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Gary R. Skuse *Editors*

Recombinant Virus Vaccines

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Preface

Since the discovery of the prophylactic effects of the cowpox virus toward variants of the variola virus in the late eighteenth century, scientists and clinicians have fought to balance the beneficial effects of viral vaccines against the potential for undesired and potentially pathogenic side effects. In the last half century or so scientists have harnessed a variety of pathogenic viruses, from a number of species, for use and study in the laboratory and the clinic. Our increased understanding of the pathology and the molecular anatomy of those viruses has enabled us to adapt them for use as recombinant expression systems for immunogens that can be used to protect hosts from infection by a wide variety of infectious agents.

This volume is intended for scientists and clinicians who are interested in learning more about and adapting methods employed in basic and biomedical research, which are directed toward understanding the development of recombinant viruses and their use as vaccine platforms. The methods and protocols contained herein involve many of the viruses currently being used for, or under development as, vaccine platforms. Throughout this work readers will find details of the use of recombinant vaccines which are employed to either produce immunogens in vitro or elicit antibody production in vivo. Within each of the parts of this work, readers will find several chapters that are grouped according to the Baltimore Classification of viruses. Taken together, the described methods should inform individuals with interests in the current methods used to generate and develop recombinant viral vaccines.

The contributors to this volume are current or nascent leaders in the field of recombinant virus vaccine development. Taken together they have provided a large number of effective protocols that can be employed or adapted as readers see fit. While an attempt has been made to be as comprehensive as possible, inevitably there are certain platforms that are not included in this collection. We sincerely hope that you find this work informative and useful in your own laboratories and that they serve to acquaint you with the current state of the art in the use of recombinant viral vaccines.

Rochester, NY, USA

Maureen C. Ferran
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Part I

Double-Stranded DNA Viruses

Chapter 1

Development of Novel Vaccines Against Infectious Diseases Based on Chimpanzee Adenoviral Vector

Chao Zhang*, Yudan Chi*, and Dongming Zhou

Abstract

Vaccination is considered to be the most effective method of preventing infectious or other diseases. Adenovirus (Ad) is one the most promising vectors in vaccine research and development. It can induce not only potent humoral but also cellular immune responses, and has therefore been widely applied in basic and translational studies. Chimpanzee Ad is a rare serotype circulating in humans. This circumvents the problem of preexisting immunity to human Ad serotypes, enhancing Chimpanzee Ad prospects in vaccine development. Here we describe experimental procedures used to generate a new generation of rabies vaccine based on a chimpanzee Ad vector, which can be extended in the development of novel vaccines against other infectious diseases.

Key words Chimpanzee adenovirus, Immune response, Vaccine, Infectious disease, Rabies

1 Introduction

Adenovirus (Ad) was first discovered in 1953 by Rowe and his colleagues [1]. It is a double stranded DNA virus with icosahedral capsids. Over the past decades, Ad-based vectors have shown great potential in gene therapy and have been used to generate recombinant vaccines against cancer or infectious diseases since the first in vivo gene transfer was performed by Rosenfeld et al. in 1991 [2–4]. Nowadays, Ad vectors are widely used as gene delivery systems due to several promising features such as high biosafety levels, broad tropism, and feasibility for scale-up production [5–7]. One of the most widely used Ad vectors originates from human serotype 5(AdHu5) [8], however, the preexisting neutralizing antibodies against AdHu5 have a high seroprevalence of 74.2% in humans [9], and the preexisting antibodies dampen the vaccination effectiveness thus restricting further application in clinical

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trials [10–12]. In order to circumvent the disadvantages of the AdHu5, the rare human serotype Ads and other Ads from nonhuman species have been developed [13–16].

Here, we use a chimpanzee-originated Ad, AdC68, as a model for the generation of Ad-based vaccines against infectious diseases. The construction of the AdC68 infectious clone is as previously described [17]. The E1 region is deleted, thus it is replication-deficient and can only replicate in E1-compensating cell lines such as HEK293 and PER. C6 [18]. In a previous study done in our laboratory, the AdC68 that expressed G protein of the rabies virus (rab.GP) was successfully constructed, expanded and purified. After testing, the rab.GP was found to be highly expressed in HEK 293 cells infected with the recombinant Ads, termed as AdC68-rab.GP. AdC68-rab.GP could elicit high levels of neutralizing antibodies against rabies virus in vaccinated mice. The generation of recombinant Ads in this study is based on the direct cloning method [17] which is simple and efficient and can be extended in the development of vaccines against other infectious diseases.

