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Ad5-nCoV vaccination could induce HLA-E restricted CD8 + T cell responses specific for epitopes on severe acute respiratory syndrome coronavirus 2 spike protein

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Abstract

Objectives: To evaluate cellular immune responses induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines immunization in population based on HLA-E-restricted CD8 $^{+}$ T cell epitopes identification. **Methods:** HLA-E-restricted SARS-CoV-2 CD8 $^{+}$ T cell nonamer peptides were predicted with software. HLA-E-transfected K562 cells binding assay was used to screen for high-affinity peptides. IFN-γ enzyme-linked immunospot assay were used to identify HLA-E-restricted epitopes. HLA-E/epitope tetramer was employed to detect the frequencies of epitope-specific CD8 $^{+}$ T cells. **Results:** Four CD8 $^{+}$ T cell epitopes on spike protein of SARS-CoV-2 restricted by both HLA-E*0101 and E*0103 were identified. HLA-E-restricted epitope specific IFN-γ-secreting CD8 $^{+}$ T cell responses could be detected in individuals vaccinated with SARS-CoV-2 vaccines. Importantly, the frequencies of epitope-specific CD8 $^{+}$ T cell in Ad5-nCoV vaccinated individual were higher than that in individuals vaccinated with recombinant protein or inactivated vaccines. Moreover, frequencies of epitope-specific CD8 $^{+}$ T cells could maintain for at least 120 days after only one dose Ad5-nCoV vaccination. While frequencies of epitope-specific CD8 $^{+}$ T cells decreased in individuals after two doses of Ad5-nCoV vaccination. **Conclusions:** These findings may contribute to more comprehensive evaluating protective effects of vaccines for SARS-CoV-2, meanwhile may provide information to characterize HLA-E-restricted CD8 $^{+}$ T cell immunity against SARS-CoV-2 infection.

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Abstract

Objectives: To evaluate cellular immune responses induced by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) vaccines immunization in population based on HLA-E-restricted CD8^{+} T cell

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Results: Four $CD8^+$ T cell epitopes on spike protein of SARS-CoV-2 restricted by both HLA-E*0101 and E*0103 were identified. HLA-E-restricted epitope specific IFN-γ-secreting $CD8^+$ T cell responses could be detected in individuals vaccinated with SARS-CoV-2 vaccines. Importantly, the frequencies of epitope-specific $CD8^+$ T cell in Ad5-nCoV vaccinated individual were higher than that in individuals vaccinated with recombinant protein or inactivated vaccines. Moreover, frequencies of epitope-specific $CD8^+$ T cells could maintain for at least 120 days after only one dose Ad5-nCoV vaccination. While frequencies of epitope-specific $CD8^+$ T cells decreased in individuals after two doses of Ad5-nCoV vaccination.

Conclusions: These findings may contribute to more comprehensive evaluating protective effects of vaccines for SARS-CoV-2, meanwhile may provide information to characterize HLA-E-restricted $CD8^+$ T cell immunity against SARS-CoV-2 infection.

Keywords:

severe acute respiratory syndrome coronavirus 2; Ad5-nCoV; vaccination; HLA-E; $CD8^+$ T cell response; epitope

Introduction

The acute infectious disease coronavirus disease 2019 (COVID-19), caused by severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2) infection, is characterized by fever and upper respiratory symptoms, and even multiple organ failure such as acute respiratory distress syndrome [1]. COVID-19 began to appear in December 2019 and posed a huge threat to global public health and human life. The World Health Organization (WHO) declared that COVID-19 has become a global pandemic in March 2020. COVID-19 spread across nearly every country. Up to now, COVID-19 has accumulated more than 661 million confirmed cases and more than 6.7 million deaths worldwide.

Vaccination is the most effective way to prevent SARS-CoV-2 infection. At present, many types of vaccines against SARS-CoV-2 have been widely vaccinated in the world and achieved certain preventive effects. Among them, recombinant SARS-CoV-2 vaccine with adenovirus type 5 vector, which was developed and produced in China (Ad5-nCoV, CanSinoBIO, China), has been proved to induce high level of both humoral and cellular immune responses [2-4]. Importantly, Ad5-nCoV has been widely vaccinated in the population and approved by WHO as COVID-19 vaccine "Emergency Use List" in May 2022 [5-7].

