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Prospects for Oral Replicating Adenovirus-Vectored Vaccines

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Abstract

Orally delivered replicating adenovirus (Ad) vaccines have been used for decades to prevent adenovirus serotype 4 and 7 respiratory illness in military recruits, demonstrating exemplary safety and high efficacy. That experience suggests that oral administration of live recombinant Ads (rAds) holds promise for immunization against other infectious diseases, including those that have been refractory to traditional vaccination methods. Live rAds can express intact antigens from free-standing transgenes during replication in infected cells. Alternatively, antigenic epitopes can be displayed on the rAd capsid itself, allowing presentation of the epitope to the immune system both prior to and during replication of the virus. Such capsid-display rAds offer a novel vaccine approach that could be used either independently of or in combination with transgene expression strategies to provide a new tool in the search for protection from infectious disease.

Keywords

replicating adenovirus vectors; oral administration; capsid-display; hexon; vaccine

Introduction

Oral delivery of immunogens to the gut is regarded as the “Holy Grail” for vaccinologists [1]. The intestine is the largest lymphoid organ and gut-associated immune cells represent up to 90% of immunocompetent cells [2]. Oral immunization offers immunological and logistical advantages including stimulation of mucosal immune responses preferentially at the site of entry for many infectious agents and ability to elicit strong systemic immunity. Oral immunization is cost effective and offers improved patient compliance due to the ease of vaccine administration, freedom from needles and from the requirement for trained medical personnel. All three oral vaccines licensed for use in the US [3] contain live virus. Live-virus vaccines add to the inherent advantages of oral immunization the ability to immunize with small (and hence less expensive) doses, and induction of a breadth of immune responses similar to those induced by natural infection. These characteristics would facilitate routine immunization and response to epidemics or pandemics [4] and make live

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oral immunization attractive in resource-poor regions, where economy and logistical tractability are critically important.

Licensed oral adenovirus (Ad) serotype 4 and 7 vaccines provide a model for use of live recombinant adenoviruses (rAds) for oral immunization. Since the 1970's, live oral Ad vaccines have been used by the United States military to prevent acute respiratory disease caused by Ad4 and Ad7 [5]. These vaccines contain lyophilized live, wild type (WT) virus incorporated into enteric tablets that protect the virus against the low pH of the stomach. After oral administration of the tablets, live virus is released into the intestine where asymptomatic replication occurs. In a single dose, the vaccines generate an immune response that was over 95% effective in preventing Ad4- and Ad7-induced respiratory illness in a clinical trial involving more than 40,000 soldiers [6–9]. The historical success of Ad military vaccines suggests great potential for recombinant vaccines using the oral replicating Ad platform.

rAds have been used to deliver vaccine antigens in over 90 pre-clinical and clinical trials [10, 11]. The rationales for use of rAd vaccines include genome stability and ease of manipulation, natural tropism for mucosal inductive sites including the gut and upper respiratory tract and ability to elicit vigorous humoral and cellular immune responses. rAds infect a broad spectrum of cells, including dendritic cells, allowing for efficient antigen presentation and can therefore also prime a robust cell-mediated response [12, 13]. However, most rAd vaccine candidates are replication defective and not intended for oral administration. Here, we review work on replicating rAd vaccines that may provide a route to effective oral immunization.

Replicating rAd Transgene Vectors as Vaccines

Most current rAd vaccine candidates are transgene expression vectors, commonly engineered to express a foreign gene inserted into early region 1 (E1) or, occasionally, early region 4 (E4) of the genome [14]. E1 and E4 are essential for viral replication, and most such rAds are replication-defective [15–17]. Extensive experience with defective recombinants in humans and animal models has shown promise in several cases [18].

