Chapter 8
Adenovirus-Based Vectors for the Development of Prophylactic and Therapeutic Vaccines

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Abstract Emerging and reemerging infectious diseases as well as cancer pose great global health impacts on the society. Vaccines have emerged as effective treatments to prevent or reduce the burdens of already developed diseases. This is achieved by means of activating various components of the immune system to generate systemic inflammatory reactions targeting infectious agents or diseased cells for control/elimination. DNA virus-based genetic vaccines gained significant attention in the past decades owing to the development of DNA manipulation technologies, which allowed engineering of recombinant viral vectors encoding sequences for foreign antigens or their immunogenic epitopes as well as various immunomodulatory molecules. Despite tremendous progress in the past 50 years, many hurdles still remain for achieving the full clinical potential of viral-vectored vaccines. This chapter will present the evolution of vaccines from "live" or "attenuated" first-generation agents to recombinant DNA and viral-vectored vaccines. Particular emphasis will be given to human adenovirus (Ad) for the

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development of prophylactic and therapeutic vaccines. Ad biological properties related to vaccine development will be highlighted along with their advantages and potential hurdles to be overcome. In particular, we will discuss (1) genetic modifications in the Ad capsid protein to reduce the intrinsic viral immunogenicity, (2) antigen capsid incorporation for effective presentation of foreign antigens to the immune system, (3) modification of the hexon and fiber capsid proteins for Ad liver de-targeting and selective retargeting to cancer cells, (4) Ad-based vaccines carrying "arming" transgenes with immunostimulatory functions as immune adjuvants, and (5) oncolytic Ad vectors as a new therapeutic approach against cancer. Finally, the combination of adenoviral vectors with other non-adenoviral vector systems, the prime/boost strategy of immunization, clinical trials involving Ad-based vaccines, and the perspectives for the field development will be discussed.

List of Abbreviations

Wt	Wild type
CTL	Cytotoxic T lymphocyte
DC(s)	Dendritic cell(s)
Ag(s)	Antigen(s)
TAA	Tumor-associated antigen(s)
HPV	Human papillomavirus
HBV	Hepatitis B virus
PEI	Preexisting immunity
Ab(s)	Antibody (antibodies)
Nab	Neutralizing antibody
AAV	Adeno-associated virus
Ad	Adenovirus
Ad5	Adenovirus serotype 5
CRAd	Conditionally replicating adenovirus
HVR	Hypervariable region
His6	A molecular tag (motif) containing 6 histidine residues
VLP	Virus-like particles
IM	Intramuscular
IV	Intravenous
IP	Intraperitoneal
SC	Subcutaneous

8.1 Introduction

Emerging and reemerging infectious diseases as well as cancer pose great global health impacts on the society. Vaccines have emerged as effective treatments to prevent or reduce the burdens of already developed diseases. This is achieved by means of activating various components of the immune system to generate systemic inflammatory reactions targeting infectious agents or diseased cells for control/

elimination. DNA virus-based genetic vaccines gained significant attention in the past decades owing to the development of DNA manipulation technologies, which allowed engineering of recombinant viral vectors encoding sequences for foreign antigens (Ags) or their immunogenic epitopes as well as various immunomodulatory molecules. Having emerged as a result of the parasite–host coevolution, viruses as vehicles for delivery and expression of Ag-encoding sequences may also function as immune adjuvants, enhancing immune responses to the transgene-expressed Ags. Despite tremendous progress in the past 50 years, many hurdles still remain for the development of virus-based vaccines and their effective clinical use.

Human adenovirus (Ad) emerged as one of the top candidate viral vectors for vaccine development owing primarily to its relatively low pathogenicity, genetic safety, and the lack of host genome integration step in its replication cycle. Other attractive features of Ad for vaccine application include its strong immune-adjuvant properties, highly efficient infection of various cell types, and vast transgene incorporation/cloning capacity. Although this chapter primarily focuses on the use of human Ad for the development of prophylactic and therapeutic vaccines, application of other viral vectors for vaccine development will also be briefly discussed. We will present vaccines first according to delivery systems/viral vector types and then with regard to their clinical applications, i.e., prophylactic or therapeutic. This will lead to detailed description of Ad as a vaccine vector, its taxonomy (serotype diversity), as well as the genomic and molecular structures. Ad biological properties related to its potential role as a vaccine vector will be highlighted along with advantages and potential hurdles for vaccine development. Along these lines, we will discuss in detail the "Ag capsid incorporation" strategy aimed at generating robust humoral Ag-specific immune responses while circumventing vector-specific preexisting immunity.

Substantial attention will be devoted to Ad vectors carrying "arming" transgenes with immunostimulatory functions as a vaccine adjuvant strategy and illustrate its use for the development of therapeutic vaccines against cancer. Retargeting of Ad vectors to specific cell surface receptors (de-targeting from natural Ad5 receptor "coxsackie/adenovirus receptor") will be discussed along with the underlying molecular technologies as a means of augmenting Ad infectivity for cancer cells and improving its gene transfer efficiency. We will also describe vaccination strategies involving combinations of Ad vectors with other (non-Ad) vaccine systems as well as the prime/boost strategy of immunization and commonly used immunocompetent animal models for immunotherapy studies and preclinical evaluation of adenoviral vaccines. Lastly, we will briefly discuss clinical trials involving Ad-based vaccines and the perspectives for the field development.

8.2 Virus-Based Recombinant Vaccines

Invention of virus-based vaccines dates back more than 200 years when Edward Jenner made a groundbreaking discovery that pus from cowpox-infected patients can elicit cross-protection of naïve subjects from much more virulent human disease smallpox (Jenner 1904). The term "vaccine" comes from the Latin word *vaccinus*, translated as "pertaining to cows," which reflects the history of the discovery. Although by the beginning of the twentieth century vaccination had already been widely used for prevention of other infectious diseases, such as diphtheria, rabies, and plague, that had nothing to do with cows, the original term remained.

Traditional concept of vaccination applies to induction of protective immunity against a given pathogen in a host achieved through intentional exposure to the natural or surrogate pathogen or Ags derived from such pathogens. The source of Ags can vary from well-defined recombinant proteins to natural proteins isolated from the pathogen or the whole pathogen. The first-generation vaccines included "live" or "attenuated" vaccines, prepared from pathogenic viruses, such as yellow fever, measles, mumps, and rubella, or bacteria, such as *Salmonella enterica enterica*, with reduced virulence. Treatment of virulent strains with temperature or chemicals allows for the generation of "inactivated" or "killed" vaccines. Those include influenza, cholera, polio, hepatitis A, and rabies vaccines as well as "toxoids," formaldehyde-inactivated toxins naturally produced by some pathogens and represented by diphtheria and tetanus vaccines. While pathogens/toxins in such vaccines are no longer infectious/toxic, i.e., biologically safe, their immunogenic properties are retained.

Advances in basic knowledge in immunology along with the development of recombinant DNA technology in the past 50 years prompted extensive efforts in vaccine development and led to second-generation vaccines, including recombinant vaccines. Recombinant DNA technology substantially expanded the repertoire of vaccine types, which may differ with regard to the form of immunological target (immunogen) and route of delivery. Recombinant vaccines comprise several distinct classes: (1) "subunit" vaccines that are typically obtained by genetic cloning and expression of immunogenic proteins or their individual subunits/domains using various expression systems, such as bacterial, yeast, or mammalian; (2) "conjugate" vaccines, a special type of "subunit" vaccines using Ags or toxoids conjugated to polysaccharides, enhancing their immunogenicity, such as *Haemophilus influenzae* type B vaccine; (3) DNA or "naked" DNA vaccines; and (4) viral-vectored vaccines. In the latter instances, foreign Ags are expressed in the host cells upon the delivery of recombinant DNA molecules coding for such Ags, such as West Nile virus experimental vaccine (Alarcon et al. 1999). In contrast to "naked" DNA vaccines, also known as "third-generation vaccines," virus-based vaccines use recombinant viral vectors as natural vehicles to deliver foreign Ag-encoding sequences. The term "vectored vaccine" is generally used for live recombinant viruses or bacteria representing a natural carrier or "vector" capable of

incorporating transgenes from a pathogen and expressing it in the host without itself causing illness. Viral vectors provide a substantially more efficient Ag delivery method, known as "transduction," as compared to "naked" DNA. Besides, naked DNA-based vaccines were found to be unable to induce strong cellular immune responses in humans. By contrast, Ags delivered in the context of viral vectors are more immunogenic than the same Ags delivered as proteins or the Ag-encoding naked DNA. This is primarily due to the ability of mammalian innate immune system to recognize viruses as danger signals through Toll-like receptors, leading to the generation/integration of innate and adaptive immune responses for a more pronounced immune efficacy against the virus.

Most virus-based vaccines contain Ag-coding sequences, replacing genes required for viral replication, and thus are replication deficient. Recently, vaccine design expanded towards replication-competent viral vectors. The advantage of replicative viruses as vectors is the dramatic (~10,000-fold) amplification of transgene and its expression because of excessive viral DNA replication. This enhances immunization efficiency and allows decreased vaccine dosing. The disadvantages include cell lysis, which typically occurs upon infection as the ultimate step in viral life cycle and may reduce the duration of transgene expression and vaccine efficacy. Furthermore, development of vaccines based on replication-competent viral vectors requires adequate animal models that should not only be immunocompetent but also fully permissive for replication of a given virus, which may not be available or cost permissive.

An important benefit of using virus-based vaccines is the ability to modulate the efficiency and cell-type specificity of an Ag-encoding transgene delivery. This can be achieved through alteration of natural tropism of viral vectors, making possible an efficient and specific transduction of specific cell population/cell type, such as cancer or Ag-presenting cells (APCs). Various strategies for transductional retargeting of viral vectors have been developed in the recent years. A large variety of viral vector systems, each with its advantages and disadvantages, have been used for vaccine development. A more detailed description of those vector systems is provided in Sect. 8.3.

Natural viral infections or viral vectors are capable of inducing strong innate as well as adaptive immune responses and establish long-term immunological memory. Preexisting immunity (PEI), particularly Ag-specific antibodies (Abs) against many human viruses typically found in patients, significantly compromises clinical efficacy of the corresponding viral vectors when used for vaccine or gene therapy applications. Many strategies have been developed to circumvent PEI to virus-based vectors, some of which will be described in other sections. Low immunogenicity of some Ags may require a more complex regimen of vaccination involving a "prime–boost" approach that may combine two different vaccine types for repeated delivery of the same Ag: a DNA vaccine followed by a viral vaccine or vice versa. Many contemporary vaccines are designed using a polyvalent approach allowing immunization against more than one Ag, serological variant (serotype) of a given pathogen, or even several different pathogens simultaneously. Optimal vaccination protocols are developed by considering various factors, such as dose, route of

administration, type of priming and boosting vectors, number of boost immunizations, and their timing.

Conventional prophylactic, i.e., preventive, vaccines against infectious diseases have been highly effective at eliminating or drastically reducing incidence of many life-threatening diseases, such as smallpox and poliomyelitis. These vaccines are administered to the host prior to an encounter with pathogenic infectious agents, such as viruses or bacteria. A single exception is the rabies vaccine, developed by Pasteur more than 100 years ago, which is administered only after exposure to the virus. However, the application of therapeutic vaccines has recently been on the rise owing to significant technological developments and comprehensive understanding of the immune system. Unlike prophylactic vaccines that achieve their disease-preventive effect by generating humoral immune responses, therapeutic vaccines often function through robust T-cell responses against key viral Ags. The contribution of humoral responses to the therapeutic efficacy of a vaccine depends on the target pathogen or disease. Therapeutic vaccines have been developed or under development for various indications, including persistent infections, such as hepatitis B and C viruses (HBV, HCV), human papillomavirus (HPV), human immunodeficiency virus (HIV), tuberculosis, malaria, as well as noninfectious chronic diseases, such as autoimmune diseases, gastric ulcers, prion-caused mad cow disease, Creutzfeldt–Jakob disease, Alzheimer's disease, and various cancers.

Immunologic control of persistent viral infections, such as HPV, HBV, and HCV causing a long-term damage to the host or the infected organ, has become the primary goal of therapeutic vaccine efforts. While HPV does not cause obvious disease, it sets the infected host at risk of developing cancers similar to HBV and HCV. While HPV, HBV, and HCV can be eliminated by the immune system after acute infection or sometimes in chronic phase, persistent HIV infection has proved more challenging. Although there are various contributing factors, the integration of HIV DNA into the genome of the virus-targeted immune T cells appears to play a determining role (Finzi et al. 1999). Unlike prophylactic vaccines where the induction of effector immune responses against pathogens is the primary requirement for vaccine efficacy, therapeutic vaccines against chronic infections and cancer are required to overcome two major obstacles to achieve efficacy: (1) induction of effector immune responses in a host that may have developed tolerance to the pathogen or cancer Ags and (2) overcoming various immune evasion mechanisms employed by the chronic infection and progressing cancer. Furthermore, therapeutic vaccines are administered to patients with suppressed immunity due to prior chemo- and/or radiation therapy treatments and in already developed disease background.

Vaccine development has gone a long way to achieving success in controlling or fully eradicating a number of fatal infectious diseases. However, despite the rapid progress in recombinant DNA technology, effective immunization against many human infectious and noninfectious diseases remains a challenge due to immune evasion achieved by the corresponding pathogens or their disabling of the immune system components crucial for vaccination mechanisms. This warrants further efforts in improving recombinant vaccines and vaccination strategies. In this

regard, viral vectors provide important advantages over recombinant DNA-based vectors.

8.3 Viral Vector Systems Used for Vaccine Development

There are two major types of vector systems for delivery of recombinant DNA (rDNA) to human tissues: nonviral and viral (Luo et al. 1999). Viral vectors provide the highest gene transfer and transgene expression efficiencies in vivo, which is the main impetus for the use of virus-based vectors in ~75 % of reported rDNA-based clinical protocols (Luo et al. 1999). A wide variety of animal viruses have been employed for the development of viral vector systems (Table 8.1). The extensive knowledge of replication, packaging, and assembly requirements for various viruses have allowed the generation of both replication-competent and replication-deficient (non-replicative) viral vectors. Furthermore, genetic manipulation of viral genomes aimed at modification of (1) viral natural tropism to achieve tissue- or cell-type specificity of viral infection and replication, (2) timing and efficiency of transgene expression, and (3) intracellular trafficking of transgene-encoded products has recently become possible. The combination of safety, specificity, and high levels of production makes viral vectors a leading choice for the expression of foreign genes in experimental and commercial applications (Levine 1987).

8.3.1 Adeno-associated Vectors

Adeno-associated virus (AAV) is a member of the *Dependovirus* genus of the *Parvoviridae* family, which includes a vast series of small viruses with a single-stranded DNA genome (Fig. 8.1). These viruses infect numerous species of mammals, including humans (Berns and Linden 1995). AAV virions are the smallest among gene therapy vectors. They have a capsid with icosahedral symmetry with a diameter of 18–25 nm, composed of only 60 proteins encoded by a single gene (the cap gene). The encapsidated AAV genome is a linear single-stranded DNA of either positive or negative polarity. A typical AAV preparation is a 50:50 mixture of virions containing DNA with positive or negative polarity (Berns 1990).

