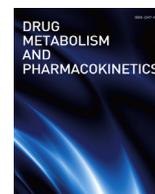




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Review article

Adenovirus vector-based vaccine for infectious diseases

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ABSTRACT

Replication-incompetent adenovirus (Ad) vectors have been widely used as gene delivery vehicles in both gene therapy studies and basic studies for gene function analysis due to their highly advantageous properties, which include high transduction efficiencies, relatively large capacities for transgenes, and high titer production. In addition, Ad vectors induce moderate levels of innate immunity and have relatively high thermostability, making them very attractive as potential vaccine vectors. Accordingly, it is anticipated that Ad vectors will be used in vaccines for the prevention of infectious diseases, including Ebola virus disease and acquired immune deficiency syndrome (AIDS). Much attention is currently focused on the potential use of an Ad vector vaccine for coronavirus disease 2019 (COVID-19). In this review, we describe the basic properties of an Ad vector, Ad vector-induced innate immunity and immune responses to Ad vector-produced transgene products. Development of novel Ad vectors which can overcome the drawbacks of conventional Ad vector vaccines and clinical application of Ad vector vaccines to several infectious diseases are also discussed.

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1. Introduction

Coronavirus disease 2019 (COVID-19) and the severe acute respiratory coronavirus 2 (SARS-CoV-2) which causes it have reminded us that infectious diseases are still a major global threat to human beings. Vaccines are a powerful tool to fight various types of pathogens and to prevent infection and progression of infectious diseases. Conventional vaccines are commonly composed of inactivated or attenuated pathogens, and proteins derived from pathogens. Conventional vaccines mimic the infection with pathogens, resulting in the induction of immune responses to pathogen-derived proteins. Although conventional vaccines have been successfully used to induce durable protective immunity against many

infectious diseases, conventional vaccine development is not a suitable approach to counter the outbreak of emerging infectious diseases caused by largely unknown pathogens. The development of conventional vaccines takes a long time, usually more than 10 years. To counter the outbreak of an emerging infectious disease, efficient and rapid production of vaccines is indispensable. In addition, mutant strains of pathogens which can escape from the effects of vaccines are often generated. Live attenuated vaccines possess a potential risk for reversion to virulence. Inactivated viral vaccines do not always induce protective effects. Cultivation and propagation of pathogens are indispensable for the production of live attenuated or inactivated vaccines, but these processes are often associated with numerous difficulties, including the requirements of facilities with a high biosafety level and large scale production. In order to circumvent these hurdles, novel approaches or platforms for vaccines are highly anticipated.

Novel types of vaccines composed of DNA or mRNA encoding pathogen proteins are expected to be increasingly used to counter emerging infectious diseases, because DNA- and mRNA-based vaccines for emerging pathogens can be rapidly produced by replacing the pathogen protein-encoding DNA or mRNA in a pre-existing platform when the outbreak of an emerging infection

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disease occurs. DNA- and mRNA-based vaccines deliver the DNA or mRNA encoding pathogen proteins to the cells following administration, leading to production of pathogen proteins in the cells and induction of corresponding immune responses to these proteins. Since DNA and mRNA are very large and highly negatively charged molecules, a drug delivery system (DDS) is required for efficient vaccination. Among the various types of DDS used for vaccines, the adenovirus (Ad) vector-based vaccines possess various properties—such as high transduction efficiencies and high titer production—that make them promising as potential vaccine vectors for emerging infectious diseases. In this review, we describe the Ad vector-based vaccines for emerging infectious diseases.

2. Basic properties of adenovirus vectors

Adenoviruses (Ads) are non-enveloped viruses containing a 35–36 kb linear double-stranded DNA genome inside an icosahedral virion of 70–90 nm in diameter (Fig. 1). Ads are isolated from a variety of vertebrate hosts, including humans, mice, dogs, and nonhuman primates. More than 70 types of human Ads have been identified, and they are classified into 7 species (A–G) [1]. Infection with Ads in humans causes cold-like symptoms, sore throat, diarrhea, and vomiting. A conventional Ad vector is composed of species C Ad serotype 5 (Ad5). The E1A gene, which is located next to the left inverted terminal repeat (ITR), is essential for virus replication because it is the first virus gene to be transcribed following infection and because the E1A protein activates transcription of the early genes of the virus. Hence, deletion of the E1A gene renders the Ad vector replication-incompetent. In addition to the E1A gene, the E3 gene is usually deleted from the virus genome to enlarge the capacity for transgene insertion. The E3 gene is dispensable for virus replication. A transgene expression cassette is inserted into the E1- or E3-deleted region. An Ad vector can be propagated in HEK293 cells, which stably express the Ad5 E1 gene proteins.

A conventional Ad vector recognizes coxsackievirus-adenovirus receptor (CAR) [2], which is an immunoglobulin superfamily

protein and mediates homotypic intercellular interactions for the formation of tight junction [3], as an infection receptor by binding of the fiber knob to CAR (Fig. 2). CAR is expressed on various types of cells, including hepatocytes, epithelial cells, and heart muscle cells. Subsequently, interaction between the Arg-Gly-Asp (RGD) motif on the penton base and $\alpha\beta3$ - and $\alpha\beta5$ -integrins occurs, followed by clathrin-mediated endocytosis [4]. Following internalization into cells, endosomal acidification triggers conformational changes of the capsid proteins, resulting in endosomal escape into the cytosol. In the cytosol, the Ad virion undergoes microtubule-mediated retrograde transport to the nuclear pore complex [5]. The Ad vector genome is then released into the interior of the nucleus, leading to transgene expression.

In addition to CAR and αv integrins, heparan sulfates have also been demonstrated to be receptors for Ad vectors [6]. Blood coagulation factor X (FX) specifically binds to the hypervariable regions (HVRs) 5 and 7 of the hexon protein with high affinity [6]. FX also binds to heparan sulfates on the cell surface. FX acts as a bridge between an Ad vector and heparan sulfates. Especially when an Ad vector is intravascularly administered, the interaction between Ad hexon proteins, FX, and heparan sulfates plays a crucial role in liver transduction. The liver is a major organ transduced by an Ad vector following intravascular administration. More than 90% of the injected dose is accumulated in the liver within 1 h following intravascular administration [7].

Several other Ad serotypes that are distinct from Ad5, including Ad serotypes 3, 11, 26, and 35, recognize CD46, desmoglein, and/or sialic acid as an infection receptors [8–12]. Since the fiber knob binds to these infection receptors, fiber-substituted Ad5 vectors containing fiber proteins of these other Ad serotypes and Ad vectors fully composed of the other Ad serotypes mediate transduction by interacting with CD46, desmoglein, and/or sialic acid [13–17]. CD46, desmoglein, and sialic acid are widely expressed on various types of cells. Such novel types of Ad vectors are a powerful tool for gene delivery to CAR-negative target cells.

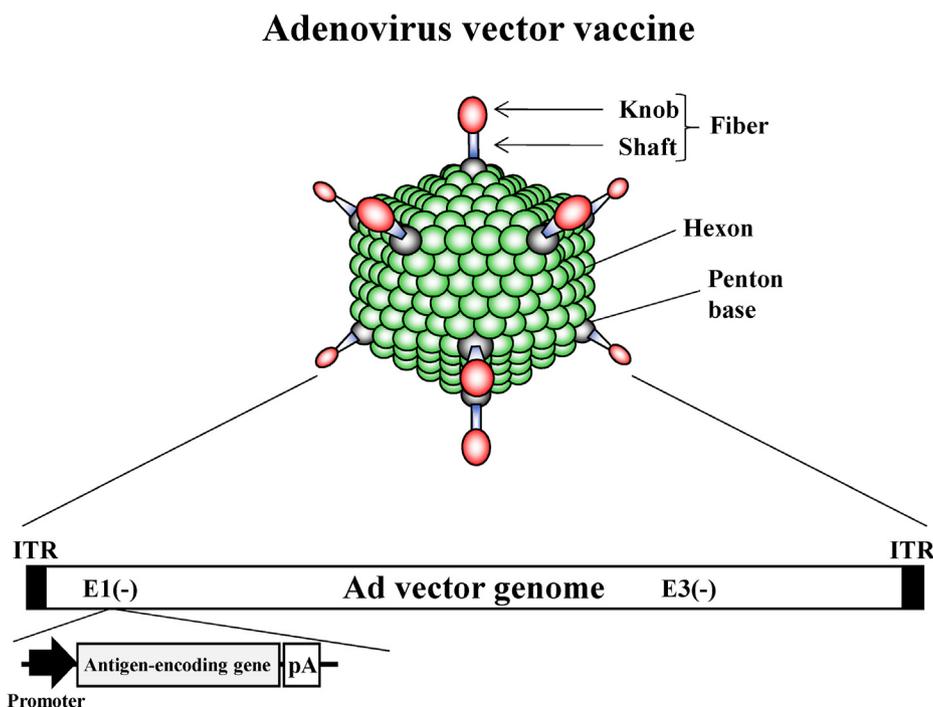


Fig. 1. Schematic diagram of an Ad vector vaccine. ITR, inverted terminal repeat; pA, poly A signal.

