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A highly conserved epitope-vaccine candidate against varicella-zoster virus induces neutralizing antibodies in mice

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ABSTRACT

Varicella-zoster virus (VZV) is a highly infectious agent of varicella and herpes zoster (HZ). Vaccination is by far the most effective way to prevent these diseases. More safe, stable and efficient vaccines, such as epitope-based vaccines, now have been increasingly investigated by many researchers. However, only a few VZV neutralizing epitopes have been identified to date. We have previously identified a linear epitope between amino acid residues 121 and 135 of gE. In this study, we validated that this epitope is highly conserved amongst different VZV strains that covered five existing phylogenetic clades with an identity of 100%. We evaluated the immunogenicity of the recombinant hepatitis B virus core (HBc) virus-like particles (VLPs) which included amino acids (121–135). VZV-gE-specific antibodies were detected in immunized mouse serum using ELISA. The anti-peptide antiserum positively detected VZV via Western blot and immunofluorescent staining assays. More importantly, these peptides could neutralize VZV, indicating that these peptides represented neutralizing epitopes. These findings have important implications for the development of epitope-based protective VZV vaccines.

1. Introduction

Varicella-zoster virus (VZV) is a member of the *human α-herpesvirus* subfamily with a double-stranded DNA genome of approximately 124,884 bp which encodes 71 open reading frames (ORFs) [1]. It is the causative agent of varicella (chickenpox) and herpes zoster (shingles, HZ). Humans are the only natural hosts for this virus. VZV causes varicella with systemic features, such as fever and a generalized pruritic vesicular rash in childhood and becomes latent in the sensory ganglia after the primary infection [2]. HZ is caused by VZV reactivation and manifests as a painful, blistering skin eruption in a unilateral and dermatomal distribution that usually lasts approximately 2 weeks, which may be followed by a common and debilitating complication, post-herpetic neuralgia (PHN), with a weakened immune system that occurs with aging [3].

Currently, two live-attenuated VZV Oka vaccines are licensed, one against varicella, another against HZ, which have remarkable and beneficial effects in preventing varicella and HZ [4,5]. With the development of biochemistry, molecular vaccinology and genetic engineering technology and increased demand for vaccine safety, the need for more safe and stable vaccines in various forms continues to grow. Plasmids containing the gene encoding the full-length or truncated form of gE (prototype DNA vaccines), which could stimulate immunity in mice, were constructed by Hasan et al. [6,7]. Mullane et al. [8] reported that the heat-treated zoster vaccine was generally safe and immunogenic in immunocompromised adults. Significantly, an adjuvanted HZ subunit vaccine based on VZV glycoprotein E (gE) was well tolerated and immunogenic, and more importantly, this vaccine induced a robust immune response and had a clinically acceptable safety [9–11]. In view of this, more safe, stable and efficient vaccines including epitope-based vaccines now more prevalent. Epitope peptides are considered to be promising candidates for new generation vaccines, especially those with conserved and neutralizing epitopes [12]. The potential advantages of epitope-based vaccines include increased safety; economical technology as they are easily produced and have simplistic compositions; the reduction of allergic reactions;

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and the ability of focus immune responses on selected epitopes [13,14].

VZV is an enveloped virus that expresses at least nine membrane proteins, of which gE is the most essential and abundant. Only a few neutralizing VZV epitopes have been identified to date. Yasushi et al. [15] reported that the gH neutralizing epitopes were conformational and comprised a cluster of the seven independent protein portions of gH. Marius et al. [16] generated a recombinant monoclonal anti-VZV antibody that recognized by a conformational epitope within the gH/L protein complex, which could effectively neutralize VZV. Residues 1-134 and 101-161 of gE were identified as immunodominant regions by Western blotting and ELISA analyses, using serum from both varicella and zoster patients [17]. Additionally, immunizations with the recombinant hybrid Ty-virus-like particles presenting the gE (aa1-134) and gE (aa101-161) regions in small animals could induce neutralizing antibody responses [18]. Furthermore, monoclonal antibody (mAb) 3B3 recognized an 11-amino acid (residues 151-161; QRQYGDVFKGD) epitope in the ectodomain of VZV gE by using the recombination PCR technique [19]. Therefore, pinpoint neutralizing epitopes and epitope-based vaccines against VZV are still lacking in-depth research. Therefore, we are interested in developing a recombinant epitope-based vaccine with neutralizing abilities against VZV.