Replication-competent transgene vectors can be constructed by careful choice of the site of transgene insertion but relatively few have been extensively investigated. Study of replicating rAd vaccines is complicated by the requirement for a host that supports viral replication if vaccines are to be evaluated under conditions that mimic their intended use in humans. Mice do not support human adenovirus replication. However, golden hamsters, cotton rats, dogs, pigs, monkeys (see below), and chimpanzees all support replication of some human Ads, providing systems that might be exploited to test replicating vaccines [19–24]. Cotton rats and hamsters have found use in characterization of replicating oncolytic adenoviruses [19, 25], and dogs have been used in evaluation of live rAd vaccines [21]. In practice, however, well-developed immunological reagents, perceived similarity of primate and human immune responses, and availability of suitable challenges to assess efficacy have restricted most studies of replication-competent rAds in permissive hosts to primates (chimpanzees or monkeys), or to human volunteers.

In early studies, replication-competent rAd7 and rAd4 expressing the hepatitis B virus surface antigen (HBsAg) were used to immunize (rAd7 HBsAg) and then boost (rAd4 HBsAg) two Ad4, Ad7-seronegative chimpanzees (rAd7/rAd4 HBsAg) by the oral route [23]. After primary vaccinations, both chimpanzees shed vaccine virus for 6–7 weeks and developed Ad7 antibodies, suggesting successful Ad7 replication in the chimpanzee gut. One developed transient seropositivity for HBsAg after the first inoculation; both developed modest titers after the second. A third chimpanzee immunized with WT Ad7 and then rAd4

HBsAg (WTAd7/rAd4 HBsAg) developed no HBsAg antibodies. Both rAd7/rAd4 HBsAg chimpanzees were protected from acute clinical disease but were not protected from infection as evident by development of antibodies against the HBV core protein in response to HBV challenge. The animal that did not seroconvert (WTAd7/rAd4 HBsAg), along with an unimmunized control, became clinically infected with HBV [23]. Three human volunteers in a small phase I vaccine trial immunized with the rAd7 HBsAg vaccine exhibited no adverse effects and shed virus between days 4 and 13 post vaccination with no evidence of person-to-person spread. Although all subjects had a significant increase in Ad7 antibodies, none made antibodies to HBsAg [26]. Protection from disease, if not infection, in chimpanzees, despite lack of seroconversion in humans, suggests potential value in using oral enteric vaccination with rAd to induce humoral immune responses to foreign pathogens.

Most animal studies of replicating rAds have been conducted in macaques. WT Ad2 and Ad5 do not replicate in monkeys, and these experiments therefore require use of an Ad5 host range mutation (*hr*404), located in the 72k DNA binding protein, that permits replication in monkey cells and macaques [24, 27]. A transgene-type rAd5 *hr*404 (rAd5hr) virus expressing the *env* and *rev* genes from SIV (Ad5hr-SIV*env/rev*) was able to replicate *in vivo* in rhesus macaques [28]. Priming orally and intranasally, followed by intratracheal immunization 12 weeks later with Ad5hr-SIV*env/rev,* generated proliferating T cells to Env and strong serum neutralizing anti-Env antibodies. Mucosal secretions also contained Env-specific IgG and IgA antibodies. Although this vaccine did not induce sterilizing immunity, it conferred acute-phase protection following intravaginal challenge with SIV [28]. Partial protection of reboosted and rechallenged transiently viremic macaques was associated with both cellular and humoral immune responses [29]. To broaden rAd-induced immunity to SIV, additional rhesus macaques were immunized simultaneously with replicating constructs expressing SIV *env, rev* and *gag* genes through oral and intranasal administration [30]. Specific T-cell responses were generated against all SIV gene products and there was a persistent response to Gag evident for more than 10 weeks post-immunization. Interestingly, immunization primed CD8+ T cells for a persistent and potent response to both dominant and subdominant epitopes [30, 31]. Intrarectal challenge with SIV demonstrated that the vaccine did not induce sterile immunity but acute viral replication was suppressed. Cellular immunity to SIV Gag and Env, along with nasal and vaginal Env-specific IgG antibodies, correlated with a significant reduction of acute phase viremia [32]. Immunized groups exhibited significant protection, with 39% of macaques having either no viremia, cleared viremia or controlled viremia at the threshold of detection 40 weeks post-challenge.