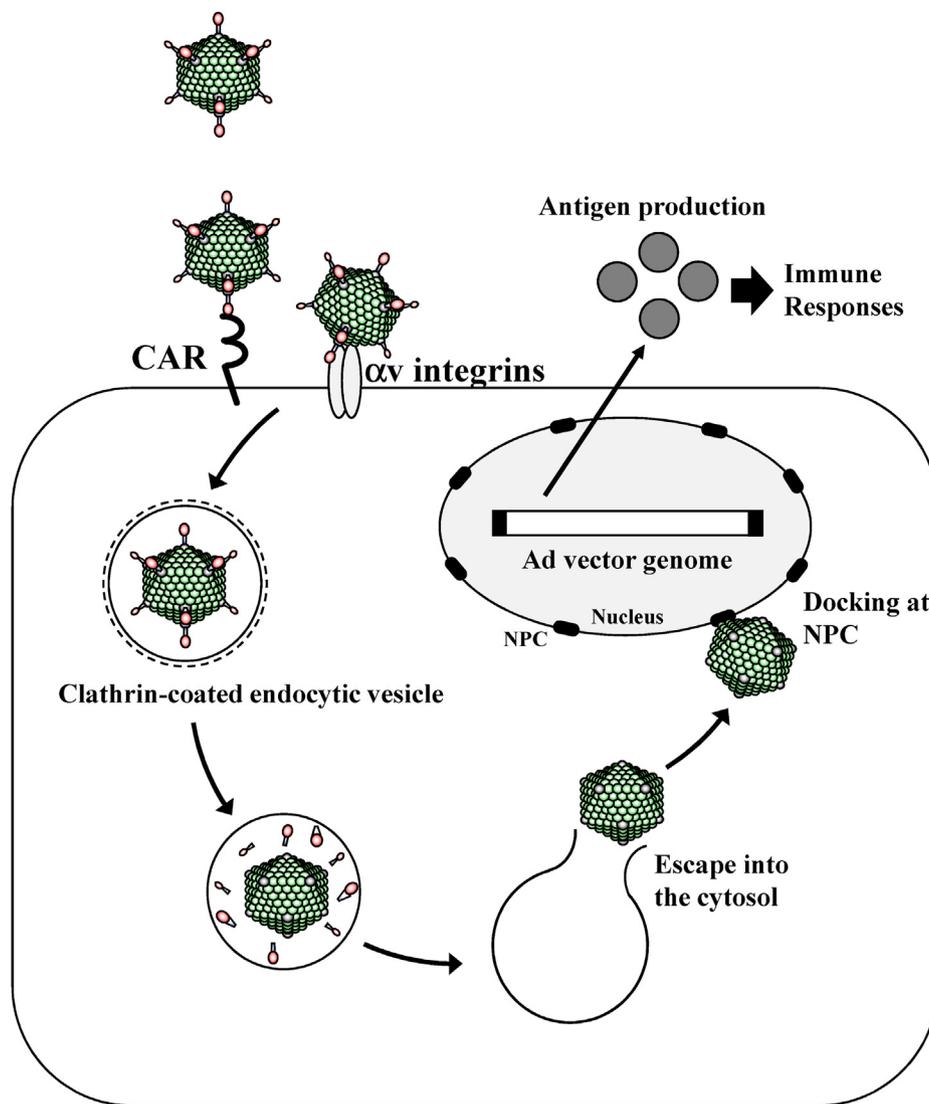


Fig. 2. Transduction pathway of an Ad vector vaccine. CAR, coxsackievirus-adenovirus receptor; NPC, nuclear pore complex.

However, it remains to be fully understood whether interaction with these infection receptors is crucial for Ad vector-induced vaccination. Mature skeletal muscle cells, which are the main target cells following intramuscular vaccination, poorly express CAR [18]. CD46 expression levels in the skeletal muscles have been shown to be lower than those in the other tissues [19,20]. In addition, interaction with the infection receptors would not be highly crucial for Ad vector-mediated vaccination following intramuscular administration. Intramuscular administration of an Ad serotype 35 (Ad35)-based vector, which recognizes CD46 as an infection receptor, mediated comparable levels of transgene expression and vaccine effects between wild-type mice expressing mouse CD46 only in the testis and human CD46-transgenic mice ubiquitously expressing human CD46 in a pattern similar to that observed in humans, when a high dose of an Ad35 vector was intramuscularly administered [21]. As described above, since CAR is mainly located on the basolateral surface, where it forms a tight junction, it is difficult to access to CAR following intranasal or intratracheal administration of an Ad vector [22]. CD46 is also a basolateral protein [23]. Further investigation into the involvement of infection receptors in Ad vector-mediated vaccination could provide important clues for the improvement of Ad vector vaccines.

3. Adenovirus vector-induced innate immunity

Activation of innate immunity is highly important for activation of adaptive immunity. Innate immunity-induced production of inflammatory cytokines and interferons (IFNs) leads to activation of immune cells, including T cells and B cells, followed by activation of adaptive immunity. Conventional vaccines often contain adjuvants which can efficiently activate innate immunity *via* stimulation of pattern recognition receptors (PPRs). An Ad vector moderately activates innate immunity without adjuvants, because the components of an Ad particle are recognized by various types of PRRs, including toll-like receptors (TLRs), retinoic acid-inducible gene-I (RIG-I)-like receptors, and cyclic guanine adenine synthase (cGAS) [24], and serve as an adjuvant. On the other hand, an Ad vector does not induce severe innate immune responses, such as cytokine storm or severe damages in the transduced cells. Ad vector-mediated innate immunity activation levels are appropriate to activate adaptive immunity to transgene products without severe side effects. The following PRR families have been demonstrated to be involved in Ad vector-induced innate immunity.

Toll-like receptors. TLRs are a major PRR family consisting of 10 toll-like receptor (TLR) members in humans and 12 members in

mice. Each TLR family member recognizes different ligands. Among the TLR family members, TLR9, which is mainly located on the endosomal membrane and recognizes unmethylated CpG-motif-containing DNA, recognizes the Ad vector genome in the endosomes following internalization of Ad vector particles into cells, leading to activation of the NF- κ B signal and IFN regulatory factor (IRF) signal [25–27]. TLR9 is mainly expressed on dendritic cells (DCs), NK cells, and macrophages. In addition to TLR9, TLR2 and TLR4 have also been shown to be involved in Ad vector-induced innate immunity [28,29]. TLR2 is mainly expressed on DCs, monocytes, and T cells and recognizes molecules with diacyl and triacylglycerol moieties, proteins and polysaccharides. Ad vector-mediated inductions of transgene product-specific IgM, IgG2, IgG3, and IgA were down-regulated in TLR2-knockout (KO) mice [28], although it is unclear which Ad vector components were recognized by TLR2. TLR4 is mainly expressed on DCs, monocytes, and T cells, and recognizes various damage-associated molecular patterns (DAMPs). Lactoferrin, which is a ligand of TLR4, binds to the capsids of Ad vectors, leading to activation of innate immunity via TLR4 [30]. Transgene product-specific IgG3 levels were lower in TLR4-KO mice than wild-type mice after intravenous injection of Ad vectors [29]. All TLR family members except for TLR3 require MyD88, which is a crucial adaptor molecule for TLR signal transduction. Both transgene product-specific antibody production and cytotoxic T lymphocyte (CTL) induction were attenuated in MyD88-KO mice following intramuscular vaccination with an Ad vector vaccine [31], indicating that TLR-MyD88 signaling is highly important for Ad vector-mediated vaccination.

cGAS. cGAS is a PRR located in the cytosol [32]. The Ad vector genome binds to and activates cGAS in the cytosol following endosomal escape into the cytosol [33]. cGAS is ubiquitously expressed in a variety of cells. Binding of Ad vector genome to cGAS results in activation of the NF- κ B signal and IRF signal. Ad vector-induced inflammatory cytokine production and expression levels of activation markers on DCs were largely reduced in cGAS-KO mice, but anti-Ad antibody production levels were comparable between wild-type and cGAS-KO mice following Ad vector vaccination [33].

