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Priming with SARS CoV S DNA and boosting with SARS CoV S epitopes specific for CD4+ and CD8+ T cells promote cellular immune responses

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Abstract

Cellular immune response plays an important role in antiviral immunity. In our previous study, immunization of mice with severe acute respiratory syndrome coronavirus (SARS CoV) spike (S) DNA vaccine could induce both humoral and cellular immunity in response to a pool of entire overlapping S peptides. Identification of functional dominant epitopes in SARS CoV S protein for T cells is crucial for further understanding of cellular immune responses elicited by SARS CoV S DNA vaccine. In present study, mice were immunized with SARS CoV S DNA vaccine. Subsequently, a pool of 17–19 mers overlapped SARS CoV S peptides, which served as immunogens, were scanned to identify the specific epitopes for T cells. Two H-2^d restricted $CD4^+$ T epitopes, N60 (S435–444) and P152 (S1111–1127), and two H-2^d restricted $CD8^+$ T cell epitopes, N50 (S365–374) and P141 (S1031–1047) were identified by three different methods, enzyme-linked immunosorbent assay (ELISA), enzyme linked immunospot assay (ELISPOT) and fluorescence activated cell sorter (FACS). The dominant $CD4^+$ T cell epitope (N60) and $CD8^+$ T cell epitope (N50) located in the receptor-binding domain (RBD) of SARS CoV S protein, which mediated virus combining and fusing to susceptible cells. Importantly, our novel finding is that mice primed with SARS S DNA vaccine and boosted with T cell epitopes (N50 and N60) could promote antigen specific $CD4^+$ and $CD8^+$ T cell immune responses. Our study provides valuable information for the design of vaccine for SARS study.

Keywords: SARS CoV; T cells; Epitope; Vaccines

1. Introduction

Severe acute respiratory syndrome (SARS) is a new infectious disease caused by a novel coronavirus named as SARS CoV [1,2], which infected 8096 people and killed 774 in 2003 [3]. SARS CoV consists of four major structural proteins: spike (S), membrane (M), envelope (E) and nucleacapsid (N). S protein is a large type I transmembrane glycoprotein responsible for receptor binding and membrane fusion [4,5]. The angiotension converting enzyme 2 (ACE-2) on the susceptible cell surface is served as a receptor for SARS CoV. And a small 193 amino acid fragment on SARS CoV residue (S318–510) was characterized as a minimal functional receptor-binding domain (RBD) [6,7]. Moreover, CD209L (L-SIGN) is another receptor for SARS CoV [8]. It has been reported that SARS CoV infection induces both humoral and cellular immunity. Neutralizing antibodies specific for SARS CoV were detected on days 5–10 after the onset of syndrome in SARS patients. The levels of antibodies peaked on days 20–30 and sustained for more than 150 days [9]. Sera from convalescent SARS patients could be

Abbreviations: SARS, severe acute respiratory syndrome; CoV, coronavirus; S, spike protein; SFU, spot forming unit; IL-7Rα, interlukin-7 receptor alpha; ODN, oligodeoxynucleotide; FACS, fluorescence activated cell sorter

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transferred to SARS patients for the treatment during the SARS outbreak [10]. At the early stage of SARS, although levels of serum cytokines including IL-2, IL-10 and IL-12 increased significantly, $CD3^+$, $CD4^+$ and $CD8^+$ T cell counts in peripheral blood decreased compared to normal individuals [11]. In addition, SARS CoV associated memory $CD4^+$ and $CD8^+$ T cells specific for SARS CoV S, N and E proteins were generated and sustained for more than 2 years [12–14].

Currently, there is no effective drug to prevent or treat SARS. Therefore, developing SARS vaccines will be an effective way to prevent SARS spreading. Several vaccines for SARS CoV have been developed and proved to be effective in animals [15]. Whole SARS CoV particles inactivated by formaldehyde, UV light or β-propiolactone could induce neutralizing antibodies in animals, and these vaccines were being tested in clinic trials in China [16]. Yang et al. reported that a candidate DNA vaccine encoding the full-length S protein induced neutralizing antibodies, which could protect mice from SARS CoV challenge [17]. After immunization with SARS CoV DNA vaccine, cellular immunity was induced by the evidence of high frequencies of IFN-γ producing effector/memory $CD4^+$ and $CD8^+$ T cells in mice [18]. Additionally, adenovirus, attenuated para-influenza virus and modified attenuated modified vaccinia virus Ankara (MVA) vaccines encoding SARS CoV S protein were used to immunize animals. Results indicated that these vaccines could elicit high titers of neutralizing antibodies [19–21].