In these studies, only 35% of macaques exhibiting rAd shedding [30], suggesting that the protocol used, bicarbonate neutralization of the stomach prior to virus delivery, might not preserve rAd infectivity. Use of enteric-coated capsules for virus administration resulted in shedding virus in stool samples of 100% of immunized macaques [33] emphasizing the importance of an optimal oral delivery method.

Recently, phase I clinical trial data has been presented for a transgene-type replication-competent rAd4 vaccine (rAd4-H5-Vtn) expressing influenza H5 hemagglutinin (HA) [34]. This virus, which induced protective immune responses in a nonpermissive mouse model [35], contains an insertion of the H5 HA gene in place of part of E3. 166 healthy volunteers received vaccine dosages ranging from 10^7 to 10^{11} recombinant virus particles (VP) [34]. Each cohort received three rAd vaccinations orally and an intramuscular boost with inactivated H5N1 vaccine. Administration of the rAd was associated with mild headache, abdominal pain, nasal congestion and diarrhea. There was no confirmed transmission of the rAd4-H5-Vtn virus to household contacts. Pre-existing antibody to Ad4 was associated with a lower immune response to the vaccine, but this effect was overcome in the high-dose cohorts of 10^{10} and 10^{11} VP. In mice, this recombinant elicits good humoral Ad4 and HA

responses but a low cell-mediated response [35]. In humans, the vaccine induced a significant level of Ad4 seroconversion and HA-specific cellular immune responses in 70% of volunteers receiving 10^{11} VP [34]. However, HA-specific antibody responses assessed by hemagglutination-inhibition (HAI) were minimal at all doses tested, with seroconversion in 4% to 19% of vaccinated volunteers. Plasma IgA ELISA titers mirrored HAI, although IgG ELISA responses indicated 50% seroconversion in the 10^{11} VP cohort. The H5 HA antigen is an intrinsically poor immunogen [36], however following boost of the inactivated H5N1 vaccine, 80% to 100% of volunteers seroconverted and 80% to 89% demonstrated antibody titers high enough to be considered protective in the 10^{10} and 10^{11} VP cohorts, respectively [34]. This indicates that although the Ad4-H5-Vtn vaccine can induce a cellular response, it is only capable of priming an HA-specific antibody response. The cellular immune response and replication of the vaccine as assessed by Ad4 seroconversion or PCR positive rectal swabs, primarily occurred after the first dose, suggesting that only one oral dose may be necessary to induce a cellular response and prime an antibody response.

The doses of Ad4-H5-Vtn required to induce vector immune responses are 100-fold (or more) greater than that in the Ad4 vaccine (10^5 – 10^7 TCID50 [5]). rAd4-H5-Vtn lacks E3, which functions in evading the host immune response [37] and may contribute to the vigor or duration of replication, and thus to the immunogenicity of the, WT vaccines. That possibility has not been experimentally addressed.

Numerous clinical trials of replicating oncolytic rAds have been conducted. In general, these studies do not include analyses of immune responses. Where vector responses have been measured they are efficiently induced [38, 39], but there are no reports of responses to transgene products.

Replicating Capsid Display rAds as Vaccines

Despite the efficacy of the oral Ad4 and Ad7 vaccines and efficient induction of antibodies against the vector, oral rAd vectors induce only modest antibody responses to transgene products in both replicating and non-replicating forms [23, 40] (Berg and Ketner, unpublished). However, a second rAd antigen expression method may offer a more potent approach to induction of humoral immunity. In capsid-display recombinants, segments of foreign antigens are incorporated into one of the capsid proteins such that they are displayed on the surface of the virus particle. Capsid-incorporated antigens are available for binding by surface antibody on B cells and can be processed by the exogenous (MHC class II) pathway. Thus, capsid display recombinants can be immunogenic without intracellular antigen expression, including in systems that do not support virus replication. Replication in a permissive host would further allow persistent antigen presentation via both the exogenous and the endogenous (MHC class I) pathways, with the potential of inducing both humoral and cellular responses. Capsid-display vectors are extremely immunogenic in mice [41–43] and therefore may offer greater efficacy in inducing humoral responses in permissive systems than do transgene rAds.