2 Materials

2.1 Molecular Cloning

1. Restriction enzymes: XbaI; NheI; PI-SceI; I-CeuI; BglII; SalI; XhoI.
2. T4 DNA ligase.
3. Competent cells: *Escherichia coli* strain DH5α cells; *Escherichia coli* strain Stbl2 cells.
4. Agarose G-10.
5. Low melting point agarose.
6. LB culture medium: yeast extract (5 g/L); tryptone (10 g/L); NaCl (10 g/L), amplicillin or kanamycin (0.1 g/L); agar (15 g/L., only be used for LB plate).
7. GelRed Nucleic Acid Gel Stain, 10,000× in DMSO (Biotium). (*see* **Note 1**).
8. KCM buffer (5×): 0.5 M KCl; 0.15 M $CaCl_2$; 0.25 M $MgCl_2$.
9. TAE Buffer (50×): 2 M Tris, 1 M acetic acid, 50 mM EDTA.
10. DNA size standard ladders.
11. NucleoBond Xtra Midi Plus (MACHEREY-NAGEL).
12. QIAprep® Spin Miniprep Kit (QIAGEN).
13. PUC57-rab.GP (codon-optimized for improving expression, Genscript).
14. pShuttle (as described in Ref. [17]).

2.2 Virus Production and Idetification

1. Chimpanzee Ad type 68 (AdC68, also called SAdV-25, ATCC, GenBank accession number: AF394196.1).
2. HEK 293 cell (ATCC, cat. no. CCL-243).
3. Cell culture reagents: Dulbecco's modified Eagle's medium (DMEM); fetal bovine serum; phosphate-buffered saline; penicillin–streptomycin 100× solution; trypsin (0.25%), phenol red.
4. Cell tranfection reagents: Opti-MEM; Lipofectamine 2000 transfection reagent (Invitrogen).
5. Virus purification reagents: Tris–HCl (1 M, pH 8.0); cesium chloride; Bio-Gel P-6DG (Bio-Rad); Liquid chromatography columns.
6. Pronase.
7. DNeasy® Blood & Tissue Kit (QIAGEN).

2.3 Immunoblotting

1. NuPAGE® Novex 10% Bis–Tris gel 1.0 mm, 10 Well (Thermo Fisher Scientific).
2. RIPA buffer: 25 mM Tris–HCl pH 7.6;150 mM NaCl, 1% (V/V) NP-40;1% (W/V) sodium deoxycholate; 0.1% (W/V) SDS.
3. Complete protease inhibitor cocktail tablets (Roche).
4. Running buffer (5×): 0.125 M Tris–HCl;1.25 M glycine;0.5% (W/V) SDS.
5. Transfer Buffer: 39 mM glycine;48 mM Tris;0.037% (W/V) SDS;20% (V/V) methanol.
6. PVDF membrane (0.45 μm filter).

2.4 Animals

ICR (4–6 weeks old) mice are purchased from Shanghai Laboratory Animal Center, China. The protocol for this animal experiment should be approved by the Institutional Animal Care and Use Committee.

3 Methods

3.1 In-Gel Ligation (See Fig. 1)

1. *Cloning the rab.GP gene into pShuttle*. Digest 500 ng of PUC57-rab.GP (Genscript) and 500 ng of pShuttle [17] with XbaI and NheI for 2 h at 37 °C, respectively. Conduct each digestion reaction in a total volume of 20 μl.
2. Run the digestion products on a 1% (W/V) low-melting point agarose gel in TAE buffer. Cut out the desired bands with a razor blade or scalpel to get the digested insert from PUC57-rab.GP and the digested backbone from pShuttle vector, respectively, and then place gel slices into Eppendorf microcentrifuge tubes. Incubate for 5 min at 65 °C. Cool for 1 min at room temperature

