



Neutralizing epitopes mapping of human adenovirus type 14 hexon

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ABSTRACT

Human adenoviruses 14 (HAdV-14) caused several clusters of acute respiratory disease (ARD) outbreaks in both civilian and military settings. The identification of the neutralizing epitopes of HAdV-14 is important for the surveillance and control of infection. Since the previous studies had indicated that the adenoviruses neutralizing epitopes were likely to be exposed on the surface of the hexon, four epitope peptides, A14R1 (residues 141–157), A14R2 (residues 181–189), A14R4 (residues 252–260) and A14R7 (residues 430–442) were predicted and mapped onto the 3D structures of hexon by homology modeling approach. Then the four peptides were synthesized, and all the four putative epitopes were identified as neutralizing epitopes by enzyme-linked immunosorbent assay (ELISA) and neutralization tests (NT). Finally we incorporated the four epitopes into human adenoviruses 3 (HAdV-3) vectors using the “antigen capsid-incorporation” strategy, and two chimeric adenoviruses, A14R2A3 and A14R4A3, were successfully obtained which displayed A14R2 and A14R4 respectively on the hexon surface of HAdV-3 virions. Further analysis showed that the two chimeric viruses antiserum could neutralize both HAdV-14 and HAdV-3 infection. The neutralization titers of anti-A14R4A3 group were significantly higher than the anti-KLH-A14R4 group ($P=0.0442$). These findings have important implications for the development of peptide-based broadly protective HAdV-14 and HAdV-3 bivalent vaccine.

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1. Introduction

Human adenoviruses (HAdVs) played an important role in a broad spectrum of illness in humans, including acute respiratory disease (ARD), pneumonia, epidemic keratoconjunctivitis and acute gastroenteritis [1,2]. HAdVs were typed and ordered into seven species (A–G) with greater than 65 genome types [3,4], and different HAdV species were associated with distinct diseases [5,6]. First discovered in the Netherlands in 1955 during an outbreak of acute respiratory disease (ARD) in military recruits [7], human adenovirus serotype 14 (otherwise known as “agent de Wit” or

HAdV-14p) was subsequently isolated during respiratory disease outbreaks among young adults [7–10]. However, in 2006–2007, a relatively rare adenovirus serotype 14 strain caused several severe lower respiratory tract disease including at least 10 deaths and 140 respiratory illnesses in New York, Oregon, Washington and Texas, in both civilian and military settings [11–16]. The outbreaks of HAdV-14 infection were then also reported in Europe and China [5,8]. The re-emerging HAdV-14 belonged to a new genome type designated “HAdV-14p1” (also known as “14a”) [17], while the most notable genetic difference between the variant and the prototype HAdV-14 strain was a deletion of 6 base pairs in the fiber knob gene [17–20].

Normal exposure to human adenoviruses leads to the presence of pre-existing immunity. Prior studies have reported a prevalence of neutralizing antibodies to HAdV-5 between 60% and 70% in some populations in Europe and USA, and up to 98% in sub-Saharan African countries and Asian (Thai) tropical countries [21,22]. As far as we know, no scientific report on the natural rates of neutralizing immunity to HAdV-14 in the human population has been published yet. According to our recent survey on large population in Guangzhou, China, the seroprevalence of neutralizing antibodies

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to HAdV-14 in healthy adults was less than 20% (data not published yet), indicating the lack of pre-existing neutralizing antibodies. The identification of neutralizing epitopes is very important in designing the HAdV vaccine [23,24], developing rapid HAdV diagnostic reagents or antiviral medicine [25]. However, no neutralizing epitope has been identified for HAdV-14. Although it is possible to locate hypervariable regions (HVRs) of HAdV by using the multiple sequence alignment method [26], the HVRs do not directly correspond to the type-specific B cell epitopes [27]. So it is necessary to analyze the 3D conformation of hexon protein which can determine accurate epitopes, but only few hexon structures have been available by X-ray crystal diffraction [28,29]. Research groups have previously predicted the B cell epitopes by using the homology modeling method to determine the 3D structure of adenovirus hexon [30–32], and then these putative epitopes have been verified by ELISA in combination with neutralization tests (NTs), indicating the reliability of this epitope mapping method [31].

