Bionanosome-based vaccines for the protection against superbacterial infection

Yoon-Keun Kim

Pohang University of Science and Technology, Korea

In post-antibiotic era, the intensive use of antibiotics has dramatically increased the frequency of resistance among human pathogens. Multidrug-resistance bacteria, so-called ESKAPE bacteria, include gram-positive bacteria, such as *Entercoccus spp*. and *Staphylococcus aureus,* and gram-negative bacteria, such as *Enterobacteriaceae (Escherichia coli, Klebsiella spp., and Enterobacter spp.), Acinetobacter baumannii,* and *Pseudomonas aeruginosa.* Outer membrane vesicles (OMV), constitutively secreted from Gram-negative bacteria, are spherical nano-meter sized bilayered proteolipids enriched with outer membrane proteins. The immunization with OMV derived from *E. coli, K. pneumoniae, E. aerogenes,* and *A. baumannii* protects lethality by each bacterium infection through the induction of both T cell and antibody responses, especially by the OMV-antigen-specific production of interferon (IFN)-γ and IL-17 from T cells. However, OMV vaccination has some drawbacks, such as safety and productivity. To overcome the drawbacks of OMV vaccines, a novel antigen delivery system, artificial vesicles (bionanosome, BNS), were prepared from *E. coli* protoplast that has their cell wall entirely removed. To evaluate immunogenic effects of the protoplast-derived bionanosome (P-BNS), GFP-encoding plasmid was inserted into *E. coli* and over-expressed, and then GFP-P-BNS was manufactured. When mice were immunized intraperitoneally with GFP-P-BNS, anti-GFP IgG and GFP-specific Th1 and Th17 cell responses were highly induced compared to immunization with GFP plus aluminum hydroxide. To make P-BNS vaccine against drug-resistant gram-negative bacteria, outer membrane proteins, which conserved across many gram-negative bacteria, were selected and then overexpressed in the P-BNS delivery system. The OMP-P-BNS vaccine protected sepsis-induced lethality by individual bacteria, including *E. coli A. baumannii*, and *P. aeruginosa*. In addition, this vaccine protected effectively sepsis-induced lethality in a cecal ligation and puncture (CLP) model, suggesting that the protective spectrum is broad. In terms of adaptive immunologic mechanism, the protective effect of OMP-P-BNS vaccine is dependent on

both IFN-gamma and IL-17, but not on IL-4. In conclusion, the P-BNS vaccine harboring bacterial surface proteins is an innovative vaccine delivery to protect against drug-resistant bacteria (superbacteria) infection and vaccination, rather than antibiotic development, is a good therapeutic strategy in post-antibiotic era.