RIG-I-like receptors. RIG-I-like receptors, including RIG-I and melanoma differentiation association gene-5 (MDA5), are also located in the cytosol [34]. RIG-I and MDA5, which are expressed in a wide variety of cells, mainly recognize pathogen-derived double-stranded RNAs, leading to activation of NF- κ B and IRF signals. Virus-associated RNAs (VA-RNAs) were shown to be recognized by RIG-I-like receptors [35,36]. VA-RNAs are approximately 160-nt long non-coding RNAs transcribed from not only the wild-type Ad genome but also the replication-incompetent Ad vector genome [35]. Ad vector-mediated activation of innate immunity in primary mouse cells genetically lacking mitochondrial antiviral-signaling (MAVS) (also called IFN- β promoter stimulator 1 (IPS-1)), which is a signal adaptor protein downstream of RIG-I and MDA5, was significantly lower than that in wild-type cells [35]. CTL induction was attenuated in the mucosal compartment of MAVS-KO mice following Ad vector vaccination [37], although transgene product-specific antibody production levels were comparable between wild-type and MAVS-KO mice following immunization with a modified Ad vector displaying the antigen epitopes [38]. These results suggested that a RIG-I-like receptor pathway is crucial for Ad vector vaccine-mediated CTL induction in the mucosal compartment.

In addition to the PRRs described above, the other PRRs, including absent in melanoma 2 (AIM2) and NALP3, have been reported to be involved in Ad vector-induced innate immunity [39,40]. As described above, the involvement of PRRs in the effects of Ad vector-mediated vaccines are highly complex and highly

dependent on the administration routes, types of antigens, injected doses, and types of Ad vectors (most of the studies described above were performed by using an Ad5 vector). Further examination is necessary to fully understand the involvement of PRRs in Ad vector-mediated vaccination.

4. Immune responses to Ad vector-produced transgene products

Ad vectors can induce transgene product-specific immune responses—that is, induction of not only antibody production, but also CTLs. Ad vector-expressing transgene products are recognized as non-self and are eliminated by the immune system. Following administration of Ad vectors, transgenes are expressed in non-immune cells (e.g., muscle cells, fibroblasts) and/or immune cells (e.g., DCs, macrophages). When Ad vector vaccines mediate transgene expression in non-immune cells, transgene products are released from the cells, followed by uptake of transgene products by antigen-presenting cells (APCs), leading to mainly production of transgene product-specific antibodies. Ad vector vaccines elicit various isotypes and subclasses of transgene product-specific antibodies following administration. In addition, intramuscular administration of Ad vector vaccines leads to induction of not only systemic humoral immunity but also certain levels of mucosal humoral immunity, although mucosal administration of an Ad vector vaccine has been shown to induce mucosal immunity more efficiently than intramuscular administration [41–43].

When the transgenes are expressed in immune cells, antigen presentation occurs in immune cells, leading to mainly CTL induction. Transgene expression in monocytes/macrophages and several DC subsets, such as CD8 α ⁺ DCs, langerin⁺ dermal DCs (dDCs), plasmacytoid DCs (pDCs), and inflammatory DCs (inf DCs) was found following intravenous, intramuscular, and intranasal administration [44–46]. Induction of CTLs is highly important to eliminate pathogen-infected cells. Moreover, an Ad vector vaccine induced CD8⁺ T cells showing polyfunctional phenotypes following intramuscular administration [47,48]. Ad vector vaccines also mediate antigen cross-presentation, a phenomenon in which exogenous *antigens* are presented on MHC class I molecules on APCs, leading to CTL induction. Quinn et al. demonstrated that Ad vector-mediated induction of systemic CTLs was diminished in basic leucine zipper ATF-like transcription factor 3 (BATF3)-KO mice [45]. BATF3-KO mice are lacking in CD8a⁺ DCs and langerin⁺ dermal DCs (dDCs), which are critical for cross-presentation of antigens to CD8⁺ T cells. These data indicate that cross-presentation of transgene products is crucial for Ad vector-mediated induction of systemic CTLs.

Ad vector vaccines induce strong CTL responses in not only the systemic compartment in but also the mucosal compartment [37,49,50]. This is an attractive advantage of an Ad vector vaccines, because pathogens often infect and invade from the mucosal compartment. Hemmi et al. suggested the following mechanism of Ad vector vaccine-mediated mucosal CTL induction following intramuscular administration as follows [49]. First, inflammatory monocytes are recruited to the muscle after intramuscular administration of Ad vectors. Then, the monocytes take up the transgene products, leading to differentiation to inf DCs. inf DCs migrate to the draining LNs (dLNs), followed by induction of T helper 17 (Th17) cells. Th17 cells migrate to the gut-mucosa, resulting in the proliferation of antigen-specific CTLs in the mucosal compartment. In addition, a chimpanzee Ad-based vector, ChAdOx1, which is used as a platform of SARS-CoV-2 vaccine, has been shown to efficiently activate mucosal-associated invariant T (MAIT) cells, which are the innate lymphoid cells in the mucosal compartment and play an important role in mucosal immunity, via

the following mechanism [46]. First, ChAdOx1 infects pDCs, leading to IFN- α production in pDCs. Then, the IFN- α produced in pDCs activates the monocytes. Finally, the activated monocytes produce interleukin (IL)-18 and tumor necrosis factor (TNF)- α , leading to activation of MAIT cells and induction of antigen-specific CTLs. On the other hand, the same study found that a conventional Ad5 vector failed to infect pDCs. TNF- α production from peripheral blood mononuclear cells (PBMCs) following treatment with an Ad5 vector was lower than that with ChAdOx1.

5. Neutralizing anti-Ad5 antibodies –the biggest hurdle for conventional Ad vector vaccines-

High seroprevalence to Ad5 in adults due to the natural infection with an Ad5 has been reported in many studies [51–55]. Indeed, more than 80% of adults possess anti-Ad5 antibodies. Neutralizing anti-Ad5 antibodies significantly inhibit the transduction with an Ad vector both by inhibiting the binding of the Ad vector to the infection receptors and by promoting the proteosomal degradation of the Ad vector, leading to a low level of vaccine effects, even when the Ad vector is locally (e.g., intramuscular) administered [52,55–57]. Neutralizing anti-Ad5 antibodies mainly recognize the hexon and fiber proteins, although neutralizing anti-Ad5 antibodies against penton base have also been detected [52,58,59]. In order to circumvent the neutralizing anti-Ad5 antibody-mediated inhibition, an Ad vector composed of rare Ad serotypes with low seroprevalences in adults, including Ad serotypes 11, 26, 35, and 48, and hexon-chimeric Ad vectors containing the hexon HVRs derived from rare Ad serotypes have been developed [14,51,54,60,61]. Hexon HVRs contain the epitopes of neutralizing anti-Ad5 antibodies [60]. Ad vectors based on non-human Ads, including chimpanzee, gorilla, and canine Ads, have also been developed [62–64]. Although non-human Ad vectors often have a problem in realizing high titer production, high titer production of Ad vectors in HEK293 cells was achieved by substituting the E4 gene with the Ad5 E4 gene in several non-human Ad vectors [65,66]. These novel Ad vectors not only can circumvent the neutralizing anti-Ad5 antibodies which are produced by Ad5 vector administration, but also are unlikely to be inhibited by pre-existing neutralizing antibodies at the prime vaccination.

In order to circumvent neutralizing anti-Ad5 antibody-mediated inhibition, other approaches have been reported. An Ad vector coated with polyethylene glycol (PEG) can escape from neutralizing anti-Ad5 antibody-mediated inhibition by blocking the binding of anti-Ad5 antibodies to the virion [67–69]. Liposome-encapsulated Ad vectors have also been demonstrated to escape from neutralizing anti-Ad5 antibodies [70–72]. Both cationic and anionic liposomes have been used for encapsulation of an Ad vector. In particular, because Ad vectors have a negative surface charge, they can be easily encapsulated by cationic liposomes simply by mixing the Ad vectors and cationic liposomes together [71]. Furthermore, recently, extracellular vesicles, such as exosomes, containing an Ad particle have recently been reported, although it remains unclear whether extracellular vesicles containing an Ad vector can circumvent the neutralizing anti-Ad5 antibodies [73–75]. Both the PEG-modified and vesicle-encapsulated Ad vectors have the advantage that they can be repeatedly administered.

