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Adenovirus-based vaccines against avian-origin H5N1 influenza viruses

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Abstract

Since 1997, human infection with avian H5N1, having about 60% mortality, has posed a threat to public health. In this review, we describe the epidemiology of H5N1 transmission, advantages and disadvantages of different influenza vaccine types, and characteristics of adenovirus, finally summarizing advances in adenovirus-based H5N1 systemic and mucosal vaccines.

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1. Introduction

Influenza A viruses are notorious for antigen drift and shift for segmented negative-sense RNA genomes, and they are named according to their two surface proteins: hemagglutinin (HA) and neuraminidase (NA), with 18 and 11 subtypes, respectively [1]. Before 1997, human infection by influenza virus was confined to H1, H2 and H3 subtypes, according to records. However, the first recorded human infection of avian influenza virus occurred in 1997 in Hong Kong, which was caused by H5N1 virus. The causal H5N1 was a reassortant with hemagglutinin (HA) from goose H5N1 and seven viral genes from other avian influenza viruses [2]. Pathogenetic and sequence analysis revealed that H5N1 belonged to the group of highly pathogenic avian influenza viruses (HPAI) based on its lethality to chickens and polybasic amino acids at the cleavage site of HA, which allowed HA processing by ubiquitously expressed proteases [3,4]. Since then, the HPAI H5N1 subtype has caused numerous outbreaks in poultry and more

than 700 human H5N1 infection cases with about 60% mortality (http://www.who.int/influenza/human_animal_interface/EN_GIP_20140727CumulativeNumberH5N1cases.pdf?ua=1). Moreover, similar to seasonal influenza viruses circulating in humans [5], the HPAI H5N1 subtype is genetically highly variable, and it has diversified into multiple phylogenetic clades over the past decade [6]. Hence, the development of vaccines against H5N1 influenza viruses is challenging.

2. Previous epidemics caused by avian-origin H5N1 influenza viruses and the progress of H5N1 vaccine development

The Asian H5N1 virus, which killed some geese, was detected in Guangdong Province, China, in 1996 [7]. In 1997, the HPAI virus subtype H5N1 caused disease in 18 patients with six deaths in Hong Kong [3]. Exposure to live poultry a week before the onset of illness was associated with disease in humans. The culling of all poultry in Hong Kong ended the first wave of H5N1, but the virus continued to circulate among avians. No further human cases occurred until 2003, when two Hong Kong residents contracted the disease, one of whom died [8]. The second wave of the Asian H5N1 epidemic occurred in

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the winter of 2003/04 in eight Asian countries, including China, Cambodia, Indonesia, Japan, Korea, Lao People's Democratic Republic, Thailand and Vietnam. The impact was particularly severe in Thailand and Vietnam, where widespread disease was reported in poultry, as well as human case fatalities [9]. From 1997 to May 2005, H5N1 viruses were largely confined to Southeast Asia, but after they had infected wild birds in Qinghai Lake, China, they rapidly spread worldwide [10,11]. Even though sustained human-to-human transmission of influenza A(H5N1) has not yet been described, it has been suggested in several household clusters [12–14]. In addition, several studies showed that airborne transmission of H5N1 can be achieved through adaptation in experimental tests [15–18]. Therefore, H5N1 remains a critical public health concern.

Vaccines are the most effective way to fight against influenza pandemic. However, H5N1 poses a particular challenge to conventional inactivated influenza virus-based vaccine as a result of its poor immunogenicity [19,20]. Even though live attenuated influenza vaccines (LAIV) are attractive for their ability to mimic the natural route of infection by which both strong systemic and mucosal immunity can be induced, LAIV is only licensed for healthy people from 2 to 49 years of age, excluding high-risk human groups. Furthermore, reversion to virulence during production may occur, and reassortment with circulating influenza viruses may happen [21]. DNA vaccine can be made rapidly and can be produced in large quantities; however, DNA vaccine alone is poorly immunogenic, and specific instrumentation is needed during immunization. Virus-like particles (VLPs)-based vaccines antigenically mimic native virions with intact biochemical activities. Khurana et al. showed that two doses of H5N1 VLPs at 15, 45, or 90 µg HA/dose induced hemagglutination inhibition (HAI), seroconversion rates of 40%, 57%, and 61%, and microneutralization (MN) of 39%, 52%, and 76%, respectively, in human [22], indicating that H5N1 VLP is, like H5N1 inactivated vaccine, poorly immunogenic. Even though subunit vaccines serve as a convenient method of generating large amounts of influenza vaccines for a potential pandemic, properties such as antigenicity and immunogenicity are indeed challenging [23]. However, adenovirus-based H5N1 vaccines are attractive based on self-adjunctivity, rapid production by the egg-independent method, and administration by various routes.