Antigen capsid-incorporation strategy is becoming promising for vaccine development against infectious agents [33,34]. Previous studies have verified that exogenous antigenic peptides can be incorporated into the hexon HVRs without compromising virus viability, and can effectively trigger a robust antigen-specific immune response [35,36]. Our group has successfully constructed a recombinant HAdV-3 vector named Ad3EGFP as a tool for viral delivery or live-vaccine construction [37]. By replacing the predicted epitopes within the HAdV-3 hexon with the corresponding epitopes from the HAdV-7 hexon, we have constructed recombinant HAdV-3/HAdV-7 bivalent vaccines candidate [38]. We also constructed a HAdV-3-based EV71 vaccine candidate using this method [39].

In the current study, we aim to investigate neutralizing epitopes of HAdV-14 by bioinformatics analysis and develop HAdV-3/HAdV-14 candidate bivalent vaccines using the recombinant HAdV-3 based vector. Four predicted neutralizing epitopes derived from HAdV-14 are synthesized and used to immunize mice. Then the four putative epitopes are verified to be neutralizing epitopes by ELISA in combination with neutralization tests. Furthermore, HAdV-14 epitopes were displayed on the surface of HAdV-3 virions, providing more profound understanding of HAdV immunogenicity and valuable information for the development of HAdV-3-based vaccines.

2. Materials and methods

2.1. Animals, cells, virus strains and plasmids

Female BALB/c mice (4–6 weeks) were purchased from Guangdong Medical Laboratory Animal Center, and housed in specific pathogen-free (SPF) conditions. The cells were grown in Dulbecco's Modified Eagle's Medium (DMEM) (Invitrogen, CA, USA) supplemented with 100 IU/ml penicillin, 100 µg/ml streptomycin and 10% (v/v) fetal bovine serum (FBS) (Invitrogen). The following plasmids and viruses used in this study were maintained in our laboratory [37,39]: the E3-defective adenovirus type 3 replication-competent plasmid pBRAdV3dE3egfp(pAd3egf) with the enhanced green fluorescent protein (EGFP) as reporter gene; the corresponding virus Ad3EGFP; shuttle vector pBRHexonL/R; HAdV-14 GZ01 strain (GenBank no. JQ824845.1) [40] was kindly provided by Professor Qiwei Zhang.

2.2. Predicting the potential neutralizing epitopes of HAdV-14 hexon

The nonconserved domains of HAdV-14 hexon proteins were determined by alignment with the available hexon protein sequences of species B human adenoviruses using the protein BLAST program (Basic Local Alignment Tool; <http://blast.ncbi.nlm.nih.gov/Blast.cgi>).

The EMBOSS antigenic tool (<http://binf.yu.edu.tw/emboss/Apps/antigenic.html>) was used to predict antigenic epitopes of the HAdV-14 hexon proteins. The potential template structures for modeling HAdV-14 hexon proteins were identified by searching them against information in the Protein Data Bank (PDB) by BLAST-P analysis. The Modeler 9v8 tool was used to model the three-dimensional (3D) structure of HAdV-14 hexon protein [41]. The antigenic epitopes that were predicted to be exposed on the capsid surface and located in nonconserved regions were selected as potential sites for recognition by neutralizing antibodies (NAbs).

2.3. Synthetic peptides

The four predicted epitope peptides were synthesized and analyzed (purity $\geq 90\%$) by high performance liquid chromatography (GL Biochem Ltd, Shanghai, China). The Cys was added on the N-terminal of synthetic peptides for conjugation. Then each peptide was chemically linked to the carrier protein keyhole limpet hemocyanin (KLH) for antibody production.

2.4. Construction of recombinant adenoviral vectors

To generate constructs containing putative HAdV-14 epitopes in different HVRs of HAdV-3, we first used overlapping PCR as previously described [39]. The corresponding primers are shown in Supplementary Table 1. The putative epitope genes were then cloned into the shuttle vector pBRHexonL/R by using ClaI and BamHI restriction enzymes. To furthermore create recombinant HAdV-3 vectors containing the predicted HAdV-14 epitopes, the shuttle vectors were digested with EcoRI and Sall, and then were cotransformed into *Escherichia coli* BJ5183 cells with the AvrII- and PacI-linearized pBRAdV3dE3egfp (pAd3egf). The obtained clones were then selected with PCR using primers HexonF/A14R1r, HexonF/A14R2r, HexonF/A14R4r or HexonF/A14R7r (Supplementary Table 1) and confirmed by both restriction digestion and sequence analysis.

Supplementary Table 1 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2015.10.117>.

