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4 Adenovirus-mediated gene therapy against viral biothreat agents

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Abstract

The common features of viral biothreat agents are highly lethal, easy to grow, and transmissible by aerosol. The examples of viral diseases caused by these agents are smallpox, viral hemorrhagic fevers, and viral encephalitis together with newly emerged viral diseases such as severe acute respiratory syndrome (SARS) and avian influenza. A deliberate release of these agents on general public or a natural outbreak could pose a great threat to the public health and global economy. Vaccine development is an important strategy to thwart the threat of these viral biothreat agents. There is an urgent need to improve

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existing vaccines against these agents and to develop new ones. Gene therapy, which introduces therapeutic genes into mammalian cells to achieve therapeutic effective, has a great potential for use as a defensive strategy against viral biothreat agents. Viral vectors that are developed in gene therapy for delivering therapeutic genes can be used for the development of vaccines against viral biothreat agents. Genes encoding protective antigens of viral biothreat agents can be carried by these viral vectors and be expressed to induce an immune response. The successful and safe use of an adenovirus vaccine to protect military trainees from acute respiratory disease has led to modify adenoviruses as vectors for vaccine development against other viral agents. Human adenovirus serotype 5 (HAd5) is the most commonly used adenovirus serotype for constructing vaccines because of its low virulence, strong induction of both humoral and cellular immunities and ease of growth in cell culture. This chapter will describe the use of HAd5 vector for the development of vaccines against viral hemorrhagic fevers, viral encephalitis, SARS and avian influenza.

Introduction

Vaccination is one of the pillars of biodefence against viral biothreat agents. Besides safety and effectiveness, an ideal biodefence vaccine should be swift to act and simple to administrate. Because it is impractical to vaccinate the public beforehand with vaccines against every possible viral biothreat agents, biodefence vaccines will be mostly used for emergency response in case of a bioterrorism attack or a natural outbreak, in which the infected people and contacts of the infected people will be vaccinated to contain the spread of viruses to the general population.

Most licensed vaccines are made either by chemically inactivated whole viruses or by live attenuated viral pathogens. Although relatively safe to use, vaccines based on inactivated viruses require large doses and multiple injections to obtain a protection. Compared to inactivated vaccines, live attenuated vaccines often give a quick and long lasting protection because these vaccines closely mimic a natural infection. Live attenuated vaccines, however, are vulnerable to genetic reversion to virulent phenotypes [1], which would be catastrophic for viral biothreat agents that often cause the most lethal infections in humans. Therefore, new approaches are needed for the development of vaccines against viral biothreat pathogens.

Gene therapy uses vectors to deliver therapeutic genes to target cells to prevent and treat disease. To this end, several viruses have been modified as vectors for gene delivery [2]. A viral vector is typically a defective virus that is capable of efficiently delivering therapeutic genes into the tissue affected by the illness, but cannot replicate itself and cause disease. Such viral vector

could also be used to deliver genes encoding the protective antigens of viral biothreat agents. The antigens expressed from a viral vector are presented to the immune system, eliciting an immune response against related viral pathogens but without the disease associated with an actual infection [3]. Thus, viral vectored vaccines combine the safety of inactivated vaccines and the rapid induction of strong immunity of live attenuated vaccines.

Several reviews have been published recently regarding the use of viral vectors for the development of vaccines against bioterrorism agents [4-6]. In this chapter, I will focus on the development of HAd5-vectored vaccines to combat the threats of viral hemorrhagic fevers, viral encephalitis, severe acute respiratory syndrome (SARS), and avian influenza.

Overview of human adenoviruses

Human adenoviruses (HAd), which consist of 51 serotypes, represent a large group in the *Adenoviridae* family. HAd are non-enveloped viruses with a double-stranded DNA genome surrounded by an icosahedral (20 faces) protein shell known as the capsid, which is largely formed by hexon, penton and fiber proteins. HAd are stable. For example, human adenovirus serotype 3 can survive up to 10 days on paper under ambient condition and serotype 2 from 3-8 weeks at room temperature [7]. The stability of HAd is an important feature for use as vaccine vectors.