3. The production of adenovirus-based vaccine and the relationship between adenovirus and the immune system

Adenoviruses are non-enveloped icosahedral, double-stranded DNA viruses. A targeted gene can be inserted into an adenovirus genome and expressed after recombinant adenovirus (rAd) infection (Fig. 1). Based on the deletion of adenovirus genome sequence, three generations of rAd are established. In the first generation, replacement of the E1 or E1/E3 gene of the adenovirus genome with a gene encoding an antigen of interest renders virus replication incompetent, but able to be produced and propagated in HEK 293 or PER.C6 cells, allowing for expression of the gene of interest by the

Wild adenovirus serotype 5 (Ad5)



First generation of rAd5



Second generation of rAd5



Third generation of rAd5



Fig. 1. Schematic illustration of wild Adenovirus serotype 5. LITR, left inverted terminal repeats; RITR, right inverted terminal repeats. PS, package signal. IG, inserted gene.

recombinant virus upon infection [24]. In order to increase cloning capacity, prolong gene expression, and reduce toxicity associated with adenovirus backbone gene expression, the second generation of rAd was developed by further deletion of E2 or E4 gene stably expressed in the production cells [25]. The third generation of rAd is helper virus-dependent and lacks nearly all viral encoding sequences, except packaging signal and two inverted terminal repeats (ITRs), enlarging cloning capacity, but reducing immunogenicity for gene therapy [26]. However, the first generation of rAd is widely used for its ease of manufacture, including production and purification, compared to the other two generations.

An important advantage for adenovirus-based vaccine is its adjuvanticity by stimulating innate immune response. Adenoviruses can infect various types of cells, such as epithelial cells, macrophage cells and dendritic cells. As shown in Fig. 2, upon infection, adenoviruses activate innate immunity in a Toll-like receptor (TLR)-dependent manner, as well as TLR-independent pathways, causing upregulation of type I interferons (IFNs) and inflammatory cytokines [27]. It was reported that neutralizing antibodies to type I IFNs could block innate and adaptive immune responses to adenoviral vectors, which showed that the induction of type I IFNs plays an important role in the adjuvanticity of adenoviruses [28]. After endocytosis of adenovirus, the genome can be recognized by TLR9, which leads to the activation of interferon regulatory factor 7 (IRF7) that promotes the induction of type I IFNs [29]. Cytosolic adenovirus genome can be recognized by DNA sensor cyclic guanosine monophosphate—adenosine monophosphate (cGAMP) synthase (cGAS) and another unknown DNA sensor that leads to activation of IRF3 and IRF7 [30,31], respectively, adding to the induction of type I IFNs to promote both cellular and humoral immune response.

4. Adenovirus-based systemic vaccines against avian-origin influenza A H5N1 viruses

Since revealing the low immunogenicity of H5N1 inactivated vaccine, adenovirus-based H5N1 vaccines have been