6. Clinical applications of Ad vector vaccines

Human immunodeficiency virus (HIV) vaccine. Since no effective vaccines for HIV have been developed to date, Ad vector vaccines for HIV have been eagerly pursued. Ad5 vector vaccines expressing HIV-1 gag/pol/nef (a 1:1:1 mixture of Ad vectors expressing each HIV-1 antigen) were tested in a double-blinded, randomized,

placebo-controlled clinical trial [76,77]. Although these Ad vector vaccines induced HIV-1-specific CD8⁺ and CD4⁺ cell responses, the vaccine group tended to exhibit higher incidence of HIV-1 infection than the control group was found. In addition, risk factors for HIV acquisition would include pre-existing immunity to Ad5 in the vaccinated group [78]. In order to circumvent such pre-existing anti-Ad5 immunity, Ad vector vaccines based on Ad serotype 26 and chimpanzee Ad for HIV-1 have been tested in clinical trials [79,80].

Zika virus vaccine. Since the outbreak of Zika virus in Brazil in 2015 [81], developmental research of Zika virus vaccines has been actively ongoing. For vaccination against Zika virus infection, genes encoding the membrane protein and envelop protein were incorporated into the Ad vector genome [82,83]. An Ad serotype 26-based vector vaccine elicited high levels of neutralizing anti-Zika virus antibodies without severe side effects in a clinical trial [84].

Influenza virus vaccine. Although conventional influenza virus vaccines, which include attenuated live virus vaccines, inactivated virus vaccines, and split virus vaccines, are widely available, the immunization efficiencies of these vaccines are relatively low [85]. In addition, conventional influenza virus vaccines are strain-specific and have a very narrow range of coverage. Since there is a global concern that emerging influenza viruses, including avian influenza virus, have the potential to cause a pandemic, a novel platform of influenza virus vaccines is being developed. A replication-incompetent Ad5 vector expressing hemagglutinin (HA) and chimpanzee Ad-based vector (ChAdOx1) expressing nucleoprotein (NP) and matrix protein-1 (M1) have been tested in clinical trials [86,87]. Furthermore, a replication-competent Ad serotype 4 (Ad4) containing the HA protein expression cassette in the E3 region showed prolonged systemic and mucosal immunity [88]. Replicating Ad4 has been orally administered to more than 10 million people as a vaccine against Ad4 respiratory disease and has shown no severe side effects [89]. Not only replication-incompetent but also replication-competent Ads would be a promising platform for Ad vector vaccines.

Ebola virus vaccine. Ebola virus disease causes severe symptoms, including high fever, headache, and diarrhea, with high mortality [90]. Ebola virus was first discovered in 1976 [91], and since its discovery, there have been several outbreaks of Ebola virus infection in Africa. Because few anti-Ebola virus agents have been approved (an exception is *Inmazeb*, a mixture of three monoclonal antibodies, which was approved by the Food and Drug Administration (FDA) as an anti-Ebola virus agent in 2020), much effort has been expended on vaccines against Ebola virus. An Ad serotype 26 (Ad26)-based vector expressing the glycoprotein of Ebola virus (Ad26.ZEBOV) has been approved in the European Union [92]. The corresponding regimen includes Ad26.ZEBOV as the prime vaccine, and the vaccinia virus encoding glycoproteins from Ebola, Sudan, Marburg, and Tai Forest viruses nucleoprotein (MVA-BN-Filo) as the booster vaccine. In addition, a chimpanzee Ad type 3 vector vaccine and Ad5 vector vaccine expressing the glycoprotein of Ebola virus were demonstrated to efficiently induce anti-Ebola virus immunity [93,94].

SARS-CoV-2 vaccine. COVID-19 is the greatest ongoing global pandemic since the influenza pandemic in 1918 (Spanish flu). Since the identification of SARS-CoV-2 in December 2019 [95], the development of vaccines against SARS-CoV-2 has progressed quickly. Four types of Ad vector vaccines for SARS-CoV-2 have been approved (Table 1): ChAdOx1 nCoV-19 (VAXZEVRIA, AZD1222) (AstraZeneca/University of Oxford, UK), Sputnik V (Gamaleya Research Institute, Russia), Ad26.COV2-S (Johnson & Johnson, Europe) and Ad5-nCoV (CanSino Biologics Inc., China). ChAdOx1 nCoV-19, Sputnik V, and Ad5-nCoV express the full-length spike protein of SARS-CoV-2, while Ad26.COV2-S expresses a pre-fusion

Table 1
Clinically approved Ad vector vaccines for COVID-19.

Ad vector vaccine	Developer	Type of Ads	Antigens	References
ChAdOx1 nCoV-19 (VAXZEVRIA, AZD1222)	AstraZeneca /University of Oxford (UK)	Chimpanzee Ad	Spike protein	[96,103]
Ad26.COVID-2-S	Johnson & Johnson (Europe)	Ad serotype 26	Spike protein	[98,104]
Sputnik V	Gamaleya Research Institute (Russia)	Ad serotype 26 (prime) Ad serotype 5 (boost)	Spike protein	[97,105]
Ad5-nCOV	CanSino Biologics Inc. (China)	Ad serotype 5	Spike protein	[99,106]

stabilized spike protein. ChAdOx1 nCoV-19 and Ad26.COVID-2-S are composed of a chimpanzee Ad type Y25, and a human Ad serotype 26, respectively, to escape from anti-Ad5 antibody-mediated inhibition. Sputnik V uses an Ad26 vector vaccine as a booster and an Ad5 vector vaccine as a prime. Ad5-nCOV is based on an Ad serotype 5. These Ad vector vaccines efficiently elicited immune responses, including induction of anti-spike protein antibodies and CD8⁺ T cell response, to SARS-CoV-2 following intramuscular administration. Clinical trial studies reported that AZD1222 and Sputnik V mediated high vaccine efficacies (AZD1222, 70.4%; Sputnik V, 91.6%) in the randomized controlled clinical trials [96,97]. Ad26.COVID-2-S also showed efficient vaccine efficacy (66.1%) [98]. More than 90% seroconversion rates were reported in the randomized controlled clinical trial of Ad5-nCOV [99]. However, ChAdOx1 nCoV-19 and Ad26.COVID-2-S have been reported to carry a potential risk of inducing thrombotic thrombocytopenia and disseminated intravascular coagulation, particularly in young adults, although the incident rates for these complications are very low [100–102]. Currently, further examination is actively underway to circumvent these side reactions.

7. Conclusions

Much of human history has been a battle against infectious diseases. Even now, there are concerns about emerging and re-emerging infectious diseases and bioterrorism, and therefore the development of a novel vaccine platform is crucial. Several Ad vector vaccines for COVID-19 have been approved and used worldwide. Worldwide use of Ad vector vaccines has revealed the large potential of Ad vectors as a vaccine platform for emerging and re-emerging infectious diseases. Moreover, recombinant Ads have been widely used as a gene delivery vehicle and oncolytic virus in clinical studies. Data obtained in clinical studies of gene therapy using Ad vectors and virotherapy using oncolytic Ads would provide important information for the improvement of Ad vector vaccines.

Author contribution

Writing-original draft; FS, MT. Writing-review and editing; FS, HM. All authors read and approved the final manuscript.

Declaration of competing interest

Authors declare no conflict of interest.