To begin an infection, adenoviral virion attaches through its fiber protein to a cellular receptor such as the coxsackievirus group B and adenovirus receptor [8]. The attachment between the fiber and the receptor allows another viral capsid protein, the penton, to bind to a second cellular receptor, the integrin [9]. Binding to integrin promotes the internalization of adenoviral viron via receptor-mediated endocytosis. Once inside the cell, a sequential disassembly of the capsid proteins allows the virion to escape from the endosome and enter the cytoplasm. The virion subsequently docks to the nuclear pore complex and injects its DNA into the nucleus to initiate viral gene expression [10].

At least 30 different mRNA species are transcribed from the HAd genome, which can be divided into three groups: early transcripts (E1A, E1B, E2, E3, and E4), delayed early transcripts (IX and IVa2), and late transcripts (L1 to L5) [7]. These transcripts are made from both strands of the viral DNA with the rightward reading strand coding for the E1A, E1B, E3, IX, and late transcripts and the leftward reading strand coding for the E4, E2 and IVa2 transcripts.

HAd cause varieties of diseases of the respiratory tract, the eye and the gastrointestinal tract. Among them is *acute respiratory disease (ARD),* a febrile respiratory illness with symptoms similar to influenza. The disease usually occurs in young adults in a closed setting such as military training

camp [11]. In the late 1960s, a live HAd vaccine was developed that was safe and reduced adenovirus-associated ARD by over 90% and ARD-related hospitalization by 50% [12, 13]. Oral administration of the vaccine produced an asymptomatic, intestinal infection while protecting the individual against ARD. The mechanism of induction of respiratory immunity through oral vaccination is unclear. It may involve the generation of serum neutralizing antibody against HAd. Alternatively, the virus might spread from the gut to the respiratory tract to induce local immunity since the live vaccine could be detected in pharynx secretions following oral administration [14].

Modification of human adenoviruses as vaccine vectors

The success of the adenovirus vaccine to protect military trainees from ARD has led to the concept of modifying HAd as vectors for vaccine delivery. HAd5 is most commonly used HAd serotype for constructing vaccines because of its low pathogenesis, ease of growth in cell culture and well-known molecular biology.

Since the size of HAd5 capsid is fixed, the amount of foreign DNA that can be packaged inside the capsid is limited, which is up to 1.2 kb without affecting the stability and infectivity of the virus [15]. To expand the packaging capacity of HAd5, the E1 and E3 coding regions of HAd5 genome are deleted, allowing for the insertion of up to 7.5 kb of foreign DNA. In addition, the deletion of E1 coding region essential for the replication of HAd5 renders the virus replication defective, enhancing the safety of the HAd5 vector. To grow the HAd5 vector, a cell line (HEK293) that provides E1 proteins essential for the vector replication was established from human embryonic kidney cells [16]. Wild-type HAd5, however, could be produced from HEK 293 cells due to the acquisition of E1 coding region by the HAd5 vector through homologous recombination between sequences in the HAd5 vector and HAd5 sequences present in HEK 293 cells. To eliminate wild-type HAd5 during vaccine preparation, PER.C6 and 911 cell lines were generated in which all the sequences homologous to HAd5 vector are deleted [17].

To efficiently stitch a piece of foreign DNA into the HA5 DNA that is about 36-kb in length, Frank Graham and colleagues developed a cloning system based on homologous recombination in mammalian cells [18]. This system involves the construction of a transfer plasmid containing an expression cassette flanked by the HAd5 sequence. The plasmid is co-transfected into cells with a plasmid containing HAd5 genome. Homologous recombination in cells allows the insertion of the expression cassette into HAd5 genome. The limitations of this system are low efficiency of homologous recombination in mammalian cells and the need for screening individual clone for the desired

recombinants. To simplify the cloning of foreign DNA into HAd5 vector, a method based on homologous recombination in bacteria was developed (Figure 1) [19, 20]. Through this method, a full-length infectious plasmid is first obtained in bacteria. Then recombinant HAd5 are produced by tranfection of the plasmid into cells.