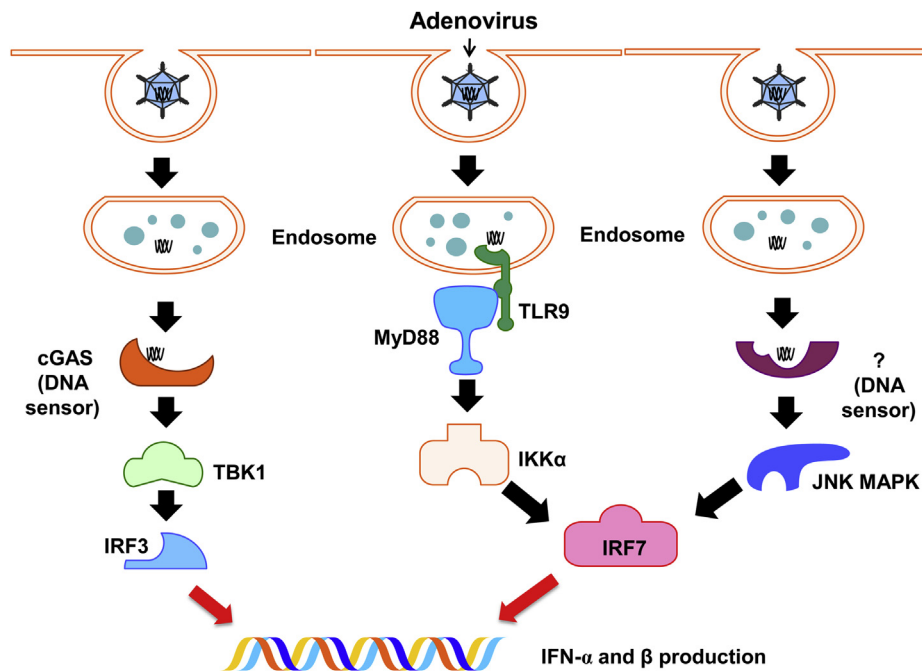


Fig. 2. Three signal pathways of IFN- α and - β production induced by adenovirus stimulation. In the endosome, the genome of adenovirus can be recognized by TLR9 which leads to IRF7 activation in MyD88- and IKK α -dependent pathways. In addition, the genome of adenovirus in cytoplasm can be recognized by cGAS and a suggested unknown DNA sensor which leads to the activation of IRF3 and IRF7, respectively, both leading to the production of IFN- α and β .

widely investigated (Table 1). In 2006, Gao et al. first reported that adenovirus serotype 5-based vectors, which expressed codon-optimized hemagglutinin (HA) gene from A/Vietnam/1203/2004(H5N1) influenza virus were prepared within 36 days after acquiring the virus sequence. Intramuscularly vaccinated mice were fully protected from challenge with the homologous virus. In addition, chickens, which had played a critical role as mediator in transmitting H5N1 to human, were completely protected from lethal challenge after a single subcutaneous immunization of the adenovirus-based H5N1 vaccine [32]. Later, it was reported that a single-dose *in ovo* vaccination with a human adenovirus vector encoding an H5N9 avian influenza virus HA not only fully protected against homologous virus, but also provided 68% protection against H5N1, demonstrating that adenovirus-based H5N1 vaccine could be administered en masse, using available robotic *in ovo* injectors [33]. The combination of different HAs in adenovirus-based H5N1 vaccine was also studied. Hoelscher et al. revealed that mice vaccinated with two adenovirus vaccines encoding hemagglutinins from clade 1 and clade 2 of H5N1 viruses were protected against both viruses with minimal interference against each other [34]. In addition, adenovirus-based multivalent vaccines encoding hemagglutinins from H5, H7 and H9 were broadly protective, and significantly high levels of HA stalk-specific antibodies were induced following immunization with the multivalent vaccines [35]. Scallan et al. reported that vaccination with H5N1 adenovirus-based vaccine by the oral route protected mice and ferret upon lethal H5N1 challenge [36]. In addition, cross-clade neutralizing antibodies were induced in ferrets immunized by the peroral route. It was also reported that DNA

prime-adenovirus boost vaccinations based on NP and M2 from H1N1 were superior to trivalent live attenuated influenza vaccines in protection against heterosubtypic H5N1 influenza challenge [37]. These cited studies show that adenovirus-based H5N1 vaccines are effective in preclinical research.

Clinical application of adenovirus-based H5N1 vaccine was also investigated. A phase I clinical study of H5N1 vaccine based on recombinant adenovirus serotype 4 showed that orally administered vaccine induced significant specific T cell response. After one boost with inactivated H5N1 vaccine, 80% for HAI and 67% for MN were induced in those vaccinated [38]. Antrobus et al. reported that a replication-deficient chimpanzee adenovirus-vectored vaccine expressing the conserved influenza antigens NP and M1 was safe and immunogenic in human [39].

Even though adenovirus-based H5N1 vaccines can induce systemic immunity, most human populations have pre-existing immunity to human adenovirus, and it is believed that pre-existing adenovirus neutralizing antibodies (vector immunity) may negatively impact the immune response to vaccine antigens when delivered by human adenovirus vectors [40]. In naive mice immunized with human adenovirus vector expressing H5N1 HA and boosted with bovine adenovirus vector expressing H5N1 HA, Singh et al. reported that the humoral responses were significantly higher than those with either alone [41]. However, immunity against non-human adenovirus will be induced after immunization with non-human adenovirus, a fact which should be taken into consideration for further immunization. Pandey et al. reported that pre-existing immunity to adenovirus can be overcome by either increasing the vaccine dose or using alternate routes of

Table 1
Selected published adenovirus-based H5N1 vaccines.

