Vaccine xxx (2018) xxx–xxx

Contents lists available at ScienceDirect

Vaccine

journal homepage: www.elsevier.com/locate/vaccine

# Penton base induces better protective immune responses than fiber and hexon as a subunit vaccine candidate against adenoviruses

Kai Hu [a,1], Ming Fu [a,b,1], Xinmeng Guan [a,b], Di Zhang [a,b], Xu Deng [a,b], Yingying Xiao [a,b], Rui Chen [a], Huan Liu [a], Qinxue Hu [a,*]

[a] State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, Wuhan 430071, China
[b] University of Chinese Academy of Sciences, Beijing 100049, China

ARTICLE INFO

*Article history:*
Received 6 April 2018
Received in revised form 26 May 2018
Accepted 28 May 2018
Available online xxxx

*Keywords:*
Adenovirus
Vaccine
Immunogenicity
Fiber
Hexon
Penton base

ABSTRACT

Human adenoviruses (AdVs) have been extensively studied as vectors for gene therapy and vaccination. However, little attention has been paid to AdV vaccine development and treatment. Currently, there is a lack of information concerning the immunogenicity of AdV major capsid proteins. Here, using AdV7 as a model, we compared the immunogenicity and protection efficacy of its three major capsid proteins in DNA forms, pFiber, pHexon and pPenton, on a mouse model. Quantification of antigen-specific antibodies showed that pHexon induced highest IgG in sera while pPenton induced highest IgA in respiratory mucosae. A neutralization assay revealed that pPenton elicited the highest neutralizing activity against the homologous AdV7 in both sera and bronchoalveolar lavages (BALs). In addition, sera and BALs from mice immunized with either of the three constructs had cross-neutralizing activities against the heterologous AdV3. Furthermore, pHexon and pPenton induced Th1/2- and Th1/17-biased cellular responses, respectively, with pFiber being the weakest in the induction of cellular responses. Virus challenge assay showed that, pPenton group had the fastest virus clearance rate, followed by pFiber and pHexon groups. Likewise, the inflammation in the lung was well controlled in pPenton group against virus challenge. Taken together, our data demonstrate that penton base is better than fiber and hexon as a vaccine candidate against AdVs. Our findings provide important information for the development of subunit vaccines against AdVs.



## 1. Introduction

Human adenoviruses (AdVs) are double-stranded non-enveloped DNA viruses that contain 51 serotypes and over 70 genotypes [1]. AdVs usually infect upper and lower respiratory tracts, gastrointestinal tract and conjunctiva, and cause mild symptoms like febrile respiratory illness (FRI), pharyngoconjunctival fever, gastroenteritis and diarrheal illness [2–4]. AdV infections in the majority of population are usually self-limited while dissemination and fatality incidents are rare. However, for children (<4 years old), immunocompromised population (e.g. HIV infected patients, organ transplant recipients) and population in closed settings (e.g. military recruits), AdV infection could pose serious health problems. Over 80% of AdV infection cases were diagnosed in young children, and these account for 5–10% of pediatric respiratory tract infections (RTIs) [5,6]. Among unvaccinated military recruits worldwide, more than 50% of FRI and pneumonia cases are associated with AdV infection [7]. While in immunocompromised population, AdV infection could cause high rates of severe respiratory failure and even pneumonia-related fatality incidents [8,9].

In contrast to the frequent outbreaks of AdV epidemics, the options for AdV prevention and treatment are limited. To date, there are no licensed drugs specifically designed to treat AdV infections. Cidofovir, a cytosine nucleotide analogue inhibiting DNA polymerase, has been the preferred treatment for severe AdV infections [10]. For prevention, the only vaccine available is a live non-attenuated vaccine formulated with live AdV-4 and AdV-7, which is only intended for military use in the United States. Although this

*Abbreviations:* AdV, adenovirus; BAL, bronchoalveolar lavage; CAR, Coxsackie and adenovirus receptor; CBA, cytometric bead array; FFU, fluorescence forming unit; FRI, febrile respiratory illness; NAb, neutralizing antibody; RTI, respiratory tract infection.

* Corresponding author at: State Key Laboratory of Virology, Wuhan Institute of Virology, Chinese Academy of Sciences, 44 Xiaohongshan Zhongqu, Wuhan 430071, China.
*E-mail address:* qhu@wh.iov.cn (Q. Hu).

[1] These two authors contributed equally to this work.

https://doi.org/10.1016/j.vaccine.2018.05.118
0264-410X/

live vaccine is efficacious, vaccine recipients and their excrements could contain live AdVs, which are contagious during immunization period [11,12]. Consequently, the development of novel preventive formulations with higher safety like subunit vaccines against AdVs is needed.

AdVs have been extensively investigated as vectors for vaccines and gene delivery [13–16]. In contrast, vaccines against AdVs themselves have received little attention. There are three major capsid proteins on AdV particles, fiber, hexon and penton base, all of which can be targeted by neutralizing antibodies (NAbs) during natural AdV infection and in immunization when AdVs are used as vectors [15]. Since pre-existed NAbs are detrimental to AdV-based vaccines and gene delivery platforms, a number of studies have been focused on the characterization of naturally occurring or AdV vectors-elicited NAbs [15,17,18]. Nevertheless, it remains inconclusive as to which AdV protein plays a predominant role in eliciting NAbs. For instance, while some studies suggested that hexon-specific NAbs exert significant neutralizing activity both *in vitro* and *in vivo*, others indicated that fiber- and penton base-specific NAbs are more frequent induced and in higher titers in humans [19–22]. Therefore, for the development of AdV subunit vaccines, it is important to understand which one of the three major capsid proteins could be the best vaccine candidate to elicit protective immune responses.