Comparative studies using lung cancer cell lines identified AAV2/1 as the most effective transducer among five adeno-associated virus serotypes: AAV2/1, AAV2/2, AAV2/4, AAV2/5, and AAV2/8 (Chen et al. 2013). AAV2 is the most commonly used serotype for transgene delivery. Although, the majority of the human population is seropositive for AAV, no significant adverse events during either pretrial efficacy studies or clinical trials involving AAV were observed (Maguire et al. 2009; Bainbridge et al. 2008). The wild-type (wt) AAV integrates into a specific region of the human chromosome 19 (between q13.3 and q13.4) upon

Table 8.1 Features of viral vectors used in gene therapy

Viral vector	Advantages	Disadvantages
Retroviral vectors	Insertion capacity for transgene <7–8 kb; stable integration into host DNA; recombinant virus titers within 10^6–10^7 pfu/ml; broad cell tropism, relatively easy manipulation of viral genome for vector engineering	Limited ability for targeting of viral infection; unable to infect nondividing cells; random integration into host genome; vector instability
Lentiviral vectors	Infect dividing and nondividing cells; stable transgene expression; transgene insertion capacity up to ~10 kb, no cytopathic effect associated with virus delivery	Can induce insertional mutagenesis; presence of regulatory (tat, rev) and accessory protein sequences in the packaging constructs
Herpesvirus vectors	Infect a wide variety of cell types, high insertion capacity (up to 50 kb); natural tropism to neuronal cells; stable viral particles allow propagation to high virus titers (10^{12} pfu/ml)	Possible toxicities; risk of recombination; no viral integration into host DNA
Poxvirus vectors	High cloning capacity allowing insertion of large DNA fragments; high transgene expression level; suited for live recombinant vaccine	Potential cytopathic effects (CPE)
Baculovirus vectors	Large insertion capacity (15 kb, up to 100 kb); very high levels of heterologous protein expression (~1 mg of protein per 1×10^6 of infected cells); production scale-up capability using high-density suspension cultures; no need for plaque purification; can be modified for transduction of mammalian cells (do not replicate but able to express the gene of interest; a very safe system)	Low cultivation temperature of insect cells (27 °C) may not be suitable for some proteins; improperly folded proteins; intracellular protein aggregates due to expression late in the infection cycle; improper glycosylation as reported for some glycoproteins
Sendai virus vectors	Capable of infecting human cell lines; low pathogenicity; powerful capacity for gene expression and a wide host range; cytoplasmic gene expression	Excessive immune responses associated with this virus administration in vivo
Epstein–Barr virus (EBV) vectors	Infects dividing and nondividing cells with preference for B cells; high transgene insertion capacity (<150 kb)	Limited access to packaging cell lines
Vaccinia virus vectors	Cytoplasmic replication mode; excellent experimental model system; broad host range; supports large insertions of foreign DNA (~25 kb)	Not suitable for large-scale, long-term expression of foreign proteins in continuous cell cultures
Adeno-associated virus (AAV) vectors	Infect dividing and nondividing cells; broad cell tropism; capability for targeted integration; low immunogenicity and pathogenicity	Limited capacity for transgene insertion (4 kb); difficulty in obtaining high titer preparations; require Ad or herpesvirus as helpers for the viral replication

(continued)

Table 8.1 (continued)

Viral vector	Advantages	Disadvantages
Adenovirus (Ad) vectors	High infectious titers (10^{12} pfu/ml); high level of transgene expression; large foreign DNA insertion capacity (7–8 kb); infects dividing and nondividing cells; safety of gene therapy applications, owing to the lack of integration in human genome	Immune response to viral proteins; lack of integration into host genome; transient gene expression

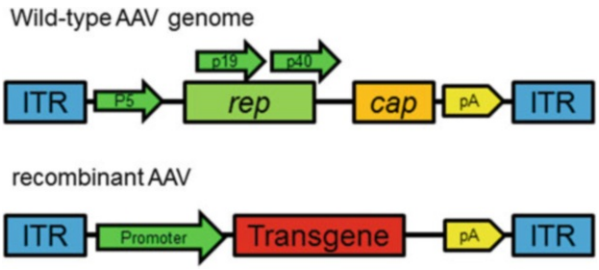

Fig. 8.1 Structure of adeno-associated virus (AAV) vectors. The wild-type AAV consists of the viral genes *rep* and *cap* coding for the different rep genes and cap (VP1, VP2, VP3) proteins, the AAV promoters (p5, p19, p40), the polyadenylation site (pA), and the inverted terminal repeats (ITR). In rAAV vectors, a transgene cassette carrying the promoter, the transgene, and the pA site are in place of the viral *rep* and *cap* genes. Figure is extensively modified from previously published work. Adapted from Walter W. and Stein U. Viral Vectors for Gene Transfer A Review of Their Use in the Treatment of Human Diseases. Drugs. 60 (2): 249-271, 2000. Adis International Limited

host cell infection (Kotin et al. 1992), whereas this ability for site-specific integration is lost in rAAV, possibly due to deletion of the viral *rep* gene. Although the integrating gene delivery systems allow for a more stable transgene expression than the episomal ones, integration of foreign DNA in the cellular chromosomal DNA is associated with the risk of insertional mutagenesis. This, in turn, may cause malignant cell transformation (Romano 2012). Therapeutic transgenes and internal promoters, regulating transgene expression in rAAV-based vectors, replace the viral *rep* and *cap* genes (Fig. 8.1) (Bartel et al. 2012). Although AAV vectors are less immunogenic than adenoviral vectors, low transduction efficiency for certain tissues, inability for targeted delivery to specific cell types, relatively low transgene-carrying capacity (~4 kb), and dependence on a helper virus for propagation limit utility of these vectors for human clinical applications.

However, several clinical trials with AAV have been performed. For example, a clinical trial in advanced cancer patients conducted in China evaluated the safety of adoptive cytotoxic T lymphocytes (CTLs) generated by the coculture with dendritic cells (DCs) transduced with rAAV encoding carcinoembryonic Ag (CEA). This study demonstrated that infusion of CEA-specific CTL was well tolerated and showed no severe adverse reactions in cancer patients (Di et al. 2012). Two phase

I clinical trials (one for rAAV2 and the other for rAAV1) and one phase II clinical trial (with rAAV1) for the alpha-1 antitrypsin gene therapy have shown promising results. However, levels of alpha-1 antitrypsin were only 3–5 % of the target range, indicating the need to increase the dose of the vector and/or gene expression levels to achieve a therapeutic range (Mueller and Flotte 2013).

8.3.2 *Adenovirus Vectors*

Ads, discovered in 1953 in human adenoid tissue (Enders et al. 1956), are non-enveloped DNA viruses carrying linear double-stranded DNA of about 35 kb in size (Fig. 8.2). Currently, over 100 types/serotypes of the *Adenoviridae* family composed of 5 genera and capable of infecting humans and a large number of different animal species are known. Human Ads belong to the *Mastadenovirus* genus with 57 characterized serotypes (Ad1–Ad57) and 7 distinct species/subgroups (A–G). They are responsible for 5–10 % of acute respiratory diseases in children and a variable number of epidemic conjunctivitis and gastroenteritis (Giacca and Zacchigna 2012). The natural tropism of human Ads for the respiratory epithelium and the conjunctiva is mainly determined by their mode of transmission rather than the molecular characteristics of the virus. Indeed, the CAR receptor-mediating cell infection by Ads is ubiquitously expressed, and most human cell types can sustain adenoviral infection and replication regardless of their proliferative state (Law and Davidson 2005). The most extensively characterized Ad types are type 2 (Ad2) and type 5 (Ad5), which are members of the C subgroup. These Ad

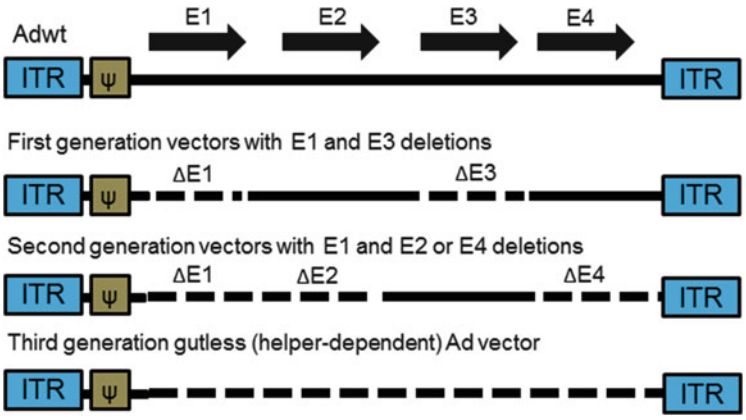

Fig. 8.2 Three generations of recombinant Ad vectors. The Ad wild type contains all early genes. The first generation lacks *E1* and *E3*; the second generation is devoid of *E1*, *E2*, or *E4*; and the third generation lacks all early genes. ITR, inverted terminal repeats; ψ, packaging signal. Figure is extensively modified from previously published work. Adapted from Walter W. and Stein U. Viral Vectors for Gene Transfer A Review of Their Use in the Treatment of Human Diseases. Drugs. 60 (2): 249-271, 2000. Adis International Limited

serotypes were used for engineering the first-generation Ad vectors since they are not associated with severe disease in humans and, therefore, suitable for in vivo applications. Another attractive property of Ads is the high efficiency with which they exploit the cellular machinery to synthesize viral mRNAs and virus-specific proteins. Given these considerations, it is not surprising that Ad vectors have become the focus of a vast series of both animal and clinical experimentations since the second half of the 1990s (Giacca and Zacchigna 2012). The attributes of Ad as well as other viral vectors have been described in detail in Table 8.1.

Three generations of replication-incompetent Ads have been described to date (Fig. 8.2). The first-generation (FG) Ads have deletion of *E1* or both *E1* and *E3* genes, which become substituted with an expression cassette typically consisting of a promoter driving a therapeutic gene and a polyadenylation signal (Danthinne and Imperiale 2000). Helper cells, which contain a genomic copy of the entire *E1* region (*E1A* plus *E1B* genes), provide in trans the *E1* proteins essential for the initiation of viral replication (Louis et al. 1997). The human embryonic kidney 293 (HEK293) cell line, stably transfected to express the viral *E1* proteins, is widely used as a helper cell line for the production of recombinant replication-deficient Ad stocks (Danthinne and Imperiale 2000). The second-generation (SG) Ad vectors were developed to lack the *E2A* and *E4* genes in addition to *E1* and *E3* genes (Fig. 8.2). The SG vectors were expected to show prolonged transgene persistence/expression due to fewer encoded Ad-specific Ags, eliciting vector-associated immune responses.

Immune responses to virus-specific genes decrease the duration of Ad-delivered therapeutic transgene expression by CTL-mediated elimination of Ad-transduced host cells (Bessis et al. 2004; Liu and Muruve 2003). The SG vector propagation depends on a helper cell line, providing in trans the missing function of the *E4* genes, which is required for Ad DNA replication and transcriptional regulation of Ad genes. The helper cell-provided functions also include transition from early to late phase of viral gene expression, viral mRNA transport, the host protein synthesis shutoff, and the assembly of the virions (Morsy and Caskey 1999). Deletion of the additional genomic regions in the SG Ad vectors did not, however, circumvent the problem of short-term transgene expression, plausibly due to the immunogenic and inflammatory potential of the residual Ad gene products in the SG vectors. Furthermore, expression of the therapeutic gene from an SG vector was reduced compared to that from a FG vector, probably due to the missing regulatory functions of some *E2* and *E4* gene products directly or indirectly upregulating expression of other virus-specific genes in the FG or the wt Ads (Giacca and Zacchigna 2012).

The third-generation of replication-deficient Ad vectors (Fig. 8.2) is characterized by the complete elimination of all viral coding regions, leaving only the inverted terminal repeats (ITRs), the transgene expression cassette, and the *psi* (ψ) packaging signal. The advantage of such vectors lies in a substantially lower immunogenicity and production of high viral titers in the 293 cell line. However, these vectors are named "gutless" or "helper dependent" as their replication depends entirely on coinfection of the packaging cell line with a helper vector

producing in trans all the required Ad proteins. These vectors are also described as "high capacity" as they can accommodate DNA sequences of up to 37 kb, thus allowing delivery of large or multiple transgenes (Alba et al. 2005; Brunetti-Pierri and Ng 2008).

Another type of Ad vectors used particularly in cancer gene therapy approaches is the conditionally replicating Ads (CRAds), which are also referred to as oncolytic Ads. CRAds infect, replicate, spread, and kill cancer cells by a natural lytic mechanism referred to as oncolysis. The general principle of these vectors is based on rendering viral replication cancer selective by mutations introduced in the Ad *E1*genes, such as a deletion of the *E1B* gene or a partial [24 base pair (bp)] deletion in the *E1A* gene. The Ad *E1B* gene product triggers proliferation of the infected cells (essential for Ad replication function) by inducing degradation of tumor suppressor p53 and the resulting block in p53-dependent apoptosis along with the activation of cell cycle signaling. The *E1B* mutation would, therefore, make an Ad unable to productively replicate in normal cells carrying a functional p53. In contrast, such a CRAd would replicate in many cancer cells where p53 signaling is disabled and the normal tumor suppression mechanism is inactive. A high percentage of tumor cells possess defective p53 and, thus, are susceptible for killing by such a CRAd (Bischoff et al. 1996), known as ONYX-015 (originally called dl1520) (Barker and Berk 1987). Another type of CRAd, carrying the abovementioned 24 bp deletion in the *E1*A pRb-binding domain, shows impaired replication in normal cells. The *E1*A pRb-binding function is required to displace the cellular transcription factor E2F from its complex with tumor suppressor pRb to induce the proliferative state of the infected cell. However, the *E1*A defect is complemented by pRb signaling defects in a number of pRb-defective cancers, making this CRAd, known as AdΔ24 (Fueyo et al. 2000) and another CRAd dl922-947 with similar *E1*A deletion (Heise et al. 2000), cancer selective.

The clinical utility of Ad-based vectors has further been improved through the generation of vectors that lose their native tropism and/or acquire cell and tissue specificity of transduction through genetic modification of viral capsid proteins. Besides targeting, Ad capsid modifications showed utility for imaging and vaccine development applications. Table 8.2 summarizes the features of Ad capsid modifications and their applications.

8.3.2.1 The "Antigen Capsid Incorporation" Strategy in Vaccine Development

Of the identified human Ad serotypes, Ad5 and Ad2 have been the most extensively used for gene therapy applications. Scientists have taken an alternative approach to conventional expression of immunization Ags in the context of viral vectors. This approach embodies genetic incorporation of Ags into viral capsids. This innovative paradigm is based on presenting Ags as components of viral capsids, achieved through genetic fusion to or incorporation in viral capsid protein(s), rather than vector-expressed transgenes. Vaccine design using Ad vectors displaying Ags on their capsid surface is known as the "Ag capsid incorporation" strategy. Whole Ags

Table 8.2 Adenovirus genetic capsid modifications and their applications in vector targeting

Capsid modification	Effect/application	References
Ad5/Ad3 serotype chimera fiber (F5/3): Ad5 fiber C-terminal "knob" domain is replaced with the Ad3 counterpart. The virus carrying such modification is referred to as Ad5/3	Enhances tumor transduction and multiple steps of Ad replication by targeting DSG2 and/or CD80/CD86; ablates CAR tropism	Stevenson et al. (1995), Krasnykh et al. (1996), Kanerva et al. (2002), Kawakami et al. (2003)
An Arg-Gly-Asp (RGD) motif incorporated into the HI loop of the fiber knob in the form of CDCRGDCFC ("RGD4C") peptide	Enhances tumor transduction via αvβ3 and/or αvβ5 integrin binding, but does not ablate CAR tropism	Dmitriev et al. (1998), Suzuki et al. (2001), Murugesan et al. (2007)
A polylysine (pK7) motif at the C-terminus of the Ad5 fiber knob alone or together with the RGD motif bearing an RGD4C peptide, incorporated into the HI loop of the Ad5 fiber knob. Vectors are referred to as Ad5pK7 and Ad5pK7/RGD, respectively	Enhanced transduction of various cell types including cancer cells through heparan sulfate proteoglycans (HSPG) or simultaneous targeting of HSPG and α_v integrins (pK7/RGD)	Wickham et al. (1997), Bouri et al. (1999), Wu et al. (2002b), Stoff-Khalili et al. (2005), Borovjagin et al. (2011)
Genetic modification of the minor capsid protein IX (pIX) by its fusion (C-terminal) to various size ligands including fluorescent (EGFP, mRFP1, mCherry) and other imaging reporter proteins such as HSV-tk or its fusions to luc and mRFP1 (HSV-tk-luc, tk-mRFP1) and metallothionein (MT)	Provides some infectivity enhancement and retargeting to various receptors (generally less efficient than corresponding fiber modifications); exposes ligand on the capsid surface and tolerates incorporation of large molecules and fusions that can be used as imaging reporters	Dmitriev et al. (2002), Vellinga et al. (2004), Le et al. (2004), Meulenbroek et al. (2004), Campos and Barry (2006), Matthews et al. (2006), Kimball et al. (2009), Borovjagin et al. (2010), Mathis et al. (2011)
Replacement of the Ad5 hexon gene with the Ad3 or Ad7 counterparts. vectors Ad5/H3 (H7)	Escape from the host neutralizing Abs against Ad5	Wu et al. (2002a), Short et al. (2010), Tian et al. (2011)
Ad5 fiber modifications replacing both the shaft and the knob domains by their counterparts from other serotypes including Ad9, Ad35, Ad41	Reduces the natural Ad5 liver tropism and in vivo (factor X-mediated) liver uptake	Shayakhmetov and Lieber (2000), Shayakhmetov et al. (2000), Nicol et al. (2004)
Incorporation of the integrin-binding peptide RGD4C at the C-terminus, in the HI loop or both locales of the Ad3 knob, in the context of F5/3 chimera fiber	Enhances cancer cell transduction, de-targets from CAR (ablated CAR tropism). Improves gene transfer to glioma	Borovjagin et al. (2005), Tyler et al. (2006)

(continued)

Table 8.2 (continued)

Capsid modification	Effect/application	References
Fiber modification by incorporation of the integrin-binding peptide RGD4C in the Ad5/35 chimera fiber knob domain	To enhance Ad5 transduction for CD46 receptor-expressing cancer cells	Murugesan et al. (2007), Matsui et al. (2011)
Ad5 fiber modification replacing its knob and part of the shaft with T4 phage-derived fibritin fused to CD40 ligand (CD40L)	To selectively target CD40-expressing cells (DC cells) for immunotherapy applications	Krasnykh et al. (2001), Belousova et al. (2003)
Fiber modification using a fiber–fibritin chimera fused to a single-chain Ab, an affibody or the *S. aureus* protein A Fc-binding domain	For targeting of Ad gene transfer to cells expressing a particular surface marker directly or via an Ab or an adapter molecule	Hedley et al. (2006a), Belousova et al. (2008), Korokhov et al. (2003)
Fiber modifications by incorporation of various size ligands in the HI loop of the Ad5 knob with or without ablation of CAR tropism	For retargeting of Ad gene transfer to cell surface markers/receptors other than CAR; to de-target from CAR	Belousova et al. (2002), Magnusson et al. (2007)
Modification of hypervariable loop 5 (HVR5) in the capsid protein hexon	To reduce/prevent Ad infection of hepatocytes; for vaccination approach	Wu et al. (2005), Vigne et al. (1999)
Modification of hypervariable loop 2 (HVR2) in the capsid protein hexon	For Ad5-based vaccine applications	Matthews et al. (2010)
Single point mutation in the hexon capsomer designed to enable defined chemical capsid modifications	To permit both de-targeting from and targeting to hepatocytes with evasion from Kupffer cell scavenging	Prill et al. (2011)

or immunogenic peptides incorporated into the viral capsid offer a potential advantage for vaccine applications. Owing to processing of the capsid-incorporated Ags through the exogenous pathways native to the Ad capsid proteins, the Ags could accrue their immunostimulatory potential. A strong humoral response against the incorporated Ags, similar to the one induced against the Ad capsid proteins, could result from the adjuvant function of the Ad vector.