SARS-CoV-2 is single positive-stranded, enveloped RNA virus that can infect many animal species and humans. The genome containing six open reading frames (ORFs), which encode non-structural proteins such as RNA dependent RNA polymerase (RdRp), and four structure proteins, namely spike protein (S), envelope protein (E), membrane protein (M) and nucleocapsid proteins (N) [8]. The S protein could induce high level of specific antibody. However, antibody responses against SARS-CoV-2 always could not be well maintained for a long time [9].

It has been reported that SARS-CoV-2-specific $CD8^+$ T cell responses in peripheral blood of COVID-19 patients were closely related to remission of the disease [10, 11]. In fact, SARS-CoV-2 specific $CD8^+$ T cell responses could be detected in acute phase of COVID-19 within 7 days after onset of symptoms, which always reached the strongest at 14 days [12]. Studies in SARS-CoV-2 infected animal models have found that although neutralizing antibodies could protect against viral attack, $CD8^+$ T cell responses could provide more important clinical protection when antibody levels were low or reduced [13]. $CD8^+$ T cell epitopes of SARS-CoV-2 restricted by classical HLA-I molecules, such as HLA-A1/ORF1a$_{1637}$, HLA-A2/S$_{269}$, HLA-A3/N$_{361}$, HLA-A24/S$_{1208}$, HLA-B7/N$_{105}$ and HLA-B40/N$_{322}$, were used to further study the effects and

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mechanisms of specific $CD8^+$ T cell responses in SARS-CoV-2 infection [14, 15]. Among them, $NP_{105\text{-}113}$ epitope restricted by HLA-B7 has been proved to induce specific $CD8^+$ T cell response with strong antiviral activity [16]. Although most studies focused on classical HLA-I restricted $CD8^+$ T cell epitopes and specific responses, non-classical HLA-I molecule HLA-E restricted $CD8^+$ T cells also play an important role for immune protection and regulation in many viral infectious diseases [17-19]. Notably, HLA-E is a ubiquitous HLA locus with only two different alleles HLA-E*0101 and HLA-E*0103, both of which have a combined frequency of over 99% in global population. The two molecules just differ in position 107 of the mature HLA-E protein (arginine for HLA-E*0101 and glycine for HLA-E*0103)[20]. It has been proposed to use HLA-E restricted $CD8^+$ T cells to treat severe COVID-19 patients in the early stage of SARS-CoV-2 infection [21]. Therefore, it is necessary to identify $CD8^+$ T cell epitopes that can overcome the polymorphism of HLA molecules. Moreover, detection of SARS-CoV-2 epitope-specific $CD8^+$ T cell responses may be important for evaluation of the protective effects induced by SARS-CoV-2 vaccines.

In this study, specific $CD8^+$ T cell responses in different vaccines inoculated populations, especially in Ad5-nCoV vaccinated subjects, were detected based on identification of four epitopes on S protein of SARS-CoV-2 restricted by both HLA-E*0101 and E*0103. These findings may provide crucial information to evaluate the effects of COVID-19 vaccines immunization in population according to HLA-E-restricted SARS-CoV-2 specific $CD8^+$ T cell responses.

Methods

Study cohort

A total of 56 samples from 43 individuals vaccinated with SARS-CoV-2 vaccines were enrolled in this study. The ages of vaccinated subjects were ranging between 21 and 32 years. The vaccinated donors who were suffering from infectious diseases or autoimmune diseases, and experiencing any inflammation or infection within a week before sampling were excluded from this study. Subjects were divided into three groups according to different types of vaccine they received, including inactivated vaccine group (CoronaVac, Sinovac Biotech, China), recombinant protein subunit vaccine group (ZF2001, Zhifei Biological, China) and adenovirus-vectored vaccine group (Ad5-nCoV, CanSino Biologics, China). The basic information of the enrolled volunteers is summarized in Table 1.