2.5. Virus rescue and preparation

To rescue the recombinant viruses, these genetic modified plasmids were linearized with AsiSI (NEB, USA). AD293 cells grown in 30-mm-diameter tissue culture dishes were transfected with 1 µg of each purified DNA using the Lipofectamine[®] 3000 (Life Tech, USA). Finally, a total of forty 100-mm-diameter dishes containing modified HAdV-3-infected AD293 cells were harvested, followed by standard cesium chloride-density gradient centrifugation as described previously [42]. The DNA of purified viruses was used for sequence analysis. The viral particle (VP) titers were determined by spectrophotometry using a conversion factor of 1.1×10^{12} VPs per absorbance unit at 260 nm. All preparations of purified chimeric viruses and Ad3EGFP used in this study had similar vp: TCID50 titer ratios of approximately $10^{6.4}:1$.

2.6. Immunization

BALB/c mice aged 4–6 weeks were injected intramuscularly (i.m.) with 5×10^9 VPs per mouse (about 10 µg total protein) recombinant adenoviruses, inactivated HAdV-14 and Ad3EGFP viron, or 50 µg KLH-conjugated HAdV-14 peptides in QuickAntibody-Mouse 5W adjuvant (Biodragon Immunotechnologies Ltd, Beijing, China), followed by one additional boost in the same adjuvant at 3-week interval. And phosphate buffered saline (PBS) was injected to mouse as the control. Two weeks after the

second immunization, serum samples were collected, pooled, heat-inactivated at 56 °C for 30 min, and then were stored at –70 °C. The animal protocol was carried out in accordance with EU Directive 2010/63/EU for animal experiments and approved by the IACUC at the Affiliated First Hospital of Guangzhou Medical University.

2.7. ELISA

Briefly, 96-well plates (Corning Inc., NY, USA) coated overnight with 100 µl/well of 1 µg/ml synthetic peptides or purified adenoviruses in carbonate–bicarbonate buffer (pH 9.6) at 4 °C. These plates were then washed with 0.05% Tween 20 in phosphate-buffered saline (PBST) and blocked for 2 h with 2% bovine serum albumin (BSA) in PBST. Sera were added in serial dilutions and incubated for 1 h. After being washed three times with PBST, the plates were added with a 1:8000 dilution of HRP-conjugated secondary antibody (CWBio Inc, Beijing, China) and incubated for 1 h. The plates were washed five times, added with tetramethylbenzidine (TMB) substrate, stopped with 2 M H₂SO₄, and the optical density (OD) was measured at 450 nm using a microplate reader (Multiskan™ MK3, Thermo Fisher Scientific Inc., USA). The endpoint titer was defined as the highest dilution at which the OD450 was at least 2-fold above that of wells receiving anti-PBS serum as negative control.

2.8. Virus neutralization tests

Neutralization tests were performed to test the neutralizing effect of antisera to HAdV-14 or HAdV-3. All of the antipeptides sera (anti-A14R1, anti-A14R2, anti-A14R4 and anti-A14R7) and anti-A14R2A3, anti-A14R4A3, were serially 2-fold diluted (1:8 to 1:16,384) in DMEM. Anti-PBS, anti-HAdV-14, anti-HAdV-3 and anti-Ad3EGFP sera were used as controls. Each dilution was mixed with virus of 100 TCID₅₀. The antibody–virus mixtures were incubated at 37 °C for 1 h and transferred to 96-well plates containing 70–90% confluent monolayers of AD293 cells. The monolayers were cultured for 96 h, after which the infection was observed by microscopy and the neutralization titers were determined as the reciprocal of the highest dilution of mice antiserum that completely inhibited visually observable cytopathic effect (CPE).

2.9. Sequence alignment

For systematic alignment on the hexon sequences of HAdV-14 strains, all the 80 sequences were downloaded from the NCBI website using the term “Human Adenovirus Type 14[Organism]”. 13 HAdV-14 sequences covering the complete hexon were selected and aligned using MEGA program. Neutralizing epitopes predicted in this study were examined for sequence similarity using the ClustalW program in MEGA.

2.10. Statistical analysis

The data are presented as means ± SEM from three independent experiments. Comparisons among groups are performed by the following non-parametric tests: Mann–Whitney *U* for two groups comparison and Kruskal–Wallis test with Dunn’s multiple comparisons for three (or more) groups comparisons. Statistical significance was defined as *P* < 0.05 using Prism 5.0 software.