The "Ag capsid incorporation" strategy has also been applied to the human rhinovirus as a vector for vaccination against HIV. A chimeric human rhinovirus HIV was shown to stimulate immunity against HIV-1 (Smith et al. 1994). In addition, combinatorial libraries of human rhinovirus capsid-incorporated HIV-1 glycoprotein 41 (gp41) epitopes were shown to induce Abs with activity that can mimic the NAb effect (Arnold et al. 2009). Preclinical and clinical development of Ad-based HIV vaccines has progressed faster than the development of other vector systems, such as human rhinovirus, owing to the tremendous flexibility of Ad vectors generally exceeding that of the rhinovirus systems. For instance, since human rhinovirus is a relatively small RNA virus, the human rhinovirus platform

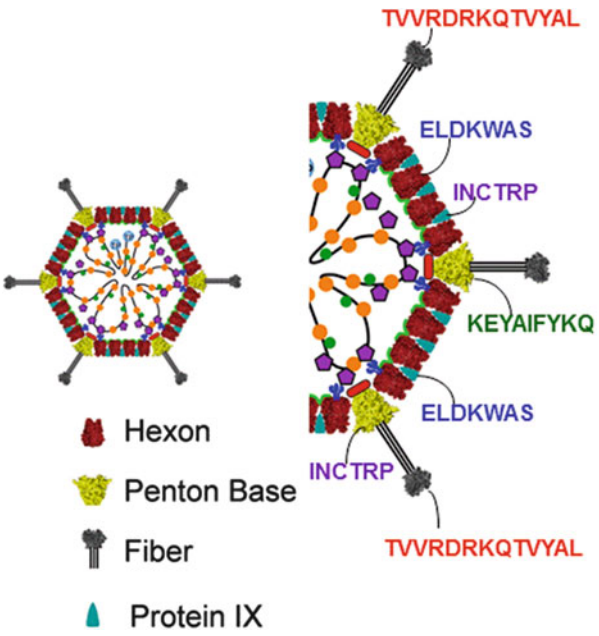

Fig. 8.3 Antigen incorporation in adenoviral structural proteins. Adenoviral capsid consists of hexon, penton base, fiber, and pIX. Antigenic epitopes can be incorporated into these capsid (structural) proteins to induce Ag-specific immune responses. For example, this figure depicts the incorporation of HIV Ags from the HIV-1 variable region 2 (TVVRDRKQTVYAL), (KEYAIFYKQ), glycoprotein 41 (ELDKWAS), and glycoprotein 120 (INCTRP). This figure is adapted from Nemerow et al. 2009. *Virology 384 (2009) 380–388,* copyright Elsevier

can only display 60 copies of a single antigenic epitope (Smith et al. 1994). In contrast, the Ad vector capsid platform could allow incorporation of HIV-1 epitopes into four distinct structural proteins/locales including hexon (Abe et al. 2009), fiber, penton base, and pIX capsid proteins (Fig. 8.3) (Matthews 2011; Nemerow et al. 2009; Matthews et al. 2013).

Although fiber (Krause et al. 2006; Shiratsuchi et al. 2010; Sharma et al. 2013), penton base (Krause et al. 2006), and pIX (Krause et al. 2006; Seregin et al. 2010a, b) have been utilized for "Ag capsid incorporation," the majority of "Ag capsid incorporation" strategies have been endeavored for the major capsid protein hexon (Fig. 8.3). Hexon is the most abundant structural protein of the Ad capsid, accounting for 63 % of its total protein mass (Rux et al. 2003; van Oostrum and Burnett 1985). Sequence analysis of hexon proteins from different Ad species revealed that, in addition to the evolutionarily conserved regions, there are also non-conserved hypervariable regions (HVRs), containing serotype-specific epitopes (Rux et al. 2003; Crawford-Miksza and Schnurr 1996). The loops at the top of the HVRs are the most pliable to modification by genetic engineering. Short heterologous peptides can be incorporated within the HVRs of the hexon without affecting the virion's stability or biological characteristics. A subset of the modifiable loops is exposed on the surface of the Ad5 capsid. HVRs 1, 2, and 5 have been utilized for

peptide or Ag incorporation (Vigne et al. 1999; Wu et al. 2005; Worgall et al. 2005, 2007; McConnell et al. 2006; Matthews et al. 2008; Palma et al. 2011).

Ad-based vaccines developed using the "Ag capsid incorporation" strategy have been tested preclinically in various disease settings (Crompton et al. 1994; Worgall et al. 2004, 2005; Krause et al. 2006; McConnell et al. 2006; Matthews et al. 2010; Matthews 2011; Gu et al. 2013). One of the first examples of "Ag capsid incorporation" used in the context of Ad was a study (Crompton et al. 1994) where an eight-amino acid sequence of the VP1 capsid protein of poliovirus type 3 was inserted into two distinct regions of the Ad2 hexon protein without affecting the propagation efficiency of one of the resulting chimeric vectors in tissue culture. Antiserum raised against the recombinant Ad vector with the polio epitope insert specifically recognized the VP1 capsid protein of polio type 3. A similar study demonstrated that His_6 epitopes could be incorporated into Ad5 hexon HVRs 1–7 (now reclassified as 1–9) without perturbing viral viability and any major biological characteristics such as replication, thermostability, or native infectivity. According to this study, His_6 appeared to be surface exposed when incorporated within HVR2 or HVR5 (Wu et al. 2005).

In an effort to create multivalent vaccine vectors using the "Ag capsid incorporation" strategy, the HVR2 and HVR5 of Ad5 have been targeted for incorporation of antigenic epitopes. To compare the flexibilities and insertion capacities, the Ad5 hexon's HVR2 and HVR5 were modified to incorporate identical epitopes of incrementally increasing sizes, ranging from 33 to 83 amino acids, within those locales. Viable vectors were produced with incorporation of 33 amino acids plus a 12-amino acid linker at HVR2 or HVR5. In addition, viable vectors were produced with incorporations of up to 53 amino acids plus a 12-amino acid linker at HVR5. The HVR5 was found to be more permissive, allowing incorporation of peptides of up to 65 amino acids. These model Ags were surface exposed as evidenced by a whole-virus ELISA analysis. In vivo immunization with these vectors demonstrated an Ag-specific immune response (Matthews et al. 2008). Although, in vivo responses were not evaluated in the context of PEI, it is possible that these vectors would indeed escape Ad5 PEI (Matthews et al. 2008).

Along these same lines, a study evaluated the ability of Ad5-based vectors expressing an HIV transgene to induce Ag-specific immune responses in the presence of Ad5 pre-immune conditions. To overcome limitations imposed by PEI to Ad5, the authors constructed vectors carrying modifications in the hexon's HVR5. This study characterized various immunological parameters associated with these vectors, such as vector neutralization, acquisition of adaptive immune response, and comparison of protective immunity. In this regard, Ad-Luc (luciferase protein expressed as a transgene in the Ad *E1* region), Ad-HisLuc (His_6 epitope presented in HVR5 region and luciferase protein expressed as a transgene), or Ad-END/AAALuc vector (containing three amino acid mutations in HVR5 and expressing luciferase protein) were administered to mice IM. The hexon-modified vector (Ad-HisLuc) generated the lowest Ad5-specific neutralizing activity, which was significantly lower than that generated by Ad-Luc at weeks 6 and 8 and by Ad-End/AAALuc vector at week 8. The individual neutralizing activity in response

to Ad-HisLuc immunization was significantly lower than that in response to immunization with Ad-Luc. Further studies support the concept that modified hexon thwarts Ad5 NAbs and promotes cellular immune responses. These findings indicate that a change in the immunogenic epitope is necessary to avoid neutralization by preexisting NAbs (Abe et al. 2009).

In a study, the first of its kind, the membrane proximal ectodomain region (MPER) of HIV gp41 protein, was incorporated in the Ad5 hexon HVR2 alone or in combination with genomic incorporation of the Gag transgene. Characteristics of the resulting vector Ad5/HVR2-MPER-L15(Gag) with respect to growth kinetics and thermostability remained unchanged as compared with peptide or Ag capsid-incorporated vectors (Matthews et al. 2006; Li et al. 2010), demonstrating that incorporation of the MPER epitope within HVR2 was not substantially detrimental to the vector's biology (Matthews et al. 2006; Li et al. 2010). This study was the first demonstration that a disease-specific Ag could be incorporated within Ad5 HVR2. Most importantly, the data demonstrated a humoral anti-HIV response in mice immunized with the hexon-modified Ad vector. Immunization with the MPER-displaying vector allows boosting, in contrast to immunization with AdCMVGag vector, possibly because the Ad5/HVR2-MPER-L15 (Gag) vector elicits less of an anti-Ad5 immune response. It is likely that the incorporation of the MPER epitope within this vector reduces the immunogenicity of the Ad5 vector. This finding is notable because HVR2 has not been fully explored for its potential use in "Ag capsid incorporation" strategies.

In a follow-up study, generation of Ad-based multivalent vectors with potential utility for vaccination against several strains of an organism or two or more distinct organisms was attempted. A multivalent vaccine based on Ad was generated that displayed Ags within hexon HVR1 and HVR2 or HVR1 and HVR5. This study focused on the generation of proof-of-concept vectors that can ultimately result in the development of multivalent vaccine vectors displaying dual Ags within the hexon of a single Ad virion/particle. These novel vectors utilize Ad5 hexon's HVR1 as an incorporation site for a seven amino acid epitope (ELDKWAS) of the HIV's gp41 MPER region (Fig. 8.3) in combination with His_6 incorporated in HVR2 or HVR5. The multivalent capsid-based vaccines incorporating HIV Ag along with His_6 within a single Ad virion/particle generated His_6 and HIV epitope-specific humoral immune responses in mice (Gu et al. 2013). This report illustrated that multivalent capsid-based vaccines are viable and immunogenic and can present different antigens within a single Ad virion/particle.

8.3.2.2 Chimeric and Rare Serotype Adenoviral Vectors for Vaccine Development

In the immediate future, it is likely that viral vector-based vaccination will become a common clinical intervention. Therefore, it has become increasingly important to design vectors that can overcome Ad5 PEI (Thacker et al. 2009; Seregin and Amalfitano 2009). Towards this end, rare and nonhuman Ad serotypes have been

genetically modified for vaccine development. Chimeric Ad vectors could consist of a subportion of the Ad5 vector genome that is replaced with genomic portions of another alternative serotype, thus creating "chimeric" Ad vectors. Alternatively, in a more extreme approach, the entire Ad vector genome could be composed of genes derived solely from alternate Ad serotypes (Noureddini and Curiel 2005; Seregin and Amalfitano 2009; Abbink et al. 2007; Youil et al. 2002; Roberts et al. 2006; Liu et al. 2009; McCoy et al. 2007). Ad hexon and fiber proteins have been manipulated genetically in chimeric strategies, primarily because these proteins are known to be the target of vector NAbs (Molinier-Frenkel et al. 2002; Roy et al. 1998; Wu et al. 2002a; Gall et al. 1996). Several chimeric fiber- and hexon-generating strategies have been employed (Table 8.2) (Seregin and Amalfitano 2009). NAbs generated against hexon HVRs account for 80–90 % of the anti-Ad NAb response, which plays a critical role in vector clearance, thereby reducing therapeutic efficacy of the vaccine vectors (Sumida et al. 2005). The importance of the HVRs as NAbs epitopes remains unclear as it relates to Ad5 and other serotypes (Yuan et al. 2009). Therefore, exact mapping of the NAb epitopes in these HVRs may be necessary to improve chimeric Ad5-based vectors (Alba et al. 2009).

One of the first studies on Ad5-based chimeric vector construction reported the replacement of Ad5 native hexon gene with the counterpart sequence of the Ad2 (Gall et al. 1998). This study was the launching point for development of other chimeric vectors. In another study, a chimeric adenoviral vector (Ad5/H3) was constructed by replacing the Ad5 hexon gene with that of the Ad3. The chimeric vector was successfully "rescued" in the HEK293 helper cell line. Ad5/H3 had a significantly slower growth profile as compared to the wt Ad5/H5 vector, indicating that the Ad3 hexon is capable of capsid incorporation and supporting encapsidation of the viral genome but with lower efficiency than the native (Ad5) hexon. The gene transfer efficiency of Ad5/H3 in HeLa cells was also lower than that of Ad5/H5. The host neutralization studies demonstrated that the NAbs against Ad5/H3 and Ad5/H5 generated in immunized C57BL/6 mice did not cross-neutralize each other in the context of in vitro infection of HeLa cells. Therefore, pre-immunization of C57BL/6 mice with one of the two types of vectors did not prevent subsequent infection with the other type, clearly demonstrating that substitution of the Ad5 hexon with the Ad3 hexon can circumvent the host neutralization of the Ad5 (Wu et al. 2002a).

More recently and along the same lines, another chimeric Ad vector, Ad3/H7, generated by replacing the Ad3 hexon gene with the hexon gene (H7) of the Ad7 was reported (Table 8.2) (Tian et al. 2011). The chimeric vectors were successfully generated in HEp-2 cells, and the Ad7 hexon-containing particles were able to encapsidate the viral genome, functioning as efficiently as the Ad3. The host vector neutralization response studies demonstrated that up to 97 % of the NAbs produced in BALB/c mice infected with both Ad7 and Ad3/H7 vectors were specific for the H7 protein in vitro. Therefore, pre-immunization of mice with one of the vectors (Ad7 or Ad3/H7) significantly prevented subsequent INL infection with the other vector in vivo. In marked contrast, pre-immunization of mice with either Ad3 or

Ad3/H7 did not prevent subsequent infection with the other vector (Tian et al. 2011).

Replacing sequences of seven HVRs in the Ad5 hexon with those of rare serotype, Ad48, resulted in a chimeric vector, Ad5HVR48 (1–7), capable of evading most of the Ad5-specific PEI in preclinical studies in mice and rhesus monkeys (Roberts et al. 2006). Ad5-based chimeric vectors in which all seven HVRs were substituted induced the same level of anti-Ag immune responses in mice with Ad5 PEI as in naïve mice. In contrast, replacing a single HVR in H5 provided only a slight enhancement of anti-Ag immune responses over those of non-chimeric Ad5 vector. Studies are underway to determine epitopes of NAbs for this vector.

Recent studies suggested that Ad5 NAb responses may be focused on one specific HVR, such as HVR1 or HVR5 (Abe et al. 2009; Crawford-Miksza and Schnurr 1996). Chimeric Ad5 vectors with subsets of H5 HVRs substituted for the Ad48 hexon HVRs were constructed and used to assess the potential of individual hexon HVRs as epitopes for Ad5 NAbs. These partial HVR-chimeric vectors were evaluated by NAb assays and immunogenicity studies with and without Ad5 PEI. Through various studies, it was demonstrated that Ad5-specific NAbs are targeted against several HVRs, indicating the need for replacing all HVRs to optimize evasion of Anti-Ad5 immunity (Bradley et al. 2012).