Several capsid proteins can display foreign epitopes, including hexon, fiber, penton base and pIX (Table 1, Figure 1A, and below). Currently, immunogenicity data is available only in mice, and conclusions therefore have been drawn only in the absence of viral replication.

Hexon (polypeptide II)

The ~960 amino acid Ad hexon protein is the most abundant of the capsid proteins, present in 720 copies per particle [44]. Analysis of hexon amino acid sequences from different serotypes revealed 9 hypervariable regions (HVRs) that diverge in sequence and length among serotypes [45]. Crystal structures of Ad2 and Ad5 hexon show that HVRs reside in

two loops that form the surface-exposed portion of hexon. HVR 1–6 are located within the DEI loop and HVR 7–9 lie within the FGI loop (Figure 1 B and D) [45, 46]. These HVRs contain serotype-specific epitopes that are primary targets of neutralizing antibodies (nAb) [47].

X-ray crystallography suggests that HVRs 2, 3, 5, 6 and 7 are unordered and protrude from the capsid surface. Virus containing insertions of His_6 peptides with flanking spacers into those HVRs are viable, with normal virion thermostability and infectivity [48]. His_6 in HVR2 or 5 is capable of binding tightly to the His_6 antibody, suggesting that the peptide is exposed on the virion when incorporated into these regions [48]. Assessed with epitopes of increasing size, HVR5 was found to accommodate a maximum of 65 amino acids, while the maximum length accommodated in HVR2 was 33 amino acids [49]. Modifications in HVR1 or 5 reduced susceptibility to neutralization by preexisting immunity (PEI) to the Ad vector [41, 50]. Substituting all the HVR loops in Ad5 with those derived from Ad43, a serotype with a low prevalence, produced a vector capable of escaping neutralization with anti-Ad5 sera from mouse, rabbit and humans [51] and that was still highly immunogenic in the presence of PEI to the WT virus.

The first capsid display recombinants incorporated 8 amino acids of the poliovirus type 3 VP1 capsid protein into regions now recognized as HVR1/2. Antiserum raised against the rAd recognized the poliovirus capsid itself [52]. Worgall et al. incorporated an immunodominant peptide from the outer membrane protein F (OprF) of *Pseudomonas aeruginosa* into HVR5 [43, 53]. Immunization with this rAd induced IgG1 and IgG2a antibody subtypes, elicited epitope-specific CD4+ and CD8+ T cell responses, and was capable of protecting 60–80% of mice from a lethal pulmonary challenge with three different *P. aeruginosa* strains. Efficacy was increased with subsequent boosts [43, 53]. In contrast, a B-cell epitope from *Bacillus anthracis* protective antigen (PA), a subunit of the lethal toxin, incorporated into HVR5, induced non-neutralizing antibodies and failed to protect against a challenge with lethal toxin [54]. The discordant results from these studies may reflect differential antibody titers or differing properties of the selected epitopes.

Subsequently, Shiratsuchi et al., inserted a B cell epitope from the circumsporozoite protein (CSP) of the murine malaria parasite *Plasmodium yoelii* into hexon HVR1 or 5 in a recombinant that also expressed CSP as a transgene [41]. The HVR1 recombinant induced high titer antibodies even in mice pre-immunized with WT Ad, suggesting that alteration of HVR1 allowed for evasion of neutralizing Ad antibodies. An rAd incorporating a B-cell epitope from *P. falciparum* CSP in HVR1 induced high-titer antibodies in mice that recognized parasites expressing the *P. falciparum* CSP and neutralized sporozoites bearing the *P. falciparum* CSP gene *in vitro* [55].