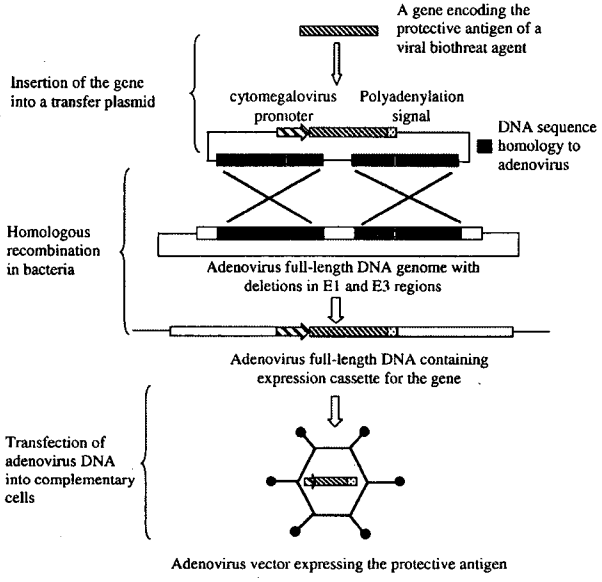

Figure 1. Generation of HAd5 vector expressing theprotective antigens of viral biothreat agents.

Advantages of human adenovirus vectored vaccines against viral biothreat agents

Vaccines against viral biothreat agents require the rapid induction of an immune response and the HAd5 vectored vaccine appears to fulfill this requirement. Vaccines delivered by the HAd5 vector induce swift and durable humoral and cellular immune responses. For instance, rhesus monkeys immunized with a single dose of the HAd5 vector encoding the gp140 envelope protein of human immunodeficiency virus (HIV) generates both gp140 antibodies and $CD8^+$ T lymphocytes specific for HIV [21]. The HAd5-mediated cellular immune response is remarkably durable, which is readily

detected as late as 151 weeks following initial vaccination. In another study, the HAd5 vector expressing the beta-galactosidase protein were used to examine the humoral and cellular immune responses to the protein [22]. A single dose injection of the vectors into mice induced a long-lasting cytotoxic T cell response against beta-galactosidase. An IgG antibody response specific for the beta-galactosidase protein was detected as early as 15 days after injection and remained stable for 6 months without boosting. The magnitude and kinetics of the cellular and humoral responses against beta-galactosidase induced by a single injection are similar to those induced by multiple injections.

The durable and swift immune responses elicted by HAd5 vector may related to its induction of memory T lymphocytes [23, 24] and its ability to deliver large amounts of antigens into the lymphoid tissues [25, 26]. In the lymphoid tissues, foreign proteins that are expressed inside the cells by the HAd5 vector are processed and presented on the cell surface to generate cytotoxic T cell responses. Foreign proteins that are released outside the cells are engulfed by antigen-presenting cells to stimulate B-cells mediated humoral response. In addition, the HAd5 capsid protein itself can act as an adjuvant to enhance immune responses by the induction of costimulators and cytokines such as type I interferons [27, 28]. Therefore, the HAd5 vector fulfills two functions – one as a vaccine carrier and another as an adjuvant for the encoded proteins.

The HAd5 vectored vaccine also induces a mucosal immunity [29]. The mucosal surface is the first line of defence against the invasion of microorganisms. One of major weapons in the mucosal defence system is the secretory IgA antibody, which blocks the attachment of viruses to the mucosal surface. Most viral biothreat agents enter the body through the mucosal surface of the respiratory tract. Therefore, vaccines eliciting mucosal immunity should limit the spread and replication of viruses at the port of entry. HAd5 infect mucosal cells of the respiratory tract and vaccine vectors made from HAd5 can deliver antigens to mucosal surface and induce the mucosal immunity. This has been demonstrated in cotton rats intranasally immunized with the HAd5 vector expressing the bovine herpesvirus glycoprotein [30]. These animals produced a strong mucosal IgA antibody response specific for the glycoprotein and were protected from the challenge of bovine herpesviruses. Similarly, when mice were intranasally immunized with an HAd5 vector expressing the rabies virus glycoprotein, they developed both serum antibody against rabies virus and secreted specific IgA antibody in the genital and intestinal tracts [31].

Similar to the adenovirus vaccine used for the control of outbreaks of ARD in military trainees, the HAd5 vectored vaccine can be formulated as oral vaccines. Oral administration of the HAd5 vectored vaccine would allow

rapid, mass vaccination during a natural outbreak or bioterrorism attack. Fooks and colleagues demonstrated that oral administration of HAd5 vectored vaccines expressing the hemagglutinin of measles virus elicited a significant protective response in mice against the challenge of measles virus [32]. This protection involves a cell-mediated immune response. Further study demonstrated that oral delivery of the HAd5 vectored vaccine is safe and well tolerated in animals [33].