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References

- Hage E, Gerd Liebert U, Bergs S, Ganzenmueller T, Heim A. Human mastadenovirus type 70: a novel, multiple recombinant species D mastadenovirus isolated from diarrhoeal faeces of a haematopoietic stem cell transplantation recipient. *J Gen Virol* 2015;96:2734–42. <https://doi.org/10.1099/vir.0.000196>.
- Bergelson JM, Cunningham JA, Droguett G, Kurt-Jones EA, Krithivas A, Hong JS, et al. Isolation of a common receptor for Coxsackie B viruses and adenoviruses 2 and 5. *Science* 1997;275:1320–3. <https://doi.org/10.1126/science.275.5304.1320>.
- Cohen CJ, Shieh JT, Pickles RJ, Okegawa T, Hsieh JT, Bergelson JM. The coxsackievirus and adenovirus receptor is a transmembrane component of the tight junction. *Proc Natl Acad Sci U S A* 2001;98:15191–6. <https://doi.org/10.1073/pnas.261452898>.
- Wickham TJ, Mathias P, Cheresch DA, Nemerow GR. Integrins alpha v beta 3 and alpha v beta 5 promote adenovirus internalization but not virus attachment. *Cell* 1993;73:309–19. [https://doi.org/10.1016/0092-8674\(93\)90231-e](https://doi.org/10.1016/0092-8674(93)90231-e).
- Greber UF, Suomalainen M, Stidwill RP, Boucke K, Ebersold MW, Helenius A. The role of the nuclear pore complex in adenovirus DNA entry. *EMBO J* 1997;16:5998–6007. <https://doi.org/10.1093/emboj/16.19.5998>.
- Waddington SN, McVey JH, Bhella D, Parker AL, Barker K, Atoda H, et al. Adenovirus serotype 5 hexon mediates liver gene transfer. *Cell* 2008;132:397–409. <https://doi.org/10.1016/j.cell.2008.01.016>.
- Sakurai F, Mizuguchi H, Yamaguchi T, Hayakawa T. Characterization of in vitro and in vivo gene transfer properties of adenovirus serotype 35 vector. *Mol Ther* 2003;8:813–21. [https://doi.org/10.1016/s1525-0016\(03\)00243-0](https://doi.org/10.1016/s1525-0016(03)00243-0).
- Gaggar A, Shayakhmetov DM, Lieber A. CD46 is a cellular receptor for group B adenoviruses. *Nat Med* 2003;9:1408–12. <https://doi.org/10.1038/nm952>.
- Segerman A, Atkinson JP, Marttila M, Dennerquist V, Wadell G, Arnberg N. Adenovirus type 11 uses CD46 as a cellular receptor. *J Virol* 2003;77:9183–91. <https://doi.org/10.1128/jvi.77.17.9183-9191.2003>.
- Wang H, Li Z-Y, Liu Y, Persson J, Beyer J, Möller T, et al. Desmoglein 2 is a receptor for adenovirus serotypes 3, 7, 11 and 14. *Nat Med* 2011;17:96–104. <https://doi.org/10.1038/nm.2270>.
- Arnberg N, Edlund K, Kidd AH, Wadell G. Adenovirus type 37 uses sialic acid as a cellular receptor. *J Virol* 2000;74:42–8.
- Baker AT, Mundy RM, Davies JA, Rizkallah PJ, Parker AL. Human adenovirus type 26 uses sialic acid-bearing glycans as a primary cell entry receptor. *Sci Adv* 2019;5:eaax3567. <https://doi.org/10.1126/sciadv.aax3567>.
- Shayakhmetov DM, Papayannopoulou T, Stamatoyannopoulos G, Lieber A. Efficient gene transfer into human CD34(+) cells by a retargeted adenovirus vector. *J Virol* 2000;74:2567–83. <https://doi.org/10.1128/jvi.74.6.2567-2583.2000>.
- Sakurai F, Mizuguchi H, Hayakawa T. Efficient gene transfer into human CD34+ cells by an adenovirus type 35 vector. *Gene Ther* 2003;10:1041–8. <https://doi.org/10.1038/sj.gt.3301959>.
- Krasnykh VN, Mikheeva GV, Douglas JT, Curiel DT. Generation of recombinant adenovirus vectors with modified fibers for altering viral tropism. *J Virol* 1996;70:6839–46. <https://doi.org/10.1128/JVI.70.10.6839-6846.1996>.
- Stone D, Ni S, Li Z-Y, Gaggar A, DiPaolo N, Feng Q, et al. Development and assessment of human adenovirus type 11 as a gene transfer vector. *J Virol* 2005;79:5090–104. <https://doi.org/10.1128/JVI.79.8.5090-5104.2005>.
- Chen H, Xiang ZQ, Li Y, Kurupati RK, Jia B, Bian A, et al. Adenovirus-based vaccines: comparison of vectors from three species of adenoviridae. *J Virol* 2010;84:10522–32. <https://doi.org/10.1128/JVI.00450-10>.
- Nalbantoglu J, Pari G, Karpati G, Holland PC. Expression of the primary coxsackie and adenovirus receptor is downregulated during skeletal muscle maturation and limits the efficacy of adenovirus-mediated gene delivery to muscle cells. *Hum Gene Ther* 1999;10:1009–19. <https://doi.org/10.1089/10430349950018409>.
- Larochelle N, Deol JR, Srivastava V, Allen C, Mizuguchi H, Karpati G, et al. Downregulation of CD46 during muscle differentiation: implications for gene transfer to human skeletal muscle using group B adenoviruses. *Hum Gene Ther* 2008;19:133–42. <https://doi.org/10.1089/hum.2007.040>.
- Johnstone RW, Loveland BE, McKenzie IF. Identification and quantification of complement regulator CD46 on normal human tissues. *Immunology* 1993;79:341–7.