The location of epitopes inserted in hexon is an important determinant of immunological properties [49]. rAds that displayed an epitope from the VP1 capsid protein of Enterovirus 71 in HVRs 1, 2, or 7 were viable and protected neonatal mice from lethal challenge through passive immunization and maternally-acquired antibodies [56]. However, antibody isotype depended on the location of the epitope insertion: insertions into HVR1 induced mostly IgG2a antibodies (Th1) while an HVR7 insertion predominantly produced IgG1 antibodies (Th2), demonstrating that insertion sites on hexon are not immunologically equivalent [56]. Similarly, insertion of the conserved extracellular domain of matrix protein 2 (M2e) of influenza A virus into variable region 1 (VR1) or VR4 of hexon of the chimpanzee-origin adenovirus SAd-V25 (AdC68), only the VR1 recombinant provided partial protection from a lethal influenza challenge [57]. Capsid display recombinants induced more robust responses than a transgene type recombinant expressing an M2e fusion protein, supporting

the hypothesis that antibody responses are best induced by antigen displayed in a repetitive and structured fashion to allow for cross-linkage of the B cell receptors.

Recent studies of rAds with modifications in two hexon HVRs have demonstrated the potential for single recombinants to elicit simultaneous antibody responses against two distinct epitopes [58]. 'Multivalent' capsid display recombinants offer potential for broadening immune responses or inducing responses to genetically variable pathogens. However, recombinants with different combinations of modified HVRs induced strikingly different responses, indicating that the design of effective multivalent hexon-modified rAds may not be straightforward.

Penton base (polypeptide III)

The penton base and fiber form the penton complex present at the 12 vertices of the capsid (Figure 1A). Each penton base monomer (~570 residues) contains an Arg-Gly-Asp (RGD) integrin-binding motif located within a flexible loop at the capsid surface [59]. An influenza A virus HA epitope inserted into the RGD loop of penton base was accessible to anti-HA antibodies, confirming surface location [60]. However, anti-HA antibodies were not detected in mice immunized with a penton base recombinant containing HA inserted into the RGD loop [60]. The insertion decreased infectivity for DC's, potentially by interfering with integrin binding, which is involved in virion internalization.

Fiber (polypeptide IV)

Fibers are homotrimers of the fiber protein (polypeptide IV) that protrude from the 12 vertices of the Ad virion and are responsible for attachment to the host cell (Figure 1A). The fiber protein has 3 domains: an N-terminal domain that attaches to the penton base, a central shaft with repeating motifs, and a C-terminal globular knob responsible for virus attachment to the host cell (Figure 1E). Ad5 fiber contains 582 amino acids and is 35–40 nM in length, but fiber length varies among serotypes due to differing numbers of repeats in the fiber shaft.

The crystal structure of the fiber knob reveals that the HI loop (Figure 1C and 1E) does not contribute to intramolecular interactions within the knob, consists mostly of hydrophilic amino acid residues, is exposed on the surface of the knob and is not involved in the formation of cell-binding sites [61]. A FLAG epitope inserted into the HI loop was also accessible to anti-FLAG antibodies, confirming that the HI loop is exposed [62]. Therefore, the HI loop is seen as particularly suitable for manipulation and most modifications initially were made at this location [60, 63]. More recently, a series of rAds with insertions of the *P. aeruginosa* OprF Epi8 epitope in fiber loops CD, DE, FG, HI and at the C terminus [64] have been examined for effects of insertions on viral growth *in vitro* and for immunogenicity. Incorporation of Epi8 into the FG and HI loops had little effect on viral growth whereas insertion into the CD and DE loops or at the C terminus strongly reduced infectivity. FG and HI loop insertions also elicited the strongest humoral and cell-mediated immune responses and were partially protective against challenge [64].

Fiber is a target for neutralizing antibodies and substitutions can contribute to evasion of PEI. For example, modification of the HI loop circumvented nAb present in ascites fluid from ovarian cancer patients [65]. Consistent with this, FG and HI loop recombinants were more effective at inducing antibody and protection in the presence of PEI than was a transgene-type recombinant expressing all of OprF [64]. The ability to manipulate fiber at multiple sites to allow for the efficacy in the presence of PEI makes fiber insertions a promising modification for capsid-display vaccines.

