International Research and Development Center for Mucosal Vaccines

Division of Clinical Vaccinology
臨床ワクチン学分野

Project Professor	Kohtaro Fujihashi, D.D.S., Ph.D.	特任教授	博士（歯学）	藤　橋　浩太郎
Project Associate Professor	Yosuke Kurashima, Ph.D.	特任准教授	博士（医学）	倉　島　洋　介

To explore new avenues for mucosal vaccine development and immune-regulation, investigators have begun to employ novel adjuvants and targeting mucosal tissues and immune cells for vaccine delivery and elucidate the mechanisms of immune-regulation in the mucosal tissues. Despite recently advanced sciences, it remains to develop effective mucosal vaccines for human use. To this end, our main task is to define the effectiveness and safety of novel mucosal vaccines including adjuvant- and delivery system-development and bring them from bench-top to practical applications.

1. Novel mucosal vaccine development for the induction of mucosal immunity in the aero-, digestive- and reproductive mucosa.

Kohtaro Fujihashi[1,2], Koichiro Suzuki[1], Rika Nakahashi[3], Yuya Murakami[1], Masao Uchida[1], Takanori Marui[1], Ai Sasou[3], Shiho Kurokawa[3], Kotomi Sugiura[3], Yoshikazu Yuki[3], and Hiroshi Kiyono[1-4] : [1]Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [2]Department of Pediatric Dentistry, The University of Alabama at Birmingham, [3]Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [4]Department of Mucosal Immunology and Immunology, Graduate School of Medicine, Chiba University, Chiba, 260-8670, JAPAN

It has been shown that oral antigen (Ag) plus adjuvant delivery for induction of immunity, as opposed to nasal delivery, is an effective non-invasive route. Further, it is well-tolerated and avoids the possibility of Ag and /or adjuvant uptake into the olfactory tissues with subsequent entry into the central nervous system (CNS). However, oral vaccines require relatively large amounts of Ag and adjuvant and are exposed to the proteolytic enzymes and lower pH of the stomach. Considerably, their efficacy limits the mainly gastrointestinal mucosa. In this regard, it is essential to develop a new generation of oral adjuvants which elicit mucosal immunity in the entire mucosal surfaces including respiratory and reproductive tracts. In order to accomplish this goal, we planned to discover novel molecules which could use potential oral adjuvant for inducing global protective mucosal immunity by using single-cell mRNA sequencing approach. We have successfully established several DNA libraries from nasopharyngeal-associated lymphoid tissues and Peyer's patches of naïve mice as well as mice given either the oral or nasal vaccine. The sequence data have been analyzed using SHIROKANE supercomputer system and we have identified several unique molecules which preferentially upregulated in the NALT of mice given nasal vaccine when compared with those in Peyer's patches of mice given an oral vaccine. We are currently testing the roles of these molecules for the induction of mucosal tissue-specific immune responses by using gain and lost function approaches.

2. Roles of Secretory IgA antibodies for the maintenance of the homeostasis in the oral cavity

Emily Chang[1], Ryoki Kobayashi[2], Kohtaro Fuji-

hashi[3,4], Masamichi Komiya[1], and Tomoko Kurita-Ochiai[2] : Departments of [1]Oral Surgery and [2]Infection and Immunology, Nihon University School of Dentistry at Matsudo, [3]Division of Clinical Vaccinology, International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [4]Department of Pediatric Dentistry, The University of Alabama at Birmingham.

Secretory IgA (SIgA) plays a central role in preventing bacterial and viral infections on mucosal surfaces by neutralizing toxins and viruses and inhibiting bacterial attachment to epithelial cells. However, the role of salivary SIgA antibodies (Abs) in regulating oral flora is still unknown. This study aimed to evaluate the association among oral bacteria, their metabolites and periodontitis in IgA-deficient (IgA KO) and wild-type (WT) control mice. Microcomputed tomography (micro-CT) analysis was used to assess alveolar bone resorption as development of periodontitis. The bacterial profiles of saliva were determined using the next-generation sequencing assays. Furthermore, the metabolites in saliva were measured and compared using CE-TOFMS. Salivary microbiota of IgA KO mice revealed a remarkably decreased frequency of Streptococcus, and increased percentages of *Aggregatibacer*, *Actinobacillus*, and *Prevotella* at the genus level when compared with those of WT. Compared to WT control mice of the same age, the level of alveolar bone loss was significantly increased in IgA KO mice, and infiltration of osteoclasts was found on the surface of the alveolar bone. The metabolome profile indicated that the metabolites of IgA KO mice had greater variability in carbon metabolic, urea cycle, and lipid pathways than WT mice. These results suggest that salivary SIgA plays an important role in regulating and maintaining normal oral microflora to prevent the development of the periodontal disease.