- [21] Verhaagh S, de Jong E, Goudsmit J, Lecollinet S, Gillissen G, de Vries M, et al. Human CD46-transgenic mice in studies involving replication-incompetent adenoviral type 35 vectors. *J Gen Virol* 2006;87:255–65. <https://doi.org/10.1099/vir.0.81293-0>.
- [22] Walters RW, Grunst T, Bergelson JM, Finberg RW, Welsh MJ, Zabner J. Basolateral localization of fiber receptors limits adenovirus infection from the apical surface of airway epithelia. *J Biol Chem* 1999;274:10219–26. <https://doi.org/10.1074/jbc.274.15.10219>.
- [23] Maisner A, Zimmer G, Liszewski MK, Lublin DM, Atkinson JP, Herrler G. Membrane cofactor protein (CD46) is a basolateral protein that is not endocytosed. Importance of the tetrapeptide FTSL at the carboxyl terminus. *J Biol Chem* 1997;272:20793–9. <https://doi.org/10.1074/jbc.272.33.20793>.
- [24] Atasheva S, Yao J, Shayakhmetov DM. Innate immunity to adenovirus: lessons from mice. *FEBS Lett* 2019;593:3461–83. <https://doi.org/10.1002/1873-3468.13696>.
- [25] Basner-Tschakarjan E, Gaffal E, O'Keeffe M, Tormo D, Limmer A, Wagner H, et al. Adenovirus efficiently transduces plasmacytoid dendritic cells resulting in TLR9-dependent maturation and IFN- α production. *J Gene Med* 2006;8:1300–6. <https://doi.org/10.1002/jgm.964>.
- [26] Cerullo V, Seiler MP, Mane V, Brunetti-Pierri N, Clarke C, Bertin TK, et al. Toll-like receptor 9 triggers an innate immune response to helper-dependent adenoviral vectors. *Mol Ther* 2007;15:378–85. <https://doi.org/10.1038/sj.mt.6300031>.
- [27] Yamaguchi T, Kawabata K, Koizumi N, Sakurai F, Nakashima K, Sakurai H, et al. Role of MyD88 and TLR9 in the innate immune response elicited by serotype 5 adenoviral vectors. *Hum Gene Ther* 2007;18:753–62. <https://doi.org/10.1089/hum.2007.016>.
- [28] Appledorn DM, Patial S, McBride A, Godbehere S, Van Rooijen N, Parameswaran N, et al. Adenovirus vector-induced inflammatory mediators, MAPK signaling, as well as adaptive immune responses are dependent upon both TLR2 and TLR9 in vivo. *J Immunol* 2008;181:2134–44. <https://doi.org/10.4049/jimmunol.181.3.2134>.
- [29] Appledorn DM, Patial S, Godbehere S, Parameswaran N, Amalfitano A. TRIF and TRIF-interacting TLRs differentially modulate several adenovirus vector-induced immune responses. *J Innate Immun* 2009;1:376–88. <https://doi.org/10.1159/000207194>.
- [30] Chéneau C, Eichholz K, Tran TH, Tran TTP, Paris O, Henriquet C, et al. Lactoferrin retargets human adenoviruses to TLR4 to induce an abortive NLRP3-associated pyroptotic response in human phagocytes. *Front Immunol* 2021;12:685218. <https://doi.org/10.3389/fimmu.2021.685218>.
- [31] Rhee EG, Blattman JN, Kasturi SP, Kelley RP, Kaufman DR, Lynch DM, et al. Multiple innate immune pathways contribute to the immunogenicity of recombinant adenovirus vaccine vectors. *J Virol* 2011;85:315–23. <https://doi.org/10.1128/JVI.01597-10>.
- [32] Ablasser A, Goldeck M, Cavar T, Deimling T, Witte G, Röhl I, et al. cGAS produces a 2'-5'-linked cyclic dinucleotide second messenger that activates STING. *Nature* 2013;498:380–4. <https://doi.org/10.1038/nature12306>.
- [33] Anghelina D, Lam E, Falck-Pedersen E. Diminished innate antiviral response to adenovirus vectors in cGAS/STING-deficient mice minimally impacts adaptive immunity. *J Virol* 2016;90:5915–27. <https://doi.org/10.1128/JVI.00500-16>.
- [34] Onomoto K, Onoguchi K, Yoneyama M. Regulation of RIG-I-like receptor-mediated signaling: interaction between host and viral factors. *Cell Mol Immunol* 2021;18:539–55. <https://doi.org/10.1038/s41423-020-00602-7>.
- [35] Yamaguchi T, Kawabata K, Kouyama E, Ishii KJ, Katayama K, Suzuki T, et al. Induction of type I interferon by adenovirus-encoded small RNAs. *Proc Natl Acad Sci U S A* 2010;107:17286–91. <https://doi.org/10.1073/pnas.1009823107>.
- [36] Minamitani T, Iwakiri D, Takada K. Adenovirus virus-associated RNAs induce type I interferon expression through a RIG-I-mediated pathway. *J Virol* 2011;85:4035–40. <https://doi.org/10.1128/JVI.02160-10>.
- [37] Shoji M, Tachibana M, Katayama K, Tomita K, Tsuzuki S, Sakurai F, et al. Type-I IFN signaling is required for the induction of antigen-specific CD8(+) T cell responses by adenovirus vector vaccine in the gut-mucosa. *Biochem Biophys Res Commun* 2012;425:89–93. <https://doi.org/10.1016/j.bbrc.2012.07.056>.
- [38] Anchim A, Raddi N, Zig L, Perrieau P, Le Goffic R, Ryffel B, et al. Humoral responses elicited by adenovirus displaying epitopes are induced independently of the infection process and shaped by the toll-like receptor/MyD88 pathway. *Front Immunol* 2018;9:124. <https://doi.org/10.3389/fimmu.2018.00124>.
- [39] Liu F, Niu Q, Fan X, Liu C, Zhang J, Wei Z, et al. Priming and activation of inflammasome by canarypox virus vector ALVAC via the cGAS/IFI16-STING-type I IFN pathway and AIM2 sensor. *J Immunol* 2017;199:3293–305. <https://doi.org/10.4049/jimmunol.1700698>.
- [40] Muruve DA, Pétrilli V, Zaisis AK, White LR, Clark SA, Ross PJ, et al. The inflammasome recognizes cytosolic microbial and host DNA and triggers an innate immune response. *Nature* 2008;452:103–7. <https://doi.org/10.1038/nature06664>.
- [41] Baden LR, Liu J, Li H, Johnson JA, Walsh SR, Kleinjan JA, et al. Induction of HIV-1-specific mucosal immune responses following intramuscular recombinant adenovirus serotype 26 HIV-1 vaccination of humans. *J Infect Dis* 2015;211:518–28. <https://doi.org/10.1093/infdis/jiu485>.
- [42] Park KS, Lee J, Ahn SS, Byun Y-H, Seong BL, Baek YH, et al. Mucosal immunity induced by adenovirus-based H5N1 HPAI vaccine confers protection against a lethal H5N2 avian influenza virus challenge. *Virology* 2009;395:182–9. <https://doi.org/10.1016/j.virol.2009.09.018>.
- [43] Bricker TL, Darling TL, Hassan AO, Harastani HH, Soung A, Jiang X, et al. A single intranasal or intramuscular immunization with chimpanzee adenovirus-vectored SARS-CoV-2 vaccine protects against pneumonia in hamsters. *Cell Rep* 2021;36:109400. <https://doi.org/10.1016/j.celrep.2021.109400>.
- [44] Patterson LJ, Kuate S, Daltabuit-Test M, Li Q, Xiao P, McKinnon K, et al. Replicating adenovirus-simian immunodeficiency virus (SIV) vectors efficiently prime SIV-specific systemic and mucosal immune responses by targeting myeloid dendritic cells and persisting in rectal macrophages, regardless of immunization route. *Clin Vaccine Immunol* 2012;19:629–37. <https://doi.org/10.1128/CVI.00010-12>.
- [45] Quinn KM, Zak DE, Costa A, Yamamoto A, Kastenmuller K, Hill BJ, et al. Antigen expression determines adenoviral vaccine potency independent of IFN and STING signaling. *J Clin Invest* 2015;125:1129–46. <https://doi.org/10.1172/JCI78280>.
- [46] Provine NM, Amini A, Garner LC, Spencer AJ, Dold C, Hutchings C, et al. MAIT cell activation augments adenovirus vector vaccine immunogenicity. *Science* 2021;371:521–6. <https://doi.org/10.1126/science.aba8819>.
- [47] Kaufman DR, Bivas-Benita M, Simmons NL, Miller D, Barouch DH. Route of adenovirus-based HIV-1 vaccine delivery impacts the phenotype and trafficking of vaccine-elicited CD8+ T lymphocytes. *J Virol* 2010;84:5986–96. <https://doi.org/10.1128/JVI.02563-09>.
- [48] Zhuang Z, Lai X, Sun J, Chen Z, Zhang Z, Dai J, et al. Mapping and role of T cell response in SARS-CoV-2-infected mice. *J Exp Med* 2021;218. <https://doi.org/10.1084/jem.20202187>.
- [49] Hemmi M, Tachibana M, Fujimoto N, Shoji M, Sakurai F, Kobiyama K, et al. T helper 17 promotes induction of antigen-specific gut-mucosal cytotoxic T lymphocytes following adenovirus vector vaccination. *Front Immunol* 2017;8:1456. <https://doi.org/10.3389/fimmu.2017.01456>.
- [50] Kaufman DR, Liu J, Carville A, Mansfield KG, Havenga MJE, Goudsmit J, et al. Trafficking of antigen-specific CD8+ T lymphocytes to mucosal surfaces following intramuscular vaccination. *J Immunol* 2008;181:4188–98. <https://doi.org/10.4049/jimmunol.181.6.4188>.
- [51] Holterman L, Vogels R, van der Vlugt R, Siewewerts M, Grimbergen J, Kaspers J, et al. Novel replication-incompetent vector derived from adenovirus type 11 (Ad11) for vaccination and gene therapy: low seroprevalence and non-cross-reactivity with Ad5. *J Virol* 2004;78:13207–15. <https://doi.org/10.1128/JVI.78.23.13207-13215.2004>.
- [52] Sumida SM, Truitt DM, Lemckert AAC, Vogels R, Custers JHHV, Addo MM, et al. Neutralizing antibodies to adenovirus serotype 5 vaccine vectors are directed primarily against the adenovirus hexon protein. *J Immunol* 2005;174:7179–85. <https://doi.org/10.4049/jimmunol.174.11.7179>.
- [53] Nwanegbo E, Vardas E, Gao W, Whittle H, Sun H, Rowe D, et al. Prevalence of neutralizing antibodies to adenoviral serotypes 5 and 35 in the adult populations of the Gambia, South Africa, and the United States. *Clin Diagn Lab Immunol* 2004;11:351–7. <https://doi.org/10.1128/cdli.11.2.351-357.2004>.
- [54] Abbink P, Lemckert AAC, Ewald BA, Lynch DM, Denholtz M, Smits S, et al. Comparative seroprevalence and immunogenicity of six rare serotype recombinant adenovirus vaccine vectors from subgroups B and D. *J Virol* 2007;81:4654–63. <https://doi.org/10.1128/JVI.02696-06>.
- [55] Lemckert AAC, Grimbergen J, Smits S, Hartkoorn E, Holterman L, Berkhout B, et al. Generation of a novel replication-incompetent adenoviral vector derived from human adenovirus type 49: manufacture on PER.C6 cells, tropism and immunogenicity. *J Gen Virol* 2006;87:2891–9. <https://doi.org/10.1099/vir.0.82079-0>.
- [56] Mallery DL, McEwan WA, Bidgood SR, Towers GJ, Johnson CM, James LC. Antibodies mediate intracellular immunity through tripartite motif-containing 21 (TRIM21). *Proc Natl Acad Sci U S A* 2010;107:19985–90. <https://doi.org/10.1073/pnas.1014074107>.
- [57] Ophorst OJAE, Radošević K, Havenga MJE, Pau MG, Holterman L, Berkhout B, et al. Immunogenicity and protection of a recombinant human adenovirus serotype 35-based malaria vaccine against *Plasmodium yoelii* in mice. *Infect Immun* 2006;74:313–20. <https://doi.org/10.1128/IAI.74.1.313-320.2006>.
- [58] Roy S, Shirley PS, McClelland A, Kaleko M. Circumvention of immunity to the adenovirus major coat protein hexon. *J Virol* 1998;72:6875–9. <https://doi.org/10.1128/JVI.72.8.6875-6879.1998>.
- [59] Cheng C, Gall JGD, Nason M, King CR, Kou P, Roederer M, et al. Differential specificity and immunogenicity of adenovirus type 5 neutralizing antibodies elicited by natural infection or immunization. *J Virol* 2010;84:630–8. <https://doi.org/10.1128/JVI.00866-09>.
- [60] Roberts DM, Nanda A, Havenga MJE, Abbink P, Lynch DM, Ewald BA, et al. Hexon-chimaeric adenovirus serotype 5 vectors circumvent pre-existing anti-vector immunity. *Nature* 2006;441:239–43. <https://doi.org/10.1038/nature04721>.
- [61] Barouch DH, Liu J, Lynch DM, O'Brien KL, La Porte A, Simmons NL, et al. Protective efficacy of a single immunization of a chimeric adenovirus vector-based vaccine against simian immunodeficiency virus challenge in rhesus monkeys. *J Virol* 2009;83:9584–90. <https://doi.org/10.1128/JVI.00821-09>.
- [62] Roy S, Medina-Jaszek A, Wilson MJ, Sandhu A, Calcedo R, Lin J, et al. Creation of a panel of vectors based on ape adenovirus isolates. *J Gene Med* 2011;13:17–25. <https://doi.org/10.1002/jgm.1530>.
- [63] Klonjowski B, Gilardi-Hebenstreit P, Hadchouel J, Randrianarison V, Boutin S, Yeh P, et al. A recombinant E1-deleted canine adenoviral vector