3. Results

3.1. Potential serotype-specific NAb epitopes for HAdV-14

Previous studies indicated that epitopes recognized by serotype-specific NAb may be exposed on the virion surface and

reside within the HVRs of hexon proteins [43–45]. The 3D model of HAdV-14 hexon was obtained by homology modeling using the known crystal structure of AdC68 hexon protein (PDB ID 2OBE) as template, and four regions within the HAdV-14 hexon (A14R1, residues 141–157; A14R2, residues 181–189; A14R4, residues 252–260; A14R7, residues 430–442) were predicted as potential neutralizing epitopes (Fig. 1).

3.2. Sequence alignment

13 sequences HAdV-14 strains containing full hexon were downloaded from NCBI and compared for homology (Supplementary Fig. 1). The alignment showed that the amino acid sequences of A14R1, A14R2 and A14R7 were identical among these strains (Supplementary Fig. 1); whereas the sequences corresponding to A14R4 were discovered heterogeneity with TEEAGNIEY vs TEEGNIEY. In the current study, the A14R4 sequence of synthetic peptide and incorporation into HAdV-3 hexon was defined as TEEAGNIEY according to the reference sequence DQ149612.1, while the HVR4 of HAdV-14 GZ01 strain used in ELISA and NT has an amino acid sequence TEEGNIEY.

Supplementary Fig. 1 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2015.10.117>.

3.3. Identification of neutralizing epitopes using synthetic peptides

To identify the responsible neutralizing epitopes, antisera generated from vaccinations with HAdV-14 were first identified by ELISAs with synthetic peptides. The synthetic peptide A55R1 was used as the control. As expected, no IgG against A55R1 was present in anti-HAdV-14 sera. Surprisingly, all four putative epitopes could bind to the anti-HAdV-14 sera but at a weak titer (Fig. 2).

Furthermore, antibody responses of mice immunized with KLH-coupled peptides were also measured by ELISAs (Fig. 3). As expected, no IgG against putative epitopes was present in anti-PBS sera as control. Fig. 3A showed the profile of reactivity of individual peptides with the antisera generated from vaccinations with HAdV-14. There was significant difference among the four anti-peptides groups (*P* = 0.0047). The IgG titers differed from 1:1600 to 1:324,000. Fig. 3B showed all the anti-peptides sera could bind to the purified HAdV-14 virions with no significant difference among the four anti-peptides groups (*P* = 0.7299).

In vitro neutralization assays were performed with serially diluted anti-peptides sera (anti-A14R1, anti-A14R2, anti-A14R4 and anti-A14R7 groups), anti-PBS and anti-HAdV-14 sera as control, neutralizing HAdV-14 cultured in AD293 cells (Fig. 4). As shown in Fig. 4, all the anti-peptides sera neutralized the HAdV-14 strain, with titers ranging from 32 to 256; whereas the control anti-PBS sera failed to neutralize HAdV-14 infections at 1:8 (the lowest dilution tested), and the neutralizing titers of anti-HAdV-14 sera was >8192. The neutralization experiments were repeated three times with nearly identical results. Statistical analyses indicated there were no significant differences in neutralizing capacity among four peptide-immunized groups (*P* = 0.5247).

3.4. Identification of neutralizing epitopes with epitope chimeric adenoviruses

In the current study, we tried to incorporate all four putative epitopes into the corresponding HVRs of HAdV-3 (Fig. 5), however, repeated attempts to incorporate the A14R1 and A14R7 into the corresponding HVR1 and HVR7 of HAdV-3 failed. In the end, we successfully incorporated A14R2 and A14R4 into the corresponding HVR2 and HVR4, respectively. The chimeric adenoviruses, A14R2A3 and A14R4A3 containing A14R2 and A14R4, respectively, were