Sample collection

Peripheral venous blood samples of SARS-CoV-2 vaccination individuals were drawn into pyrogen-free blood collection tubes with citrate as an anticoagulant. Peripheral blood mononuclear cells (PBMCs) were isolated using a standard Ficoll-Hypaque (Sigma-Aldrich, MO) density gradient centrifugation.

Epitopes prediction

The Immune Epitope Database and Analysis Resource (IEDB), NetMHC4.0 Server and NetMHCpan 4.1 Server are used to predict binding of peptides to MHC molecule of known sequence using artificial neural networks (ANNs). Specifically, nine-mer peptide as the peptide length option was selected. HLA-E*0101 or HLA-E*0103-restricted SARS-CoV-2 nine-mer peptide epitopes were predicted by inputting 1,273 amino acids of S protein sequence, 75 amino acids of E protein sequence, 222 amino acids of M protein sequence and 419 amino acids of N protein sequence of SARS-CoV-2 prototype strain Wuhan-Hu-1 (GenBank accession number: NC_045512.2) into the databases, respectively.

There is only HLA-E*0101 allele option could be selected in NetMHC4.0 Server database, while both HLA-E*0101 and E*0103 alleles could be selected in NetMHCpan 4.1 Server database under the loci or species option with HLA-E. Both the two methods specified if a sequence is a strong MHC binder (SB) or a weak MHC binder (WB) based on a %Rank score. By default, %Rank $< 0.5\%$ and %Rank $< 2\%$ thresholds are considered for detecting as SB and WB for HLA-E molecule.

Peptides synthesis

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The predicted SARS-CoV-2 nine-mer epitopes were synthesized at least 90% purity as assessed by high-performance liquid chromatography and mass spectrometry (ChinaPeptides, Shanghai, China). Peptides from HLA-Cw*$03_{3\text{-}11}$ or HLA-A*$02_{3\text{-}11}$ leading sequences VMAPRTLIL or VMAPRTLVL, which have been confirmed to be the HLA-E-restricted epitopes, were synthesized and used as positive control. The peptide melanoma antigen-encoding gene (MAGE-1) with sequence EADPTGHSY, which couldn't bind to HLA-E molecules, was synthesized and used as the negative control. All the peptides were stored at 1 mM concentration at -70°C and repeated freeze-thawing was avoided.

K562 cell lines transfected with HLA-E molecule

An immortalized chronic myelogenous leukemia cell line K562 with MHC molecules expression deficiency was obtained from ATCC (CCL-243). We stably transfected K562 cell lines with single allele HLA-E*0103 or HLA-E*0101 to generate mono-allelic cell lines with lentivirus transfected methods. Stably transfected cells showed strong expression (>95%) of HLA-E*0103 or HLA-E*0101 according to flow cytometry (FCM) analysis.

K562/HLA-E cells binding assay

The stable transformed K562/HLA-E*0103 or K562/HLA-E*0101 cells were respectively incubated with 10 μM each indicated peptide and 1 μM human β2-microglobulin (β2m, Sigma) in serum-free RPMI 1640 medium for 16 h at 26°C with 5% CO2, while K562/HLA-E cells with no peptide incubation under the same culture conditions were used as blank control. These cells binding with peptides were further incubated at 37°C for 2 h for thermal stability. We detected the expression of HLA-E molecules on surface of K562/HLA-E cells by staining with PE-labeled anti-HLA-E monoclonal antibody (mAb) (3D12; BioLegend, USA), using ACEA NovoExpress system (Agilent Technologies, USA). The results are presented as the fluorescence index (FI). FI [?] 1 represents high-affinity peptide, indicating that the stable combination of the peptide with HLA-E molecules on surface of K562/HLA-E cells could increase the mean fluorescence of the HLA-E molecules by at least one-fold.

Isolation of $CD8^+$ T cells with magnetic beads kits

The $CD8^+$ T cells from PBMCs were isolated by magnetic streptavidin nanobeads of Biolegend MojoSort^{TM}Isolation Kits. Incubating PBMCs with biotin antibody cocktail followed by incubation with magnetic streptavidin nanobeads. Targeted cells were depleted by magnetically labeled fraction using a magnetic separator, while untouched $CD3^+CD8^+$ cells were collected.