Fiber modifications intended to redirect or ablate virus binding to specific cellular receptors have also been explored [43, 66–68]. However, immunogenicity generally is not addressed in those studies.

pIX (polypeptide IX)

pIX (approximately 140 amino acids) is present in about 240 copies per virion. Trimers of pIX contribute to stability of the virus particle [69, 70]. The C-terminus of pIX is exposed on the surface of the virion and has been used as a substrate on which to attach large polypeptides including fluorescent proteins, fully functional enzymes and foreign antigens in viable rAds [71–75]. pIX fusions containing the envelope protein gp70 of the Friend murine leukemia virus (FV) [75] and the *Yersinia pestis* V and F1 capsular antigens [42] induced high-titer antibodies. The ability of pIX to accommodate very large proteins makes it an attractive site for display of conformational epitopes.

Comparative Immunogenicity

The immunogenicity of influenza A virus HA epitopes inserted into various Ad capsid proteins has been compared [60]. Insertion sites included hexon HVR5, the RGD loop of penton base, the HI loop of the fiber knob and the C terminus of pIX. All HA insertions were located on the virion surface, however, an anti-HA antibody demonstrated strongest binding to HA incorporated into hexon. Infection of A549 cells and DCs showed that HA incorporation into hexon interferes minimally with virus entry *in vitro*, whereas incorporation into fiber knob, pIX and penton base partially reduced the intracellular Ad genome copy numbers following infection. The humoral immune response was strongest against the hexon insertion when immunizing with the same number of particles but fiber was the most immunogenic when controlling for the number of HA copies per virion [60]. A comparison of an ovalbumin (OVA) epitope inserted into the fiber HI loop or hexon HVR5 indicated that fiber insertions were better detected in native virions and triggered a more dramatic increase in anti-OVA antibody responses upon re-administration [63].

Pre-existing Immunity and Replicating rAds

Antibodies to many Ad serotypes are prevalent in the human population. PEI to the vaccine serotype can interfere with a robust immune response against the foreign antigen even in non-replicating rAds [23, 76], although mucosally administered replication-defective rAd vaccines have elicited transgene-specific antibodies despite the presence of PEI, and homologous serotype boosts can be effective [32, 77, 78]. Importantly, if capable of suppressing the growth of viable rAds, PEI might mitigate the inherent advantage of vaccine vector replication after administration of a low dose [79], and live rAds thus may be more sensitive to PEI than their defective counterparts. PEI can be addressed by use of uncommon human adenovirus serotypes or viruses from other species [80, 81] [82] as vectors. Additionally, as noted above, modifications to both hexon and fiber have been shown to reduce susceptibility to PEI [41, 50, 51] and properly-designed capsid display rAds therefore may be inherently resistant to PEI. Limited experience with replicating rAds has provided some data on the effects of PEI [34], but this topic must be more thoroughly addressed.

Safety of Replicating rAds

Concerns have been expressed over the safety of replicating vaccines due to the possibility of inducing disease in the immunocompromised and to the possibility of unintentional spread to contacts. Systemic adenovirus infections can be fatal in people who are profoundly immunocompromised, for example, in the course of bone marrow transplantation [83, 84]. Further, Ad is commonly present in AIDS-related deaths, although it is not generally believed that it was the cause [85, 86]. Clearly, live rAds cannot be administered to the

severely immunodeficient. However, unwitting administration of the military vaccine to a small number of recruits with early HIV infection produced no observed ill effects, nor did the concurrent HIV infection prolong shedding, suggesting a small margin of safety in that population [87]. Transmission of the oral military Ad vaccine did occur, but required intimate contact, as it was not observed among recruits in the barracks [88, 89], and no confirmed transmission of the rAd4-H5-Vtn virus to contacts was observed in its recent clinical trial [34]. This aspect of use of live vaccines, rAd or others, must be carefully investigated.

