUNE RESPONSE TO VECTORS

After systemic administration of adenovirus, a large proportion of vector is sequestered by the liver. Subsequent uptake by resident macrophages results in the rapid release of large quantities of inflammatory cytokines (Muruve et al., 1999). Thus, in addition to the adaptive immune response brought about by low-level expression of viral genes, a substantial innate immune response is triggered on virus administration. Activation of the innate immune system is stimulated by the virus particle and therefore is not dependent on transcription from viral DNA. This response can cause inflammation of both target and surrounding tissues, resulting in considerable loss of transduced cells, and can also lead to severe systemic toxicities (reviewed by Liu and Muruve, 2003). Furthermore, high titers of antibodies against capsid proteins, either preexisting because of previous exposure to natural virus or generated as a result of vector administration, may inhibit subsequent dosing with the same vector. A number of strategies are being developed that aim to avoid antibody-mediated vector neutralization in order to allow for repeated dosing. These include the use of vectors derived from serotypes that do not cross-react, the use of adenoviruses from other species (e.g., chimpanzee), and coadministration of vectors with immunosuppressive drugs (Mastrangeli et al., 1996; Mack et al., 1997; Fitzgerald et al., 2003; Reyes-Sandoval et al., 2004). Another strategy that has also shown promise is to coat vectors with inert chemicals such as polyethylene glycol (Croyle et al., 2001, 2002). In addition to problems associated with the production of neutralizing antibodies, loss of transgene expression can occur via the actions of antiviral cytokines. The mechanisms by which transcription is turned off are not well understood, although there is evidence that cytokines can interfere with the function of some commonly used promoters (Qin et al., 1997).

SUMMARY AND FUTURE DIRECTIONS

In the early days of gene therapy, adenovirus vectors were looked on with great excitement as potential tools for almost any therapeutic application. The potency of the immune response to first-generation vectors, in retrospect, might have been anticipated because it was known that the requirement of the E1 region for replication was not absolute (Gaynor and Berk, 1983; Imperiale et al., 1984). Nevertheless, the utility of adenovirus for future gene correction strategies may be bounded if advances in the development of helper-dependent vectors continue at their present rate, resulting in increased removal of helper virus contamination. Similarly, vectors carrying mutations in E2 genes show great promise. In the meantime, adenovirus is arguably the vector of choice for treatment of localized cancer, as the results of ongoing clinical trials are encouraging. Additional modifications to the tropism of the virus may be required, however, before adenovirus will be useful for systemic treatment of metastatic disease. Perhaps the greatest obstacle to both systemic delivery and long-term gene correction will be the innate immune response, to which even helper-dependent vectors are susceptible. Although the previous 50 years have provided considerable knowledge of adenovirus biology, we clearly need to continue our study of this intriguing virus and how it interfaces with the host in order to make it a more generally useful tool for gene therapy.

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ADENOVIRUS IN GENE THERAPY


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