K562/HLA-E cells pre-incubation with peptides

Before incubation with peptides, K562/HLA-E*0103 cells were incubated at 26 for 24 hours to adapt to the condition for antigen presentation in advance. K562 cells were then incubated with peptides constructed (ul/10 cells) at 26 for 19h for the next assay.

Ex Vivo **ΙΦΝ-γ ενζψμε-λινκεδ ιμμυνοσποτ ασσαψ (ΕΛΙΣΠΟΤ)**

The human IFN-γ ELISPOT kit (DAKEWE Biotech Company, China) was used to detect the capacity of IFN-γ production by $CD8^+$ T cells stimulated with specific antigenic peptides presented by K562/HLA-E cells as previously. The spots representing peptide-specific IFN-γ-producing $CD8^+$ T cells were counted using an automatic ELISPOT reader (Cellular Technology Limited, USA). Adjusted spot-forming cells (SFCs) after subtracting negative values are expressed as SFCs/10^6 cells.

Peptide/HLA-E*0103 tetramer staining

Two peptides with high binding affinity S17 and S19 were selected for construction of peptide/HLA-E tetramer labeled with PE by Epigen Biotech (Nantong, China). The PBMCs of the SARS-CoV-2 vaccinated individuals were stained with each PE-labeled peptide/HLA-E tetramer for 10 min, and subsequently stained with FITC-labeled anti-human CD8 mAb (clone SK1, Biolegend, US) and APC-labeled anti-human CD3 mAb (clone OKT3, Biolegend, US). Approximately 300,000 cells gated on $CD3^+$ cells were captured. The

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gate for $CD8^+$ $tetramer^+$ T cells was set up by matching non-tetramer staining isotype control (BioLegend). Compensation controls were checked regularly to avoid false-positive results and individually determined for each experimental setup.

Statistical analysis

Statistical analysis was performed using GraphPad Prism software, version 6 (GraphPad; La Jolla, CA, USA). The Mann–Whitney *U* test was used for parameter comparison between two subject groups. The frequencies of epitope-specific $CD8^+$ T cells are presented as median with 95% confidence interval (CI95%) or median with range.*P* -Value (two-tailed) below 0.05 (*P* [?] 0.05) was considered to be statistically significant.

Results

Thirty-six HLA-E-restricted peptides on SARS-CoV-2 were predicted and synthesized

HLA-E*0101 or HLA-E*0103-restricted peptides on SARS-CoV-2 structure protein S protein, E protein, M protein and N protein were predicted. According to the binding affinity, thirty-six predicted nonapeptides on SARS-CoV-2 with strong binding or weak binding ability in either of the two databases were selected. A total of 36 SARS-CoV-2-derived HLA-E-restricted nonapeptides with the detail information including 25 peptides on S protein, one peptide on E protein, six peptides on M protein and four peptides on N protein were listed in Table S1.

Four nonapeptides of SARS-CoV-2 exhibited high binding affinity to bothHLA-E*0103 and HLA-E*0101 molecules

Then we used the K562/HLA-E cell binding assay to further screen the high binding affinity SARS-CoV-2 nonapeptides to HLA-E*0103 or HLA-E*0101 molecules, respectively. Among the 36 predicted HLA-E restricted SARS-CoV-2 peptides, six peptides including S7, S10, S13, S15, S19 and S25 could increase the expression of HLA-E*0101 on K562/HLA-E cell surface characterized by fluorescence index (FI) [?] 1, indicating a high binding affinity to HLA-E*0101 molecule (Figure 1A). Moreover, four of the 23 predicted peptides including S7, S13, S19 and S25 could also increase the HLA-E*0103 expression on K562/HLA-E cell surface with FI [?] 1, suggesting a high binding affinity to HLA-A*0103 molecule as well (Figure 1B). Therefore, the four nonapeptides S7, S13, S19 and S25 of SARS-CoV-2 exhibited high binding affinity to both HLA-E*0103 and HLA-E*0101 molecules. However, there may be slight difference of the binding affinity among these peptides according to the FI value. Specifically, S19 showed higher binding affinity to HLA-E*0103 molecule, while S7 and S15 exhibited higher binding affinity to HLA-E*0101 molecule. Detailed information for the screened peptides with high binding affinity were summarized in Table S2.

