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## Development and evaluation of *Staphylococcus epidermidis* DNA, mRNA and protein vaccines

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**Objectives:** The traditional antibiotic and drug-release treatments are ineffective against biomaterial infections due to the inherent resistance of bacterial biofilms to antibiotics and host defense. A long-term immune response to specific bacterial colonization may hold the promise for life-time prevention of biomaterial infections. In this study, we targeted *Staphylococcus epidermidis* (*S. epidermidis*)- one of the leading nosocomial pathogens, and developed the DNA, mRNA and protein vaccines expressing the Accumulation Associated Protein (AAP)- a *S. epidermidis* protein employed to form biofilms on the first stage of *S. epidermidis* infection, and investigated the potential of these vaccines against biomaterial associated infections in vitro and in vivo.

**Methods:** The gene fragment encoding a 128 aa G5 domain of AAP was cloned into the plasmidsp VAX1, pGEM4z-A64 and pET21, yielding pVAX/aap as the DNA vaccine; pGEM/aap, the precursor for the mRNA vaccine; and pET21/aap to express AAP as the protein vaccine. C57B/6 mice were divided into six groups, and subcutaneously injected with 25 µg naked DNAaap, 25µg DNAaap+PEI, 5µg naked mRNAaap, 5µg mRNA aap+PEI, 100 µg protein AAP, or 100µg protein OVA as a control at week 0, 2 and 3. Serum was collected for IgG, IgG1 and IgG2a antibody analysis by ELISA, as well for biofilm inhibition assays. Splenocytes were isolated for AAP-specific IFN ELISA and flow cytometry analysis.

**Results:** Strong anti-*S. epidermidis* antibody response were developed in protein AAP-immunized mice. Mice immunized with the AAP protein vaccine developed dramatically highest anti-*S. epidermidis* IgG antibody response compared to other groups of mice. Biofilm inhibition assays further indicated that the high levels of antibodies could inhibit *S. epidermidis* RP62A biofilm formation in a dose-dependent pattern (Fig.1). We observed a relative weak anti-AAP and *S. epidermidis* IgG responses in Nucleic Acid vaccine groups, while the naked DNAaap induced slightly higher antibodies. IgG1 and IgG2a antibody ratio analysis indicated that the protein vaccine induced Th2 immune responses, whereas the pDNA/mRNA vaccines promoted Th1-biased immunity. Significant AAP-specific IFN $\gamma$  responses were induced in DNA- and mRNA-aap immunized mice. Though relative weak antibody responses in nucleic acid vaccine groups, mice received either DNA- or mRNA-aap vaccines, whether naked or complexed with PEI, developed strong antigen-specific IFN $\gamma$  responses in splenocytes. Though there were no significant difference between the naked DNA and the DNA+PEI vaccines, we observed a significant higher IFN $\gamma$  response in the mRNA+PEI group of mice compared to the naked mRNA group. These results were consistent with the data obtained from flow cytometry analysis of AAP-activated CD4<sup>+</sup> IFN $\gamma$ <sup>+</sup> and CD8<sup>+</sup> IFN $\gamma$ <sup>+</sup> splenocytes.

**Conclusions:** Our study validated the potential of *S. epidermidis* DNA/mRNA/protein vaccines in developing both antibody and cellular immune responses against *S. epidermidis* biofilm formation.

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