Liver sequestration of Ad5-based vectors is another substantial drawback that hinders systemic applications of Ad5-based therapies. Previous studies demonstrated that the human coagulation factor X (FX) binds to the Ad5 hexon through a direct interaction between its Gla domain and the hexon HVRs, leading to Ad5 liver uptake/sequestration following its systemic delivery (Waddington et al. 2008; Kalyuzhniy et al. 2008). The binding affinities for FX vary among Ad serotypes and may account for the differences in their hepatocyte transduction efficiencies previously observed in vivo. While Ad2 and Ad5 bind factor X with the highest affinity, weak or no binding was observed for Ad9, Ad35, Ad48, and Ad51. The hexon–FX interaction has been demonstrated for multiple human Ad serotypes, showing diversity in the affinity levels. The domains and amino acid sequences in the HVRs are integral for high-affinity interaction with FX; however, several aspects of this binding and its mechanism remain uncertain (Alba et al. 2009).

Utilization of vectors derived entirely from alternative human Ad serotypes (including Ad26 and Ad35) have also shown great promise, particularly in terms of ability to deliver transgenes (Abbink et al. 2007; Liu et al. 2008a, 2009; Barouch et al. 2004). Vectors based on Ads, which normally infect nonhuman species, have also shown a great promise. These nonhuman Ad vectors have been developed from numerous species, including canine, bovine, porcine, and chimpanzee (Bangari and Mittal 2006). For example, vectors have been recently developed from chimpanzee Ads C1 or C8 (AdC). Initially AdC vectors gained attention since human sera fail to significantly neutralize them (Roy et al. 2004). Notably, unlike some other serotypes, the *E1*-deleted mutant of AdC7 is easily propagated in vitro (Kobinger et al. 2006). An AdC7 vector expressing the SARS coronavirus Ag elicited higher B- and T-cell responses as compared to Ad5 vector carrying the same transgene in

mice with Ad5 PEI (Zhi et al. 2006). Additional promising results were seen with an AdC7 vector for another infectious disease. In this regard, a single injection of AdC7 encoding the *Ebola* glycoprotein provided protection from a lethal challenge, unlike the corresponding Ad5 vector (Kobinger et al. 2006).

It is important to note that several Ad epitopes recognized by T cells are conserved among a broad range of human and nonhuman primate-derived Ads. Therefore, it is possible that T cells in patients with Ad5 PEI will also recognize vectors derived from the alternate Ad species (Calcedo et al. 2009; Leen et al. 2004; Tang et al. 2006; Joshi et al. 2009). Since NAbs against bovine Ad3 (BAd3) have not been reported in humans, BAd3 have been evaluated for vaccine applications. In a mouse model, a single immunization of BAd3 encoding the HA Ag of H5N1 influenza induced greater levels of Ag-specific cellular immunity than the corresponding Ad5 vector, and this property was not compromised by Ad5 PEI (Singh et al. 2008). Importantly, mice with Ad5 PEI that received a prime–boost regimen of BAd3-Ad5 vectors encoding HA were fully protected from lethal flu virus challenge. In contrast, mice treated by a homologous Ad5-Ad5 prime–boost regimen were not protected. Consequently, Ads of nonhuman species may induce strong responses and provide immune protection comparable or superior to those of Ad5, while retaining protective potential in the presence of Ad5 PEI. The use of alternative serotype Ads thus allows for improved induction of immune responses to vector re-administration in host that have Ad5 PEI (Abbink et al. 2007; Liu et al. 2009; McCoy et al. 2007). As a result of these earlier studies, alternative serotypes vectors have been now tested in patient populations for HIV vaccine development (Barouch et al. 2011). In addition, human clinical trials evaluating Ad26 as a vaccine agent against HIV have been initiated.

In spite of the benefits associated with the use of alternative serotype Ad vectors, those vectors have several limitations including the likelihood of causing adverse effects in humans. One limitation of alternative Ad serotype usage is that some of them are unable to induce adequate levels of transgene expression in infected cells and are less amenable to large-scale purification (Thacker et al. 2009). With respect to Ad immune response, humans have evolved under continuous exposure of their populations to human Ad species, as opposed to Ad species infecting nonhuman hosts. Consequently, it is possible that the human system of innate immunity reacts to the capsid proteins of nonhuman Ad species in a different way. It is also plausible that the human immune system induces a more robust innate response when challenged with different xenotype or rare serotype Ads than upon exposure to Ad serotypes native to humans (hAd2/5). Recently it has been demonstrated that the innate immune response to capsid proteins of alternative serotype Ads has not only been substantially more robust as compared to Ad5 but in some cases can cause toxicity in animal models (Abbink et al. 2007; Appledorn et al. 2008; Hartman et al. 2008; Hensley et al. 2007). Alternative serotype vectors have intrinsically different tropism than Ad5 resulting in quite different biodistribution of these vectors in vivo. Over the last decade, Ad5-based vectors have been proven to be safe in humans and animals. The knowledge gained from such safety studies must be taken into account when testing chimeric or alternative serotype-based vectors.

8.3.2.3 Replicating Ad Vectors as Vaccines

The majority of the current Ad vaccine candidates are represented by transgene-expressing vectors, commonly engineered to express a foreign gene inserted into early (*E*) regions 1, 3, or 4 of the Ad genome (Small and Ertl 2011). Genes of the Ad5 *E1* and *E4* regions are essential for viral replication, and the majority of Ad vectors lacking those are replication deficient (Huang and Hearing 1989; Weinberg and Ketner 1986; Jones and Shenk 1978). A substantial amount of research has been performed with replication-deficient vectors in humans and animals, showing promise in several cases (Sullivan et al. 2000). Study of replicating Ad vaccines is difficult because the host/animal model system must support Ad replication if the vaccines are to be evaluated under conditions recapitulating their intended use in humans (Deal et al. 2013). Mice do not support human Ad replication. However, Syrian hamsters (Thomas et al. 2006a), cotton rats, dogs, pigs, monkeys, and chimpanzees partially support replication of some human Ad vectors. These animals, therefore, offer fully immunocompetent model systems that can be exploited to evaluate replicating vaccines (Thomas et al. 2006a; Pacini et al. 1984; Chengalvala et al. 1991; Jogler et al. 2006; Lubeck et al. 1989; Klessig and Grodzicker 1979). While cotton rats and Syrian hamsters have been extensively utilized for evaluation of replication properties of oncolytic Ad vectors (Thomas et al. 2006a; Toth et al. 2005), dogs have been typically used to characterize Ad-based vaccines (Chengalvala et al. 1991). With respect to evaluation of replicative vectors in the context of primate models, it has been difficult to assess vaccine efficacy and translate in vivo findings to the human clinical context due to the differences between primate and human immune systems.

Replication-competent Ad serotypes 7 (rAd7) and 4 (rAd4) expressing the HBV surface Ag (HBsAg) were used to prime (rAd7 HBsAg) and then boost (rAd4 HBsAg) two Ad4/Ad7-seronegative chimpanzees via an oral administration (Lubeck et al. 1989). After primary immunization, both chimpanzees shed vaccine virus for 6–7 weeks and developed Ad7-specific Abs, suggesting successful Ad7 replication in the animals. One chimpanzee developed transient seropositivity for HBsAg after prime, and both chimpanzees developed modest Ab titers after the boost immunization. A third chimpanzee primed with wt Ad7 and then boosted with rAd4HBsAg did not develop HBsAg Abs. Both rAd7/rAd4 HBsAg chimpanzees were protected from acute clinical disease, but not from infection, as evident by development of Abs against the HBV core protein in response to an HBV challenge. The animal that did not seroconvert (wtAd7/rAd4HBsAg), along with an unimmunized control, became clinically infected with HBV (Lubeck et al. 1989).

Three human participants of a phase I vaccine trial were immunized with the rAd7 HBsAg vaccine and exhibited no adverse effects. They shed the virus between days 4 and 13 post-vaccination with no evidence of person-to-person spread. Despite the fact that all the patients showed a significant increase in Ad7 Abs, none generated Abs to HBsAg (Tacket et al. 1992). Protection from the disease, if

not infection, in chimpanzees, regardless of lack of seroconversion in humans, suggests a potential value of using oral enteric vaccination with rAd to induce humoral immune responses to foreign pathogens.

8.4 Adenovirus-Based Prophylactic and Therapeutic Vaccines for Infectious Diseases

8.4.1 Prophylactic Vaccines Against Pandemic and Seasonal Influenza

Vaccines against infectious diseases that have been currently licensed for use in patients include heat-inactivated or killed whole organism vaccines, microbial extracts, purified or recombinant proteins, DNA vaccines, virus-like particles (VLPs), or recombinant viruses. While many diseases have been controlled or essentially eradicated owing to vaccination, the goal to generate community immunity for a wide variety of diseases remains elusive due to a number of problems associated with current vaccines (Zhang et al. 2011). A substantial amount of time and research efforts are directed towards determining which strains of influenza (flu) to include in the flu vaccine each year. In most years the vaccine matches quite closely to the current disease-causing strains of flu, while sometimes, despite all of the research efforts, the vaccine is not a good match and fails to provide adequate protection (Duda 2014). Since fewer flu B cases have been identified as compared to flu A cases, there are two strains of influenza A, H1N1 and H3N2, and one strain of influenza B used in the current flu vaccine. The H3N2 strain of influenza A used in the vaccine is a good match to the H3N2 strain causing the majority of flu illnesses in the United States. During the 2012/2013 flu season, there have been two illness-causing influenza B strains. One of those strains was well matched by the flu vaccine, but the other was not. Therefore, patients who were vaccinated with this season's flu vaccine are likely to be well protected, while those who happened to contract the other strain of flu B that was not part of the vaccine may not have a good protection. According to the Centers of Disease Control and Prevention, the flu vaccine has been about 61 % effective at protecting people from flu during the 2013–2014 mid-season for all age groups (CDC 2014). This number represents quite an impressive success rate for a vaccine. However, considering the emergence of new seasonal strains, the battle against flu should continue by changing the vaccine strain composition. In this regard, the development of a universal vaccine, capable of providing a broader spectrum of protection against the disease, would greatly benefit the community and is a task of highest priority.

Traditional inactivated or live attenuated vaccines are fairly effective in protecting the population against seasonal flu by targeting a viral Ag, hemagglutinin (HA). However, in case of a pandemic caused by a new strain of influenza with high mortality rate in humans, such as H5N1, it is difficult to produce adequate amounts

of an effective vaccine in a timely manner using the conventional egg-based production system because: (1) it takes at least 4 months to produce the first vaccine after the identification of a new potential strain (Emanuel and Wertheimer 2006); (2) H5N1 viruses are extremely lethal to personnel, requiring biosafety level 3 containment facilities for the vaccine production; and (3) H5N1 viruses do not replicate well in chicken embryos, resulting in low yield of the virus for vaccine development (Smith et al. 2008). In addition, the supply of eggs for vaccine production might be compromised during an H5N1 pandemic due to high mortality of the affected chickens. Overall, both inactivated and live attenuated H5N1 vaccines are only mildly immunogenic in humans, requiring high doses of Ag, multiple cycles of vaccination, and/or the use of adjuvants, further complicating the process (Adams and Sandrock 2010; Rockman and Brown 2010; Clayville 2011). Furthermore, the live attenuated influenza vaccine is only approved for healthy people of ages 2 through 49 and excludes high-risk populations. Generally, the licensed platforms for manufacturing of the existing seasonal influenza vaccines are not ideal for an H5N1 pandemic scenario as experienced in the 2009 H1N1 influenza pandemic (Haaheim et al. 2009). Therefore, it is urgent to explore alternative vaccine strategies for pandemic influenza, capable of preventing or controlling H5N1 infection in a timely manner. Numerous vaccine types with egg-independent production technologies, such as mammalian cell-based vaccines, recombinant protein-based vaccines, virus-like particle-based vaccines, DNA vaccines, bacterial-vectored vaccines, and viral-vectored vaccines, have been comprehensively studied as alternative options (Haaheim et al. 2009; Singh et al. 2010; Horimoto and Kawaoka 2009). Included in the list of possible alternative strategies are recombinant Ad vector H5N1 vaccines, which are promising candidates capable of inducing a rapid and long-term cross-protective immunity against frequently evolving H5N1 viruses (Tutykhina et al. 2011; Tang et al. 2009; Vemula and Mittal 2010; Toro and Tang 2009; Lambe 2012).

Comprehensive studies have shown that Ad vector-based vaccines induce humoral and cellular immune responses superior to those of recombinant protein vaccines, plasmid-based DNA vaccines, and other recombinant vector systems currently available (Abbink et al. 2007; Naslund et al. 2007; Barefoot et al. 2008). The natural tropism of Ad5 vectors for the respiratory tract makes them particularly desirable for intranasal (INL) vaccination against pathogens (e.g., influenza virus) that preferentially invade the mucosa. INL immunization with Ad-based vector vaccines through the use of a nasal spray could be advantageous since immunization becomes simple, practical, economical, and well suited for mass vaccination plans. In this context, Ad-based vectors are well suited for vaccine development against influenza that infects upper respiratory track. The effectiveness of Ad-based influenza vaccines has been extensively evaluated against various influenza virus subtypes with major focus on H5N1.

Immunization with Ad-based vaccines using different routes and dose regimens has been shown to have a significant effect on the type and strength of the induced immune responses (Holst et al. 2010; Kaufman et al. 2010; Suda et al. 2011; Steitz et al. 2010). For example, parenteral administration is associated with diminished

vaccine efficacy due to the presence of preexisting anti-Ad immunity. In marked contrast, vaccination via alternative routes was shown to overcome PEI against Ad5-based vectors (Van Kampen et al. 2005; Song et al. 2010; Holman et al. 2009; Appledorn et al. 2011; Xiang et al. 2003; Pandey et al. 2012). INL immunizations can overcome some effects of PEI against Ad5 vectors, inducing substantial immune response against encoded Ags and providing protection against challenge pathogens in mice, rabbits, and nonhuman primates (Xiang et al. 1996; Shi et al. 2001; Yu et al. 2008; Croyle et al. 2008; Xu et al. 2009; Richardson et al. 2011). INL and IM immunization with an Ad, expressing HA protein from A/Hong Kong/156/97 virus, has been shown to confer effective protection against lethal challenges with the heterologous (A/Hong Kong/483/97) and (A/Vietnam/1203/04) H5N1 influenza viruses in the absence of strong humoral neutralizing responses against A/Vietnam/1203/04 virus (Hoelscher et al. 2006). These studies thus demonstrate that Ad-vectored vaccines generate a robust cellular immunity against influenza that has the benefit of conferring broader protection against continuously evolving H5N1 viruses. The cellular immune response likewise plays an important role in virus clearance and promotes early recovery from infection (Thomas et al. 2006b; Lin et al. 2010).

An Ad vaccine encoding the influenza HA gene is able to stimulate cross-protective immunity between different subtypes of avian influenza virus (Wei et al. 2010; Toro et al. 2008; Shmarov et al. 2010). This suggests that Ad-based vaccination may induce secretion of Abs against conserved epitopes of the HA molecules from different strains and/or subtypes (Du et al. 2010), providing cross-protection against divergent influenza viruses. Mounting evidence suggests that Ad-based nasal influenza vaccines induce greater Ag-specific IgA and IgG responses in the respiratory tract. These vaccines could also provide more virus-specific activated T cells in the lung and better protection than IM-administered Ad vaccines (Shmarov et al. 2010; Park et al. 2009; Price et al. 2009). This is significant because mucosal immunity can potentially provide cross-protection against different strains of influenza (Hasegawa et al. 2009; Tamura et al. 2005; Ichinohe et al. 2007; Perrone et al. 2009; Lau et al. 2012; Gustin et al. 2011). More importantly, immune responses have been found in human clinical trials where human subjects could be safely and effectively immunized with Ad-vectored nasal influenza vaccines in the presence of preexisting anti-Ad5 immunity (Van Kampen et al. (2005) and data from a recent and yet unpublished Ad H5N1 nasal vaccine phase I clinical study involving 48 patients).

The HA glycoprotein is the primary flu vaccine target that stimulates higher levels of HA inhibition and neutralizing Ab (NAb) titers and stronger cellular immune responses and confers better protection against homologous or heterologous H5N1 virus challenge compared to neuraminidase-, nucleoprotein-, and matrix protein 1/2-based flu vaccines (Rao et al. 2010; Patel et al. 2009; Chen et al. 2009b; Nayak et al. 2010). Nevertheless, a monovalent HA Ad-based flu vaccine may not provide adequate protection against a broad range of heterologous strains of H5N1 influenza viruses that are currently classified into more than ten antigenically unique clades on the basis of phylogenetic analysis of their HA genes

(Continued evolution of highly pathogenic avian influenza A (H5N1): updated nomenclature 2012).

Several strategies have been undertaken to broaden the protection against pandemic flu strains, potentially pathogenic to both animals and humans, and combat the disease (Zhang 2012). These strategies include: (1) co-immunization with multivalent Ad vectors expressing HA glycoproteins or other Ags derived from different clades, (2) generation of Ad vectors expressing HA protein with NA protein and/or other highly conserved influenza Ags, (3) the use of rare Ad serotypes, and (4) different combinations of prime–boost vaccination with Ad-based H5N1 vaccines. The above strategies are not mutually exclusive and have been tested in animals to evaluate the efficacy of Ad-based vaccines against H5N1 isolates from various clades.