Human adenovirus vectored vaccines against viral biothreat agents

Viral hemorrhagic fevers

Viral hemorrhagic fevers are characterized by fever, a bleeding diathesis and circulatory shock [34]. The disease can be caused by a group of viruses from families of *Arenaviridae*, *Bunyaviridae*, *Filoviridae*, and *Flaviviridae*. Ebola and Marburg viruses from the filovirus family are the most extensively studied viruses of viral hemorrhagic fevers. These viruses are highly lethal with a mortality rate of 90%. Ebola virus (EBOV) consists of four distinctive strains: *Sudan Ebolavirus* (SEBOV), *Zaire Ebolavirus* (ZEBOV), *Reston Ebolavirus* (REBOV), and *Ivory Coast Ebolavirus* (ICEBOV). ZEBOV and SEBOV attribute to all human outbreaks and death [35]. EBOV is transmitted through direct contact with infectious blood, secretions, or other body fluids. Although the first case of EBOV outbreak was reported in 1976, a potential reservoir of this virus is still unknown. A recent survey of wild animals in Gabon and the Republic of the Congo showed fruit bats can carry EBOV without showing any signs of infection, indicating that these animals may act as a reservoir for this deadly virus [36].

EBOV infection is a large concern for travelers, military personnel and people living in Ebola endemic areas. The virus also presents a threat to the public as a potential biothreat agent and is listed, together with smallpox and anthrax, as category "A" bioterror agents by the Centers for Disease Control and Prevention (CDC) (http://www.bt.cdc.gov/agent/agentlist-category.asp).

In the last several years, vaccine candidates based on HAd5 vector have been developed for EBOV and shown a great promising to combat the EBOV infection. Sullivan et al constructed an HAd5 vector encoding the glycoprotein (GP) from the ZEBOV [37]. A regimen of DNA immunization and boosting with the HAd5 vector induced cellular and humoral immune responses in non-human primates. These vaccinated animals survived a lethal dose challenge of a highly pathogenic, wild-type ZEBOV virus with no clinical signs of infection for more than six months and no detectable virus in the blood. In contrast, unvaccinated animals died less than one week after the challenge. This regimen of DNA priming and the HAd5vectored vaccine boosting, however,

requires more than six months to complete the vaccination. The same research team later demonstrated that vaccination of non-human primates with a single dose of HAd5 vectors encoding GP and nucleoprotein generated Ebola-specific CD8(+) T-cell and antibody responses and protected these animals from either a low or high dose challenge of the ZEBOV [38]. A recent study showed that an HAd5 vector expressing GP alone is sufficient to protect non-human primates against a lethal challenge of ZEBOV and that a dose of as low as 10^{10} HAd5 vector particles is effective [39].

One of problems for developing Ebola vaccines is that the vaccine made against one strain of EBOV is not effective against other strains [40]. To overcome this problem, HAd5 vector has been used to make the bivalent vaccine that is able to protect against different strains of EBOV. Vaccination of mice with a bivalent HAd5 vectored vaccine co-expressing GPs of SEBOV and ZEBOV generated antibody and cell-mediated immune responses specific to both EBOV strains [41]. Challenge of the vaccinated mice with ZEBOV showed a 100% protection. Due to the lack of a mouse model for SEBOV, the results for the SEBOV challenge are unavailable. However, a nonhuman primate model for SEBOV has been established [40] and it will be interesting to see if the bivalent vaccine is also effective against the infection of SEBOV.

Vaccine candidates based on the HAd5 platform are also developed for cross-protection against different strains of Marburg virus (MARV), another deadly virus from the filovirus family. Recombinant HAd5 expressing the viral GPs from either the Ci67, Ravn or Musoke strain of MARV were constructed [42]. Mice given the recombinant viruses generated both antibodies and cytotoxic T lymphocytes specific to Musoke strain GP and Ci67 strain GP, respectively. Antibody responses were also shown to be cross-reactive across the MARV strains but not cross-reactive to EBOV. A bivalent HAd5-vectored vaccine was made to express the GP fusion protein derived from both Musoke and Ci67 strains of MARV [43]. Vaccination of mice and guinea pigs with the vaccine led to efficient production of specific antibodies against MARV. Guinea pigs injected with the vaccine are 100% protected against lethal challenges of the Musoke, Ci67 and Ravn strains of MARV.