Conclusion

Replication-competent transgene or capsid display rAds delivered orally to the gut mucosa offer an unconventional immunization approach. Transgene rAds have been shown to induce a robust cellular-mediated immune response, and capsid-display rAds promise to induce strong humoral responses. Critically, transgene and capsid display designs possess complementary immunological characteristics and can be combined in single rAds [41], and such hybrid rAds offer a potential route to greater potency than either approach alone. Multiple-antigen hybrid rAds, in particular, may be capable of increasing the breadth of immune responses to pathogens with a high mutation rate, such as influenza A, or a complex biology, such as malaria. Continued innovation in vaccine research is critical in order to control diseases such as HIV, influenza and malaria that have proven refractory to conventional immunization strategies, and replicating rAds have earned their place among the novel strategies worthy of exploration.

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Abbreviations in order of appearance

Ad	Adenovirus
rAd	recombinant adenovirus
WT	wild-type
E1	early region 1
E4	early region 4
HBsAg	hepatitis B virus surface antigen
***hr*404**	host range mutation in DNA binding protein
rAd5hr	recombinant Ad 5 with *hr*404 mutation
Ad5hrSIV*env/rev*	Ad5 with *hr*404 mutation expressing *env* and *rev* genes of SIV
rAd4-H5-Vtn	recombinant Ad4 expressing influenza H5 hemagglutinin
HA	influenza hemagglutinin
VP	virus particles
MHC class II	major histocompatibility complex II
MHC class I	major histocompatibility complex I

HVRs	hypervariable regions
nAb	neutralizing antibodies
PEI	preexisting immunity
OprF	outer membrane protein F of *Pseudomonas aeruginosa*
PA	protective antigen of *Bacillus anthracis*
CSP	*Plasmodium* circumsporozoite protein
M2e	influenza A matrix protein 2 extracellular domain
VR1	variable region 1
RGD	Arg-Gly-Asp motif
FV	Friend murine leukemia virus
OVA	ovalbumin

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Highlights

- Live oral adenovirus (Ad) vaccines have proven safe and effective.
- Live recombinant Ads can express antigens as transgenes or by capsid display.
- Ad recombinants are highly immunogenic
- Live Ad recombinants induce potent cell-mediated responses in permissive animals
- Capsid display recombinants promise good humoral responses.