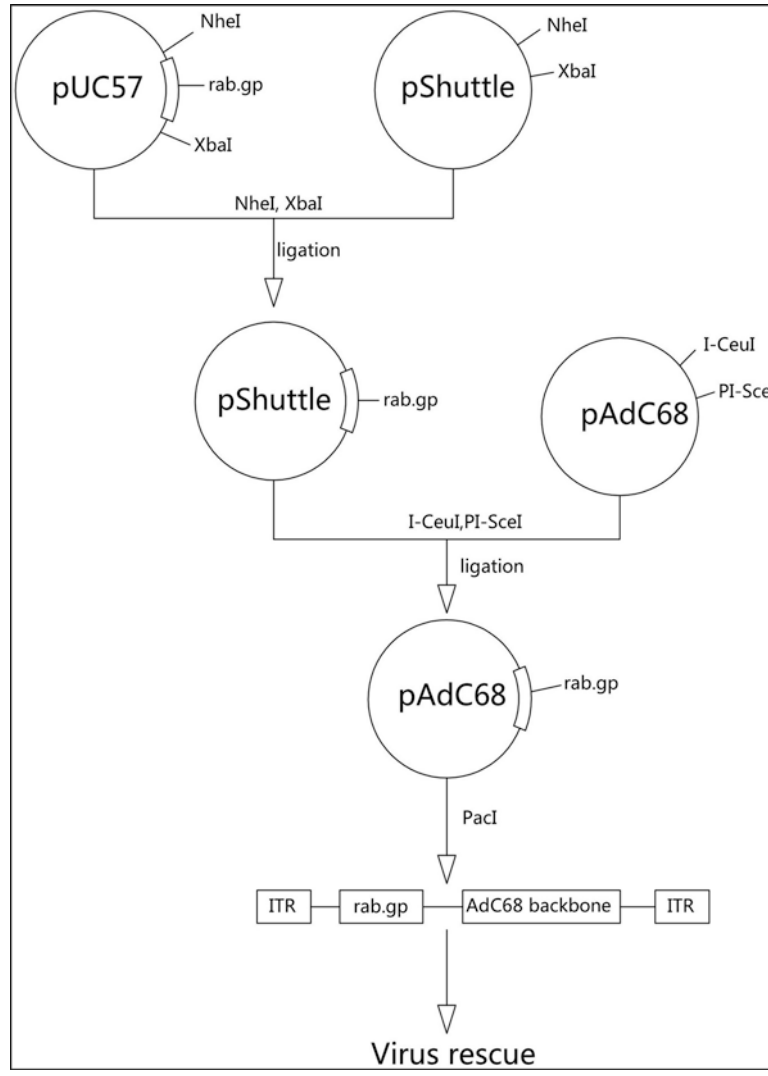

Fig. 1 Flowchart of the construction of pAdC68-rab.GP

(*see* **Note 2**). Set up the in-gel ligation with a total volume of 20 μl; use 4 μl of backbone in liquefied gel, 12 μl of insert in liquefied gel, and mix both with 1 μl T4 DNA ligase. Incubate at 16 °C overnight (*see* **Note 3**).

3. Melt the ligation products for 5 min at 65 °C, and then dilute in 180 μl of 1× KCM buffer (*see* **Note 4**), cool the system at room temperature for 1 min (*see* **Note 5**). Transform 50 μl of diluted ligation product into 100 μl of DH5α competent cells (transforming efficiency ≥10^9 CFU/μg), and then incubate on ice for 30 min. After that, perform the heat shock at 42 °C for 30 s, and spread the transformation mix onto a kanamycin-containing LB plate. Incubate plates for 14 h at 37 °C.

4. Pick up several colonies and culture each of them in 5 mL LB selective medium for 12 h in a shaker at 37 °C and 0.9 × *g* shaking speed. Extract the plasmid DNA by QIAprep® Spin Miniprep Kit based on manufacturer's instructions. Identify the plasmids by restriction enzyme digestions with Nhe1 and XbaI, respectively; choose the right clone, so the pShuttle-rab.GP was successfully generated.
5. *Clone the rab.GP gene into AdC68 vector*; digest 1 μg of the AdC68 plasmid and 1 μg of pShuttle-rab.GP with I-CeuI and PI-SceI, respectively. Conduct each reaction in a total volume of 20 μl and incubate for 4 h at 37 °C.
6. Run the digestion products on 1% (W/V) low-melting point agarose gel in TAE buffer. Cut out the desired bands with a razor blade or scalpel to get the digested insert from pShuttle-rab.GP and the digested backbone from AdC68 vector, and then place gel slices into Eppendorf microcentrifuge tubes. Incubate for 5 min at 65 °C. Cool for 1 min at room temperature. Set up the in-gel ligation with a total volume of 20 μl; use 4 μl of backbone in liquefied gel, 12 μl of insert in liquefied gel and mix both with 1 μl T4 DNA ligase. Incubate at 16 °C overnight (*see* **Note 6**).
7. Melt the ligation products for 5 min at 65 °C, and then dilute in 180 μl of 1× KCM buffer, cool the system at room temperature for 1 min. Transform 50 μl of diluted ligation product into 100 μl of Stbl2 competent cells (transforming efficiency $\geq 10^9$ CFU/μg) with heat shock as described in **step 3**, and spread the transformation mix onto an ampicillin-containing LB plate. Incubate plates for 24 h at 30 °C (*see* **Note 7**).
8. Pick up several colonies and culture each of them in 5 mL LB selective medium for 12 h in a shaker at 30 °C and 0.6 × *g* shaking speed (*see* **Note 7**). Extract each plasmid DNA by QIAprep® Spin Miniprep Kit based on manufacturer's instructions. Identify the plasmids by restriction enzyme digestions with BglII, SalI, and XhoI, respectively. Run the digested products on 1% agarose gel and verify the bands by electrophoresis (*see* Fig. 2a). Choose the right clone, so the AdC68-rab.GP vector (pAdC68-rab.GP) was successfully generated.
9. Select one correct clone and culture it in 200 mL LB medium for 20 h in a shaker at 30 °C and 0.6 × *g* shaking speed. Extract plasmid DNA using NucleoBond Xtra MidiPlus based on manufacturer's instructions.

3.2 Virus Rescue, Expansion, Purification (See Note 8)

1. *Virus rescue*. Seed HEK 293 cells on a 6-well plate 1 day before transfection, and culture cells overnight to 80–85% confluency at 37 °C and 5% CO_2 in DMEM with 10% FBS and 1× penicillin–streptomycin solution.