Staphylococcus epidermidis Vaccines against Biomaterial Associated Infections

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Introduction: Medical-device based biofilm infections account for ~60-70% of all hospital-acquired infections (nosocomial infections), which causes 99,000 deaths and $4.5-11 billion medical costs each year in the United States. The inherent resistance of bacterial biofilms to antibiotics and host defense highlights the need for more effective preventive strategies beyond the traditional antibiotic and drug-release treatments. Our strategy is to promote a long-term immune response against specific bacterial attachment and/or colonization of implanted biomaterials by impregnating biomaterials with bacterial vaccines. Since *Staphylococcus epidermidis (S. epidermidis)* is one of the leading nosocomial pathogens, in this study, we specifically developed *S. epidermidis* protein/plasmid DNA (pDNA)/mRNA vaccines expressing the Accumulation Associated Protein (AAP) – a *S. epidermidis* protein employed to form biofilms in the first stage of *S. epidermidis* infection, and investigated the potential of these novel vaccines against biomaterial associated infections *in vitro* and *in vivo*.

Material and Methods: *Development of three S. epidermidis vaccines.* The gene fragment encoding a 128aa G5 domain of AAP was cloned into the plasmids pET21, pVAX1 and pGEM4z-A64, yielding pET21/aap to express AAP as the protein vaccine, pVAX/aap as the DNA vaccine, and pGEM/aap, the precursor for the mRNA vaccine. The protein AAP was expressed in *E. coli* BL21 (DE3) and purified using Talon single step column kit before emulsified with incomplete Freund's adjuvant. The DNA aap (pVAX/aap) was prepared using the Endo-free plasmid mega prep kit (Qiagen), and the mRNA aap was prepared by *in vitro* transcribing of pGEM/aap using the mMESSAGEmMACHINE high yield RNA transcription kit (Ambion). The pDNA- and mRNA-aap were complexed with JetPEI™ (Polyplus transfection) at an N/P ratio of 8. *Animal Study*. C57B/6 mice were divided into six groups, and subcutaneously injected with 100μg protein OVA as a control (group 1), 100μg protein AAP (group 2), 25μg naked DNAaap (group 3), 25μg DNAaap+PEI (group 4), 5μg naked mRNAaap (group 5), or 5μg mRNAaap+PEI (group 6) 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 IFNγ ELISA and Intracellular Cytokine Staining (ICS) analysis.

Results & Discussions: *A strong anti-S. epidermidis antibody response was developed in protein-immunized mice.* Mice immunized with the AAP protein vaccine developed the 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. We observed a relative weak anti -AAP and -*S. epidermidis* IgG responses in NA 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 IFNγ responses were induced in NA-immunized mice.* Though relative weak antibody responses in NA vaccine groups, mice received either pDNA- or mRNA-aap vaccines, whether naked or complexed with PEI, developed strong antigen-specific IFNɣ responses in spenocytes. Though there were no significant difference between the naked DNA and the DNA+PEI vaccines, we observed a significant higher IFNγ response in the mRNA+PEI group of mice compared to the naked mRNA group. These results were consistent with the data obtained from ICS analysis of AAP-activated CD4+IFNγ+ and CD8+ IFNγ spelnocytes.

Conclusions: Our study validated the potential of *S. epidermidis* protein/pDNA/mRNA vaccines in developing both antibody and cellular immune responses against *S. epidermidis* biofilm formation, and provided a novel beneficial alternative to current antibiotic therapies against biomaterial associated infections.