Immunization route	Types of adenovirus	Immunogen	Protective effects	Reference
Intramuscular route	Adenovirus serotype 5	HA, NP and M2 from (A/Thailand/1(KAN-1)/2004(H5N1)	100% protection against H5N1 in mice and ferret	[63]
Intramuscular route	Adenovirus serotype 5	M2 consensus sequence	100% protection against H1N1 and 80% protection against H5N1 in mice	[64]
Intramuscular route	Chimpanzee adenovirus simian adenovirus 24	NP from A/Puerto Rico/8/34(H1N1)	Partial protection against H5N1 in mice	[65]
Intramuscular route	Adenovirus serotype 5	HA from A/HongKong/156/1997(H5N1)	100% protection against H5N1 in mice	[66]
Intramuscular route	Bovine adenovirus subtype 3	HA from A/Hong Kong/483/97(H5N1)	100% protection against H5N1 in mice	[41]
Intramuscular route	Adenovirus serotype 5	NP and M2 from A/Puerto Rico/8/34(H1N1)	100% protection against H1N1 and 80% protection against H5N1 in mice	[37]
Intramuscular route	Porcine adenovirus 3 and adenovirus serotype 5	HA from A/Hanoi/30408/2005(H5N1)	100% protection against H5N1 in mice	[67]
Intramuscular route	Adenovirus serotype 5	HAs from A/Vietnam/1203/04(H5N1) and A/Indonesia/05/05(H5N1) and NP from A/Vietnam/1203/04(H5N1)	100% protection against H5N1 in mice	[34]
Intramuscular and subcutaneous route	Adenovirus serotype 5	HA from A/Vietnam/1203/2004(H5N1)	100% protection in mice and chicken	[32]
Intramuscular and intranasal route	Adenovirus serotype 5	NP and M2 from A/PR/8/34 (H1N1)	100% protection against H5N1 in mice	[68]
Intramuscular and intranasal route	Adenovirus serotype 5	HA from A/Hong Kong/213/2003(H5N1) and M1 and M2 from A/Vietnam/1203/2004(H5N1)	100% protection against H5N1 in mice	[50]
Aerosolized intranasal route	Adenovirus serotype 5	HA from A/Indonesia/5/2005(H5N1)	100% protection against H5N1 in ferret	[49]
Intranasal route	Adenovirus serotype 4	HA from from A/Vietnam/1194/2004(H5N1)	100% protection against H5N1 in mice	[69]
Intranasal route	Adenovirus serotype 5	NP from A/PR/8/34 (H1N1)	100% protection against H1N1, 100% protection against H3N2 and 40% protection against H5N1	[51]
Intranasal route	Adenovirus serotype 5	NP from A/PR/8/34 (H1N1) and consensus M2	100% protection against virulent H5N1, H3N2 and H1N1 in mice	[52]
Intraperitoneal route	CAdVax vector	HA, NA, and M1 from A/chicken/Thailand/CH-2/2004 (H5N1) and A/South Carolina/1/18 (H1N1)	100% protection against H5N1 in mice	[70]
In ovo route	Adenovirus serotype 5	HA from A/turkey/Wisconsin/68 (H5N9)	68% protection against H5N1 and 100% protection against H5N2 in chicken	[33]
Oral route	Adenovirus serotype 5	HA from A/Indo/05/2005 (H5N1)	100% protection against H5N1 in mice and ferrets	[36]
Oral route	Adenovirus serotype 4	HA from A/Vietnam/1194/2004(H5N1)	80% for HAI seroconversion and 67% for MN seroconversion in human	[38]

vaccination in mice [42]. However, whether these approaches will work in human needs further investigation.

5. Adenovirus-based mucosal vaccines against avian-origin influenza A H5N1 viruses

Mucosal immunization via nasal route could induce both cellular and humoral immune response generating not only mucosal cytotoxic lymphocytes and IgG, but also sIgA (secretory IgA), which effectively prevented penetration of pathogen inside the cells and its dissemination throughout the human body [43–45]. However, mucous membranes of the upper respiratory tract exhibited low epithelium permeability [46], thus, free antigens, such as proteins and peptides, are unable to induce strong immune response. Adenovirus-based vaccines are indeed satisfactory in mounting mucosal immune response for high transduction efficiency in various cell types, such as epithelial cells or dendritic cells, and the activation of innate immune response [47].