Viral encephalitis

Viral encephalitis is characterized by fever, persistent headache, confusion or agitation, difficulty walking, and seizures. In severe cases, persistent neurological damage and death may occur. Many viruses can cause viral encephalitis. For biodefence alphaviruses from *Togaviridae* family, including Venezuelan equine encephalitis virus (VEEV), eastern equine encephalitis virus (EEEV) and western equine encephalitis virus (WEEV), are the most important because these viruses are relatively stable in natural environment, they are highly infectious by aerosol, they are easy to produce in large

quantities, and they can be used as either incapacitating or lethal agent [44]. The CDC has classified these viruses as category "B" bioterrorism agents.

VEEV, EEEV, and WEEV cause encephalitis in humans with different mortality rates [45]. EEEV is the most virulent with case fatality rate of 30% to 40%. In fatal cases, patients usually die within 2 to 10 days after the onset of encephalitis. Compared to EEEV, WEEV appears to be less virulent with case fatality rate of 10%. The fatal encephalitis often occurs in infants and in young children. VEEV usually cause an acute incapacitating illness with fever, chills, headache, muscle pain, diarrhea and vomiting, which appear 2 to 5 days after exposure to the virus. The encephalitis occurs usually in children with a case fatality rate of less than 1%.

Current biodefence against VEEV, EEEV, and WEEV are inadequate. No antiviral drugs are available and treatment only focuses on easing clinical symptoms. A live attenuated VEEV vaccine, designated as TC-83, was developed by serial passage of a virulent VEEV Trinidad donkey strain in fetal guinea pig heart cells. The vaccine protects laboratory workers from infection [46]; however, 15% to 30% of vaccine recipients developed fever, malaise and headache and half of these are so severe that bed rest is required. Investigational vaccines derived from killed EEEV and WEEV are available only for laboratory workers at risk of exposure to these viruses. These vaccines require multiple injections and annual boosters. Therefore, new approaches are urgently needed to develop anti-viral drugs and vaccines for the alphaviral encephalitis.

Alphaviruses share common structural elements: an envelope containing glycoprotein-bearing lipid bilayer, a capsid, and inside the capsid, a positive-sense, single-stranded RNA genome [47]. Similar to the cellular mRNA, the alphavirus RNA is capped with a 7-methylguanosine at its 5' terminus and is polyadenylated at its 3' terminus (Figure 2). The 5' two thirds of the viral RNA genome encodes the nonstructural proteins required for transcription and replication of the viruses. The 3' one third of the viral genome encodes the capsid and envelope glycoproteins. The envelope proteins are encoded by a subgenomic mRNA and are derived by proteolytic cleavage of the E3-E2-6K-E1 polypeptide [48]. E2 is initially synthesized as a precursor protein, PE2, which consists of E3 and E2, followed by cleavage of E3 from PE2 by furin-like protease activity. Subsequently, the E2 forms heterodimer with E1 and the complex is transported to the cell surface to become the envelope of the virus. Studies from VEEV have demonstrated that the envelope proteins are the major determinants for the induction of immune protection against the virus [49-51].

HAd5-vectored vaccines expressing the envelope glycoprotein (E3-E2-6K) of VEEV vaccine strain TC-83 have been made and tested for their ability to protect mice against airborne challenge of different strains of VEEV [52].

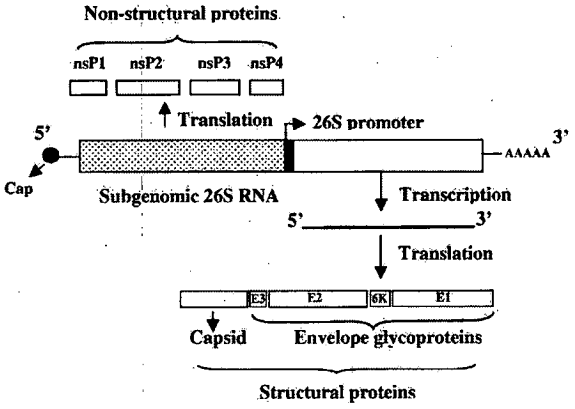

Figure 2. Organization of the alphavirus genome and synthesis of viral proteins.

