論文の内容の要旨

論文題目　Nanogel-based PspA Intranasal Vaccine Prevents Invasive Disease and Nasal Colonization by Pneumococcus
（ナノゲルタンパク質デリバリーを用いた PspA 経鼻ワクチンによる肺炎球菌感染症および鼻腔内定着に対する抑制効果）

氏名　こん　いるぎゅう
孔　壹奎
Kong Il Gyu

The use of polysaccharide-based injected multivalent pneumococcal conjugate vaccines has diminished the number of fatal infections with pneumococci expressing each of the polysaccharides present in the vaccine. However, *Streptococcus pneumoniae* remains a problematic pathogen because of the large number of different capsular polysaccharides compatible with virulent disease in man. Clinical demand to overcome these problems has prompted the preclinical development of the universal serotype-independent pneumococcal vaccines that are based on a surface protein common among all strains. Pneumococcal surface protein A (PspA) has emerged as a promising candidate protein, expressed on the surfaces of all capsular serotypes of *S. pneumoniae*, and is able to elicit protective immune responses in mice and parenteral immunization of humans can elicit antibodies capable of protecting from fatal infections. Including *S. pneumoniae*, respiratory pathogens are colonized at the nasal mucosa without progressing to diseases, and shed from one to another to be transmitted from carrier to the susceptible host. In this context, nasal vaccine is an advantageous route of administration since it is mimicking natural infection to induce the protective immunity in the respiratory tract. Moreover, nasal immunization has been elucidated to be an effective way of immunization to induce antigen-specific immunoglobulin (Ig) A in the respiratory tract. After nasal vaccination, IgA-producing cells are effectively induced and they secrete IgA into the secretion. Thus, the nasal intranasal vaccination route is

an improved route for preventing colonization of the nasal cavity by pneumococci. Several studies have confirmed the efficacy of PspA as a nasal vaccine antigen by co-administration of PspA with a mucosal adjuvant such as cholera toxin (CT) or cholera toxin subunit B to mice. The mice subsequently mounted antigen-specific immune responses in not only the systemic compartment but also the respiratory mucosal compartment where initial bacterial colonization occurs. However, a leading obstacle to the practical use of nasal vaccine with a protein-based pneumococcal antigen is the need to co-administer a biologically active toxin-based mucosal adjuvant (e.g., CT) for effective induction of antigen-specific immune responses.

In this study, 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). First of all, the efficacy of the nanogel-based PspA nasal vaccine (cCHP-PspA) was tested in murine pneumococcal airway infection models. Intranasal vaccination with cCHP-PspA induced protective immunity against pneumococci. After the lethal challenge, the survival rate of the cCHP-PspA-vaccinated group was 100%, as was that for PspA-CT-vaccinated mice. In contrast, most of the mice nasally immunized with PspA alone (survival rate, 0%) or PBS (20%) died within 8 days of challenge with *S. pneumoniae* Xen10. The cross-protective effect of PspA was also observed. When mice intranasally immunized with cCHP-PspA (family 1, clade 2) were challenged with different subtype of the pneumococcal strain 3JYP3670 expressing PspA of clade 4 (family 2), the cCHP-PspA immunized mice were also protected, while the other groups of mice immunized with PspA alone or PBS were not. *In vivo* imaging of pneumococcal lung infection further revealed no sign of infection in the lungs of mice nasally immunized with cCHP-PspA. Nasal vaccination with cCHP-PspA enhanced bacterial clearance from bronchoalveolar lavage fluid (BALF) and lung. Bacterial numbers in nasal washes (NWs) and the nasal passage were significantly decreased (approximately 100-fold lower) in mice immunized with cCHP-PspA nasal vaccine compared to two control groups of mice nasally administered with PspA alone or PBS.

When T cells responses were examined, nasal cCHP-PspA induced higher levels of interleukin (IL)-17 in $CD4^+$ T cells from the spleen, cervical lymph nodes and nasal passages. In the cCHP-PspA-vaccinated group also produced high levels of IL-4 and IL-13, the hallmark cytokines of Th2-type immune response, but only scant amounts of interferon γ (Th1). These results show the potential of a cCHP-PspA nasal vaccine as an advanced pneumococcal vaccine that can induce Th17 response which has been shown to be involved in the induction of anti-pneumococcal immunity together with Th2-type immune response associated with the induction of protective antibody. PspA-specific IgG

responses in the systemic compartment were thus significantly higher in the mice immunized with nasal cCHP-PspA than in those given PspA only. Intranasal vaccination with cCHP-PspA induced PspA-specific mucosal IgA antibodies in the nasal secretions. In addition, BALF samples from mice nasally vaccinated with cCHP-PspA contained PspA-specific IgA antibodies, and PspA-specific IgG antibodies were detected at high titers in both the NWs and the BALF of mice nasally immunized with cCHP-PspA.

