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A recombinant trivalent vaccine candidate against human adenovirus types 3, 7, and 55

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ABSTRACT

Human adenoviruses types 3 (HAdV-3), 7 (HAdV-7) and 55 (HAdV-55) are major pathogens of acute respiratory infections (ARI) in children and adults. More than one type of HAdV can infect patients simultaneously, and the infections are sometimes fatal. However, there is currently no vaccine approved for general use in children and adults. Thus, development of a multivalent HAdV vaccine to combat HAdV infection becomes imperative. In this study, we constructed a new recombinant trivalent human adenovirus vaccine (rAdMHE3-h55), which expresses the hexon protein of HAdV-55 in the E3 region of rAdMHE3, a previously prepared bivalent vaccine candidate against HAdV-3 and HAdV-7. The results of in vitro neutralization assays indicate that rAdMHE3-h55 can induce the production of neutralizing antibodies against HAdV-3, HAdV-7, and HAdV-55 in mice. Furthermore, immunization with the recombinant trivalent vaccine candidate completely protected the mice challenged with HAdV-3, HAdV-7, orHAdV-55, respectively, showing lower lung viral loads and less lung Pathological changes was compared with those in unvaccinated mice. The current findings contribute to the development of a new adenovirus vaccine candidate and also advance this construction method for the generation of recombinant adenovirus vaccines. In conclusion, our recombinant trivalent vaccine rAdMHE3-h55 can provides protection against challenge with HAdV-3, HAdV-7, or HAdV-55 in mice. Future work of optimizing this vaccine candidate may lead to a more effective way of preventing respiratory diseases caused by common human adenoviruses.

1. Introduction

Human adenovirus (HAdV), which was first isolated in the Netherlands in 1953 [1], plays an important role in abroad spectrum of illnesses in humans [2–4]. Common clinical symptoms of HAdV infection include pneumonia, sore throat, acute otitis media, and fever, and the majority of cases have gastrointestinal symptoms. Previous work indicates that 9% and 3.2% of respiratory tract infections in children and adults, respectively, are caused by HAdV [5,6]. In 2011, there was a communitywide adenovirus outbreak in Taiwan, and the infection rate of adenovirus among all respiratory viruses increased from a baseline of 0–5% up to 10% [7].

Adenoviruses are non-enveloped, icosahedral, double-stranded DNA viruses. There are at least 69 known types of HAdVs, and they are divided into seven groups named A–G [8]. The B1 adenoviruses HAdV-3 and HAdV-7, which cause acute respiratory diseases, have occurred epidemically and caused outbreaks in North America, Asia, and Europe [9–14]. HAdV-3 accounted for most (74%) of the HAdV cases, but HAdV-7 was also detected; although HAdV-7 had accounted for only 0.3% of cases in 2008, this percentage increased to 10% in 2011 [15–17]. HAdV-55 was first isolated in 2005, and this pathogen caused outbreaks of re-emerged ARD in Singapore that year [18]. In 2006, HAdV-55 caused an outbreak in Shanxi Province of China [19,20], after which this pathogen apparently re-emerged among military and civilian populations in many provinces of China [21–25]. A study found that HADV-3, HAdV-7, and HAdV-55 were the main types of adenovirus involved in this outbreak [26–30].

Vaccination is the most effective method by which to prevent viral infection. Notably, natural infection with adenovirus can produce persistent, type-specific immunity, and the resulting anti-adenovirus antibodies can provide long-term and stable protection against HAdVs of the same type. Until now, the American military is the only group in the world using oral enteric HAdV-4 and

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HAdV-7 vaccines. After the U.S. military stopped administering these vaccines in 1999, the infection rate of adenovirus increased, so they resumed using them in 2011 [31–33]. The successful application of HAdV-4 and HAdV-7 vaccines in the U.S. military has proven the effectiveness of immunization with an adenovirus vaccine. However, the vaccine used by the U.S. military contains live attenuated strains, which may increase the risk of recombination of the vaccine strains to create more violent and pathogenic viruses. Thus, it is critical to develop a polyvalent adenovirus vaccine that is economical, efficacious, and safe because this type of vaccine will be important for the prevention and treatment of adenovirus infections, especially in crowded, susceptible populations [34].

Using genetic engineering technology to develop new HAdV vaccines are being vigorously explored in recent years. Most studies in this field have focused on the development of recombinant bivalent vaccines [35]. The HAdV capsid protein hexon is one of the major antigens which can induce neutralizing antibodies (NAbs). Two strategies are commonly used for generating recombinant adenovirus vaccines: (1) change the highly variable region (HVR) to construct a recombinant hexon protein, and (2) expression of hexon in the E3 region of another HAdV genome. Using the first strategy, we once successfully constructed a recombinant HAdV-3/HAdV-7 bivalent vaccine candidate named rAdMHE3 by replacing the predicted epitopes within the HAdV-3 hexon with the corresponding epitopes from the HAdV-7 hexon [35]. In the present study, we constructed a recombinant trivalent HAdV vaccine candidate, rAdMHE3-h55 that expresses the hexon of HAdV-55 in the E3 region of the bivalent vaccine rAdMHE3. Our in vitro and in vivo studies show that rAdMHE3-h55 could not only induce neutralizing antibodies against HAdV3, HAdV7 and HAdV55, but also provide protection against challenge with HAdV-3, HAdV-7, or HAdV-55 in mice.