There has been a significant progress during the past decade in the field of Ad vector H5N1 flu vaccines. However, these vaccines must circumvent several challenges before they can be considered a suitable alternative to current licensed vaccines. One major drawback that candidate vaccines must overcome before their licensure is the lack of information related to correlation between the vaccine-induced immunity and their disease protection efficacy (Madore et al. 2010). In addition, the standardization of immune assays used in the assessment of innate and adaptive immune response is essential for the comparative analysis of such vaccines. Recently, Ad5 PEI has been thought to diminish vaccine efficacy. Emerging data from clinical trials suggest that this limitation can be overcome by increasing the vaccine dose (Catanzaro et al. 2006) or by using INL route of vaccination (Van Kampen et al. 2005). However, more clinical data is needed to clarify the influence of PEI on the Ad-vectored vaccines.

8.4.2 Prophylactic Vaccines Against Human Immunodeficiency Virus

Since the beginning of the HIV epidemic, nearly 70 million people have been infected with the virus, and approximately 35 million have died of AIDS. The estimates are that 34.0 million people were living with HIV at the end of 2011. After decades of efforts, we have seen dramatic progress in treating AIDS, but the disease has still not been eradicated (Li et al. 2013; Duerr et al. 2006). Current treatment options involve combinations (or "cocktails") of at least three different kinds of antiretroviral agents; however, the results are far from being satisfactory. Therefore, there is an urgent need to develop effective prevention and treatment options for AIDS. In this context, development of effective vaccines has been the main focus of the governments, academic institutions, and the industry worldwide.

There has been a rapid increase in preventive candidate vaccines against HIV utilizing various strategies, including DNA, protein, and viral vector-based vaccines (Gamble and Matthews 2010). Initial efforts were focused on the

development of traditional (first-generation) vaccines using inactivated or attenuated HIV virions. However, the advancements in molecular engineering and gene-based vector development have led to the emergence of viral vector-based vaccines, eliciting robust immune responses against HIV (Casimiro et al. 2003). The generation of humoral as well as cellular immunity, particularly involving CTL with long-lasting memory, is the desired features of HIV vaccines. The role and the importance of NAbs as well as CTL responses in preventing and controlling HIV infection have already been demonstrated (Sha et al. 2004). Among all viral-vectored vaccine candidates (Table 8.1), replication-defective Ad5 is the most widely used due to its proven efficacy in producing high titers of Ag-specific Abs and strong CTL responses, as well as high safety of intramuscular (IM) or subcutaneous (SC) administration (McElrath and Haynes 2010; McElrath et al. 2008).

There have been several clinical trials using Ad5 vector-based vaccines against HIV. A multiclade vaccine (MRKAd-5 HIV-1) containing clade B gag/pol/nef genes was tested in both phase I (Merck 16) (Priddy et al. 2008) and phase IIb (HVTN 502/Step) clinical trials (Buchbinder et al. 2008). In the phase I trial, the MRKAd5 trivalent vaccine was generally well tolerated and induced cell-mediated immune responses against HIV-1 peptides in most healthy adults. Another multiclade and multivalent recombinant Ad5-vaccine, VRC-HIVAD014-00VP, which contained a clade B gag-pol gene insert as well as the envelope gene inserts from three major HIV clades (A, B, and C), was tested in a phase I clinical trial (HVTN 054) (Peiperl et al. 2010). The VRC-HIVAD014-00VP vaccine was determined to be safe and highly immunogenic following a single-dose immunization in human volunteers without preexisting Ad NAbs.

While the Merck vaccine was highly immunogenic, it induced only narrow responses generating a median ≤ 1 T-cell response per participant (McElrath et al. 2008). The HVTN 502/Step phase IIb trial was terminated after an interim analysis showing that the tested vaccine neither reduced the rate of HIV-1 incidence nor plasma viremia after infection (McElrath et al. 2008; Buchbinder et al. 2008). Extensive work has been performed to elucidate potential causes of vaccine failure. Although preliminary results suggested a reverse correlation between the natural Ad5 Ab titers and the vaccine efficacy, subsequent analyses failed to support such a correlation (Hutnick et al. 2009; O'Brien et al. 2009). The results of the abovementioned vaccine trials (using two vaccines) have been extensively evaluated to determine if (1) vaccine-induced immune responses are arbitrarily distributed across vaccine inserts or clustered into immunodominant epitope hotspots; (2) the immunodominance patterns, observed in these trials, differed from each other; (3) vaccination-induced epitope hotspots overlap with those induced by a natural HIV-1 infection; (4) immunodominant hotspots correspond to the evolutionarily conserved regions of the HIV genome; and (5) epitope prediction methods can be used to identify these hotspots. It was concluded that the observed immune responses clustered into the epitope hotspots in all three vaccine trials, while some of these hotspots were not the same as in natural chronic infection. There were significant differences between the immunodominance patterns revealed in each trial. Furthermore, in some trials such differences were observed even between

different groups of participants receiving the same vaccine. Some of the vaccine-induced immunodominant hotspots were found in highly variable regions of the HIV genome. The latter was most evident for the MRKAd-5 HIV vaccine. Finally, epitope prediction methods can partially estimate the region of vaccine-induced epitope hotspots. These findings have potential implications for vaccine design and suggest a framework by which different vaccine candidates can be compared in early phases of evaluation (Hertz et al. 2013). Additional post-trial data analyses would be informative for the design of effective vaccines, in general, and Ad-based HIV vaccines, in particular.

In spite of tremendous progress, the design of effective Ad-based vaccines still has major drawbacks. In particular, PEI to Ad vectors facilitates induction of strong Ad-specific Ab and CTL responses following IM or SC vaccine administration, which result in diminished expression of transgene-encoded Ags and compromised Ag immunity. To circumvent the effects of PEI, various techniques have been employed individually or collectively. For instance, a prime–boost heterologous vaccination approach involving priming with DNA vaccine and boosting with Ad-vectored vaccine has shown a great promise (Nabel 2002; Lo et al. 2008; Park et al. 2003; Qiu and Xu 2008). The superior efficacy of heterologous prime–boost strategy over homologous prime–boost approach in eliciting immunity against a variety of pathogens and tumors has been established (Park et al. 2003; Kim et al. 2007; Schneider et al. 1998) and demonstrates the ability of heterologous prime–boost regimen to induce $CD4^+$ Th1 responses (Park et al. 2003).

8.4.3 *Adenovirus-Based Vaccines Against Potential Bioterrorism Agents*

A number of highly contagious infectious diseases with limited or no preventive or therapeutic treatments can be potentially used as weapons for bioterrorism (Boyer et al. 2005). A vaccine (AdsechPA) based on *E1*/*E3*-deleted human Ad vector has been developed to encode *B. anthracis's* protective Ag (PA) for inducing host immune defenses against the corresponding pathogen. AdsechPA was assessed for its ability to evoke anti-PA immune responses to protect mice against lethal dose of the *B. anthracis* toxin by comparison with a vaccine based on purified PA protein combined with a commonly used adjuvant Alhydrogel (rPA/Alhydrogel). AdsechPA vaccine developed protective immune response in immunized mice faster than the rPA/Alhydrogel vaccine. By 11 days, 27 % of the AdsechPA-immunized mice demonstrated protective immune responses, whereas no rPA/Alhydrogel-immunized mice survived the challenge. This study was able to demonstrate that an Ad vector encoding a secreted PA can elicit a rapid, robust protective immune response to lethal toxin, the primary mediator of anthrax pathogenesis (Tan et al. 2003).

In 2006 the "Ag capsid incorporation" strategy (see Sect. 8.3.2.1) was utilized to construct anthrax-specific Ad vaccine vectors. Vectors with chimeric hexon incorporating *B. anthracis's* PA induced formation of PA-specific Abs in mice but failed to yield protection against the anthrax toxin (lethal factor) challenge (McConnell et al. 2006). In contrast to these findings, a prior study employing an "Ag capsid incorporation" strategy demonstrated protection against *P. aeruginosa* challenge (Worgall et al. 2005, 2007). Briefly, this study described incorporation of a neutralizing epitope from the *P. aeruginosa's* outer membrane protein F (OprF) into the HVR5 of the Ad5 hexon, which increased the Ab response, consisting of both IgG1 and IgG2a subtypes in BALB/c mice (Worgall et al. 2005). Furthermore, the mice immunized with the virus containing the OprF epitope on the capsid achieved 60–80 % survival rate upon pulmonary challenge with *P. aeruginosa*.

An Ad-based vaccine has also been constructed to protect against plague-causing bacteria *Y. pestis*. One critical requirement for a vaccine against the plague is its ability to prevent the *Y. pestis's* effector proteins from entering macrophages. Because "V" Ag of the bacteria plays a key role in this process, an Ad-based vaccine vector (AdsecV) was designed to encode the Ag as a fusion with a signal sequence for its extracellular secretion. High anti-V IgG titers were induced in immunized mice 2 weeks following a single IM administration of the vaccine and continued to rise through 4 weeks post-immunization. A single IM dose of AdsecV protected mice from a lethal INL challenge with *Y. pestis*, whereas no mice were immunized with the control vector (Chiuchiolo et al. 2004). Recently the "Ag capsid incorporation" strategy was utilized to construct vaccine vectors for protection against *Y. pestis* infections. Ad vectors displaying *Y. pestis*'s V Ag or F1 capsular Ag on the capsid surface elicited a higher V- or F1-specific response, allowing boosting and better protection against a lethal challenge than that produced by vaccination with V or F1 proteins and conventional adjuvants (Boyer et al. 2010).

8.4.4 Therapeutic Vaccines Against Human Immunodeficiency Virus

To date, there has been one semi-successful HIV prophylactic trial, (RV144). The tested vaccine was comprised of four doses of recombinant canary pox priming immunogen, ALVAC-HIV (vCP1521), and two doses of AIDSVAX B/E, recombinant HIV-1 gp120 proteins from HIV-1 subtype B and circulating recombinant form 01_AE (CREF01_AE). The RV144 HIV-1 trial was the first to demonstrate evidence of protection against HIV-1 infection with an estimated vaccine efficacy of 31.2 % (Rerks-Ngarm et al. 2009). In the absence of a vaccine that can prevent HIV-1 infection, there are still many benefits to be realized from generation of a therapeutic vaccine. A therapeutic vaccine would be beneficial if it were able to increase the viral threshold titer necessary for infection, increase the time to clinical

manifestation of virus, control viral load after infection, and reduce the chance of secondary transmission (McMichael 2006; Emini and Koff 2004; Thorner and Barouch 2007; Robinson and Amara 2005; Sekaly 2008; Gamble and Matthews 2010). A therapeutic HIV vaccine could induce this type of response and would invariably decrease contagiousness, the need for costly and potentially dangerous antiretroviral treatments, and the number of opportunistic infections in patients. While the effect of controlling the normal HIV-1 pathology with therapeutic vaccines would be favorable for individual patients as well as society at large, the effect of preventing HIV-1 infections in humans with a prophylactic vaccine is also broadly appealing and of the utmost importance (Beena et al. 2013).

Therapeutic HIV vaccines are designed specifically for HIV-positive individuals, who have an uncompromised immune system that is capable of generating effective anti-HIV immune responses. Therefore, clinical trials for therapeutic vaccines are recruiting volunteers with $CD4^+$ T-cell counts greater than 250 cells/mm^3, and most studies require a $CD4^+$ T-cell count greater than 350 cells/mm^3. Patients with compromised immune system may be unable to produce a good immune response to a therapeutic HIV vaccine and are therefore not eligible for these trials. Furthermore, most of the trials require that therapeutic vaccine recipients continue taking antiretroviral drugs during the study.

While multidrug therapy has improved the prognosis for subjects infected by the virus, it has not eliminated the infection. Immunological therapies, including therapeutic vaccines, are needed to complement drug therapy in the search for a "functional cure" for HIV. DermaVir (Genetic Immunity Kit, Budapest, Hungary and McLean, Virginia, USA), an experimental HIV/AIDS therapeutic vaccine, combines three key elements of a rational therapeutic vaccine design: a single pDNA immunogen expressing 15 HIV Ags, a synthetic pDNA nanomedicine formulation, and a DC-targeting topical vaccine administration. DermaVir alone or in combination with antiretroviral drugs was evaluated in chronically SIV-infected macaques. DermaVir provided virological, immunological, and clinical benefit for SIV-infected macaques during chronic HIV infection and AIDS. In combination with antiretroviral drugs, DermaVir augmented SIV-specific T-cell responses and enhanced control of viral load rebound during treatment delays. The data also indicated the feasibility of therapeutic immunization even in immune-compromised hosts and suggested that DermaVir can supplement antiretroviral drugs to sustain suppression of HIV-1 replication (Lisziewicz et al. 2005). DermaVir's novel mechanism of action involves vaccine transportation by epidermal Langerhans cells to the lymph nodes to express the pDNA-encoded HIV Ags and induce precursor/memory T cells with high proliferation capacity. This effect has been consistently demonstrated in mouse, rabbit, primate, and human subjects. Safety, immunogenicity, and preliminary efficacy of DermaVir have been clinically observed in HIV-infected human subjects. The DermaVir technology platform for DC-based therapeutic vaccination might also offer an innovative treatment paradigm for cancer and infectious diseases (Lori 2011).

8.5 Adenovirus-Based Prophylactic and Therapeutic Vaccines for Cancer

8.5.1 Adenoviral Vectors in Cancer Therapy

Antitumor potential of human Ad was discovered soon after its isolation in 1953 (Rowe et al. 1953) by observation of tumor regression in clinical cases of cervical carcinoma following Ad inoculation (Huebner et al. 1956). However, Ad emerged as a potential therapeutic agent for cancer only decades later, after the groundbreaking developments in recombinant DNA technology. Several clinical studies using Ad vectors for cancer therapy have been conducted in the past decade. Some of those led to the clinical development of Ad vector-based products for treatment of various cancers, such as Advexin® and Gendicine® encoding wt tumor suppressor p53 under control of cytomegalovirus or Rous Sarcoma Virus promoters, respectively (Raty et al. 2008). Remarkably, in 2003 Gendicine® was approved in China as the first gene therapy medicine in the world (Toth et al. 2010). Onyx-015, an Ad2/Ad5 hybrid oncolytic virus replicating selectively in p53-defective cancers, became the first engineered replication-selective virus to be used in humans (Liu et al. 2008b). In 2005 a similar virus (H101) in combination with chemotherapy was approved by the Chinese State Food and Drug Administration for the treatment of refractory nasopharyngeal (head and neck) cancer (Liu and Kirn 2008).

The current use of human Ad as a vector for cancer therapy applications can be divided into six major categories: (1) suicide gene therapy also known as gene-directed enzyme prodrug therapy (GDEPT), (2) suppressor replacement gene therapy, (3) RNA interference (RNAi)-based gene therapy, (4) anti-angiogenic therapy, (5) oncolytic virotherapy, and (6) cancer immunotherapy. Briefly, the GDEPT approach utilizes the concept of foreign (bacterial or viral) enzyme-mediated intracellular conversion of nontoxic compounds (prodrugs), compatible with systemic administration, into cytotoxic metabolites capable of blocking DNA replication or transcription/protein synthesis in target (cancer) cells upon vector-mediated delivery of the foreign enzyme-encoding transgenes ("suicide" genes). Due to inability of cellular enzymes to use such prodrugs as substrates, the cytotoxic effect is achieved specifically in target (cancer) cells expressing the delivered therapeutic transgenes. Suppressor replacement and RNAi-based therapies represent variations of the gene therapy approach aimed at replacing mutated/inactivated tumor suppressors or disrupting tumor-promoting factors, respectively. In the former instance, a tumor suppressor gene p53 mutated in many cancer cell types is replaced by its functional (wt) counterpart, whereas in the latter instance disruption of mRNA encoding a tumor-promoting factor (hTERT, HER2, etc.) by RNAi, delivered in the context of an Ad vector, is aimed at inhibiting tumor progression. Similarly, the anti-angiogenic therapy utilizes a gene therapy approach for systemic or intratumoral delivery and expression of anti-angiogenic factors, inhibiting tumor vasculature formation and thereby growth of primary tumors and metastases (Sharma et al. 2009).

In contrast to the above gene delivery-based strategies, the oncolytic virotherapy approach utilizes natural ability of replication-competent Ad for lytic destruction of infected cells, whereby intracellularly produced viral progeny gets released from the infected cells and spread around a tumor mass in consecutive rounds of Ad replication cycle. This is achieved by cancer targeting of Ad replication process. Cancer selectivity of Ad replication is rendered by two major genetic strategies of blocking Ad DNA replication in normal (non-cancer), but not in malignant cells. Those involve engineering mutations (deletions) in DNA replication-controlling immediate early (*E1*) Ad genes to inactivate their crucial biological function of inducing proliferation of infected cells (see also Sect. 8.3.2) (Bischoff et al. 1996; Heise et al. 2000; Fueyo et al. 2000) or replacing the native promoter of *E1* genes with tumor-specific promoters (TSPs) selectively activated in cancer cells (Ko et al. 2005).