To address the concern about the potential for antigen deposition and accumulation in central nervous system after nasal immunization, I instilled ^{111}In-labelled PspA alone or in complex with cCHP (^{111}In-labelled cCHP-PspA) into the nasal cavities of mice. Whereas the nasal passages in the mice treated with ^{111}In-labelled cCHP-PspA had higher radioactivities of than did those treated with ^{111}In-labelled PspA alone from 6 hours after administration, there was no accumulation of ^{111}In-labelled PspA in the olfactory bulbs or brain through the 48 hour-observation period. These findings further supports the benefits of the cCHP delivery system for prolonged vaccine antigen exposure at the mucosal surfaces of the upper respiratory tract, and furthermore, cCHP is a safe and effective vaccine antigen delivery system. Further study revealed that the cCHP vaccine delivery system enabled prolonged antigen exposure at the nasal epithelium, allowing continuous antigen uptake by nasal dendritic cells located in the epithelial layer and lamina propria of the nasal passages for the initiation of antigen-specific immune responses.

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. Chemokines are known to play pivotal roles in the complex migratory pathway of leukocyte trafficking in mucosal immune system by regulating leukocyte trafficking through signaling to a group of seven transmembrane G protein-coupled receptors. Among them, CCL28/CCR10 interaction is known to be critical to IgA-producing cell trafficking from the inductive site (e.g., mucosa-associated lymphoid tissue) to effector sites including salivary gland and tonsil. However, in the upper respiratory tract, which is the natural invasion site for numerous air-borne pathogens and the most front line of the respiratory immune system, little is known about the respiratory imprinting system by chemokines and chemokine receptors for the migration of nasally-induced IgA^+ cells, even though the nasal route is a favorable way of vaccination to prevent respiratory infectious diseases. To elucidate the mechanism how IgA^+ cells are recruited in the nasal effector site after cCHP-PspA nasal immunization, I focused on the characterization of chemokine receptor expression on IgA^+ cells and their ligand expression in the nasal passage after cCHP-PspA nasal immunization.

My results showed that CCL28 was required for the recruitment of $IgA^+B220^+CD138^+$ plasmablasts expressing its receptors, CCR10 and CCR3, to the nasal passage after nasal cCHP-PspA

immunization. More specifically, my findings demonstrated that 1) cCHP-PspA nasal immunization induced the recruitment of CCR10 and CCR3 positive IgA^+ cells to the mouse nasal passage, 2) CCL28 was expressed and produced at high levels in the epithelial cells of the mouse nasal passage and secreted in NW, 3) cCHP-PspA nasal immunization potentiated CCL28 expression in the mouse nasal passage and 4) CCL28/CCR10 and CCR3 interaction was essential for the migration of IgA^+ cells to the nasal passage. Indeed, *in vivo* blocking of CCL28 during cCHP-PspA immunization suppressed the migration of IgA^+ plasmablasts to the nasal passage leading to the reduction of nasal PspA-specific IgA production.

In summary, these studies demonstrated the efficacy of cCHP antigen-delivery system for the induction of antigen-specific IgA antibody producing cells in nasal cavity. 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 the cCHP-PspA-vaccinated mice survived against lethal challenge with S. pneumoniae, and mice immunized with intranasal cCHP-PspA had less colonization and invasion of pneumococcal organisms in the respiratory tract. Intranasal administration of cCHP-PspA resulted in enhanced PspA-specific Th17 response, mucosal IgA, and systemic IgG antibody responses that have been shown to involve in the protective immunity against pneumococcus. To my knowledge, this study is the first to show the efficacy of a nasal vaccine not only for the induction of protective immune responses but also for the prevention of nasal colonization by a single protein antigen (PspA) without adding any biologically active adjuvant. In addition to showing the efficacy of intranasal cCHP-PspA vaccine against pneumococcus, my investigation demonstrated that cCHP-PspA nasal immunization 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.