2. Materials and methods

2.1. Viruses and cells

All the adenoviruses were cultured in AD293 cells, which were purchased from ATCC and subsequently kept in our lab. All plasmids and viruses used in this study (described below) were maintained in our laboratory, was provided by State Key Laboratory of Respiratory Disease. AD293 cells were cultured in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, CA, USA) with Penicillin-G 100 U mL^{-1}, streptomycin 100 μg mL^{-1} and 10% (v/v) fetal bovine serum (FBS) (Invitrogen). Adenovirus was purified by standard CsCl gradient centrifugation. HAdV-3 GZ-1 (GenBank No. DQ099432), a clinical isolate in our lab, was cultured in AD293 cells. HAdV-7-CQ1198 (GenBank no. JX625134.1) was provided by the Children's Hospital, Chongqing Medical University (Chongqing, China). HAdV-55-Shanxi-Y16 (GenBank No. KF911353.1) was kindly provided by Dr. Lin Chen of Guangzhou Medical University (Guangzhou, China). *Escherichia coli* BJ5183 and Top 10cells were purchased from Takara (Dalian, China).

2.2. Generation of the trivalent vaccine strain rAdMHE3-h55 against HAdV3, HAdV7 and HAdV55

The recombinant trivalent adenovirus vaccine strain rAdMHE3-h55 was generated on the basis of a recombinant bivalent HAdV vaccine strain against both HAdV 3 and HAdV 7, which was produced previously in our lab [35]. The detailed procedures were described as follows.

2.2.1. Construction of the bivalent virus vector pBRAd-MHE3

Construction of pBRAd-MHE3 was described in detail by Qiu et al. [35]. The pBRAd-MHE3 encodes the genome of HAdV3 GZ-01 with its hexon HVR5 gene fragment replaced by the corresponding gene fragment of HAdV7, an enhanced green fluorescent protein (GFP) gene, and with the E3 region deletion (Fig. 1A). Before transfection, the plasmid pBRAd-MHE3 was digested with Rsr II and purified using a gel extraction kit (Takara) according to the manufacturer's instruction.

2.2.2. Construction of pSKE3LR-h55 vector

To facilitate the hexon gene fragment of HAdV55 integrated into pBRAd-MHE3 vector, the shuttle plasmid pSKE3LR was constructed as described preciously [36]. Briefly, the PCR generated E3L and E3R fragments was cloned into pBluescript II SK, and digested with Age I and EcoR I before sub cloning. The whole HAdV-55 hexon (h55) gene fragment was amplified from HAdV-55-Shanxi-Y16 by

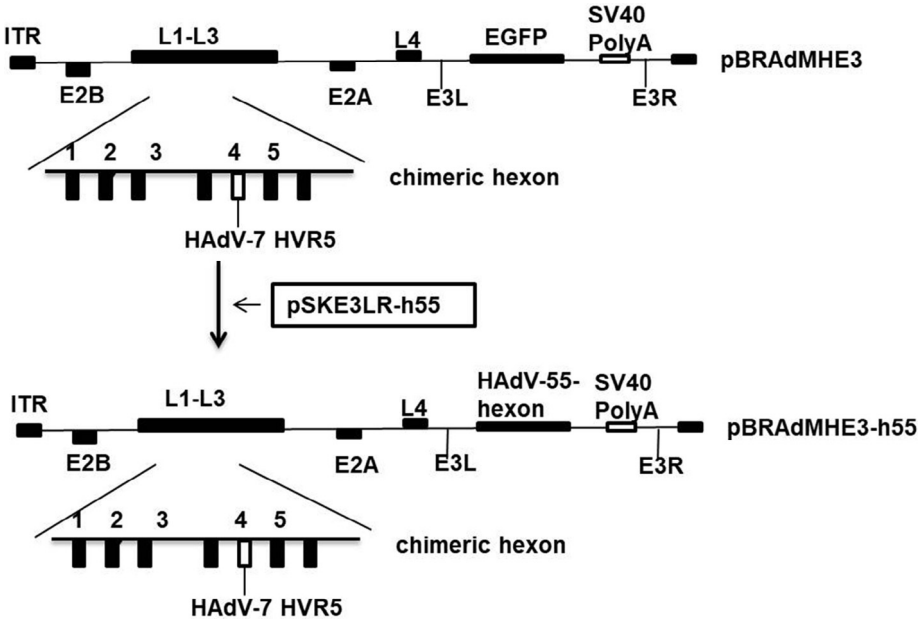

Fig. 1. Schematic depiction of the construction of pBRAdMHE3-h55. ITR, inverted terminal repeat.

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