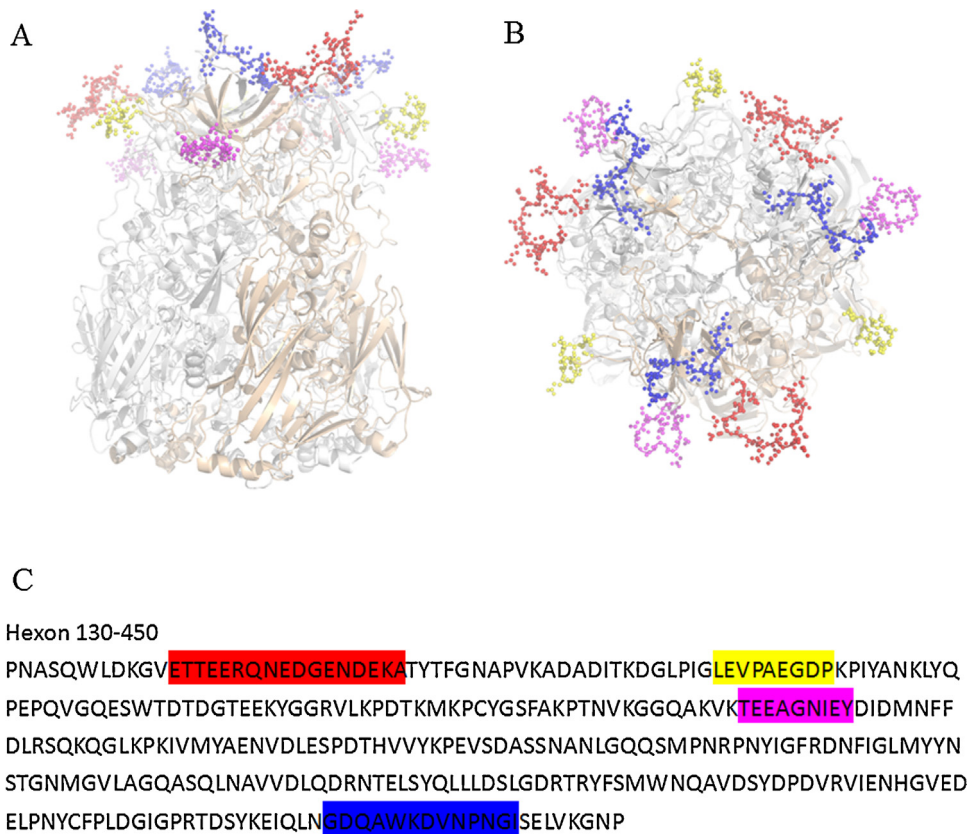


Fig. 1. Predicted epitopes in the tower regions of hexon protein of HAdV-14. A14R1, A14R2, A14R4 and A14R7 were colored by red, yellow, magenta and blue respectively (side view: A, top view: B) and their corresponding locations were labeled on the primary amino acid sequence of hexon (C). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

obtained successfully. The silver staining of gels demonstrated the complement of capsid proteins of chimeric viruses A14R2A3 and A14R4A3 were equivalent to those of Ad3EGFP (Supplementary Fig. 2). Sera immunized with hexon-modified chimeric adenoviruses

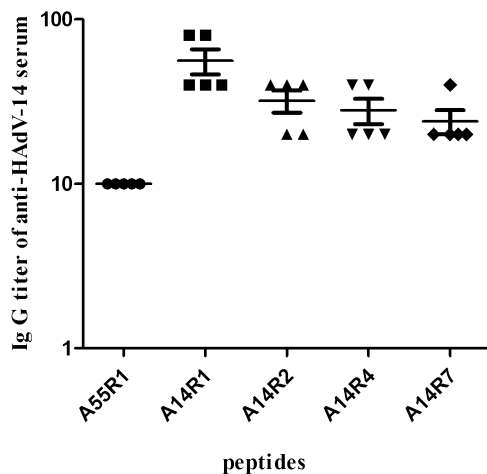


Fig. 2. ELISA of peptides with antiserum against HAdV-14. Sera from mice boosted with the HAdV-14 strain were collected for ELISA binding assays. The wells were coated with synthetic peptide A14R1, A14R2, A14R4 and A14R7 or A55R1 as control. The vertical axis represented the dilution factor (diluted from 1:20 to 1:160). Each symbol represented a mouse, and the line indicated the geometric mean titer (GMT) of the group. Results were expressed as the mean \pm SEM from three independent experiments, and comparisons among groups were performed by Kruskal–Wallis test with Dunn's multiple comparisons. The synthetic peptide of A55R1 (EGVKNGEERVTEEEEN) encoding partial protein sequences within HAdV-55 hexon, was used as control. The antisera which did not show any reactivity at 1:20 (the lowest dilution tested) were assigned a titer of 1:10 for GMT computation.

were used as the primary antibody for the ELISA analysis. As expected, anti-PBS sera or anti-Ad3EGFP sera did not react with any peptide in the ELISA. The anti-A14R2A3 and anti-A14R4A3 sera recognized the corresponding A14R2 and A14R4 peptides respectively, with no significant difference ($P=0.9025$) discovered (Supplementary Fig. 3).