- capable of transduction and expression of a transgene in human-derived cells and in vivo. *Hum Gene Ther* 1997;8:2103–15. <https://doi.org/10.1089/hum.1997.8.17-2103>.
- [64] Farina SF, Gao GP, Xiang ZQ, Rux JJ, Burnett RM, Alvira MR, et al. Replication-defective vector based on a chimpanzee adenovirus. *J Virol* 2001;75:11603–13. <https://doi.org/10.1128/JVI.75.23.11603-11613.2001>.
- [65] Dicks MDJ, Spencer AJ, Edwards NJ, Wadell G, Bojang K, Gilbert SC, et al. A novel chimpanzee adenovirus vector with low human seroprevalence: improved systems for vector derivation and comparative immunogenicity. *PLoS One* 2012;7:e40385. <https://doi.org/10.1371/journal.pone.0040385>.
- [66] Luo S, Zhang P, Ma X, Wang Q, Lu J, Liu B, et al. A rapid strategy for constructing novel simian adenovirus vectors with high viral titer and expressing highly antigenic proteins applicable for vaccine development. *Virus Res* 2019;268:1–10. <https://doi.org/10.1016/j.virusres.2019.05.008>.
- [67] Matsui H, Sakurai F, Katayama K, Yamaguchi T, Okamoto S, Takahira K, et al. A hexon-specific PEGylated adenovirus vector utilizing blood coagulation factor X. *Biomaterials* 2012;33:3743–55. <https://doi.org/10.1016/j.biomaterials.2012.02.011>.
- [68] Suzuki-Kouyama E, Katayama K, Sakurai F, Yamaguchi T, Kurachi S, Kawabata K, et al. Hexon-specific PEGylated adenovirus vectors utilizing avidin-biotin interaction. *Biomaterials* 2011;32:1724–30. <https://doi.org/10.1016/j.biomaterials.2010.10.060>.
- [69] O'Riordan CR, Lachapelle A, Delgado C, Parkes V, Wadsworth SC, Smith AE, et al. PEGylation of adenovirus with retention of infectivity and protection from neutralizing antibody in vitro and in vivo. *Hum Gene Ther* 1999;10:1349–58. <https://doi.org/10.1089/10430349950018021>.
- [70] Fukuhara H, Hayashi Y, Yamamoto N, Fukui T, Nishikawa M, Mitsudo K, et al. Improvement of transduction efficiency of recombinant adenovirus vector conjugated with cationic liposome for human oral squamous cell carcinoma cell lines. *Oral Oncol* 2003;39:601–9. [https://doi.org/10.1016/s1368-8375\(03\)00047-2](https://doi.org/10.1016/s1368-8375(03)00047-2).
- [71] Yotnda P, Chen D-H, Chiu W, Piedra PA, Davis A, Templeton NS, et al. Bilamellar cationic liposomes protect adenoconstructs from preexisting humoral immune responses. *Mol Ther* 2002;5:233–41. <https://doi.org/10.1006/mthe.2002.0545>.
- [72] Zhong Z, Shi S, Han J, Zhang Z, Sun X. Anionic liposomes increase the efficiency of adenovirus-mediated gene transfer to coxsackie-adenovirus receptor deficient cells. *Mol Pharm* 2010;7:105–15. <https://doi.org/10.1021/mp900151k>.
- [73] Ran L, Tan X, Li Y, Zhang H, Ma R, Ji T, et al. Delivery of oncolytic adenovirus into the nucleus of tumorigenic cells by tumor microparticles for virotherapy. *Biomaterials* 2016;89:56–66. <https://doi.org/10.1016/j.biomaterials.2016.02.025>.
- [74] Kakiuchi Y, Kuroda S, Kanaya N, Kumon K, Tsumura T, Hashimoto M, et al. Local oncolytic adenovirotherapy produces an abscopal effect via tumor-derived extracellular vesicles. *Mol Ther* 2021. <https://doi.org/10.1016/j.jymthe.2021.05.015>.
- [75] Zhang Y, Wu J, Zhang H, Wei J, Wu J. Extracellular vesicles-mimetic encapsulation improves oncolytic viro-immunotherapy in tumors with low coxsackie and adenovirus receptor. *Front Bioeng Biotechnol* 2020;8:574007. <https://doi.org/10.3389/fbioe.2020.574007>.
- [76] Buchbinder SP, Mehrotra DV, Duerr A, Fitzgerald DW, Mogg R, Li D, et al. Efficacy assessment of a cell-mediated immunity HIV-1 vaccine (the Step Study): a double-blind, randomised, placebo-controlled, test-of-concept trial. *Lancet (Lond. Engl.)* 2008;372:1881–93. [https://doi.org/10.1016/S0140-6736\(08\)61591-3](https://doi.org/10.1016/S0140-6736(08)61591-3).
- [77] McElrath MJ, De Rosa SC, Moodie Z, Dubey S, Kierstead L, Janes H, et al. HIV-1 vaccine-induced immunity in the test-of-concept Step Study: a case-cohort analysis. *Lancet (Lond. Engl.)* 2008;372:1894–905. [https://doi.org/10.1016/S0140-6736\(08\)61592-5](https://doi.org/10.1016/S0140-6736(08)61592-5).
- [78] Gray G, Buchbinder S, Duerr A. Overview of STEP and Phambili trial results: two phase IIb test-of-concept studies investigating the efficacy of MRK adenovirus type 5 gag/pol/nef subtype B HIV vaccine. *Curr Opin HIV AIDS* 2010;5:357–61. <https://doi.org/10.1097/COH.0b013e32833d2d2b>.
- [79] Baden LR, Stieh DJ, Sarnecki M, Walsh SR, Tomaras GD, Kublin JG, et al. Safety and immunogenicity of two heterologous HIV vaccine regimens in healthy, HIV-uninfected adults (TRAVVERSE): a randomised, parallel-group, placebo-controlled, double-blind, phase 1/2a study. *Lancet HIV* 2020;7:e688–98. [https://doi.org/10.1016/S2352-3018\(20\)30229-0](https://doi.org/10.1016/S2352-3018(20)30229-0).
- [80] Hayton E-J, Rose A, Ibrahimsa U, Del Sorbo M, Capone S, Crook A, et al. Safety and tolerability of conserved region vaccines vectored by plasmid DNA, simian adenovirus and modified vaccinia virus ankara administered to human immunodeficiency virus type 1-uninfected adults in a randomized, single-blind phase I trial. *PLoS One* 2014;9:e101591. <https://doi.org/10.1371/journal.pone.0101591>.
- [81] Bogoch II, Brady OJ, Kraemer MUG, German M, Creatore MI, Kulkarni MA, et al. Anticipating the international spread of Zika virus from Brazil. *Lancet (Lond. Engl.)* 2016;387:335–6. [https://doi.org/10.1016/S0140-6736\(16\)00080-5](https://doi.org/10.1016/S0140-6736(16)00080-5).
- [82] Xu K, Song Y, Dai L, Zhang Y, Lu X, Xie Y, et al. Recombinant chimpanzee adenovirus vaccine AdC7-M/E protects against Zika virus infection and testis damage. *J Virol* 2018;92. <https://doi.org/10.1128/JVI.01722-17>.
- [83] Guo Q, Chan JF-W, Poon VK-M, Wu S, Chan CC-S, Hou L, et al. Immunization with a novel human type 5 adenovirus-vectored vaccine expressing the premembrane and envelope proteins of Zika virus provides consistent and sterilizing protection in multiple immunocompetent and immunocompromised animal models. *J Infect Dis* 2018;218:365–77. <https://doi.org/10.1093/infdis/jiy187>.
- [84] Salisch NC, Stephenson KE, Williams K, Cox F, van der Fits L, Heerwegh D, et al. A double-blind, randomized, placebo-controlled phase 1 study of Ad26.ZIKV.001, an ad26-vectored anti-zika virus vaccine. *Ann Intern Med* 2021;174:585–94. <https://doi.org/10.7326/M20-5306>.