ΗΛΑ-Ε ρεστριστεδ επιτοπες οφΣΑΡΣ-ὃ˝-2 ωερε ιδεντιφιεδ βψ ινδυςινγ σπεςιφις ΙΦΝ-γ-προδυςινγ ˆΔ8$^+$ Τ ςελλ ρεσπονσες

We next assessed the capacity of each peptide to elicit epitope-specific $CD8^+$ T cell responses by detecting the secretion of IFN-γ *in vitro* in SARS-CoV-2 vaccinated subjects. The results showed that four peptides (S7, S13, S19, S25) could effectively elicit epitope-specific IFN-γ-secreting $CD8^+$ T cell responses in peripheral blood of vaccinated subjects (Figure 2A), indicating that the four SARS-CoV-2 peptides could be defined as HLA-E restricted $CD8^+$ T cell epitopes. Specifically, the median (range) was 108 (13-685) SFC/10^6 PBMCs for peptide S7, 87 (10–516) SFC/10^6 PBMCs for peptide S13, 102 (15–588) SFC/10^6 PBMCs for peptide S19 and 104 (8-591) SFC/10^6 PBMCs for peptide S25. However, there was only 11 out of 56 samples collected showed the HLA-E restricted epitopes-specific $CD8^+$ T cell responses. Moreover, the frequencies of IFN-γ-secreting $CD8^+$ T cell responses showed no difference among the four HLA-E restricted SARS-CoV-2 epitopes (Figure 2B).

The frequencies of HLA-E restricted SARS-CoV-2 epitopes-specific $CD8^+$ T cells could be detected in peripheral blood of vaccinated individuals

The SARS-CoV-2 epitopes S7 and S19 were selected to construct HLA-E/peptide tetramer to accurately detect the frequencies of epitope-specific $CD8^+$ T cells in the peripheral blood of SARS-CoV-2 vaccinated

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subjects. The frequencies of both epitope S7 and epitope S19-specific $CD8^+$ T cells could be detected (Figure 3A). The frequency of epitope S7-specific $CD8^+$ T cells in 56 samples ranged from 0.020% to 1.120% (median values: 0.11%, CI95%: 0.1234%-0.2398%), while the frequency of epitope S19-specific $CD8^+$ T cells ranged from 0.020% to 0.74% (median value: 0.10%, CI95%: 0.1280%-0.2156%). Notably, there was no difference of the frequencies of epitope-specific $CD8^+$ T cells between the two epitopes in each SARS-CoV-2 vaccination group (Figure 3B). When compared the frequencies of epitope-specific $CD8^+$ T cells among three different of SARS-CoV-2 vaccines immunization groups for each epitope, the frequencies of epitope-specific $CD8^+$ T cells in individuals receiving adenovirus-vectored vaccine group seemed higher than that in inactivated vaccine group and recombinant protein subunit vaccine group (Figure 3C).

The frequencies of epitope-specific $CD8^+$ T cells were decreased in the individuals receiving two doses of SARS-CoV-2 vaccination than only one dose

The frequencies of epitope-specific $CD8^+$ T cells in individuals who received only one dose of Ad5-nCoV vaccine were further analyzed according to the time interval between the latest vaccination and sample collection. The results showed that the frequencies of epitope-specific $CD8^+$ T cells were elevated from the time less than 30 days to the time 30 days-60 days, then decreased at the time 60 days-90 days, and again elevated at the time 90 days-120 days, even more than 120 days. The trends of the frequencies of epitope-specific $CD8^+$ T cells at different time quantum was similar between the epitope S7 and epitope S9 (Figure 4A-4B). Then we compared the frequencies of epitope-specific $CD8^+$ T cells in individuals who received different doses of Ad5-nCoV vaccination. Notably, the frequencies of epitope-specific $CD8^+$ T cells in subjects with two doses vaccination were lower compared with that in subjects with only one dose vaccination (P <0.05 for epitope S7) (Figure 4C-4D).