3. Stratified layer analysis revealsintrinsic hormone stimulates cryptal mesenchymal cells forcontrolling mucosal homeostasis

Seiichi Matsumura[1–3], Yosuke Kurashima[1,2,4,5,7], Sayuri Murasaki[2], Masako Morimoto[1], Fujimi Arai[2], Yukari Saito[1], Nana Katayama[2], Dayoung Kim[2], Yutaka Inagaki[6], Takahiro Kudo[3], Peter Ernst[5,7,8], Toshiaki Shimizu[3], Hiroshi Kiyono[2,4,5] : [1] Department of Innovative Medicine, Graduate School of Medicine, Chiba University, [2]Department of Mucosal Immunology, The University of Tokyo, Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [3]Department of Pediatrics, Juntendo University Faculty of Medicine [4]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [5]Division of Gastroenterology, Department of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines (CU-UCSD cMAV), University of California, San Diego, [6]Center for Matrix Biology and Medicine, Graduate School of Medicine, Tokai University, [7]Division of Comparative Pathology and Medicine, Department of Pathology, University of California, San Diego, [8]Center for Veterinary Sciences and Comparative Medicine, University of California, San Diego.

Mesenchymal cells in the crypt play indispensable roles in the maintenance of intestinal epithelial homeostasis through their contribution to the preservation of stem cells. However, the acquisition properties of the production of stem cell niche factors by the mesenchymal cells have not been well elucidated, due to technical limitations regarding the isolation and subsequent molecular and cellular analyses of cryptal mesenchymal cells. To evaluate the function of mesenchymal cells located at the large intestinal crypt, we established a novel method through which cells are harvested according to the histologic layers of the mouse colon, and we compared cellular properties between microenvironmental niches, the luminal mucosa and crypts. The gene expression pattern in the cryptal mesenchymal cells showed that receptors of the hormone/cytokine leptin were highly expressed, and we found a decrease in Wnt2b expression under conditions of leptin receptor deficiency, which also induced a delay in cryptal epithelial proliferation. Our novel stratified layer isolation strategies thus revealed new microenvironmental characteristics of colonic mesenchymal cells, including the intrinsic involvement of leptin in the control of mucosal homeostasis.

4. Orally desensitized mast cells form a regulatory network with Treg cells for the control of food allergy

Yoshihiro Takasato[1,2], Yosuke Kurashima[1,3-7], Masahiro Kiuchi[8], Kiyoshi Hirahara[8], Sayuri Murasaki[1,4], Fujimi Arai[1,4], Kumi Izawa[9], Ayako Kaitani[9], Kaoru Shimada[1,4], Yukari Saito[3], Shota Toyoshima[10], Miho Nakamura[1], Kumiko Fujisawa[1], Yoshimichi Okayama[10], Jun Kunisawa[4,7], Masato Kubo[11,12], Naoki Takemura[3,4,13], Satoshi Uematsu[3,4,14], Shizuo Akira[15,16], Jiro Kitaura[9], Takao Takahashi[2], Toshinori Nakayama[8], and Hiroshi Kiyono[1,4-6,8] : [1]Department of Mucosal Immunology, The University of Tokyo Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [2]Department of Pediatrics, Keio University School of Medicine, [3]Department of Innovative Medicine and Mucosal Immunology, Graduate School of Medicine, Chiba University, [4]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo,

[5]Division of Gastroenterology, Department of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines (CU-UCSD cMAV), University of California, San Diego, [6]Institute for Global Prominent Research, Chiba University, [7]Laboratory of Vaccine Materials, Center for Vaccine and Adjuvant Research and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition, [8]Department of Immunology, Graduate School of Medicine, Chiba University, [8]Atopy Research Center, Juntendo University Graduate School of Medicine, [9]Allergy and Immunology Research Project Team, Research Institute of Medical Science, Center for Allergy, Center for Medical Education, Nihon University School of Medicine, [10]Laboratory for Cytokine Regulation, RIKEN Center for Integrative Medical Sciences, [11]Division of Molecular Pathology, Research Institute for Biomedical Science, Tokyo University of Science, [12]Laboratory of Bioresponse Regulation, Graduate School of Pharmaceutical Sciences, Osaka University, [13]Department of Immunology and Genomics, Osaka City University Graduate School of Medicine, [14]Laboratory of Host Defense, WPI Immunology Frontier Research Center, Osaka University, [15]Department of Host Defense, Research Institute for Microbial Diseases, Osaka University,

Oral immunotherapy (OIT) is an effective approach to controlling food allergies. Although the detailed molecular and cellular mechanisms of OIT are unknown currently, they must be understood to advance the treatment of allergic diseases in general. To elucidate the mechanisms of OIT, especially during the immunological transition from desensitization to allergy regulation, we generated a clinical OIT murine model and used it to examine immunological features after OIT. We found that in mice that completed OIT successfully, desensitized mast cells (MCs) showed functionally beneficial alterations, such as increased expression of regulatory cytokines and enhanced expansion of regulatory T cells. Importantly, regulatory-T-cell-mediated inhibition of allergic responses was decreased in mice in which desensitized MCs were depleted during OIT. Collectively, these findings show that the desensitization process modulates the activation of MCs, leading directly to enhanced induction of regulatory-T-cell expansion and promotion of clinical allergic unresponsiveness. Our results suggest that efficiently inducing regulatory MCs is a novel strategy for the treatment of allergic diseases.

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