In the current study, using AdV7, one of the three most common AdVs, as a model, we systematically compared the immunogenicity of its three major capsid proteins, fiber, hexon and penton base on a murine model, and investigated their potential as subunit vaccines against AdVs.

## 2. Materials and methods

### 2.1. Ethical statement

BALB/c mice were purchased from Beijing HFK Biotechnology. All animal experiments were approved by the Institutional Ethical Review Board of the Wuhan Institute of Virology, Chinese Academy of Sciences, and performed in accordance with the guidelines of the Hubei Laboratory Animal Science Association.

### 2.2. Cells, viruses and plasmids

293 T and HeLa cells were purchased from American Type Culture Collection (ATCC) and cultured in DMEM supplemented with 10% FBS and antibiotics. Human AdV 3 (ATCC-VR-3) and AdV7 (ATCC-VR-7) were purchased from ATCC and propagated according to the manufacturer's instructions, titrated, aliquoted and stored in −80 °C before use. The coding sequences of fiber, hexon and penton base were amplified from the total DNA extracted from AdV7-infected cells, and ligated into pcDNA3.1(+) (Thermo Scientific). Sequence verified clones were designated as pFiber, pHexon and pPenton, respectively. Plasmids used for prokaryotic expression were amplified from pFiber, pHexon and pPenton, and ligated into pET28a (Novagen) and designated as pET-Fiber, pET-Hexon and pET-Penton, respectively. Primers used for the amplification are listed in Table S1.

### 2.3. In vitro expression

293 T cells were transfected with pcDNA3.1, pFiber, pHexon or pPenton using Lipofectamine 2000 (Thermo Scientific) according to the manufacturer's instructions. Forty-eight hours post transfection, cells were harvested and lysed with cell lysis buffer supplemented with protease inhibitor cocktail (Roche). Cleared cell lysate supernatants were separated by SDS-PAGE and transferred onto a PVDF membrane. After blocking in 5% non-fat milk, the membrane was probed with anti-His-HRP (Thermo Scientific) or mouse anti-GAPDH (Santa Cruz) followed by m-IgGκ BP-HRP (Santa Cruz). After incubation, the membrane was extensively washed with PBST. Immune-bands were visualized by addition of Immobilon Western Chemiluminescent HRP Substrate (Millipore) and recorded by X-ray film (Thermo Scientific). Bands were semi-quantified by densitometry analysis using Image J (version 1.51j8, National Institutes of Health), with the target gene expression relating to GAPDH being calculated by the density ratio of target gene/GAPDH.

### 2.4. Mouse immunization, challenge and sampling

Mouse immunization was performed as previously described with modifications [23–25]. In brief, inbred female BALB/c mice (6–8 wk old) were immunized intramuscularly with endotoxin-free pcDNA3.1 (negative control), pFiber, pHexon and pPenton, respectively. All plasmids were administrated twice at the dose of 30 μg/mouse in two-week intervals. One week after the final administration, sera and bronchoalveolar lavages (BALs) were collected for antibody titration and virus neutralization, while splenocytes were collected for the measurement of Th1/2/17 cytokines. Virus challenge assay was performed as previously described with modifications [26]. In brief, one week after the final immunization, mice were anesthetized and challenged with $10^{10}$ fluorescence forming units (FFUs) AdV7 intranasally. Day 0, 1, 3, 5 and 10 after challenge, mice were sacrificed and virus titer in the lung was determined by Light Diagnostics™ Adenovirus DFA Kit (Merck Millipore) according to the manufacturer's instructions.

### 2.5. RT-PCR

Three days after mice being injected with pcDNA3.1, pFiber, pHexon or pPenton, mice were sacrificed and quadricep muscles were harvested. Total RNA was extracted using RNeasy Mini Kit (Qiagen) according to the manufacturer's instructions. cDNA was synthesized using High-Capacity cDNA Reverse Transcription Kit (Thermo Scientific) and used as the template for amplification of fiber, hexon and penton base. Primers used for amplification are listed in Table S2.

### 2.6. Prokaryotic expression and purification of fiber, hexon and penton base proteins

Prokaryotic expression and purification of proteins were performed as previously described with modifications [27]. In brief, pET-Fiber, pET-Hexon and pET-Penton were transformed into *E. Coli* BL21 (New England BioLabs) and protein expression was induced by IPTG (Sigma-Aldrich) stimulation. After stimulation, bacteria were harvested and sonicated. Fiber and penton base in the soluble fraction and hexon in the insoluble fraction were collected for purification. Purification of fiber and penton base in the soluble fraction was performed by nickel-charged chelating Sepharose Fast Flow column (GE Healthcare) on the ÄKTA protein purification systems (GE Healthcare). For the purification of hexon, insoluble fraction was first dissolved in dissolving buffer (5 mM imidazole, 0.5 M NaCl, 2 mM Tris-HCl, 8 M urea, pH 7.4), and purified by nickel-charged chelating Sepharose Fast Flow column. Purified hexon was then refolded in refolding buffer (5 mM imidazole, 0.5 M NaCl, 2 mM Tris-HCl, descendent urea concentrations, pH 7.4) using Zeba desalting spin columns (Thermo Scientific). All purified proteins were exchanged into PBS using desalting spin columns. Purity was confirmed by Coomassie Brilliant Blue staining and Western blot (Fig. S1). Protein concentration was measured

Please cite this article in press as: Hu K et al. Penton base induces better protective immune responses than fiber and hexon as a subunit vaccine candidate against adenoviruses. Vaccine (2018), https://doi.org/10.1016/j.vaccine.2018.05.118