Supplementary Figs. 2 and 3 related to this article can be found, in the online version, at <http://dx.doi.org/10.1016/j.vaccine.2015.10.117>.

To detect whether chimeric viruses immunization could induce neutralizing antibody responses against HAdV-14 virus, we analyzed the neutralization titers of anti-A14R2A3 and anti-A14R4A3 sera against HAdV-14 or HAdV-3 (Fig. 6). In vitro neutralization assays were performed with serially diluted antisera from 8 to 8192, while anti-AD3EGFP or anti-HAdV-14 sera were used as controls. As expected, the anti-AD3EGFP sera failed to neutralize HAdV-14 infections at the lowest dilution tested. Both the A14R2A3 and A14R4A3 antisera could neutralize the HAdV-14 strain infection, with titers ranging from 32 to 128 (Fig. 6). No significant difference was discovered between the anti-A14R2A3 and anti-A14R4A3 groups (Fig. 6A) ($P=0.0597$) or between anti-A14R2 (Fig. 4) and anti-A14R2A3 (Fig. 6A) groups against HAdV-14 ($P=0.0930$). Further analysis showed that the neutralization titers of anti-A14R4A3 (Fig. 6A) group were significantly higher than the anti-A14R4 (Fig. 4) group ($P=0.0442$), which indicated that the hexon-modified virus could elicit stronger neutralizing antibodies against HAdV-14 than KLH coupled peptides.

To further determine whether the insertion of the heterologous epitopes in the recombinant HAdV-3 would weaken its immunogenicity against HAdV-3, the neutralizing titers of anti-A14R2A3 and anti-A14R4A3 sera against HAdV-3 were tested, while anti-HAdV-14 sera were used as control (Fig. 6B). The results showed

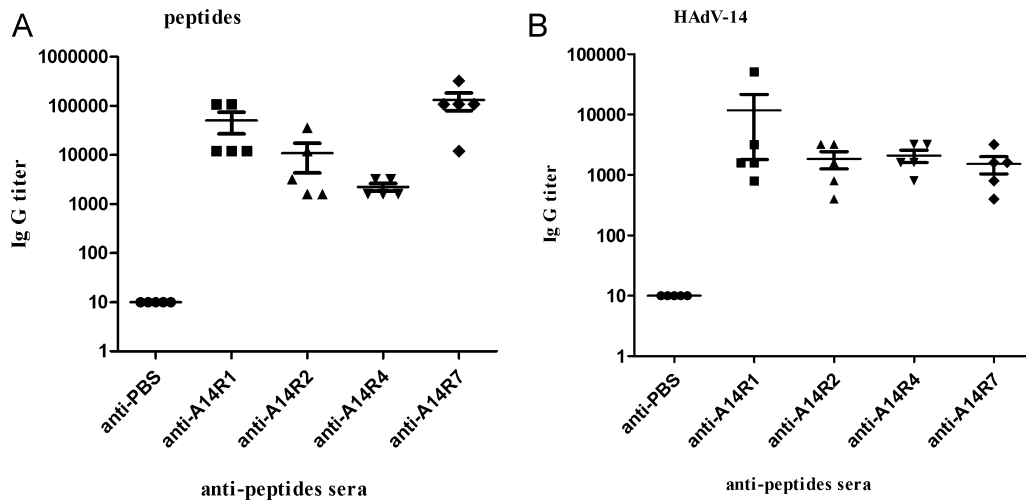


Fig. 3. IgG titers of anti-peptides sera by ELISA. Sera from mice boosted with the predicted KLH coupled epitopes were collected for ELISA binding assays. (A) The wells were coated with synthetic peptide. (B) The wells were coated with HAdV-14 strain. The vertical axis represented the dilution factor (diluted from 1:20 to 1:1,024,000), and anti-PBS serum was used as control. Results were expressed as the mean \pm SEM from three independent experiments, and comparisons among groups were performed by Kruskal–Wallis test with Dunn’s multiple comparisons. Antisera which did not show any reaction at 1:20 (the lowest dilution tested) in ELISA were assigned a titer of 10 for GMT computation.