Discussion

SARS-CoV-2 vaccines inducing specific antibody production, meanwhile the specific antiviral T cell responses may present better protective effects for people. Therefore, exploring the vaccine-induced SARS-CoV-2-specific $CD8^+$ T cell responses based on $CD8^+$ T cell epitopes might be one of the important approaches. In this study, we identified four nonamer epitopes on SARS-CoV-2 restricted by HLA-E molecule, which could elicit epitope-specific $CD8^+$ T cell responses in SARS-CoV-2 vaccinated population. Importantly, the HLA-E-restricted SARS-CoV-2 epitope-specific $CD8^+$ T cells showed high frequencies in Ad5-nCoV vaccinated individuals. The frequencies of epitope-specific $CD8^+$ T cells were decreased in the individuals receiving two doses of Ad5-nCoV vaccination than only one dose. These results may greatly advance the understanding of the cellular immune defense against SARS-CoV-2 infection, meanwhile contribute to more comprehensive evaluating the protective effects of vaccines for SARS-CoV-2 covering all the HLA-diverse populations.

The most ideal result after infection or vaccination is highly protective and lasting immunity, so as to establish a high level of immunity of the population. The large-scale inoculation of SARS-CoV-2 vaccine in population has greatly reduced the rates of infection and severity. T cell response plays a key role in vaccine mediated protection. It has been confirmed that vaccines that could induce SARS-CoV-2 specific T cell response could effectively control infection, avoid severe tissue damage, and significantly reduce hospitalization rate and mortality. In clinical trials of SARS-CoV-2 mRNA vaccine (BNT162b2; Modern VRC) or adenovirus vector vaccine (AdV5; ChAdOx), SARS-CoV-2 specific $CD8^+$ T cells could secrete high level of IFN- γ, which was similar to the level of specific $CD8^+$ T cell response in patients with COVID-19. The inoculation of SARS-CoV-2 mRNA vaccine or adenovirus vector vaccine could induce high frequency of $CD8^+$ T cell response specific to S protein with $CCR7^-CD45RA^+$effect phenotype, meanwhile could induce persistent memory $CD8^+$ T cells [3, 22-24]. Moreover, the strong SARS-CoV-2 specific T cell responses could be detected in naturally infected individuals after vaccination. Our results showed that the HLA-E restricted SARS-CoV-2 epitopes-specific $CD8^+$ T cell responses could be detected in the subjects vaccinated with adenovirus vector vaccine Ad5-nCoV, further indicating that Ad5-nCoV inoculation could induce effective T cell responses in the population.

HLA-E has been proved to present epitope to interact with $\alpha\beta$TCR on $CD8^+$ T cells in several infectious

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diseases. Since HLA-E is a ubiquitous HLA allele locus in the population, HLA-E could be used as the first choice in more specific and effective peptide vaccine design and research. The current research mainly focuses on the mechanism of HLA-E mediated NK cell function in the process of SARS-CoV-2 infection. It has been found that SARS-CoV-2 could induce HLA-E expression on the surface of pulmonary epithelial cells, which could bind peptides derived from S protein and interact with CD94/NKG2A on NK cells [25]. The peptide derived from nsp13 of SARS-CoV-2 presented by HLA-E could not interact with NKG2A on NK cells, thus mediating the killing effects of NK cells to virus-infected target cells [26]. Therefore, it is necessary to identify HLA-E restricted epitope of SARS-CoV-2, which would provide information for people who suffered from COVID-19 disease or vaccinated with SARS-CoV-2 vaccine. Based on the general rule of epitope binding motifs, nonapeptides which could be presented by HLA-E molecule always showed mainly anchor residues methionine (M), isoleucine (I) or leucine (L) at position 2, and isoleucine (I) or leucine (L) at position 9. Therefore, the six SARS-CoV-2 nonapeptides we identified with residue M/I/L at position 2 and residue L at C-terminal were conformed the general rule of HLA-E-restricted peptides. Notably, our study found that two epitopes S7 and S19 on SARS-CoV-2 restricted by HLA-E could also be resented by HLA-A*02 reported in other studies [14]. Considering that 86% sequence was similar between HLA-E and HLA-A*02 molecules, and HLA-A*02 molecules may share binding peptides with HLA-E molecule. Therefore, this is a common phenomenon that HLA-E*01 and HLA-A*02 molecules always could present the same epitopes such as influenza $M_{159\text{-}167}$, $M_{158\text{-}166}$ and Epstein-Barr virus $BZLF_{139\text{-}147}$ due to conserved deep pockets [27]. The co-presentation of antigen epitopes by both HLA-E*01 and HLA-A*02 seems to be more important in inducing $CD8^+$ T cell response for HLA-E restriction epitopes.