Adenovirus-based mucosal vaccines have achieved promising results. Hoelscher et al. showed that intranasal immunization with adenovirus serotype 5 expressing H5HA induced both humoral and cell-mediated immunity against H5HA, especially interferon-gamma-secreting CD8⁺ T cell response [48]. In order to elicit high levels of potent and durable humoral and cellular responses in the lower airways, Song et al. demonstrated that aerosolized recombinant adenovirus encoding H5HA completely protected ferrets against H5N1 [49]. The results of adenovirus vaccine-based influenza conserved protein are encouraging. Park et al. reported that mucosal immunity induced by adenovirus-based H5N1 vaccine, including H5HA and two conserved influenza proteins, M1 and M2, confers protection against a lethal H5N2 avian influenza virus challenge [50]. Importantly, a single intranasal, but not sublingual, immunization of adenovirus expressing NP from H1N1 provided 100% protection against H1N1, 100% protection against H3N2, and 40% protection against H5N1 [51]. Also, a single-dose mucosal immunization with adenovirus vaccine encoding NP and M2 provided rapid and full protection against virulent H5N1, H3N2 and H1N1 viruses [52]. These results suggest that adenovirus-based vaccines can serve as a platform for HA and influenza conserved proteins through mucosal immunization [53].

Adenovirus-vectored nasal vaccines can also bypass pre-existing immunity against adenovirus, a weakness of all viral vectors [42]. In addition, intranasal immunization of adenovirus-vectored vaccines through the use of a nasal spray would be suitable for mass vaccination by its simplicity and practicality. However, clinical data of mucosal adenovirus H5N1 vaccine in human is not yet available.

6. Conclusions and perspectives

Since 1997, human infection of avian influenza virus other than H5N1 has also been reported [54]. However, H5N1 continues to play a major role in human infection of avian influenza viruses for its high mortality rate. In addition, given

its widespread presence among avians, the potential of human-to-human transmission cannot be ignored. Vaccination is the most effective way to prevent a potential H5N1 pandemic. By its avian origin, high mortality rate, and poor immunogenicity, H5N1 vaccines based on traditional methods, such as those used against seasonal influenza, are unsatisfactory. Therefore, new vaccination methods are needed to compensate for the weaknesses of inactivated H5N1 vaccine.

Adenovirus-based H5N1 vaccines are an attractive alternative since they (i) serve as an adjuvant for H5N1 by activating innate immunity, (ii) are egg-independent, and (iii) can be administered by various routes, especially the nasal route, which endows the vaccine with strong mucosal immunity and the convenience of application for mass vaccination. Therefore, adenovirus-based H5N1 vaccines, especially mucosal vaccine, may be an effective weapon against a new outbreak of H5N1. However, problems, such as pre-existing immunity to human adenovirus and the question of whether immunity against non-human adenovirus after immunization with non-human adenovirus in human will impede further immunization, should be taken into account, and other strategies should be developed. We have previously reported that a recombinant adeno-associated virus (AAV) encoding receptor binding domain of SARS-CoV spike protein induced SARS-CoV-specific IgG antibody with neutralizing activity [55,56]. Other studies suggested that activation of the immune system by the transgene product following AAV-mediated gene transfer might be easier to control than that following adenovirus-mediated gene transfer, indicating that AAV-based vaccines lead to mild immune response [57]. Therefore, the combination of adenovirus and AAV in H5N1 vaccine may result in a synergy that achieves an optimal immune response.

Adenovirus-based vaccines may also apply to the new emerging viruses, such as H7N9 and MERS-CoV [58,59]. Like H5N1, H7N9 is of avian origin [60,61], which indicates that an egg-independent vaccine should be developed. In addition, H7N9 causes more serious infection in older people who respond less effectively to vaccines; thus, immunization with adenovirus-based H7N9 may be more efficacious in persons above the age of 65. An adenovirus-based MERS vaccine, especially the RBD region [62], may be effective and, most importantly, can be administered through both intramuscular and intranasal immunization. In conclusion, adenovirus-based vaccines are promising for both new emerging and reemerging viruses.

Conflict of interest

The authors have declared that no conflict of interests exist.

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