[課程―2]

審査の結果の要旨

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Combining the accumulated data on the pneumococcal vaccine efficacy to overcome the drawbacks of current pneumococcal polysaccharide vaccines and the new technical strategies to improve the current vaccination system, I developed a pneumococcal vaccine that combines the advantages of pneumococcal surface protein A (PspA) with a nontoxic nasal vaccine-delivery system based on a nanometer-sized hydrogel (nanogel) bearing a cationic cholesteryl-group-bearing pullulan (cCHP). It was applied to the murine pneumococcal airway infection models as a new formulation of nasal vaccine. The results demonstrated in my research were as follows;

1. The cCHP-PspA intranasal immunization induced protective immunity against pneumococci. After the lethal challenge, the survival rate of the cCHP-PspA-vaccinated group was 100%, showing cross-protectiveness. *In vivo* imaging of pneumococcal lung infection further revealed no sign of infection in the lungs of mice nasally immunized with cCHP-PspA. Importantly, mice immunized with intranasal cCHP-PspA had less colonization and invasion of pneumococcal organisms in the respiratory tract.

2. Cytokine assay of the T cell culture supernatant revealed that intranasal administration of cCHP-PspA resulted in enhanced PspA-specific Th17 response with Th2 responses. After intranasal immunization with cCHP-PspA, mucosal IgA, and systemic IgG antibody responses were strongly induced. The cCHP-PspA intranasal immunization is supposed to be beneficial as a new vaccine formulation because Th17, mucosal IgA and systemic IgG immune responses have been shown to involve in the protective immunity against pneumococcus.

3. To address the concern about the potential for antigen deposition and accumulation in central nervous system after nasal immunization, PspA tracing was performed by using radioactive PspA or fluorescence-conjugated PspA in complex with cCHP after intranasal

administration. The cCHP vaccine delivery system enabled prolonged antigen exposure at the nasal epithelium, allowing continuous antigen uptake by nasal DCs located in the epithelial layer and lamina propria of the nasal passages for the initiation of antigen-specific immune responses without CNS accumulation of PspA.

4. To understand the molecular and cellular mechanism of the cCHP-PspA nasal vaccination induced protective immunity against the pneumococcal infection, I examined how the IgA^+ plasmablasts were induced by the cCHP-PspA nasal immunization. cCHP-PspA nasal immunization potentiates CCL28 expression in the mouse nasal passage and induced the recruitment of CCR10 and CCR3 positive IgA^+ cells to mouse nasal passage. By the *in vivo* blocking experiment, I demonstrated that CCL28/CCR10 and CCR3 interaction is essential to the migration of IgA^+ cells to the nasal passage after intranasal cCHP-PspA immunization.

My current study demonstrated the efficacy of cCHP antigen-delivery system for the induction of protective immune response against pneumococcus. I made the effort for the application of cCHP-based vaccine delivery system for the respiratory infection (e.g., *Streptococcus pneumoniae*) and in parallel, I tried to shed light on the basic mechanism that explains how nasally-induced IgA^+ cells are migrated into the nasal passage. My study thus demonstrated that cCHP-PspA nasal immunization mounted strong protective immunity both in the mucosal and systemic compartemtns, and it induced $CCR10^+/CCR3^+$ IgA^+ cells and enhanced CCL28 production in the nasal epithelium. The CCL28/CCR10 and CCR3 interaction-mediated airway imprinting system is essential to the homing of IgA^+ plasmablasts to the nasal passage. By providing new insights into how cCHP-PspA vaccine works, this study provided a basic and scientific platform for further possibility of testing the effectiveness of cCHP-PspA in non-human primates in order to advance its applicability in human, and my contributions are worthy of PhD degree.