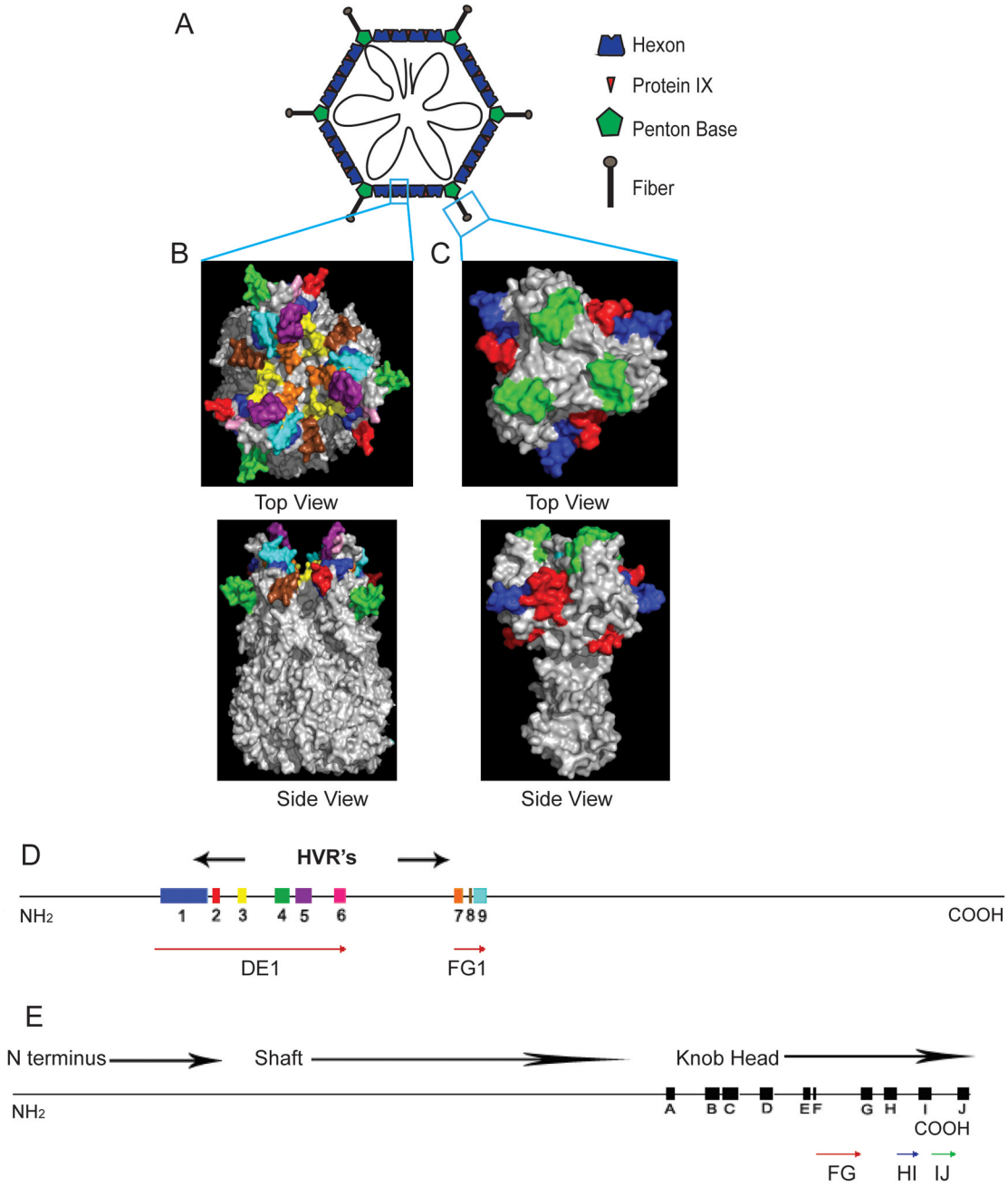

Figure 1.

Adenovirus Capsid Structure A) Cartoon diagram of an adenovirus particle depicting capsid proteins and DNA. B) Surface model of the trimeric Ad5 hexon protein showing the HVR regions. The amino acid location of the region is located in parenthesis. Blue: HVR1 (137–181), red: HVR2 (187–193), yellow: HVR3 (211–218), green: HVR4 (247–260), purple: HVR5 (267–282), brown HVR6 (304–315), orange: HVR7 (418–428), pink: HVR8 (435–436), cyan: HVR9 (440–451). Produced using program PYMOL and PDB 3IYN [97]. C) Surface model of the trimeric Ad2 penton knob and shaft. Red: FG loop (488–515), blue: HI loop (537–549), green: IJ loop (558–572). Produced using program PYMOL and PDB 1QIU

[98]. D) Map of the Ad5 hexon protein with labeled HVRs E) Map of the fiber protein with labeled β-strands in black boxes and FG, HI and IJ loops indicated with colored arrows

Table 1

Capsid Protein Insertion Sites

Capsid Protein	Insertion Sites	Maximum Foreign Epitope Insertion Size[b]	References
Hexon	HVR1	24	[41, 50–52, 55, 56, 58]
	HVR2	45	[48, 49, 51, 52, 56, 58, 90, 91]
	HVR3	6	[48, 51]
	HVR4	15	[51, 56]
	HVR5	66	[43, 48–51, 53, 54, 56, 58, 60, 63]
	HVR6	6	[48, 51]
	HVR7	15	[48, 51, 56]
	HVR8	--	[51]
	HVR9	--	[51]
	VR1[a]	24	[57]
	VR4[a]	24	[57]
Penton	RGD loop	9	[60]
Fiber	C terminus	14	[64, 92–94]
	CD loop	14	[64]
	DE loop	14	[64]
	FG loop	16	[64, 95]
	HI loop	22	[43, 60, 62–68, 95]
	IJ loop	16	[95]
pIX	C terminus	393	[42, 60, 71–75, 96]

[a] non-human adenovirus serotype with only 5 variable regions

[b] size in amino acids