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研究課題名	Development of mucosal vaccines designed for infant based on the understanding of the infantile nasopharyngeal and gut mucosal immune system		
研究代表者	Ernst Peter ( カリフォルニア大学サンディエゴ校・教授 )		
	受入教員	石井 健 ( 東京大学医科学研究所・教授 )	
	分担者	Chu B. Hiutung ( University of California, San Diego (UCSD) · Assistant Professor )	
	分担者	Laure Campillo-Gimenes ( University of California, San Diego (UCSD) · Project Scientist )	
	分担者	Jesus Rivera-Nieves ( University of California, San Diego (UCSD) · Professor )	
	分担者	Mitchell Kronenberg ( University of California, San Diego (UCSD) · Professor )	
研究組織	分担者	Hiroshi Kiyono ( Chiba University/Future Medicine Education and Research Organization · Distinct Professor )	
	分担者	Kohtaro Fujihashi ( Chiba University Hospital/Department of Human Mucosal Vaccinology · Project Professor )	
	分担者	Yosuke Kurashima ( Chiba University / Inovative Medicine · Associate Professor )	
	分担者	Daisuke Tokuhara ( Wakayama Medical University / Department of Pediatrics · Professor )	
	分担者	Muneki Hotomi ( Wakayama Medical University / Department of Otolaryngology · Professor )	
	分担者	Tomoya Tsuchihashi ( Wakayama Medical University · Assistant professor )	

Peter B. Ernst

#### IMSUT International Joint Usage/Research Center Project <International>

# Joint Research Report (Annual/Project Completion)

### **Project Completion Report**

#### Report

## Nasal Norovirus vaccine containing zymosan induces antigen-specific IgA antibody responses in infant mice.

We examined the effectiveness of nasal immunization of virus-like particle (VLP) derived from Norovirus (GII.4) with or without zymosan as an adjuvant in infantile and adult BALB/c mice. As a result, VLP-immunized infantile mice weakly induced VLP specific fecal IgA compared with those of VLP-immunized adult mice (Figure 1). On the other hand, nasal immunization of VLP with zymosan effectively enhanced the induction of VLP-specific fecal IgA compared with those of adult mice (Figure 1). In addition, we clarified that zymosan enhanced the uptake of VLP not by macrophages but by dendritic cells at the nasal epithelium in infant mice (Figure 2). These results demonstrated that zymosan can be used as the mucosal vaccine adjuvant especially for children. We will further elucidate the mechanisms of zymosan-induced enhancement of infantile mucosal immunity and will clarify the protective effectiveness of zymosan/VLP nasal vaccine against Norovirus infection. Our results will contribute to develop effective nasal vaccines using zymosan as an adjuvant against various mucosal infections (e.g. Norovirus and RS virus) for children.

Figure 1. Zymosan effectively enhances induction of VLP-specific fecal IgA in infant mice.

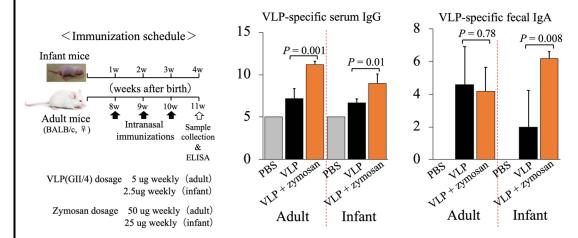
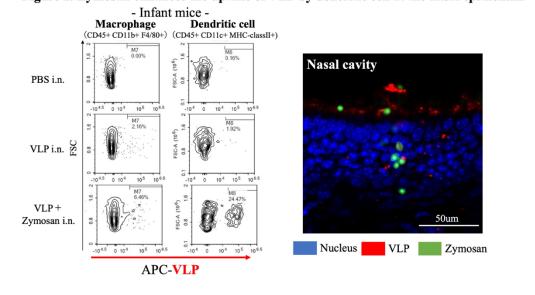
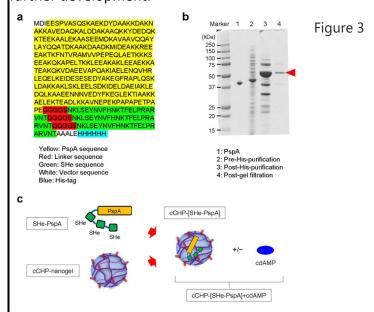


Figure 2. Zymosan enhances the uptake of VLP by dencritic cell at the nasal epithelium



## 2. Nasal cCHP-based RSV vaccine is safe and elicits protective immunity.

Respiratory syncytial virus (RSV) is a leading cause of upper and lower respiratory tract infection, especially in children and the elderly. Various vaccines containing the major transmembrane surface proteins of RSV (proteins F and G) have been tested; however, they have either afforded inadequate protection or are associated with the risk of vaccine-enhanced disease (VED). Recently, F protein–based maternal immunization and vaccines for elderly patients have shown promising results in phase III clinical trials, however, these vaccines have been administered by injection. Here, we examined the potential of using the ectodomain of small hydrophobic protein (SHe), also an RSV transmembrane surface protein, as a nasal vaccine antigen (Figure 3). A vaccine was formulated using our previously developed cationic cholesteryl-group-bearing pullulan nanogel as the delivery system, and SHe was linked in triplicate to pneumococcal surface protein A as a carrier protein (Figure 3). Nasal immunization of mice and cotton rats induced both SHe-specific serum IgG and mucosal IgA antibodies, preventing viral invasion in both the upper and lower respiratory tracts without inducing VED (S. Umemoto, et al.). Moreover, nasal immunization induced greater protective immunity against RSV in the upper respiratory tract than did systemic immunization (S. Umemoto, et al.), suggesting a critical role for mucosal RSV-specific IgA responses in viral elimination at the airway epithelium. Thus, our nasal vaccine induced effective protection against RSV infection in the airway mucosa and is therefore a promising vaccine candidate for further development.



Umemoto, S., Nakahashi-Ouchida, R., Yukim Y., Kurokawa, S., Machitam T., Uchida, Y., Mori, H., Yamanoue, T., Shibata, T., Sawada, S.I., Ishige, K., Hirano, T., Fujihashi, K., Akiyoshi, K., Kurashima, Y., Tokuhara, D., Ernst, P.B., Suzuki, M., and Kiyono, H. Cationic-nanogel nasal vaccine containing the ectodomain of RSV-small hydrophobic protein induces protective immunity in rodents. NPJ Vaccines. Jul 24;8(1):106, 2023. doi: 10.1038/s41541-023-00700-3.

The pancreas contains exocrine glands, which release enzymes (e.g., amylase, trypsin, and lipase) that are important for digestion and islets, which produce hormones. Digestive enzymes and hormones are secreted from the pancreas into the duodenum and bloodstream, respectively. Growing evidence suggests that the roles of the pancreas extend to not only the secretion of digestive enzymes and hormones but also to the regulation of intestinal homeostasis and inflammation (e.g., mucosal defense to pathogens and pathobionts). Organ crosstalk between the pancreas and intestine is linked to a range of physiological, immunological, and pathological activities, such as the regulation of the gut microbiota by the pancreatic proteins and lipids, the retroaction of the gut microbiota on the pancreas, the relationship between inflammatory bowel disease, and pancreatic diseases. We herein discuss the current understanding of the pancreas-intestinal barrier axis and the control of commensal bacteria in intestinal inflammation (Figure 4).

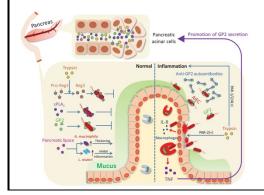


Figure 4.