The ultimate goal of cancer immunotherapy is to elicit endogenous immune responses against developing or well-established tumors. The ineffectiveness of antitumor immune responses, caused by the establishment of immunosuppression in the tumor microenvironment during oncogenesis, indicates that the immune system needs to be modulated in a very specific way to effectively suppress tumor formation. Immunotherapy relies on preformed effector mechanisms and is well suited for the treatment of established tumors. In this context, cancer vaccines based on whole tumor cells, DCs pulsed with tumor-associated Ags (TAAs), or TAA-based subunit vaccines with immune adjuvants have recently emerged as important modalities to combat cancer. In summary, viral vector-based vaccines represent an important treatment modality owing to their high practical value, versatility in Ag delivery, robust natural adjuvanticity, and intrinsic capability for oncolysis.

8.5.2 Adenovirus-Based Cancer Vaccines

Increased understanding of the immune system and developments in recombinant DNA technology led to the emergence of innovative vaccine strategies and the notion that cancer vaccines could become a common treatment modality in the next decade. The recent licensure of DC-based vaccine Provenge® for the treatment of prostate cancer in humans along with the approval of Oncept™ as therapy for oral melanoma in dogs lends support to the clinical promise of cancer vaccines (Aurisicchio and Ciliberto 2011). Ad-based cancer vaccines represent an important and rapidly developing branch of the cancer vaccinology field. Ad vectors emerged as promising gene therapy vectors and recombinant vaccine carriers owing to their well-characterized molecular genetics, high yield propagation capacity amenable to pharmaceutical scale production, and biological characteristics (Choi and Yun 2013). Many studies have shown that Ad-based vaccines are more efficient in generating antitumor immunity than vaccines based on other delivery systems (Basak et al. 2000; Okur et al. 2011). However, PEI to human Ad2 and 5 and

their natural liver tropism substantially compromised the utility of these viruses for vaccine development. These hurdles justify implementation of various strategies for "shielding" of hAd vectors from PEI, including utilization of alternate Ad serotypes, xenotypes, or chimeric Ad vectors (Hedley et al. 2006b) described in previous Sects. 8.3.2.1 and 8.3.2.2). The "shielding" approach involves hAd capsid genetic and chemical modification strategies as well as the use of hAd vectors in conjunction with cell-based therapies (ex vivo pre-loaded DCs). Administration of hAds through mucosal surfaces, preventing rapid vector clearance by immune system, represents a powerful alternative to the vector "shielding" approach.

To bypass the problem of PEI to hAd2/5 in human populations, alternative serotypes from humans or nonhuman primates have been employed for vaccine development. Human Ad35 has been proposed as an alternative to hAd5 for vaccine delivery because of its low seroprevalence. However, hAd35-based vectors demonstrated lower immunological potency as compared to hAd5 vectors in mice and nonhuman primates. Similar results were obtained for hAd11, hAd24, and hAd34 (Barouch et al. 2004; Lemckert et al. 2005). Since PEI in humans does not cross-neutralize ovine, chimpanzee, canine, porcine, or fowl Ads (Aurisicchio and Ciliberto 2011), nonhuman Ad species, capable of efficiently transducing some types of human and murine cells in culture, gained attention as potential vectors for cancer vaccine development. Most importantly, cancer vaccines based on chimpanzee Ads, such as ChAd3, have been shown to induce immune responses comparable to hAd5 serotype-based vectors, break tolerance to self-tumor Ags, overcome hAd5 PEI, and achieve antitumor effects (Peruzzi et al. 2009).

8.5.2.1 Prophylactic Adenoviral Cancer Vaccines

Prophylactic cancer vaccines utilize immunomodulatory mechanisms, leading to the development of adaptive immune responses against TAAs. Most important of those is the generation of TAA-specific $CD8^+$ T memory cells, which undergo rapid activation and acquisition of effector function should cancer cells expressing such TAAs emerge in the host. In addition, prophylactic cancer vaccines can elicit humoral immune responses characterized by production of TAA-specific Abs. The latter may play some role in the containment of primary tumor development as well as in preventing recurrence of previously eradicated tumors. However, a pivotal role in vaccine-based cancer prevention belongs to TAA-specific CTL-based cellular immunity (Finn and Forni 2002).

Adenoviral Vaccines Targeting Tumor-Associated "Self" Antigens

Many tumor Ags are simply normal, non-mutated tissue differentiation Ags, that are seen as "self" by the host immune system. Immunization with these "self" Ags could induce autoimmunity. Tumor prevention by stimulation of TAA-specific immunity and breaking the immunologic tolerance has been evidenced in numerous

transgenic mouse models overexpressing heterologous genes for TAAs, such as rat HER2/neu (Hutchinson and Muller 2000), human CEA (Kass et al. 1999; Mizobata et al. 2000), and human carcinoma-associated mucin (MUC1) (Taylor-Papadimitriou and Finn 1997; Rowse et al. 1998). The deliberate induction of self-reactivity using a recombinant viral vector has been shown to result in tumor destruction, and $CD4^+$ T lymphocytes were found to be an integral part of this process (Overwijk et al. 1999). Thus, vaccine strategies targeting tissue differentiation ("self") Ags may be valuable in the prevention/treatment of cancers arising from differentiated cells and tissues/organs, such as melanocytes, prostate, testis, breast, and ovary.

Studies in animal models have demonstrated that cancer vaccines are most effective in protection from tumor challenge or in prevention of tumor occurrence in genetically predisposed animals. In contrast, cancer vaccines have shown limited therapeutic efficacy against established tumors in animal models, which reflected their failure to demonstrate objective antitumor responses in most human clinical trials (Finn and Forni 2002). The superior effectiveness of cancer prevention versus therapy is not surprising, given the condition of the immune system prior to vaccination: neither impaired by tumor- and chemotherapy-induced suppression nor tolerant to TAAs in the established tumor environment.

A novel recombinant vector based on rAd40 encoding mouse mesothelin (Msln) has been described as an effective prophylactic cancer vaccine against the formation of metastatic lesions of pancreatic cancer in the corresponding mouse tumor model. Intravenous (IV) administration of rAd40 resulted in Msln delivery and expression in wider range of mouse organs as compared to conventional Ad5, distributed mainly to the liver, spleen, and lungs. Besides, rAd40 showed reduced levels of liver transduction or inflammatory responses, resulting in reduced liver toxicity relative to Ad5. Msln vaccination has been reported to enhance antitumor effects against Msln-expressing tumors via Msln peptide-specific $CD8^+$ T-cell-mediated immunity (Hung et al. 2007; Miyazawa et al. 2011). In line with this finding, a one-time IV administration of rAd40-Msln not only prevented growth of the primary tumors in the Ag-specific manner but also blocked metastases formation, indicating that rAd40-Msln vaccination is a promising approach to stimulate both transgene- and tumor-specific immunity (Yamasaki et al. 2013).

Guanylyl cyclase C (GUCY2C) is an autoantigen principally expressed by intestinal epithelium and universally by primary and metastatic colorectal tumors. Immunization with Ad expressing the structurally unique GUCY2C extracellular domain [GUCY2C(ECD)] produced prophylactic and therapeutic protection against GUCY2C-expressing colon cancer metastases in mice without collateral autoimmunity (Snook et al. 2007, 2008). The mechanism of this protection involves lineage-specific induction of Ag-targeted $CD8^+$ T cells, without $CD4^+$ T cells or B cells (Snook et al. 2012).

Recombinant Ad vaccine expressing a kinase-inactive mutant form of human HER2 used for immunization of BALB/c wild type (WT) or HER2 transgenic mice protected WT mice from the HER2-expressing mouse carcinoma D2F2/E2. Half of the HER2 transgenic mice were protected fully and long-term after preventive

one-time IP vaccination, whereas tumor growth in mice that eventually developed neoplastic lesions was delayed. Protection in WT and HER2 transgenic mice was associated with high or low levels of IgG2a antibodies, respectively. This study also evidenced that CTLs were induced in WT but not in HER2 transgenic mice and defined a critical requirement for NK cells in vaccine-induced protection against HER2-expressing tumors (Triulzi et al. 2010). A similar study demonstrated that vaccination with Ad vector Ad-HER2-ki encoding a full-length HER2, mutation-inactivated (non-oncogenic) for kinase function, resulted in robust polyclonal immune responses to HER2 in tolerant animal models and translated into strong and effective antitumor responses in vivo (Hartman et al. 2010).

Ad vector encoding human mucin (MUC1), a tumor antigen expressed in breast, pancreatic, and ovarian cancers, has been developed for transduction of DCs, which specifically stimulated autologous peripheral blood lymphocytes upon vector-mediated gene transfer, substantially improved by Ad relative to liposome transfection (Pecher et al. 2001). Ad vaccines encoding other TAAs are mentioned below (see Sect. 8.5.2.2).

Vaccines Against Virally Induced Cancers

The success in vaccine development against infectious diseases has been recently translated to prophylactic vaccines against virally induced neoplasms. The US Food and Drug Administration (FDA) has approved a preventive vaccine against HBV, causing 80 % of liver cancers, and two prophylactic vaccines against HPV: Gardasil® and Cervarix®, protecting against infection by the two types of HPV (type 16 and 18) closely associated with cervical and some head and neck cancers. While both HPV vaccines were derived from VLPs, composed of a single HPV protein, the alternate genetic vaccine approaches utilizing hAd vector for the delivery and expression of HPV or HBV Ags have been recently endeavored as well. Some of those studies are briefly described below followed by other examples of most recent vaccine strategies for cancer prevention.

Cervical cancer, whose causal association with genital HPV infection has been firmly established (Steller 2002; Walboomers et al. 1999), remains a leading cause of cancer-related mortality in women. The E6 and E7 genes of high-risk HPV types (HPV-16 and -18) encode oncoproteins capable of immortalizing human keratinocytes (Pecoraro et al. 1989) by altering cell growth regulation through inactivation of p53 and Rb tumor suppressors, respectively (Munger et al. 1992; Dyson et al. 1989). Oncoproteins E6 and E7, selectively retained and expressed in cervical tumors, are attractive targets for development of cancer vaccine for prevention of cervical cancer.

A recombinant Ad vector encoding mutant HPV-16 E6/E7 cassette has been described by Wieking and colleagues (Wieking et al. 2012) as a promising vaccine against HPV-positive head and neck cancers. Those engineered mutations render full-length forms of both E6 and E7 (E6Δ/E7Δ) non-oncogenic while preserving their antigenicity. At all dosages, mice inoculated with the Ad5 E6Δ/E7Δvector

completely cleared E6/E7-expressing HPV-positive head and neck squamous cell carcinoma tumors implanted 2 weeks after either intratracheal or submucosal Ad vaccine inoculation, with significant E6/E7-specific IFN-γ production. This result suggested that immunization with HPV-16 E6/E7 oncoproteins can be an effective method of protecting a host from E6/E7-expressing head and neck squamous cell carcinoma (Lee et al. 2008; Wieking et al. 2012). A non-oncogenic HPV-16 E6/E7 vaccine enhances treatment of HPV expressing tumors.

Calreticulin (CRT), an abundant 46 kDa Ca^{2+}-binding protein localized to the endoplasmic reticulum (ER), is considered to be related to the HSP family (Nash et al. 1994). The protein has been shown to aid in Ag presentation and can be complexed with peptides in vitro to elicit peptide-specific $CD8^+$ T-cell responses (Basu and Srivastava 1999). A replication-deficient Ad vector expressing a CRT/E7 fusion gene (Ad-CRT/E7) has been constructed to achieve higher efficiency of Ag delivery and improve Ag presentation of E7 protein through CRT. The ability of Ad-CRT/E7 to induce Ag-specific immunotherapeutic activity against E7-expressing tumors was explored in a mouse model. Vaccination with Ad-CRT/E7 induced stronger E7-specific immune responses (i.e., T-cell proliferation, IFN-γ production, and cytotoxicity) as compared to Ad vector expressing E7 alone. The Ad-CRT/E7 vector provided complete protection in mice challenged with E7-expressing TC-1 tumors and generated long-term immune memory that controlled recurrences. Most importantly, vaccination of tumor-bearing mice with Ad-CRT/E7 resulted in complete tumor regression in all tumor-bearing animals. Taken together, these results demonstrated that Ad-CRT/E7 is an effective vaccine to prevent E7-expressing cervical tumors and could potentially be clinically effective for the treatment of already established disease (Gomez-Gutierrez et al. 2007).

Antitumor immunity has also been reported with an Ad-based vaccine encoding the HPV E7 or ectodomain (ecd) of human epithelial self-Ag hMUC-1 genetically fused to 209-amino acid ecd of the CD40L. This vector also contained the human growth hormone signal sequence (sig) at the N-terminus of the ecdhMUC1-ecdCD40L fusion. MUC-1 is expressed from birth in normal epithelial cells but diffusely upregulated on epithelial surfaces in 90 % of cancers of the breast, ovary, colon, and lung. SC injection of the Ad-sig-E7/ecdCD40L vector into C57BL/6 mice generated a $CD8^+$ T-cell-dependent immune resistance to the growth of TC-1 tumors for up to 1 year. A robust growth suppression of hMUC-1-positive syngeneic tumors was also observed upon SC administration of the Ad-sig-ecdhMUC-1/ecdCD40L vector in a 100 % of hMUC-1-transgenic mice, initially immunologically unresponsive to the hMUC-1-positive syngeneic cancer cells. Thus, immunization with the sig-ecdhMUC-1/ecdCD40L fusion overcomes anergy to syngeneic tumors in this transgenic mouse model (Zhang et al. 2003).

Yet another protein fusion strategy was used for the enhancement of HPV E7 Ag immunogenicity. A fusion of the HPV16 E7 oncoprotein (either full or truncated) to the C-terminus of HBsAg was delivered in the context of non-replicative Ad5 vector using low immunization doses (10^6 infectious units per dose). The HBsAg/E7 fusion protein assembled efficiently into VLPs, which stimulated Ab responses against both the carrier and the foreign Ags and induced an E7-specific $CD8^+$

cytotoxic T-cell response. The Ab and the T-cell responses in this case were significantly stronger than those induced by a control Ad vector expressing wild-type E7 and were not affected by preexisting immunity against either HBsAg or Ad5. Mice vaccinated with Ad5 vectors encoding HBsAg/E7 developed Ab and CTL responses against both E7 and HBsAg. However, the Ab (IgG) titers were higher for E7 than for HBsAg (Baez-Astua et al. 2005).

In summary, Ad vectors show great potential as prophylactic cancer vaccines not only for immunization against virally induced neoplasms (HPV and HBV) but also against self-TAAs, due to the robust adjuvant properties of Ads and their ability to break immunologic tolerance to "self" Ags. However, the resulting induction of autoimmunity may present a substantial challenge to treatment of some patients. Besides, PEI to hAd2/5 in humans and efficient liver uptake can become major obstacles in hAd-based vaccination due to rapid clearance of the vector and possible toxicities. The above factors, however, can be circumvented by using vectors based on alternate Ad serotypes or nonhuman Ad species, implementation of genetic and/or chemical Ad shielding technologies, and utilization of alternate (mucosal) routes of vaccine delivery.

8.5.2.2 Therapeutic Cancer Vaccines

A broad variety of delivery vehicles, including hAd vectors, are currently being evaluated as therapeutic vaccines for treatment of established tumors. Although many of these approaches have failed to generate significant therapeutic benefits in clinical settings, the efficacy of therapeutic vaccines has been gradually improving, which in most cases is a result of expanding diversity of vaccination strategies and treatment combinations. Therapeutic anticancer vaccines have evolved from immunotherapy that can be used to prime–boost tumor-specific immune responses. Vaccines are typically used in combination with other cancer therapy strategies, such as immunomodulation, oncolytic virotherapy, DC-targeting/activation, and anti-angiogenesis to achieve a clinical response. This is because immune response to TAAs in the established tumor microenvironment is counterbalanced by various immune evasion mechanisms of which $CD4^+/CD25^+/FoxP3^+$ T regulatory cells (Tregs) play a central role. Tregs are critical for tolerance to self-Ags and play a major role in determining the effectiveness of anticancer vaccines. Therefore, strategies that target or overcome various immune evasion mechanisms may work in synergy with therapeutic vaccines for an effective clinical response (Yaddanapudi et al. 2013). For example, recombinant antibodies (αCTLA-4, αCD137, αCD3), cyto/chemokines (IL-15, LIGHT, mda-7), or costimulatory ligands (CD80) delivered through Ad-mediated gene transfer to tumors cannot overcome the immune evasion mechanisms (resistance) in both breast and cervical cancer models, as none of the Ad vectors displayed any significant therapeutic effect. However, the combination of Ad.αCTLA4 vector with systemic depletion of Tregs, using anti-CD25 Ab (breast cancer model) or low-dose cyclophosphamide (cervical cancer model), resulted in a significant tumor growth delay in vivo mediated by NKT cells and $CD8^+$ T cells (Liu et al. 2011).

