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Review

# 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.



**Contents**



*Abbreviations:* 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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## 1. 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 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 1970s, 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 preclinical 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.

## 2. 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,



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