- [85] *Influenza vaccine for 2020-2021*. *Med Lett Drugs Ther* 2020;62:145–50.
- [86] Coughlan L, Sridhar S, Payne R, Edmans M, Milicic A, Venkatraman N, et al. Heterologous two-dose vaccination with simian adenovirus and poxvirus vectors elicits long-lasting cellular immunity to influenza virus A in healthy adults. *EBioMedicine* 2018;29:146–54. <https://doi.org/10.1016/j.ebiom.2018.02.011>.
- [87] Van Kampen KR, Shi Z, Gao P, Zhang J, Foster KW, Chen D-T, et al. Safety and immunogenicity of adenovirus-vectored nasal and epicutaneous influenza vaccines in humans. *Vaccine* 2005;23:1029–36. <https://doi.org/10.1016/j.vaccine.2004.07.043>.
- [88] Matsuda K, Migueles SA, Huang J, Bolkhovitinov L, Stuccio S, Griesman T, et al. A replication-competent adenovirus-vectored influenza vaccine induces durable systemic and mucosal immunity. *J Clin Invest* 2021;131. <https://doi.org/10.1172/JCI140794>.
- [89] Gaydos CA, Gaydos JC. Adenovirus vaccines in the U.S. military. *Mil Med* 1995;160:300–4.
- [90] Dhama K, Malik YS, Malik SVS, Singh RK. Ebola from emergence to epidemic: the virus and the disease, global preparedness and perspectives. *J Infect Dev Ctries* 2015;9:441–55. <https://doi.org/10.3855/jidc.6197>.
- [91] Johnson KM, Lange JV, Webb PA, Murphy FA. Isolation and partial characterisation of a new virus causing acute haemorrhagic fever in Zaire. *Lancet (Lond. Engl.)* 1977;1:569–71. [https://doi.org/10.1016/S0140-6736\(77\)92000-1](https://doi.org/10.1016/S0140-6736(77)92000-1).
- [92] Volkman A, Williamson A-L, Weidenthaler H, Meyer TPH, Robertson JS, Excler J-L, et al. The Brighton Collaboration standardized template for collection of key information for risk/benefit assessment of a Modified Vaccinia Ankara (MVA) vaccine platform. *Vaccine* 2021;39:3067–80. <https://doi.org/10.1016/j.vaccine.2020.08.050>.
- [93] Stanley DA, Honko AN, Asiedu C, Trefry JC, Lau-Kilby AW, Johnson JC, et al. Chimpanzee adenovirus vaccine generates acute and durable protective immunity against ebolavirus challenge. *Nat Med* 2014;20:1126–9. <https://doi.org/10.1038/nm.3702>.
- [94] Zhu F-C, Hou L-H, Li J-X, Wu S-P, Liu P, Zhang G-R, et al. Safety and immunogenicity of a novel recombinant adenovirus type-5 vector-based Ebola vaccine in healthy adults in China: preliminary report of a randomised, double-blind, placebo-controlled, phase 1 trial. *Lancet (Lond. Engl.)* 2015;385:2272–9. [https://doi.org/10.1016/S0140-6736\(15\)60553-0](https://doi.org/10.1016/S0140-6736(15)60553-0).
- [95] Chan JF-W, Kok K-H, Zhu Z, Chu H, To KK-W, Yuan S, et al. Genomic characterization of the 2019 novel human-pathogenic coronavirus isolated from a patient with atypical pneumonia after visiting Wuhan. *Emerg Microb Infect* 2020;9:221–36. <https://doi.org/10.1080/22221751.2020.1719902>.
- [96] Emary KRW, Golubchik T, Aley PK, Ariani CV, Angus B, Bibi S, et al. Efficacy of ChAdOx1 nCoV-19 (AZD1222) vaccine against SARS-CoV-2 variant of concern 202012/01 (B.1.1.7): an exploratory analysis of a randomised controlled trial. *Lancet (Lond. Engl.)* 2021;397:1351–62. [https://doi.org/10.1016/S0140-6736\(21\)00628-0](https://doi.org/10.1016/S0140-6736(21)00628-0).
- [97] Logunov DY, Dolzhenko IV, Shcheblyakov DV, Tukhvatulin AI, Zubkova OV, Dzharullaeva AS, et al. Safety and efficacy of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine: an interim analysis of a randomised controlled phase 3 trial in Russia. *Lancet (Lond. Engl.)* 2021;397:671–81. [https://doi.org/10.1016/S0140-6736\(21\)00234-8](https://doi.org/10.1016/S0140-6736(21)00234-8).
- [98] Sadoff J, Gray G, Vandebosch A, Cárdenas V, Shukarev G, Grinsztejn B, et al. Safety and efficacy of single-dose Ad26.COV2.S vaccine against covid-19. *N Engl J Med* 2021;384:2187–201. <https://doi.org/10.1056/NEJMoa2101544>.
- [99] Zhu F-C, Guan X-H, Li Y-H, Huang J-Y, Jiang T, Hou L-H, et al. Immunogenicity and safety of a recombinant adenovirus type-5-vectored COVID-19 vaccine in healthy adults aged 18 years or older: a randomised, double-blind, placebo-controlled, phase 2 trial. *Lancet (Lond. Engl.)* 2020;396:479–88. [https://doi.org/10.1016/S0140-6736\(20\)31605-6](https://doi.org/10.1016/S0140-6736(20)31605-6).
- [100] Tølbøll Sørensen AL, Rolland M, Hartmann J, Harboe ZB, Roed C, Jensen TØ, et al. A case of thrombocytopenia and multiple thromboses after vaccination with ChAdOx1 nCoV-19 against SARS-CoV-2. *Blood Adv* 2021;5:2569–74. <https://doi.org/10.1182/bloodadvances.2021004904>.
- [101] Greinacher A, Thiele T, Warkentin TE, Weisser K, Kyrle PA, Eichinger S. Thrombotic thrombocytopenia after ChAdOx1 nCoV-19 vaccination. *N Engl J Med* 2021;384:2092–101. <https://doi.org/10.1056/NEJMoa2104840>.
- [102] Hwang J, Lee SB, Lee SW, Lee MH, Koyanagi A, Jacob L, et al. Comparison of vaccine-induced thrombotic events between ChAdOx1 nCoV-19 and Ad26.COV2.S vaccines. *J Autoimmun* 2021;122:102681. <https://doi.org/10.1016/j.jaut.2021.102681>.
- [103] van Doremalen N, Lambe T, Spencer A, Belij-Rammerstorfer S, Purushotham JN, Port JR, et al. ChAdOx1 nCoV-19 vaccination prevents SARS-CoV-2 pneumonia in rhesus macaques. *BioRxiv Prepr Serv Biol* 2020. <https://doi.org/10.1101/2020.05.13.093195>.
- [104] Mercado NB, Zahn R, Wegmann F, Loos C, Chandrashekar A, Yu J, et al. Single-shot Ad26 vaccine protects against SARS-CoV-2 in rhesus macaques. *Nature* 2020;586:583–8. <https://doi.org/10.1038/s41586-020-2607-z>.

- [105] Logunov DY, Dolzhikova IV, Zubkova OV, Tukhvatulin AI, Shcheblyakov DV, Dzharullaeva AS, et al. Safety and immunogenicity of an rAd26 and rAd5 vector-based heterologous prime-boost COVID-19 vaccine in two formulations: two open, non-randomised phase 1/2 studies from Russia. *Lancet (Lond. Engl.)* 2020;396:887–97. [https://doi.org/10.1016/S0140-6736\(20\)31866-3](https://doi.org/10.1016/S0140-6736(20)31866-3).
- [106] Zhu F-C, Li Y-H, Guan X-H, Hou L-H, Wang W-J, Li J-X, et al. Safety, tolerability, and immunogenicity of a recombinant adenovirus type-5 vectored COVID-19 vaccine: a dose-escalation, open-label, non-randomised, first-in-human trial. *Lancet (Lond. Engl.)* 2020;395:1845–54. [https://doi.org/10.1016/S0140-6736\(20\)31208-3](https://doi.org/10.1016/S0140-6736(20)31208-3).