Recently, another advance in improving efficacy of therapeutic vaccines has been made by specifically targeting intracellularly expressed TAAs to exosomes taking advantage of the ability of the factor V like C1C2 domain of lactadherin to specifically address proteins to exosomes. Adenoviral vectors expressing the extracellular domains of CEA and HER2 coupled to the C1C2 domain demonstrated significant improvement in Ag-specific immune responses to each of these Ags in naïve and tolerant transgenic animal models and significantly enhanced therapeutic antitumor effects in a human $HER2^+$ transgenic tumor model in mice (Hartman et al. 2011), suggesting that exosomal targeting could improve future antitumor vaccination.

Cancer Vaccines Using Immunomodulatory Factors

Ad-based cancer vaccines often combine delivery and expression of TAAs along with various immunostimulatory molecules, such as IL-2, IL-6, IL-12, IL-18, GM-CSF, TNF-α, IFN-α, IFN-γ (Waldmann 2006; Windbichler et al. 2000; Porta et al. 2007; Robertson and Ritz 1996; Tanaka et al. 1997; Hwang et al. 2004; Wright et al. 1999; Santodonato et al. 2001; Odaka et al. 2002; Khorana et al. 2003), and various chemokines (Lapteva et al. 2009; Namkoong et al. 2014). Apart from cytokines and chemokines, certain membrane-bound receptors are also required for efficient activation of immune response. In particular, T cells following recognition of foreign Ags in context of major histocompatibility complex (MHC) molecules ("signal 1") require another signal ("signal 2") delivered by costimulatory receptors of the CD28 and the TNFR superfamilies for activation. These two signals are then accompanied by a third signal ("signal 3"), in the form of cytokines. T cells receiving all three signals undergo proliferation, acquisition of effector function, and establishment of long-term memory (Lanzavecchia and Sallusto 2005). The ligands for CD28, CD80 and CD86, and TNFRα, such as 4-1BBL, OX-40L, are expressed by APCs (Choi et al. 2006; Greenfield et al. 1998; Iwakami et al. 2001; Yoshida et al. 2003; Loskog et al. 2004). Tumors, particularly those of non-hematopoietic origin, do not express costimulatory ligands and as such exploit the lack of costimulation as an effective means of immune evasion (Allison and Krummel 1995). Therefore, the use of cancer vaccines in combination with the aforementioned immune modulators has the potential to augment effector immune responses while overcoming various tumor immune evasion mechanisms for a pronounced antitumor immune response with clinical benefits (Putzer et al. 1997; Choi et al. 2006; Habib-Agahi et al. 2007).

Oncolytic Adenovirus-Based Cancer Vaccines

Therapeutic cancer vaccines in conjunction with oncolytic viral vectors (see Sects. 8.3.2 and 8.5.1) represent another attractive cancer treatment modality. Conditionally replicating Ad (CRAd) vectors proved to be a powerful tool for

tumor regression not only due to their direct lytic effect on infected cancer cells but also to the immune responses against TAAs, released upon CRAd-mediated oncolysis and naturally presented to immune system by macrophages and DCs at the site of tumor destruction. Thus, replicative Ad vectors, especially oncolytic vectors "armed" with transgenes encoding immunomodulatory factors, play an important role as adjuvant components of therapeutic cancer vaccines. It should be noted, however, that expressing TAAs in the context of highly potent oncolytic Ads may not be always an optimal vaccination strategy because of potentially short duration of transgene expression in the CRAd-infected cells due to the rapidly developing cytopathic effect leading to oncolytic cell destruction. Furthermore, attempts have been made to augment the immunotherapeutic potential of oncolytic Ads by vector incorporation of heat shock proteins as transgenes. Since heat shock proteins act as chaperones facilitating protein folding and translocation, their overexpression in cancer cells augments adaptive antitumor T-cell immunity by stimulating the processing and presentation of TAAs to T cells via APCs (Wang et al. 2001, 2006).

Dendritic Cell-Based Cancer Vaccines

Ad-based vaccines can be used in conjunction with APCs, particularly with DCs. DCs are highly efficient "professional" APCs that process and load intracellularly expressed TAAs onto both MHC I and MHC II molecules, thus allowing appropriate and relatively persistent induction of Ag-specific $CD8^+$ as well as $CD4^+$ T-cell responses. Ad vectors have been extensively used to transduce and modify DCs, either for effective TAA delivery and presentation to effector T cells or for their activation, i.e., induction of DC-mediated Ag-specific immune responses. Upon activation DCs migrate to the draining lymph nodes (LNs) and simultaneously upregulate costimulatory molecules and cytokines to prime both T helpers (Th) and CTLs in paracortical areas of LNs (Fig. 8.4). DCs thus provide a link between innate and adaptive immunity. A number of studies have demonstrated that ex vivo transduction of DCs with Ad vectors encoding TAAs and their expression in DCs results in induction of antitumor immunity upon DC inoculation back in tumor-bearing animals (Xia et al. 2006). Immune activation of DCs can be induced by delivering immunostimulatory factors or by genetic modification of the Ad5 capsid fiber protein (Worgall et al. 2004), for instance, with CD40 ligand, which binds to CD40 receptor, highly expressed on DCs (Fig. 8.4) (Belousova et al. 2003). Besides, Ad5 vectors exhibit adjuvant effect on their own and are capable of inducing DC activation and maturation upon transduction (Geutskens et al. 2000; Kanagawa et al. 2008).

Another important advantage of using DC-based vaccines is their susceptibility for ex vivo treatment with Ad vectors, circumventing the problem of Ad5-associated PEI encountered in vivo in human clinical trials. However, this treatment approach is laborious and costly. For this reason, specific targeting of

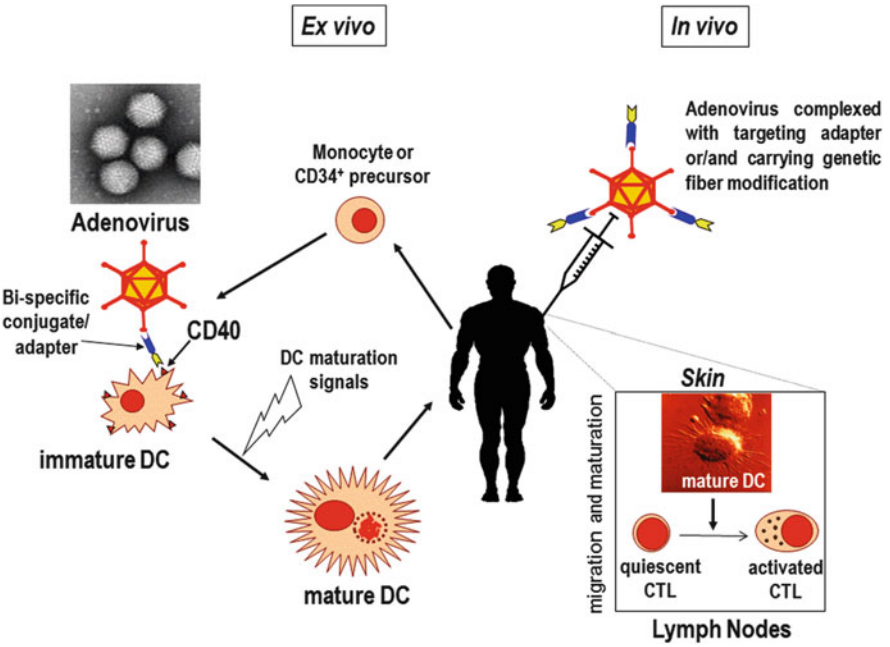

Fig. 8.4 Ex vivo and in vivo strategies for DC-based vaccines. Ad-based vaccines can be targeted to CD40 receptor expressed on DCs via an Ad5 fiber genetic modification or bi-specific conjugate/adapter molecules or combination thereof. In the ex vivo approach, patient-isolated DCs are transduced with Ad vaccines in a clinical laboratory settings and treated with maturation factors before infusing back into the patient. For in vivo vaccination, retargeted Ad-vectored vaccines with genetically modified fiber and/or complexed with adapter is injected intradermally into the patient to target DCs in their natural environment. This leads to antigen uptake by DCs and maturation and migration to lymph nodes where DCs activate T cells for the generation of CTL responses

DCs in vivo (mucosal tissues or skin) may serve a better treatment alternative (Fig. 8.4).

The major route of the receptor-dependent cell attachment of human Ads involves CAR, whose expression on immune cells, including DCs, is typically not detectable or actively downregulated. Various receptors expressed on the DC surface, such as CD40, TLR4, and DC-SIGN, can be utilized to achieve transductional retargeting of Ad vectors. Ad retargeting to these receptors would potentially allow effectively and specifically transduce DCs not only ex vivo but also in vivo (Geijtenbeek et al. 2000; Korokhov et al. 2005).

In order to infectivity-enhance/retarget Ad vectors for/to DCs, a number of fiber-modification strategies have been developed. These can be classified into genetic, nongenetic (bi-specific adapter based), and a new strategy combining the two. The genetic strategies (Table 8.2) introduce modifications in the Ad5 fiber protein C-terminal knob domain by: (1) incorporation of short peptide ligands (RGD4C, pK7), (2) knob-swap between different Ad serotypes (Ad5/3, Ad5/35 fiber

chimeras), or (3) whole knob replacement by artificial fusion molecules embodying the essential fiber trimerization motif and a receptor-binding motif (see also Table 8.2). This motif could be in the form of: (1) a targeting ligand (CD40L) (Belousova et al. 2003), (2) an Fc-binding domain of the *Staphylococcus aureus* protein A (Korokhov et al. 2003), (3) a single-chain Ab (scFv) (Hedley et al. 2006a), or (4) an affibody molecule with a given Ag-binding specificity (Belousova et al. 2008). Fiber incorporation of the Ad3 or Ad35 knobs or the CD40L has been shown to improve DC transduction efficiency, allowing selective delivery of Ags in vitro and in vivo using a human skin explant model (van de Ven et al. 2009; de Gruijl et al. 2006). The adapter-based strategies utilize engineered bi-specific fusion molecules bridging Ad capsid protein fiber and the targeted receptor on DCs (see Fig. 8.4). There are two types of engineered bivalent adapters for targeting CD40 receptor. The first type is represented by a chemically conjugated bi-specific Ab consisting of a Fab fragment of the Ad5 fiber knob-specific Ab "1D6.14" and anti-CD40 monoclonal Ab (mAb) "G28-5" (Tillman et al. 1999). The second type is a recombinant fusion between a soluble CAR (sCAR) and the CD40-specific, G28-5 mAb-derived single-chain Ab (scFv) (Pereboev et al. 2002; Hangalapura et al. 2012). Subsequently the latter adapter was improved by fusing CAR with CD40L via a trimerization motif-containing linker (Pereboev et al. 2004). The "combined" strategy utilizes a genetically modified fiber, fused to the *S. aureus* protein A Fc-binding domain (see above) in conjunction with a protein-fusion adapter, composed of Fc-binding domain portion of the human IgG1 fused to a CD40-specific scFv, derived from the G28-5 anti-CD40 Ab. This bi-specific adapter can bridge the fiber-modified Ad particles with the CD40 receptors on DCs (Korokhov et al. 2003). A similar strategy was used to target Ad5 to DCs via a DC-specific receptor DC-SIGN (Korokhov et al. 2005).

A very recent study assessed the ability of DC-based vaccines to induce a long-lasting tumor-specific CTL response in either prophylactic or therapeutic applications in spontaneous syngeneic murine tumor models (Ricupito et al. 2013). The authors found that priming with a DC-based vaccine induced a long-lasting CTL response in wt mice, while homologous boosting better sustained the pool of central memory T cells associated with potent protection against B16F1 melanoma challenge. Appropriate timing of booster vaccination was also critical. Conversely, prime–boost vaccination proved to be of no advantage or even detrimental in therapeutic settings in B16F1 and transgenic adenocarcinoma of the mouse prostate (TRAMP) models, respectively. These results indicate that booster vaccinations impact antitumor immunity to different extents, depending on their application (prophylactic or therapeutic), and suggest evaluating the need for boosting in any given cancer patient depending on the state of the disease (Ricupito et al. 2013).

The target genes transferred into the DCs by viral vectors, including Ads, fall into two categories: TAAs and immunomodulatory proteins, such as cytokines or costimulatory molecules. A variety of TAAs delivered to DCs via an Ad vector have been shown to boost immune responses to tumor cells (Gallo et al. 2005; Xia et al. 2006). Replication-deficient recombinant Ads, encoding human gp100 or MART-1 melanoma Ag, were used to transduce human DCs ex vivo in model

systems for cancer vaccine therapy. Vector-transduced DCs stimulated MART-$1_{(27-35)}$- or gp100-specific tumor-infiltrating T lymphocytes to produce IFN-γ and induced the peptides-specific, MHC class I-restricted CTLs within PBL from normal donors in vitro (Butterfield et al. 1998; Linette et al. 2000). Furthermore, a protective response to a lethal tumor challenge of unmodified murine B16 melanoma cells was observed upon vaccination of immunocompetent mice with bone marrow-derived murine DCs transduced with AdVMART-1 vector (Ribas et al. 2000). Genetic immunization using DCs transduced ex vivo with an hAd5 expressing the HER2/neu gene has also been demonstrated to induce $CD4^+$ and $CD8^+$ T-cell-based immunity against a breast tumor cell line overexpressing HER2/neu (Chen et al. 2001).

To augment the ability of DCs to present TAAs, a strategy of DC modification allowing them to constitutively express immunomodulatory proteins such as cytokines and chemokines has been developed. DCs genetically modified to express T-cell stimulatory factors/cytokines, such as GM-CSF, TNF-α, IL-12, SLC, lymphotactin, and CD40L, could possess adjuvant-like properties useful in the treatment of tumors as long as sources of TAA are available (Kirk and Mule 2000). A combined immunotherapy including gene therapy and DC vaccines would have some advantages over each modality administered as a monotherapy. Such combined cancer immunotherapy may include DC-based vaccines and Ad-mediated cytokine gene therapy with TNF-α or CD40 (Liu et al. 2004).

Combining an Ag-specific immunization with immunostimulation, using interleukins as vaccine adjuvants, is a widely used strategy in therapeutic vaccine development. A combined injection of mice with adenoviral vectors carrying either IL-12 or HPV-16 E7 oncogene (TAA) induced significant antitumor immunity. IL-12 is known to induce cellular immune responses, suppressing tumor growth and expression of E7. The utility of E7- (AdE7) and/or IL-12-encoding (AdIL-12) Ads for protection against TC-1 tumors was assessed using an animal tumor model. The protective effect of AdIL-12/AdE7 combination was significantly more profound than that of each vaccine alone and also resulted in regression of 9 mm-sized tumors in approximately 80 % of the experimental animals as compared to the "no vector" control group. Serum levels of E7-specific Ab and INF-γ production as well as T helper cell-proliferative responses were found to be significantly higher in mice coinfected with AdIL-12 and AdE7 than in experimental groups receiving injection of either AdIL-12 or AdE7 vaccines. CTL responses were only exhibited by the AdIL-12/AdE7 co-injected group, suggesting that the tumor suppression effect was mediated primarily by $CD8^+$ and, to a lesser extent, by $CD4^+$ T cells (Jin et al. 2005).

In another study, DCs from hepatocellular carcinoma patients were transduced ex vivo with Ad vector encoding α-fetoprotein (AFP) as TAA and human IL-2 genes and showed expression of both proteins. The DCs expressing AFP and IL-2 (AFP/IL-2-DCs) enhanced cytotoxicity of CTLs and significantly increased the production of IL-2 and IFN-γ compared with AFP-DC or plain (untransduced) DC controls. In vivo data from a mouse model suggested that immunization with AFP/IL-2-DCs enhances Ag-specific antitumor efficacy more potently than

immunization with IL-2-DCs or AFP-DCs individually. These findings provide a potential strategy to improve the efficacy of DC-based tumor vaccines (Yang et al. 2012).

A new cancer vaccine based on a truncated form of survivin (devoid of its anti-apoptosis function) as tumor Ag, combined with IL-2, was built and tested in DNA prime–rAd boost regimen for prophylaxis of melanoma cancer. While immunization with the DNA vaccine alone resulted in a weak immune response and modest antitumor effect, the tumor inhibition ratio upon DNA vaccine combination with IL-2 increased to 89 % and further increased to nearly 100 % by rAd–survivin boosting. Complete tumor rejection was observed in 5 out of 15 mice. Efficacy of the vaccine administered therapeutically was enhanced by nearly 300 % when combined with carboplatin. These results indicate that vaccination with a truncated survivin vaccine using DNA prime–rAd boost combined with IL-2 as adjuvant and carboplatin represents an attractive strategy for overcoming immune tolerance to tumors and holds potential therapeutic benefits for melanoma therapy (Zhang et al. 2012).

