**Application of Dendritic Cell-Activating All-in-One Vaccine Platform**

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Dendritic cells (DCs) are professional antigen-presenting cells (APCs) that play a crucial role in priming and activating T cell responses. Activation of DCs via pattern recognition receptors (PRRs) leads to the upregulation of costimulatory molecules and cytokines, which enhance antigen-specific immune responses. DCs represent a major target for PRR-activating vaccine adjuvants. Therefore, targeted delivery and intracellular transmission of protein antigens to DCs could significantly enhance T cell-mediated immune responses. Flagellin, a unique PRR agonist, is capable of activating both extracellular Toll-like receptor 5 (TLR5) and intracellular NOD-like receptor family CARD domain-containing protein 4 (NLRC4) inflammasome pathways. Engineered cell-permeable flagellin has the potential to activate both pathways simultaneously. Furthermore, flagellin can be covalently fused with antigens to create built-in adjuvanted vaccines. Our previous studies have demonstrated that Vibrio vulnificus FlaB serves as an excellent adjuvant in combination with various vaccine antigens against pathogens and cancer. In this study, we report a protein-based all-in-one engineered vaccine platform consisting of a DC-targeting peptide, optimized antigen, and an inflammasome-activating built-in adjuvant to efficiently enhance cross-presentation and subsequent robust T cell activation. Initially, we identified a novel peptide (DCpep6) through in vivo phage biopanning that specifically binds to and enters CD11c+ cells. Subsequently, we engineered an all-in-one vaccine molecule composed of DCpep6 (D), an optimized antigen (X), and a built-in flagellin adjuvant (F). The resulting DXF vaccine was stably expressed, and each component retained its functionality. In vivo administration of DXF led to rapid biodistribution to draining lymph nodes and internalization into CD11c+ cells. Immunization with DXF elicited strong T cell responses and conferred long-term memory. Importantly, the DXF-mediated immune response was dependent on NLRC4, as it was abolished in NLRC4-deficient mice. In conclusion, we propose a protein-based all-in-one vaccine platform that intracellularly delivers both antigen and inflammasome activator to DCs, resulting in long-lasting immune responses. This platform holds great promise for the development of effective vaccines against various pathogens and cancers