Mice, intranasally vaccinated three times with the HAd5-based VEEV vaccine, survived the challenge of Trinidad donkey strain of VEEV, which is the original virulent virus used for making the live TC-83 vaccine. The HAd5-based vaccine, however, conferred less protection against other VEEV strains. Therefore, the gene encoding envelope glycoproteins from other strains may be needed to incorporate into the HAd5 vector for the protection against multiple VEEV strains. Because DNA vaccines encoding envelope proteins of VEEV only provided partial protection against the airborne challenge of VEEV, an recombinant HAd5 vaccine expressing the E3-E2-6K of VEEV was used in a prime-boost vaccination regimen [53]. Boosting with the HAd5-based vaccine after DNA vaccination enhanced Th2-type IgG response and neutralizing antibody production. In addition, the prime-boost regimen significantly increased protection against the airborne challenge of VEEV.

Currently, adenovirus-vectored vaccine candidates against WEEV and EEEV have not been reported. Similar to VEEV, WEEV consists of multiple strains. A recent study in a mouse lethal challenge model demonstrated that these strains can be classified into two a high-virulence group, consisting of strains California, Fleming and McMillan, and a low-virulence group, consisting of strains CBA87, Mn548, B11, Mn520 and 71V-1658 [54]. A DNA vaccine was constructed expressing both the capsid and envelope glycoproteins of 71V-1658 strain of WEEV [55]. The efficacy of the vaccine was tested in mice and showed 100% protection against the challenge of WEEV 71V-1658 strain. However, only 50% protection was achieved against the high-virulence strain Fleming. No antibody response could be detected after the DNA vaccination. However, a cytotoxic T lymphocyte against the E2

envelope glycoprotein was detected, suggesting that the protective immunity conferred by the DNA vaccine is related to a cell-mediated immune response.

SARS

SARS is an emerging infectious disease, which first appeared in China's Guangdong province in late 2002. Within weeks, the viruses spread to 29 countries and resulted in more than 8,000 cases with more than 800 deaths worldwide [56, 57]. Although the epidemic was officially contained by July 2003 through strict isolation of patients, it is not clear whether the virus will re-emerge in humans. Because of its ease of dissemination and potential for high morbidity and mortality, SARS-associated coronavirus (SARS-CoV), the pathogen of SARS, is considered as a potential bioterrorism agent (http://www.bt.cdc.gov/agent/agentlist-category.asp).

SARS-CoV was transmitted among humans by droplets and fomites. The disease began with an influenza-like illness with headache, muscle pain, and fever, often followed by acute atypical pneumonia, respiratory failure, and death. Patients that recovered from SARS develop neutralizing antibodies against SARS-CoV. The antibodies became detectable at 5-10 days after the onset of symptoms, and their levels peaked at 20-30 days and then were sustained for more than 150 days [58].

The virion of SARS-CoV contains a 29-kb-long, positive-strand RNA, which binds to the nucleocapsid (N) protein to form a helical capsid [57]. The capsid is contained within a viral membrane that consists of the viral surface spike protein (S), envelope protein (E), and membrane glycoprotein (M). The S protein, forming the peplomers on the virion surface, is responsible for the corona- or crown-like morphology of the virus. During the viral infection, the S protein mediates the binding of virus to a cellular receptor, angiotensin-converting enzyme 2, resulting in the entry of the virus into host cells [59, 60]. Monoclonal antibodies raised against the S protein potently neutralize SARS-CoV infection and reduced disease severity and the number of viruses in animals challenged with SARS-CoV [61-63]. These studies indicate that the S protein could be a major candidate for vaccine development [64].

The N protein of SARS-CoV, which plays important roles in viral pathogenesis, replication, and RNA packaging, is another potential candidate for vaccine development [65]. The N protein is one of the immunodominant antigens of SARS-CoV and the antibodies to the N protein were highly detectable in SARS patients [66]. Although these antibodies have no virus-neutralizing activity in vitro, there is evidence that the protein may provide in vivo protection by the induction of SARS-CoV-specific $CD8^+$ T cells and a long persistence of memory T-cell response [67, 68].

HAd5 vectors expressing the S or N proteins of SARS-CoV have been evaluated as vaccine candidates against SARS. A replication-defective HAd5 vector expressing the SARS-CoV N protein has been constructed [69]. Mice immunized with the vector generated potent SARS-CoV-specific humoral and T cell-mediated immune responses. However, a recent study showed that compared with a whole killed vaccine, HAd5-vectored SARS vaccines are less effective in inhibiting SARS-CoV replication in the murine respiratory tract [70]. Titres of serum neutralizing antibodies induced by the HAd5-vectored vaccines were significantly lower than those induced by the killed vaccine, which could be related to the less protection afforded by the HAd5-vectored vaccines.