BALB/c mice immunized with SARS CoV S peptides specific for B cells (S91–102, S424–435, S458–469 and S1065–1076) with Freund adjuvant could trigger a rapid and highly effective immune response in vivo [22]. Meanwhile, four human HLA-0201 restricted $CD8^+$ T cell epitopes S411–420, S978, S1203 and SSp-1 were demonstrated in recovered SARS patients [23–25].

In order to identify the epitopes of SARS CoV S antigen for T cells, mice were immunized with SARS CoV DNA vaccines. One hundred and sixty nine SARS CoV S peptides were mapped for specific stimulation of T cells to produce IFN-γ. Our data confirmed previous studies [19] and demonstrated that two dominant $H\text{-}2^d$ restricted epitopes N50 and N60 were specific for $CD8^+$ and $CD4^+$ T cells, respectively. Importantly, our novel finding is that mice boosted with T cell epitopes could specifically and significantly increase the cellular immune responses of both $CD4^+$ and $CD8^+$ T cells, suggesting that epitopes for T cells could be used for vaccination in SARS study.

2. Materials and methods

2.1. Mice

Female BALB/c and C57BL/6 mice, 6–8 weeks old, were purchased from Zhongshan University Animal Center (Guangzhou, China) and maintained in animal care facility under pathogen-free conditions.

2.2. SARS CoV S DNA vaccine, S peptides and CpG ODN sequence

Plasmids encoding severe acute respiratory syndrome coronavirus (SARS CoV) spike (S) protein was constructed as described [17] and kindly provided by Dr. Gary J. Nabel from the Vaccine Research Center (VRC), National Institute of Allergy and Infectious Diseases (NIAID), National Institutes of Health (NIH), Bethesda, MD, USA. Plasmid DNA was purified by plasmid purified kit (QIAGEN). The 260/280 ratios ranged from 1.8 to 2.0. The endotoxin content from purified plasmid DNA was below 10 EU/ml in which the level had no effect on the immune response. Seventeen to nineteen-mer peptides with 10 amino acids (aa) overlap spanning the entire SARS CoV S protein were synthesized and kindly provided by Dr. Richard A. Koup from VRC, NIAID, NIH, Bethesda, MD, USA. In total, there are 169 peptides. Pools (1–8) of 20 consecutive peptides were prepared, and pool 9 contained 9 peptides (P161–169). Peptides were dissolved in DMSO at 25 mg/ml and used at the final concentration of 1 μg/ml (for each) in all experiments, except for the dose response studies. Peptides N50 (S365–374, KCYGVSATKL) and N60 (S435–444, NYNYKYRYLR) were synthesized and kindly provided by School of Pharmaceutical Sciences, Sun Yat-sen University, Guangdong, China. The CpG oligodeoxynucleotide (ODN) 1826 (TCC ATG ACG TTC CTG ACG TT) was synthesized by Sangon Corp. (Shanghai, China).

2.3. Antibodies

Purified anti-CD28 and anti-CD16/CD32, PerCP conjugated anti-CD4, FITC conjugated anti-CD62L, PE conjugated anti-CD8, APC-Cy7 conjugated anti-CD8, PE or APC conjugated anti-IFN-γ, FITC conjugated anti-IL-2 and isotype-matched control monoclonal antibodies (mAbs) were purchased from BD/PharMingen (San Diego, CA). FITC conjugated anti-IL-7R mAb was obtained from eBioscience (San Diego, CA).

2.4. Immunization

BALB/c and C57BL/6 mice were injected intramuscularly (i.m.) with 50 μg truncated SARS CoV S plasmid DNA in 100 μl PBS in the left thigh. Mice were boosted twice with 3-week intervals. In DNA prime-peptides boost experiments, BALB/c mice were immunized twice with 50 μg SARS CoV S plasmid DNA with 3-week intervals in the left thigh. Three weeks later, mice were boosted twice with 3-week intervals by subcutaneous (s.c.) injection of peptides (N50 and N60, 50 μg of each) with or without 25 μg CpG ODN at the base of tail. PBS and CpG ODN were administrated alone as controls. Each experiment was repeated 2–3 times with consistent results and 3–5 mice were used in each experiment.