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A novel peptide isolated from phage library to substitute a complex system for a vaccine against staphylococci infection

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Abstract

Staphylococcus aureus is a major human pathogen and many of the strains are resistant to conventional antibacterial treatment. The bacteria cause disease largely due to the production of multiple toxins, whose synthesis is controlled by an RNA molecule termed RNAIII. The production of RNAIII is induced by quorum sensing systems, one of them containing the protein RNAIII activating protein (RAP). Here we show that we partially purified supernatant of *S. aureus*, used this fraction to vaccinate mice, and selected antibody-binding peptides by phage display. Mice were vaccinated with the various peptides and challenged with *S. aureus*. One of the binding peptide termed R13 induced a protective immune response. Western blot analysis showed that the anti-R13 antibodies specifically bind to native or recombinant RAP. Mice vaccinated with R13 were protected and protection was sustained for the duration of the 6-month study period. Our results show that R13 could be used as a long-term effective protective-peptide-vaccine to prevent *S. aureus* infections and once again show that targeting the RAP quorum sensing system is an effective approach to preventing staphylococcal infections. In addition our studies show that selection of specific protective peptides by phage display using sera induced by complex antigens is a rapid and effective way to identify the protective antigen and select for a peptide vaccine.

Keywords: *Staphylococcus aureus*; TRAP; Peptide vaccine; Long-term; Phage display

1. Introduction

Staphylococcus aureus is a Gram-positive bacterium that can cause many different types of diseases, ranging from minor skin infection to life-threatening deep infections such as pneumonia, endocarditis, meningitis, postoperative wound infections, septicemia, and toxin shock syndrome [1]. The emergence of drug resistance has made many antibiotics ineffective.

Many diseases caused by *S. aureus* have been associated with the toxins the bacteria produce [1]. The ability of the bacteria to express toxic exomolecules is due to a complex gene regulatory system. Until now, two staphylococcal quorum sensing systems (SQS1 and SQS2) have been described [2]. SQS 1 is composed of RAP [3–5] and its target molecule TRAP [2]. RAP can induce the histidine phosphorylation of TRAP. The phosphorylation of TRAP leads to the synthesis of SQS2 by an unknown mechanism [2,5]. SQS 2 comprises the molecules encoded by *agr* (Agr A–D) [6,7]. AgrD is a propeptide that yields an autoinducing peptide (AIP) that can induce the phosphorylation of AgrC to lead to the production of RNAIII [6,7]. RNAIII upregulates the expression of *S. aureus* exotoxins. Mice vaccinated with RAP were shown to be protected from a *S. aureus* infection [3]. RAP binding

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peptides screened from a random phage-displayed peptide library inhibit *S. aureus* pathogenesis in vivo [8]. The purification of RAP was difficult and there was some argument on the existence of RAP [9,10]. In 2003, Korem et al. characterized RAP as an orhtholog of the ribosomal protein L2 [11].

Peptide vaccines based on relevant epitopes of protective antigens, besides being cost-efficient to prepare, can be used to direct the immune response to cellular or humoral immunity by selection of specific T- and B-cell epitopes [12,13]. Coupling of such peptides to carriers and/or the employment of appropriate adjuvants may help enhance the specific immune response [13]. Peptide vaccines are attractive also because they may avoid toxic effects associated with the intact immunogen and could be designed to emphasize the immunogenic elements that may be hidden in the native antigen [13].

Here we identified a minotope of RAP (the ribosomal protein L2), and show that antibodies against it interfere with RNAIII production and protect mice from a *S. aureus* infection.

2. Methods

2.1. Bacterial strains, peptide phage display library and animals

Wild type S. aureus lab strain RN6390B were our lab strain. Ph.D™ phage peptide library purchased from New England BioLabs. The complexity of the library was 2.7×10^9 and the titer was 1.5×10^{13}/μl. *E. coli* ER2537 was used as the host strain for the phage library (New England BioLabs). *E. coli* BL21 was our lab strain used in the expression of protein (Beijing Institute of Basic Medical Sciences, China). *E. coli* GI826 was purchased from Invitrogen Corporation. Immuocompetent hairless BALB/c mice (20–25 g, outbred, male) and Balb/c mice (20–25 g, outbred, male) were provided by Beijing Research Center of Animals.

2.2. Partial purification of RNAIII activating proteins (RAPs)

To purify RAPs, *S. aureus* RN6390B (5×10^8 cells/ml) were grown from the early exponential phase in CY broth for 6 h to the post exponential phase of growth. Bacteria were removed by centrifugation for 10 min at 10,000 rpm, Culture supernatant was concentrated by a 10-kDa-cutoff membrane (Millipore) at 4 °C. Concentrated post-exponential supernantants were boiled for 10 min to eliminate heat-lablile proteins, centrifuged at 4 °C, 12,000 rpm for 10 min (what g xxx), and soluble material (3 ml) was applied to a FPLC gel filtration column S300 (16/60, Amersham Pharmacia) in presence of buffer (50 mM Tris–Cl, pH 8.0). One milliliter eluted fractions were concentrated 10 times by lyophilization.

2.3. Activation of RNAIII synthesis

One milliliter of sample (peak 1, peak 2, in 0.5 M Tris–Cl pH 8.0) were added to early exponential *S. aureus* RN6390B cells (4.5 ml) and growth continued at 37 °C for 30 min. Cells were collected and total RNA extracted as described [8]. The same amount of RNA was applied on a 1% formaldehyde agrose gel. The gel was northern blotted by dry transfer and the membrane was hybridised with the RNAIII specific DNA probe labelled by Prime-a-Gene labelling System (Promega) [8]. The membrane was autoradiographed.

2.4. Expression and purification of recombinant RAP (rL2)

Primers corresponding to the 5′ and 3′ of *rblB*, the gene encoding for L2 protein in *S. aureus* strain RN6390B were designed [11] to amplify the complete gene, using *S. aureus* strain RN6390B chromosomal DNA as the template. Amplified gene was cloned to the pET-28a vector (Invitrogen) that contains a six-histidine tag at the 5′ end of the insert gene. The synthesis of recombinant protein was induced by 0.5 mM IPTG for 3 h at 37 °C. rL2 protein was purified using a nickel column according to the manufacturer's instruction.

2.5. Production and purification of polyclonal anti-RAPs antibodies

New Zealand white female rabbits were first immunized by subcutaneous injection of 1 ml of immunogens (0.1 mg of RAPs in PBS mixed with complete Freund's adjuvant). Repeated immunizations were performed by injection of mixtures of RAPs and incomplete Freund's adjuvant at the 4th and 7th week. On the 8th week, sera were collected and analyzed by ELISA against injected recombinant proteins. Anti-RAPs antibodies were purified by applying the sera to an affinity column (rProteinA FF resin, Amersham Pharmacia) according to the manufacturers instructions. Antibodies were eluted with 0.2 M Gly–HCl (pH 3.0).

2.6. Selection of binding peptides by phage display

The procedure of selection was in accordance with the protocol of Ph.D™ phage peptide library kit (New England BioLabs). For each round, phages (1×10^{11}) were applied to the target protein pre-coated in a 96-well plate (10 μg/well). The level of specific phage enrichment was calculated by ratio of input and output as described [8]. After three rounds of biopanning, positive phage clones were selected and their DNA were sequenced.

2.7. Enzyme-linked immunosorbent assay (ELISA)

The specific binding of positive phage clones to polyclonal anti-RAPs antibodies was tested by ELISA. Briefly,