HAd5-vectored SARS vaccine was also tested in a large animal model. Gao et al constructed recombinant HAd5 encoding the S or N protein of SARS-CoV Urbani strain and tested immunogenicity of these vaccine candidates in monkeys [71]. The vaccinated monkeys all had an antibody response against the S protein and a T-cell response against the N protein. The antibody isolated from vaccinated monkeys neutralized SARS-CoV in vitro.

One of safety issues regarding the development of SARS vaccines is the vaccine-induced infection-enhancing antibodies and inflammatory responses [72]. Antibodies that neutralized the S protein have been found to enhance the entry of SARS-CoV into the cells [73]. Ferrets vaccinated with recombinant vaccinia viruses expressing the S protein of SARS-CoV developed a more rapid and vigorous neutralizing antibody response than control animals after challenge with SARS-CoV; however, the vaccinated animals also showed stronger inflammatory responses in liver tissue [74]. These results warrant further investigation of the safety of the HAd5-vectored SARS vaccines in large animal models.

Avian influenza

Avian influenza is another newly emerging infectious disease that poses a threat to public health. The disease ranges from an asymptomatic or mild infection to an acute, fatal infection in avian species, such as chickens, turkeys, and migratory waterfowl. The causative agent of avian influenza, the H5N1 avian influenza A virus, caused outbreaks in domestic poultry markets in Hong Kong in 1997, and subsequent poultry-to-human transmission of the virus resulted in 18 confirmed human cases with six deaths [75-77]. Since December 2003, the H5N1 infections in animals have been reported in Asia, Africa, the Pacific, Europe, and the Near East. By June, 2006, the virus had killed 130 of the 228 infected humans (http://www.who.int/csr/disease/avian_influenza/country/cases_table_2006_06_20/en/index.html).

Because the H5N1 infection among domestic and wild birds is not expected to diminish in the near future, human cases resulting from direct contact with infected birds will likely continue to occur.

The H5N1 virus causes a range of clinical outcomes in humans, from mild infections to severe respiratory illness and death. Complications in severe cases include acute respiratory distress syndrome, leukopenia, lymphopenia, hemophagocytosis, and multiorgan dysfunction. The wider tissue tropism of the virus also results in infections of gastrointestinal system, liver, and kidney.

Recently, a rare instance of human-to-human transmission was reported in a large family cluster in Indonesia [78]. However, there are no signs that the virus is becoming more virulent The H5N1 virus attaches predominantly to type II pneumocytes, alveolar macrophages, and nonciliated bronchiolar cells in the human lower respiratory tract, which may explain the localization and severity of H5N1 viral pneumonia in humans [79]. On the molecular level, human and avian influenza viruses use different cellular receptors for binding to host cells. The human virus preferentially recognizes sialic acid linked to galactose by an α-2,6 linkage (SAα2,6Gal) that is present mainly on the surface of epithelial cells in the bronchi; on the other hand, the avian virus preferentially recognize sialic acid linked to galactose by an α-2,3 linkage (SAα2,3Gal) mainly on alveolar cells [80]. Because the H5N1 viruse can replicate efficiently only in cells in the lower region of the respiratory tract, this could explain why human-to-human transmission of the virus so far has been rare. There are concerns, however, that genetic reassortment between human and avian influenza A virus genes could render the virus to bind SAα2,6Gal that is present in the upper region of the respiratory tract, where the virus can grow in a large quantity and readily transmit among huamns by sneezing and coughing to trigger a pandemic.

Using mathematical simulation models, two research groups demonstrated that prompt vaccination could significantly reduce the severity of a possible influenza pandemic [81, 82]. Currently, most influenza virus vaccines consist of formaldehyde-inactivated viruses grown from embryonated eggs [83, 84]. This manufacturing process is time-consuming. If it is used for making vaccines against the possible pandemic strain of H5N1, it may require many months before such vaccines could be produced in a large quantity and made avialable for the mass immunization in humans. Since the traditional method for the development of influenza vaccines is unlikely to be effective for prompt vaccine production during a pandemic, attemps have been made to develop alternative strategies such as reverse genetics [85, 86] and recombinant DNA vaccines [87, 88] for vaccine production.