Our group previously generated a VZV-gE-specific complement-dependent mAb (named 4A2) by immunization with a recombinant gE antigen expressed by insect cells and the 4A2 epitope was identified as the amino acid sequence (SAQEDLGDDTGIHIV) [20]. In this study, we validated the gE-epitope that we identified and found that it was highly conserved. Then, we generated a fusion protein with 149 aa of the hepatitis B virus core (HBc) protein and the epitope, and confirmed that this protein was able to self-assemble into virus-like particles (VLPs) by electron microscopy. We further found that the VZV gE-specific IgG antibody in mice immunized with chimeric VLPs HBc-gE (aa121-135) were not lower than purified rgE protein and heated-inactivated vOka groups, in high-dose groups. More importantly, we proved that these chimeric particles were assessed for an ability to induce neutralizing antibodies to gE-specific epitope in mice. Our results suggest that chimeric HBc particles carrying the neutralizing epitope of gE can induce protective immunity against VZV. These findings have important implications for the development of epitope-based protective VZV vaccines

2. Materials and methods

2.1. Ethics statement

Female BALB/c mice were purchased from shanghai SLAC Laboratory Animal Co., Ltd. All animal experiments were performed in accordance with protocols approved by the Xiamen University Institutional Animal Care and Use Committee and were approved by the Xiamen University Laboratory Animal Management Ethics Committee.

2.2. Cells and virus

Human acute retinal pigment epithelial cells (ARPE-19) were grown as previously described [20]. The VZV strain (v-Oka) was propagated by cocultivating infected cells with uninfected cells and harvested at 90% virus-induced cytopathic effect (CPE), which usually occurred at 3 days post-infection (dpi). The infected cells were rinsed with phosphate-buffered saline (PBS) and scraped into 1 mL of protection buffer (9% sucrose, 25 mM histidine, 150 mM NaCl; pH 7.35). Then, the cells were stored at −80 °C. A fresh aliquot was thawed to make cell-free virus for the subsequent tests. The live attenuated vOka vaccine (lot number: RS201510001) was provided from Beijing Wantai Biological Medicine Co. The heat-inactivated vOka was prepared by treatment of the live attenuated varicella virus at 56 °C for 30 min and tested by immunofluorescence assay to confirm the loss of infectivity.

2.3. Epitope alignment

To assess the degree of homology of the epitope recognized by 4A2, 136 VZV strains, which covered five existing phylogenetic clades that were available from GenBank, were selected and their gE amino acid sequences were aligned and analyzed using Clustal W within the DNASTAR software (version 7.0, Madison, WI). Weblogo (http://weblogo.berkeley.edu/) was used to generate the sequence logo to visualize the conservation of the epitope sequence.

2.4. Peptide synthesis

A set of 15 synthetic peptides based on the gE (aa121-135) peptide of gE was synthesized at MMG Co. (Germany), and contained single alanine substitutions at each amino acid position to identify key binding residues that were recognized by 4A2.

2.5. Recombinant protein productions

The synthesized gE (aa121-135) gene fragment was annealed by upstream primer (5′-GATCCTCTGCACAGGAGGATCTTGGGGACGATACGGGCATCCACGTTATCG-3′) and downstream primer (5′-AATTCGATAACGTGGATGCCCGTATCGTCCCCAAGATCCTCCTGTGCAGAG-3′), and cloned into a BamH I and EcoR I digested pC149/mut plasmid vector [21] to create the HBc-gE (aa121-135) plasmid. For cloning and expressing studies, the *Escherichia coli* strain, ER2566 (DE3), was used. Recombinant HBc-gE (aa121-135) and HBc (aa1-149) (pC149/mut) proteins were expressed and purified as previously described [21].

2.6. SDS-PAGE and Western blot analyses

The purified recombinant proteins or v-Oka-infected whole-cell lysates were subjected to 15% or 12% SDS-PAGE gels and transferred to polyvinylidene difluoride (PVDF) membranes. For Western blotting, anti-HBc mAb (Beijing Wantai Biological Medicine Co., China), anti-VZV-gE mAb 4A2 (National Institute of Diagnostics and Vaccine Development in Infectious Diseases, China), anti-β-tubulin mAb 7B9 (Abcam, Cambridge, UK) and anti-HBc-gE (aa121-135) peptide mouse serum were used. Briefly, the membranes were incubated with 5% skim milk in Tris-buffered saline (TBS) for 1 h at 37 °C, and then incubated with mAb or antiserum for 1 h at 37 °C. After three washes with TBST (0.05% Tween 20 in TBS), the membranes reacted further with horseradish peroxidase (HRP)-conjugated goat anti-mouse secondary antibodies (Sigma–Aldrich, St. Louis, MO, USA) for 1 h at 37 °C and were visualized with bromochlorindole phosphate/nitro blue tetrazolium substrate (BCIP/NBT).

2.7. Electron microscopy and 3D models

The recombinant proteins were examined using negative-stain EM. A sample was diluted to 0.8 mg/mL, applied to 200 mesh carbon-coated copper grids for 5 min, and the sample was negatively strained with 2% uranyl acetate. Samples were evaluated with a JEM2100HC transmission electron microscope (JEOL, Tokyo, Japan) at 200 kV. The three-dimensional structural models of the chimeric VLPs were generated with the HBV capsid (PDB: 4G93) crystal structure as the template.

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