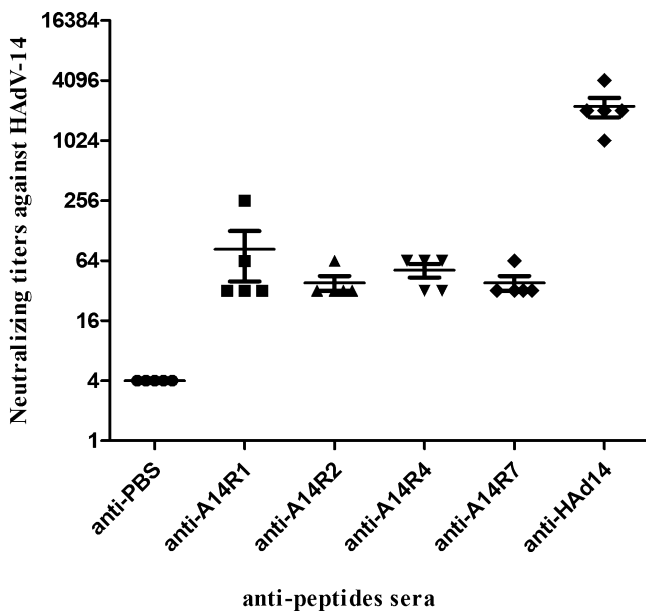


Fig. 4. Neutralization titers of anti-peptides sera against HAdV-14. Sera from mice boosted with the predicted KLH coupled epitopes were collected for NTs. Anti-PBS serum was used as the negative control. Results were expressed as the mean \pm SEM, and comparisons among groups were performed by Kruskal–Wallis test with Dunn’s multiple comparisons. Antiserum which did not show any reaction at 1:8 (the lowest dilution tested) in NTs was assigned a titer of 4 for GMT computation.

- 1) HVR1:¹³⁶IVTETTEERQNEDEKATTTNT¹⁵¹.....
- 2) HVR2:¹⁶⁴KEGLQIGLEVPAGEGDPKPIYADK¹⁸⁷.....
- 3) HVR4:²⁴³KVKTEEAGNIEYDID²⁶⁰.....
- 4) HVR7:⁴²²IKVKEDQAWKDVNPNQNEIAI⁴⁴⁶.....

Fig. 5. Amino acid residues of the predicted HAdV-14 epitopes marked in red, yellow, magenta and blue, incorporated into corresponding HVRs of HAdV-3 hexon. The numbers show the positions of the amino acid residues in the HAdV-3 hexon. Of which chimeric A14R2A3 and A14R4A3 were successfully rescued, while A14R1A3 and A14R7A3 were failed to be rescued. (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

that both the A14R2A3 and A14R4A3 antisera neutralized the HAdV-3 strain, with titers ranging from 512 to 8192, and no difference was found when compared with the anti-AD3EGFP sera group ($P=0.1301$).

4. Discussion

For adenovirus vaccine design, rapid diagnostic reagents development and anti-adenovirus medicine preparation, it is important to determine the neutralizing epitopes of pathogenic adenovirus types. However, to our knowledge, among the 65 adenoviruses, very little is currently known about the type-specific neutralization antibody recognition sites [38,43]. It has been previously reported that hexon is the major target of type-specific adenovirus NAbs [44]. Because hexon is a protein homotrimer, the 3D conformation of hexon protein can provide relevant information to obtain accurate epitope information. In previous study, the 3D structure of HAdV-3 hexon and the epitopes within the hexon were successfully predicted using the homology modeling method in combination with a bioinformatics epitope screening algorithm [31]. In the current study, we used a similar method to determine the 3D model of HAdV-14 hexon and the four neutralizing epitopes within the hexon were predicted. Then these candidate epitopes were successfully validated by ELISA and neutralization tests (NTs), demonstrating the reliability of this structure-based epitope screening method.

In recent years, the antigen capsid-incorporation strategy has offered a novel and exciting approach for adenovirus-based vaccine design because of the plasticity of the adenoviruses. This approach might induce multivalent immunity against various infectious diseases or pathogens of multiple serotypes [46,47]. In the current study, we tried to incorporate the four putative epitopes A14R1, A14R2, A14R4 and A14R7 into HAdV-3 vectors, but only the A14R2 and A14R4 were successfully represented on the surface of HAdV-3 vectors. Strikingly, the GMT of neutralizing titers of anti-A14R4A3 (Fig. 6A) against HAdV-14 were 2-fold higher than anti-A14R4 (Fig. 4) group, with significant difference ($P=0.0442$). This indicates that the hexon-modified virus could elicit stronger neutralizing antibodies against HAdV-14 than KLH coupled peptides. These data illustrated the advantage of the potent immunologic properties