It has been proved that receptor-binding domain (RBD) on S protein contains a variety of conformational epitopes that could induce high levels of antibody production [8], especially specific antibody with neutralizing activity. In addition, S protein also contains multiple dominant T cell epitopes, which could induce effective T cell immune response [28]. Therefore, S protein is the primary antigen used in development of SARS-CoV-2 vaccines. RBD region of S protein has become the core target for research of therapeutic neutralizing antibody and the mechanisms for specific T cell responses. HLA-E restricted specific $CD8^+$ T cell epitopes identified in this study were all from S protein of SARS-CoV-2. Although these epitopes aa269-aa277, aa576-aa584, aa958-aa966 and aa1185-aa1193 were not locus within the RBD region (aa329-aa521), they are still important for the potential applications in future.

In fact, although SARS-CoV-2 non-structural protein (nsp) and open reading frames (ORFs) such as ORF3, nsp3, nsp4 and nsp12 showed low levels in SARS-CoV-2 infected cells, they also contain very important $CD8^+$ T cell epitopes. For example, ORF9b derived epitopes could induce high-level virus specific $CD8^+$ T cell responses in COVID-19 patients, confirming good immunogenicity of epitopes *in vivo* [29]. Therefore, identification of HLA-E restricted epitopes on nsp and ORFs of SARS-CoV-2 also make sense for research the effects of $CD8^+$ T cells in further study.

In conclusion, HLA-E-restricted SARS-CoV-2 epitope-specific $CD8^+$ T cell responses could be detected in Ad5-nCoV vaccinated population. Importantly, the levels of epitope specific $CD8^+$ T cell responses could maintain for a long time after only one dose vaccination. The study may be a good supplement for evaluation of vaccination effects of Ad5-nCoV from the perspective of T cell responses. However, HLA-E-restricted $CD8^+$ T cell responses induced in naturally infected individuals after vaccination still need to be investigated in the future.

Conflict of Interest

The authors have no competing interests to declare.

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Ethical Approval statement

The study was conducted in accordance with the Declaration of Helsinki, and approved by the Institutional Review Board of the Fourth Military Medical University. The research involving humans and human materials was also approved by the Ethical Review Board of the Fourth Military Medical University (KY20212112-C-1), and the related data were anonymized before use.

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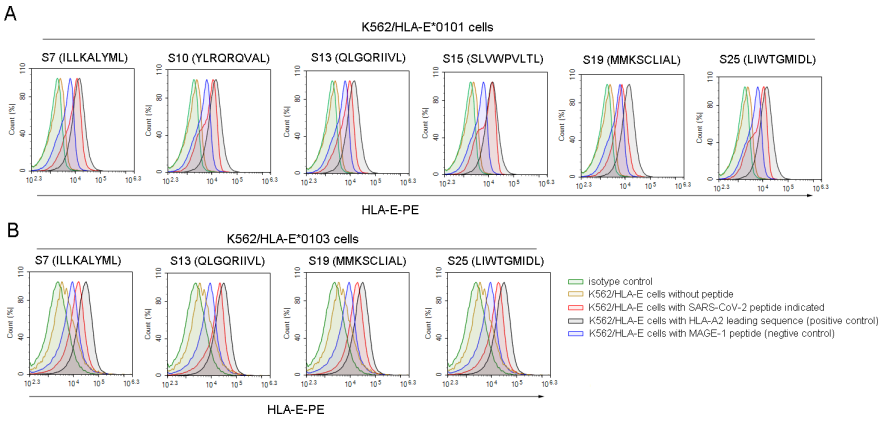

