論文の内容の要旨

論文題目： **Immune Regulation by Cytokines and Chemokines following Nasal Vaccination**

（経鼻ワクチンにおけるサイトカインとケモカインによる免疫制御）

氏名　朱 善伊（JOO SUNYI）

Mucosal vaccination, especially through nasal administration, is a promising alternative to conventional parental vaccination for preventing infectious disease. In addition to its remarkable ability to elicit systemic immune responses, nasal vaccination offers strong immune protection in both the respiratory and vaginal mucosae. Despite the recognized advantages of nasal vaccination, the molecular and cellular mechanisms for the induction of antigen-specific immune responses and cellular migration pathways from nasal to reproductive mucosal mucosa after the nasal delivery of antigen are largely unknown. This dissertation study is aimed toward improving our understanding of specific mucosal cytokine and chemokine signaling pathways involved in the induction of antigen (Ag)-specific immune response after nasal vaccination, including Ag-specific IgA antibody responses and mucosal crosstalk between the nasal and reproductive compartments.

Thymic stromal lymphopoietin (TSLP) is an interleukin (IL)-7-like cytokine and is involved in Th2-type immune responses, in which myeloid dendritic cells (DCs) are known to be its primary target. However, whether TSLP and its receptor TSLPR are involved in the induction and regulation of antibody (Ab) production after mucosal immunization has been unknown. Here I address the critical role of TSLP–TSLPR signaling in the induction of Ag-specific IgA responses after nasal immunization. To this end, I nasally immunized TSLPR knock-out (KO) and wild-type (WT) mice with pneumococcal surface protein A (PspA) and cholera toxin (CT) to reveal the role of TSLP in the induction of Ag-specific IgA Abs and protective immunity against pneumococcal bacteria (e.g., Streptococcus pneumoniae). In addition, I used an in vitro DC–B cell co-culture system to study the contribution of TSLP to the induction of IgA antibodies.

After nasal immunization with PspA plus CT, the expression of TSLP was enhanced in respiratory mucosal tissue and TSLPR expression was increased in mucosal $CD11c^+$ cells (containing both DCs and macrophages), compared with those in mice immunized with PspA alone. In addition, PspA-specific IgA responses—but not IgG Ab responses—were significantly decreased after nasal immunization with PspA plus CT in both the serum and mucosal secretions of TSLPR-KO mice compared with WT mice. Moreover, mucosal CD11c+ cells isolated from nasal and lung tissues of TSLPR-KO mice nasally immunized with PspA plus CT were less activated and exhibited markedly reduced expression of IgA-enhancing cytokines (e.g., APRIL, BAFF, and IL-6) compared with those from equivalently immunized WT mice. Furthermore, the use of an in vitro DC–B cell co-culture system demonstrated that nasal $CD11c^+$ cells—but not splenic $CD11c^+$ cells—were responsive to TSLP for the induction of IgA production in an IL-6-dependent manner. These results suggest that TSLP–TSLPR signaling is pivotal in the nasal-immunization–induced respiratory-IgA–mediated immunity against pathogenic pneumococcal infection.

In addition to the respiratory mucosa, nasal vaccination effectively induces Ag-specific immune responses in female genital tissues. However, the cellular and molecular aspects of immuno-biological mechanisms through which nasal vaccination induces Ag-specific immune responses in distant reproductive mucosal tissue remain to be elucidated. Here I address the role of selective signaling through chemokine ligand–receptor interaction on the effective reproductive homing pathway initiated by nasal vaccination. To this end, I used a nasal immunization model comprising live attenuated thymidine-kinase-deficient herpes simplex virus 2 (HSV-2 TK^-) and a genital HSV-2 infection system. For immunological analyses, I used HSV-2-specific interferon (IFN)-γ-ELISPOT, fluorescence-activated cell sorting (FACS), and qualitative polymerase chain reaction (qPCR) assays to compare control and nasally immunized groups to elucidate the reproductive imprinting chemokine and receptor involved in the migration of Ag-specific immune cells from the nasal cavity to reproductive tissue.

After nasal immunization with HSV-2 TK^-, the expression of C-C chemokine receptor (CCR) 5 in $CD4^+$ T cells was significantly upregulated in both the nasal Ag-priming site and vaginal tissue. In addition, all ligands of CCR5 showed increased expression in vaginal tissue; in particular, C-C chemokine ligand (CCL) 5 expression was highly enhanced in vaginal tissue after nasal immunization with HSV-2 TK^-. Furthermore, CCR5 deficiency and CCL5 blocking both significantly diminished Ag-specific IFN-γ-secreting effector cell responses in vaginal

tissue after nasal immunization. In an adoptive transfer model, effector cells generated in CCR5-KO mice failed to migrate into vaginal tissue and therefore were not protective against lethal HSV-2 virus genital infection. The production of CCL5 in vaginal tissue was induced by IFN-γ-producing effector cells that had migrated into the vagina after nasal immunization. These results indicate that the CCR5–CCL5 axis is required for the migration of nasally primed HSV-2-specific effector cells from the nasal mucosa to the vagina.

In conclusion, my dissertation thesis research provides new insights into the molecular and cellular mechanisms underlying the nasally induced Ag-specific IgA antibody responses mediated by the TSLP–TSLPR system. My results also increase our understanding of the nasal-to-reproductive mucosal migration of Ag-specific immune cells that is controlled through the CCL5–CCR5 interaction. This study thus highlights the importance of new strategies that activate specific cytokine–chemokine interactions for the initiation of antigen-specific IgA antibody responses and mucosal homing pathways for the design of nasal vaccines against respiratory and sexually transmitted infectious diseases.