Vaccine Strategies Targeting Tumor Angiogenesis

Zhao and colleagues employed a conventional Ag immunization approach to induce angiogenic effect in tumors. These authors used a recombinant hAd encoding VE-cad, a transmembrane glycoprotein located at junctions between endothelial cells. VE-cad (VEc) is the most endothelial cell-specific cadherin that plays a critical role in regulating the process of normal and pathological angiogenesis (Vestweber 2008; Hendrix et al. 2001; Liao et al. 2002). To enhance Ag delivery to DC and macrophages, the recombinant AdVEc virus was coupled ex vivo with mannan under oxidizing conditions. Mannose receptors (MRs, CD206), a new family of multilectin receptor proteins, are the most ubiquitous receptors expressed on APCs, including DCs and macrophages. In addition to its role as a DC-targeting ligand, oxidized mannan coupled with Ad particles could act as an immunologic adjuvant, contributing to the ability of mature DCs to generate primary T-cell responses (Zhao et al. 2011). Vaccination with AdVEc–mannan complex (AdVEc-m) suppressed tumor angiogenesis and reduced density of tumor vasculature, resulting in both prophylactic and therapeutic effects on tumor growth and prolongation of mouse survival. Animals injected with AdVEc-m developed VE-cad-specific Ab as well as CTL responses. Depletion of $CD4^+$ or $CD8^+$ T lymphocytes in vivo impaired tumor suppressive activity of the AdVEc-m vaccine, suggesting important role of $CD8^+$ cytotoxic T cells in the tumor regression mechanism (Zhao et al. 2011).

Heterologous Prime–Boost Cancer Vaccination Strategies

A large number of Ad vectors encoding various TAAs (both tissue differentiation Ags and viral Ags/oncoproteins) have been developed to date for cancer vaccine

applications alone or in combination with adjuvants (immunomodulatory molecules) and/or other vaccine types (DNA, other viral vectors, proteins, DC vaccines) in a variety of heterologous prime–boost vaccination regimens. Examples of such TAA immunizations include: HER2/neu protein against breast cancer (Ren et al. 2012; Chen et al. 2011); Epstein–Barr virus (EBV) nuclear Ag-1 (EBNA1) (Smith et al. 2012) or LMP1/LMP2 (Chia et al. 2012) against nasopharyngeal carcinoma tightly associated with EBV infections; guanylyl cyclase C, a cancer mucosa Ag for prevention of primary and metastatic colorectal tumors (Snook et al. 2012); a prostate-specific Ag (PSA) (Lubaroff et al. 2009) or a combination (protein fusion) of PSA and prostate stem cell Ag for treatment of prostate cancer (Karan et al. 2011); a CEA for treatment of patients with advanced colorectal cancer (Mori et al. 2009; Morse et al. 2013) or its combination with HER2/neu (Diaz et al. 2013); a human telomerase reverse transcriptase (hTERT) (Liao et al. 2012; Chen et al. 2009a); and many others.

Therapeutic efficacy of homologous and heterologous prime–boost vaccine strategies against the 5T4 oncofetal antigen was evaluated by Ali and colleagues in an elegant study using 5T4 Ag-expressing replication-defective Ad (Ad5T4) and retrovirally transduced DC lines (DCh5T4) in a subcutaneous B16 melanoma model. All vaccine combinations tested could provide significant tumor growth delay. While DCh5T4 prime–Adh5T4 boost regimen was the best for tumor prophylaxis, it did not demonstrate any therapeutic efficacy in mice with established tumors. In contrast, Adh5T4 prime–DCh5T4 boost vaccination was the best regimen for tumor therapy. The authors concluded that prior immunization with Adh5T4 can condition the mice to induce 5T4-specific Th1 immune responses, which can be sustained and subsequently boosted with DCh5T4. In contrast, prior immunization with DCh5T4 augments Th2 immune responses (already induced by B16h5T4 tumor growth itself), such that a subsequent vaccination with Adh5T4 cannot elicit tumor regression, whose success is dependent on altering the polarizing immune responses from Th2 to Th1. Interestingly, depletion of CD25+ Tregs after tumor challenge, but before immunization, restored therapeutic efficacy (Ali et al. 2007).

A novel strategy of therapeutic immunization against prostate cancer using delivery of human prostate-specific TAA (hPSMA) to DCs by a CD40-targeted Ad vector was recently proposed by Williams and colleagues as an efficient means for DC activation and hPSMA presentation to T cells. A mouse model of prostate cancer was developed by the authors through generating clonal derivatives of the mouse RM-1 prostate cancer cell line expressing human PSMA. To maximize Ag presentation by DCs, expression of both MHC class I and TAP proteins was induced in RM-1 cells by transduction with another Ad vector encoding IFN-γ (Ad5-IFNγ). Administering hPSMA-expressing DCs as well as direct intraperitoneal injection of the Ad5-hPSMA vector resulted in high levels of tumor-specific CTL responses against RM-1-PSMA cells pretreated with Ad5-IFNγ. The CD40 targeting significantly improved the therapeutic antitumor efficacy of the PSMA-encoding Ad vector when combined with Ad5-IFNγ in the mouse model of human prostate cancer (RM-1-PSMA) (Williams et al. 2012). Some of those strategies have been evaluated in phase I/II clinical trials summarized in Table 8.3.

Table 8.3 Clinical trials with adenovirus-based cancer vaccines

Vaccine type/name; targeted antigen(s)	Vaccination regimen (prime–boost)	Trial phase (patients involved)	Immune response to vaccine	Clinical outcome	References
DCs transduced with Ad5 (Δ*E1*) vector expressing Gp100 and MART-1 melanoma Ags	Ex vivo transduction of autologous DCs with the Ad vector and patient infusion every 2–3 weeks	Phase I, 12 melanoma patients	Immunologic end points for this phase 1 trial are not available	Three patients developed leukoderma but experienced disease progression (no tumor eradication)	Tsao et al. (2002)
AdV-MART1/DC melanoma vaccine: DCs transduced with Ad5 (Δ*E1*) vector expressing MART-1 melanoma Ag	Ex vivo transduction of autologous DCs with the Ad vector followed by intradermal infusion	Phase I/II trial, 23 patients; 14 patients received all three scheduled DC vaccines	Activated both Th and CTL in vivo. Significant $CD8^+$ and/or $CD4^+$ MART-1-specific T-cell responses seen in 6/11 and 2/4 patients, respectively; $CD8^+$ and $CD4^+$ T-cell responses to additional Ags noted in two patients	No systemic grade II–IV toxicities. Worsening vitiligo in one patient. No hypersensitivity to 10^2–10^4 vp dose. This DC vaccine was safe and immunogenic	Butterfield et al. (2008)
Ad5(Δ*E1*) vector expressing PSA (Ad/PSA) for hormone-refractory metastatic prostate cancer	Single SC vaccine injection at one of three dose levels as an aqueous solution or suspended in Gelfoam matrix	Phase I trial, 32 patients with hormone-refractory metastatic prostate cancer	Anti-PSA T-cell responses in 68 % patients; PSA Ab produced in 34 % patients	55 % patients survived longer; decrease in anti-PSA Ab but increase in T-cell responses; no serious adverse events	Lubaroff et al. (2009)
INGN-225, a DC-based vaccine; transduced with wt p53-encoding Ad5 (Adp53)	Three doses of INGN-225 injected intradermally every 2 weeks	Phase I/II trial, 54 patients with SCLC	Specific anti-p53 immune response seen in 41.8 % with overall post INGN-225 response in 51.5 % patients; immune response data available in 29 patients (14 positive, 15 negative)	INGN-225 was well tolerated; toxicity grade ≤ 2, appears to sensitize SCLC for chemotherapy	Chiappori et al. (2010)

DCs transduced with Ad5/F35 (Ad35 fiber) encoding a truncated LMP1 (ΔLMP1) and full-length LMP2 (Ad-ΔLMP1-LMP2) Ags of EBV-positive NPC	Ex vivo transduction of DCs with the Ad vector with subsequent infusion into the patient; IL-2 immunomodulation	Phase II trial, 16 patients with metastatic NPC	Positive reactions to transduced DCs in 9 (75 %) out of 12 patients at the time of third vaccination; positive DTH reactions in 4/8 patients after fifth vaccination with smaller magnitude; 3/8 patients had positive reactions	No significant toxicity; three patients had clinical responses including one with partial response (for 7½ months) and two with stable disease (for 6½ and 7½ months	Chia et al. (2012)
T-cell-based vaccine (LMP1/EBNA1-specific T-cell lines) obtained by ex vivo transduction with Ad*E1*-LMPpoly vector encoding EBNA1 fused to multiple $CD8^+$ T-cell epitopes from EBV LMP1 and LMP2	Adoptive transfer with EBV-specific T cells using 3–8 infusions of 2.3×10^7 cells (median) in vitro; T-cell cultures with IL-2-supplemented medium	Phase I trial, 24 NPC patients with (16) or without/minimal (6) - EBV-specific T-cell expansion	Transient increase in the frequencies of LMP1- and 2- and EBNA1-specific T-cell responses after adoptive transfer to be associated with grade I flu-like symptoms and malaise; time to progression from 38 to 420 days	Adoptive immunotherapy with Ad*E1*-LMPpoly vaccine is safe and well tolerated and may offer clinical benefit to patients with NPC	Smith et al. (2012)
1. DNA vaccine (V930): HER2-ECDTM + CEA-LTB; 2. Bicistronic Ad vaccine (V932): Ad6 (Δ*E1*) HER2-ECDTM/CEA-LTB. (Targeting HER2 and CEA)	*Study 1*: V930 alone using electroporation or *Study 2*: in combination with V932. V930 prime–V932 boost	Phase 1, *study 1*: 28 stage II–IV patients with solid HER2 and/or CEA tumors; *Study 2*: 11 patients, 6 from study 1 and 5 new	No CMI augmentation to HER2/CEA, measurable Ab and CMI to LTB	Well tolerated without any serious adverse events. Prime–boost strategy did not augment any detectable CMI to HER2 or CEA	Diaz et al. (2013)

DCs dendritic cells, *CMI* cell-mediated immune response, *HER2-ECDTM* extracellular and transmembrane domains of human HER2, *CEA* carcinoembryonic antigen, *LTB* B subunit of *Escherichia coli* heat-labile toxin, *CEA-LTB* CEA fused to LTB, *IHC* immunohistochemistry, *CLL* chronic lymphocytic leukemia, *PFS* progression-free survival, *MART* melanoma antigen recognized by T cells, *DTH* delayed-type hypersensitivity, *SCLC* small cell lung cancer, *NPC* nasopharyngeal carcinoma, *SC* subcutaneous, *LMP* latent membrane proteins, *IL*-2 interleukin-2, *Ag* antigen

Finally, to assure high level of clinical efficacy, the field of cancer vaccine development has recently moved towards personalized, i.e., patient-specific approach. An example of such contemporary cancer vaccine-based therapy is the recently FDA-approved first ever vaccine for therapy of cancer called sipuleucel-T also known under commercial name of Provenge® (Dendreon, Inc.). Although formulation of this vaccine does not involve Ad-based gene delivery, it is important to mention here with regard to the underlying approach the clinical outcomes and historical significance of the treatment approval. This cell-based vaccine is created by isolating APCs from individual patient's peripheral blood mononuclear cells and culturing them with a fusion protein called PA2024, consisting of PAP prostatic acid phosphatase (PAP; an Ag that is expressed on the majority of prostate cancer cells but not on non-prostate tissues), linked to cytokine GM-CSF (PAP-GM-CSF). APCs cultured in the presence of PAP-GM-CSF display increased amounts of costimulatory molecules on their surface and constitute the active component of sipuleucel-T. These activated APCs are eventually infused back into the APC donor patient for treatment. In a multicenter, randomized, placebo-controlled clinical trial, sipuleucel-T increased the overall survival of metastatic castration-resistant prostate cancer patients by 4 months, however failed to induce tumor regression. Although the exact therapeutic mechanism remains elusive, the treatment appears to favor older patients. On the downside, the underlying personalized approach makes the treatment laborious, technically complex, and extremely expensive (Small et al. 2006; Gulley and Drake 2011).

While there are some obstacles that have been identified in the use of Ads as cancer vaccine carriers, extensive knowledge of Ad biology has enabled researchers to develop methods to effectively overcome these obstacles. As a result, Ad vectors have moved to the forefront of tumor vaccinology and are showing substantial promise as vehicles for cancer Ag delivery in conjunction with oncolytic therapy, immunotherapy, cell-based therapy, and other approaches.

8.6 Concluding Remarks

Ad-based vectors have exceptional ability to stimulate cellular immune responses against the protein product of the transgene, a feature that served the main impetus for the preferential use of these vectors for the development of therapeutic vaccines. In the past 20 years, recombinant Ad vectors expressing a variety of Ags have been constructed and tested for immunization in various settings. However, Ad infection induces pathogenic inflammatory responses in immunocompetent hosts, including humans, even if the viral vector does not replicate, as early Ad gene expression alone is responsible for the pathogenic reaction. The inflammation consists of an early phase, in which TNF-α plays a major role and a late phase consisting of an extensive T-cell response (Ginsberg 1996; Imler 1995). In addition, PEI to Ads is a major impediment for vectors derived from common human serotypes, such as 5 or 2. Preexisting NAbs against these serotypes can be found in up to 90 % of human

adults depending on age and geographic region (Chen et al. 2010). Preexisting NAbs limit Ad-delivered transgene expression, thereby reducing the immune efficacy of the vaccine to the target Ag. A series of approaches have been entertained to circumvent the negative impact of preexisting NAbs, which include genetic modifications of Ad vectors or the use of those isolated from different species, such as chimpanzees (Fitzgerald et al. 2003). Another strategy to avoid vector PEI is to genetically alter the natural tropism of the vector to improve its selective targeting to CD40 receptor on DCs in situ, thus making generation of autologous DCs more efficient and inexpensive (Hangalapura et al. 2012).

One of the important features of Ad-based vectors is the induction of strong $CD8^+$ T- and B-cell responses. The T-cell response is long lasting with minimal contraction and activated cells remaining in effector and effector memory stages for months (Tatsis et al. 2007). Although the basis of this observation is not known, it may reflect that Ad vectors, similar to wt viruses, persist mainly in T cells and remain transcriptionally active.

Ad vectors also allow the genetic incorporation of foreign sequences into the viral capsid proteins that may allow the expression of gene products on the surface in a repetitive and orderly fashion, thereby generating high-affinity B-cell responses (Matthews et al. 2008; Pichla-Gollon et al. 2009; Wu et al. 2005). The noninvasive mucosal vaccination of Ad vectors minimizes systemic inflammation, because preexisting Ad5 immunity does not interfere appreciably with the potency of an Ad-vectored nasal vaccine. Nasal administration of Ad vectors encoding Ags derived from selected pathogens not only provides a painless and practical means of immunization but also generates rapid and sustained protection against the pathogens. Importantly, Ad particles alone without transgene expression were shown to induce an anti-influenza state in the airway. Ad-vectored vaccines can also be used for mass immunization of animals with important application to veterinary medicine (Zhang et al. 2011).

Over the past two decades, virus chimera technologies have boosted the development of novel therapeutic Ads for applications in gene therapy and oncolysis. These strategies of developing virus chimerism address the most relevant drawbacks to clinical implementation of virus-based therapies, including those that have been identified very recently such as blood coagulation factor-mediated liver tropism of Ads (Waddington et al. 2008; Kalyuzhniy et al. 2008). Safety and increased therapeutic efficiency in clinical settings have already been demonstrated by several chimeric Ads (Koski et al. 2010; Pesonen et al. 2010). All these new insights and developments led to a high number of possible combinatorial treatment modalities and delivery schemes. This calls for careful preclinical evaluation in relevant ex vivo human models and transitional in vivo tumor models, before clinical translation. To bring optimized combinations of retargeted Ad configurations and adjuvants in optimized heterologous prime–boost schedules to the clinic will be a major effort both in terms of time and cost (de Gruijl and van de Ven 2012). We consider that the most promising approaches to Ad-based immune response are (1) incorporation of heterologous Ag into Ad capsid, (2) a DC-targeted Ad vaccines, and (3) an oncolytic Ads armed with both Ag and

immunomodulatory transgenes. Therefore, it is reasonable to view certain properties and components of the immune system as allies, rather than enemies, taking advantage of them for improving efficacy of cancer and other disease-targeted vaccines. In this regard, by making a part of the solution what has previously been viewed as a problem, we expect to observe more Ad-based cancer vaccines achieving clinical efficacy in the years to come.

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