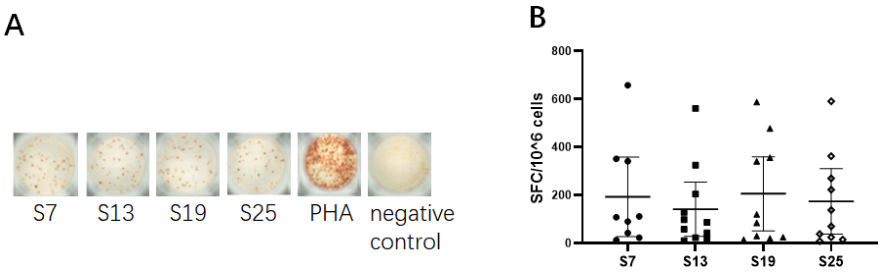

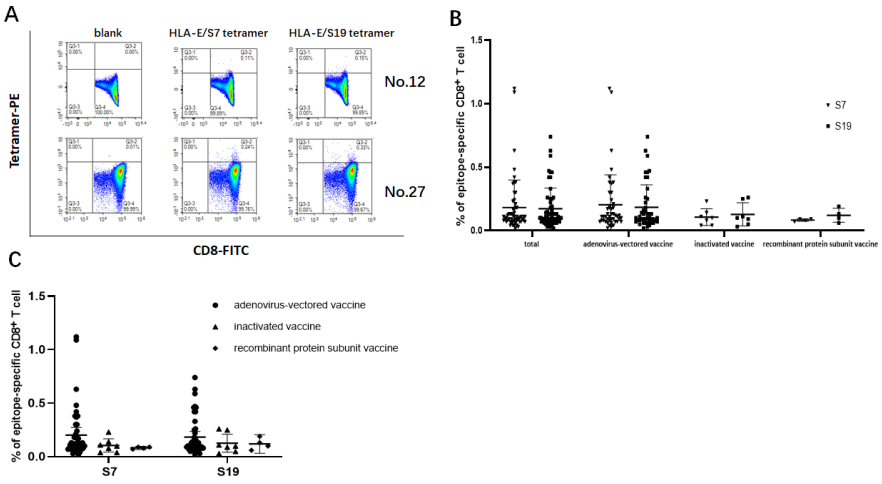

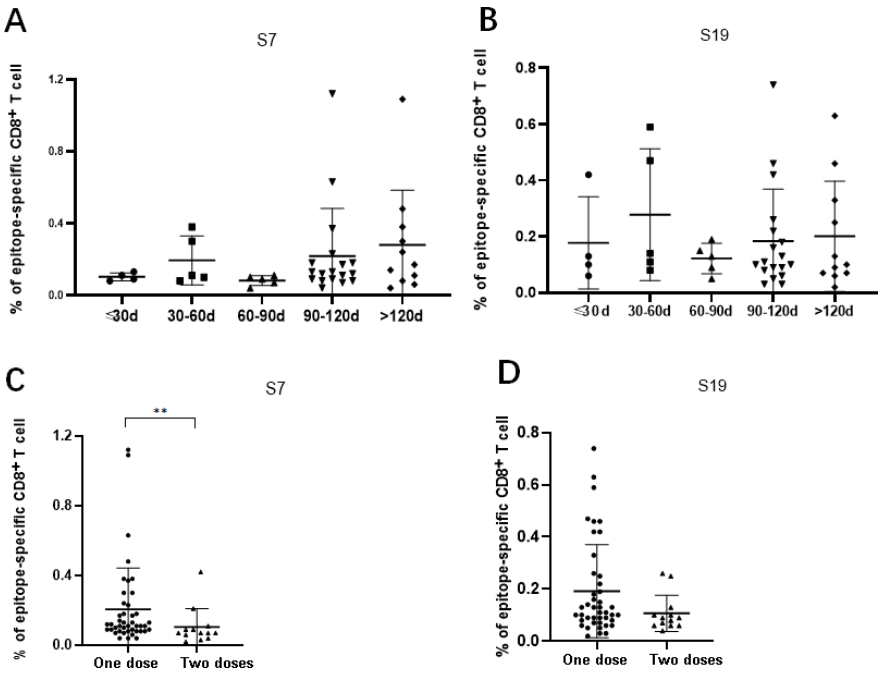

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