International Vaccine Design Center

Division of Mucosal Vaccines (New Dimensional Vaccine Design Team)

新次元ワクチンデザイン系・粘膜ワクチン分野

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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 recent 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.

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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 a 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 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. Our results showed that one of these molecules is indeed up-regulated in NALT and the reproductive tract. We confirmed that mice deficient with this molecule showed reduced levels of antigen-specific IgA antibody responses in the vaginal washes despite intact levels of serum IgG titers. We are currently testing chemokine receptor expression which involved for the regulation of antigen specific IgA responses.

2. Human salivary protein-derived peptides specific-salivary SIgA antibodies enhanced by nasal double DNA adjuvant in mice play an essential role in preventing *Porphyromonas gingivalis* colonization *in vitro*

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We have previously shown that fimbriae-bore from *Poryphyromonas gingivalis* (Pg), one of the putative periodontopathogenic bacteria specifically bound to a peptide domain (stat23, prp21) shared on statherin or acidic proline-rich protein 1 (PRP1) molecule of human salivary proteins (HSPs). In this study, we first investigated whether nasal administration of double DNA adjuvant (dDA) consisting of DNA plasmid expressing Flt3 ligand and CpG oligodeoxynucleotide plus sta23 or prp21 peptide as an antigen (Ag) in mice could enhance stat23- or prp21-specific secretory IgA (SIgA) antibody (Ab) responses in the saliva of mice. Significant elevated levels of salivary SIgA Ab to stat23 or prp21 in mice given nasal stat23 or prp21 with dDA were seen compared to those in mice given Ag alone. Of interest, mice given the mixture of stat23 and prp21 (double Ags) plus dDA, nasally, resulted in stat23- and prp21-specific salivary SIgA Ab induction, which is mediated through significantly increased numbers of $CD11c^+$ dendritic cells populations and marked Th1 and Th2 cytokines production by $CD4^+$ T cells in the mucosal inductive and effector tissues. Furthermore, when mice were nasally immunized with double Ags plus dDA, stat23- and prp21-specific salivary SIgA Ab responses were enhanced, and the SIgA Ab-enriched saliva showed significantly reduced numbers of live Pg cells binding to human whole saliva-coated hydroxyapatite beads (wsHAPs) as compared with those in mice given double Ags alone or naïve mice. Additionally, saliva from IgA knock-out mice given nasally double Ags plus dDA indicated no decrease of live Pg binding to wsHAPs. These findings show that HSP-derived peptides-specific salivary SIgA Abs induced by nasal administration of stat23 and prp21 peptides plus dDA, play an essential role in the preventing Pg attachment and colonization on the surface of teeth, suggesting that the SIgA may interrupt and mask fimbriae-binding domains in HSPs on the teeth.

3. Utilizing mast cells in a positive manner to overcome inflammatory and allergic diseases

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Mast cells (MCs) are immune cells widely distributed in the body, accompanied by diverse phenotypes and functions. Committed mast cell precursors

(MCPs) leave the bone marrow and enter the blood circulation, homing to peripheral sites under the control of various molecules from different microenvironments, where they eventually differentiate and mature. Partly attributable to the unique maturation mechanism, MCs display high functional heterogeneity and potentially plastic phenotypes. High plasticity also means that MCs can exhibit different subtypes to cope with different microenvironments, which we call "the peripheral immune education system". Under the peripheral immune education system, MCs showed a new character from previous cognition in some cases, namely regulation of allergy and inflammation. In this review, we focus on the mucosal tissues, such as the gastrointestinal tract, to gain insights into the mechanism underlying the migration of MCs to the gut or other organs and their heterogeneity, which is driven by different microenvironments. In particular, the immunosuppressive properties of MCs let us consider that positively utilizing MCs may be a new way to overcome inflammatory and allergic disorders.

Intestinal homeostasis and inflammation: gut microbiota at the crossroads of pancreas–intestinal barrier axis

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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 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. Thus, the pancreas–intestinal barrier axis and the control of commensal bacteria in intestinal inflammation need to be further elucidated.

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