Rapid production of recombinant DNA vaccines based on HAd5 vectors has been reported. Andrea Gambotto and colleagues constructed a HAd5

vector expressing the full-length haemagglutinin (HA) gene from an H5N1 virus isolated in Vietnam during the 2003–2005 outbreak [89]. The time for the construction of such HAd5 vector only took 36 days. Mice immunized twice with the vector were fully protected from challenge with a lethal dose of H5N1 virus. Moreover, it required only a single-dose subcutaneous vaccination to completely protect chickens from an intranasal challenge that killed all unvaccinated chickens within 2 days. A similar HAd5-vectored H5N1 vaccine was made by researchers from the CDC [90]. When compared to a recombinant subunit H5N1 vaccine, the HAd5-based vaccine induced a three- to eight-fold increase in H5N1 specific cellular immune response. These studies demonstrate the feasibility of using HAd5 vectors for the development of H5N1 vaccines in the event of the pandemic because their production is fast and does not rely on fertilized eggs.

Obstacle of human adenovirus vecotred vaccine against biothreat agents

A major obstacle of using HAd5 for making vaccines against biothreat agents is the preexisting anti-vector immunity in human populations. An analysis of serum samples from humans indicated that prevalence of neutralization antibodies specific to HAd5 can be as high as 85% of tested samples [91]. The anti-vector immunity can limit the effectiveness of HAd5-vectored vaccine and prevent subsequent use of the same vector for booster [92, 93]. In addition, animal study showed that preexisting immunity increased liver toxicity of HAd5 vector [94].

Several strategies have been developed to overcome the problem of preexisting immunity to HAd5 [95]. One is to use other HAd serotypes that have low seroprevalnce in the human population or to use non-human adenoviruses. For example, HAd serotype 35, which has less than 20% seroprevalence in the human population [91], was modified into a vaccine vector. Gene transfer with the vector was not impaired by the preexisting anti-HAd5 immunity [96]. Adenoviruses isolated from a wide range of animal species have been explored as vaccine delivery vectors [97]. Replication defective adenoviral vectors based on chimpanzee adenoviruses were developed to express protective antigens [98]. Despite the presence of the preexisting anti-HAd5 antibodies, these vaccines stimulated robust T and B cell responses to ZEBOV and SARS-CoV in mice and completely protected the mice and guinea pigs from a lethal challenge of ZEBOV [99, 100].

Another strategy to overcome the problem of the preexisting immunity to HAd5 is to modify the adenoviral hexon protein [101]. Because the hexon is the main capsid protein responsible for anti-HAd5 immunity [93, 102], molecuar modification of the hexon should cirvcumvent this problem. Diane

Roberts and colleagures recently reported the construction of the chimeric HAd5 vector in which the antigenic regions of the HAd5 hexon protein were replaced with the corresponding regions from HAd serotype 48, which has a low seroprevalence in humans [103]. The Gag protein of simian immunodeficiency virus expressed by the chimeric vector showed similar immunogenicity in mice and rhesus monkeys to that expressed by parental HAd5 vector. The presence of the high levels of the preexisting anti-HAd5 immunity did not affect the immunogenicity of the chimeric vector. This report demonstrated that key neutralizing epitopes on the surface of adenoviral capsid proteins can be removed to make recombinant adenoviral vectors resistant to the neutralization by preexisting anti-vector immunity.

Finally, HAd5 vector can be insulated from the neutralization of preexisting anti-vector immunity through encapsulation. Yotnda and co-workers encapsulated HAd5 vector using bilamellar cationic liposomes [104]. The encapsulated HAd5 vector resisted the neutralization by anti-HAd5 antibodies. Encapsulation of adenovirus vectors into biodegradable alginate microparticles also can circumvent the vector-specific immune response [105].

Conclusion

Vaccines are the cornerstone for the protection of the public from the attack of viral biothreat agents. There is an urgent need to improve existing vaccines against these agents and to develop new ones. An ideal vaccine against viral biothreat agents should be safe, easy to deliver, provide long-lasting protection, and require only one or a few doses to be effective. A vaccine platform based on adenovirus vectors could fulfill most of these requirements. Adenovirus vectors are relatively safe. Vaccines delivered by adenovirus vectors induce mucosal immunities that prevent replication of viral biothreat agents at the site of entry. Adenovirus-vectored vaccines are easy to produce and manufacture as an oral vaccine. Promising results have been demonstrated in animal models for these vaccines against several viral biothreat agents; however, problems of preexisting immunity to adenovirus vectors need to be solved before adenovirus-vectored vaccines can be used in humans.

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