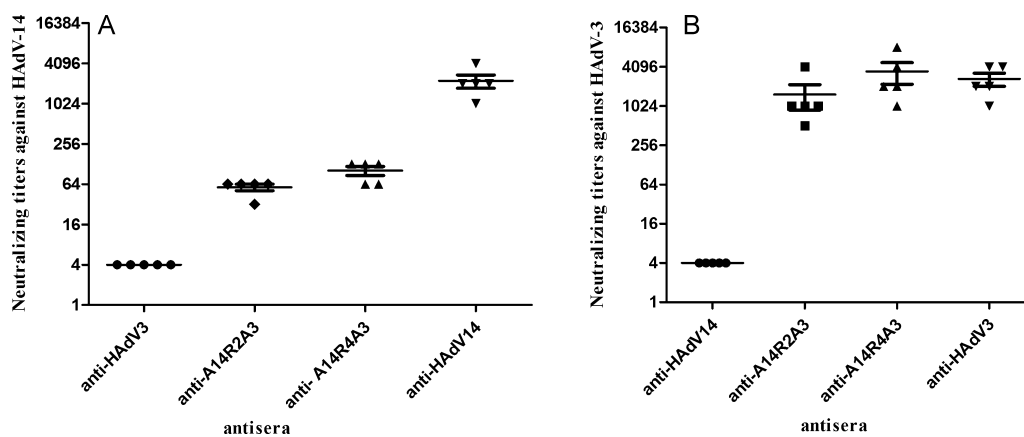


Fig. 6. Neutralization titers of anti-A14R2A3 and anti-A14R4A3 serum against HAdV-14 (A) or HAdV-3 (B). Sera from mice boosted with chimeric virions A14R2A3 and A14R4A3 were collected for neutralization tests against both HAdV-3 and HAdV-14. Anti-HAdV-3 or anti-HAdV-14 serum was used as control. The vertical axis represented the dilution factor (diluted from 1:8 to 1:16,384). Results were expressed as the mean \pm SEM, and comparisons among groups were performed by Mann–Whitney *U* test. Antiserum which did not show any reaction at 1:8 (the lowest dilution tested) in NTs was assigned a titer of 4 for GMT computation.

of the adenovirus hexon for presenting peptide epitopes to the immune system.

In the current study, we used DQ149612 as a reference sequence of HAdV-14 to synthetic peptides. By sequence alignment of 13 representative HAdV-14 strains derived from NCBI, we found there were variations only within A14R4 sequence, which were $^{252}\text{TEEAGNIEY}^{260}$ vs $^{252}\text{TEEGNIEY}^{260}$ with only two amino acids replacement. The synthetic peptide A14R4 sequence and the peptide incorporated in HAdV-3 was TEEAGNIEY, however, the HAdV-14 GZ01 strain used in the neutralization tests encoding TEEGNIEY. The results showed that the antibody induced by synthetic peptide A14R4 or epitope chimeric adenovirus A14R4A3 could detect and neutralize the HAdV-14 GZ01 strain (Supplementary Fig. 3B and Fig. 6A), indicating the antibody induced by TEEAGNIEY was universal for all HAdV-14 strains.

In conclusion, we have successfully predicted four neutralizing epitopes of HAdV-14 using the homology modeling method and proven the correctness of epitopes prediction by ELISA assays and neutralization tests. Then we successfully incorporated A14R2 and A14R4 derived from HAdV-14 into the HVR2 and HVR4 region of HAdV-3 hexon and presented it on the surface of virions using the antigen capsid-incorporation strategy. The chimeric virions elicited neutralizing antibodies which were able to confer good in vitro protection against both HAdV-14 and HAdV-3 in AD293 cells (Fig. 6). Taken together, the current findings contribute not only to the development of HAdV-14 vaccine candidates or rapid adenovirus diagnostic reagents, but also to the construction of new gene delivery vectors. In the next step, we will set out to create novel multivalent HAdV-3 vaccine vectors presenting several B-cell epitopes derived from pathogenic adenovirus types that would yield optimal vaccine efficacy.

Authors' contribution

MQ performed most of the experiments and drafted the paper. TXG designed the experiments and edited the paper. JZX and LQ performed some neutralization tests. HJF performed the hexon protein modeling. LXM prepared some viruses. LQM and ZR supervised the work and edited the final version of this manuscript. All authors read and approved the final version of the manuscript.

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Conflicts of interest: There are no conflicts of interest.

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