IMSUT Distinguished Professor Unit

Division of Stem Cell Therapy
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We are working on uncovering new diseases, elucidating the causes of disease, and developing therapeutic modalities by connecting the knowledge and methodology of basic science including immunology, molecular biology, cell biology, and developmental engineering with clinical medicine. Our ultimate goal is to contribute to establishing new frontiers of stem cell therapy and to make clinical applications of stem cells a reality.

1. An interspecies barrier to tetraploid complementation and chimera formation

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To study development of the conceptus in xenogeneic environments, we assessed interspecies chimera formation as well as tetraploid complementation between mouse and rat. Overall contribution of donor PSC-derived cells was lower in interspecies chimeras than in intraspecies chimeras, and high donor chimerism was associated with anomalies or embryonic death. Organ to organ variation in donor chimerism was greater in interspecies chimeras than in intraspecies chimeras, suggesting species-specific affinity differences among interacting molecules necessary for organogenesis. In interspecies tetraploid complementation, embryo development was near normal until the stage of placental formation, after which no embryos survived.

2. Generation of Vascular Endothelial Cells and Hematopoietic Cells by Blastocyst Complementation

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In the case of organ transplantation accompanied by vascular anastomosis, major histocompatibility complex mismatched vascular endothelial cells become a target for graft rejection. Production of a rejection-free, transplantable organ, therefore, requires simultaneous generation of vascular endothelial cells within the organ. To generate pluripotent stem cell (PSC)-derived vascular endothelial cells, we performed blastocyst complementation with a *vascular endothelial growth factor receptor-2* homozygous mutant blastocyst. This mutation is embryonic lethal at embryonic (E) day 8.5－9.5 due to an early defect in endothelial and hematopoietic cells. The *Flk-1* homozygous knockout chimeric mice survived to adulthood for over 1 year without any abnormality, and all vascular endothelial cells and hematopoietic cells were derived from the injected PSCs. This approach could be used in conjunction with other gene knockouts which induce organ deficiency to produce a rejection-free, transplantable organ in which all the organ's cells and vasculature are PSC derived.

3. Intra-embryo Gene Cassette Knockin by CRISPR/Cas9-Mediated Genome Editing with Adeno-Associated Viral Vector

Naoaki Mizuno,[1] Eiji Mizutani,[1] Hideyuki Sato,[1] Mariko Kasai,[1] Aki Ogawa,[1] Fabian Suchy,[2] Tomoyuki Yamaguchi,[1] and Hiromitsu Nakauchi[1,2]: [1]Division of Stem Cell Therapy, Institute of Medical Science, University of Tokyo, [2]Institute for Stem Cell Biology and Regenerative Medicine, Department of Genetics, Stanford University School of Medicine

Intra-embryo genome editing by CRISPR/Cas9 enables easy generation of gene-modified animals by non-homologous end joining (NHEJ)-mediated frameshift mutations or homology-directed repair (HDR)-mediated point mutations. However, large modifications, such as gene replacement or gene fusions, are still difficult to introduce in embryos without costly micromanipulators. Moreover, micromanipulation techniques for intra-embryo genome editing have been established in only a small set of animals. To overcome these issues, we developed a method of large-fragment DNA knockin without micromanipulation. In this study, we successfully delivered the knockin donor DNA into zygotes by adeno-associated virus (AAV) without removing the zona pellucida, and we succeeded in both large-DNA fragment knockin and whole exon exchange with electroporation of CRISPR/Cas9 ribonucleoprotein. By this method, we can exchange large DNA fragments conveniently in various animal species without micromanipulation.

Publications

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The mucosal immune system not only senses pathogenic antigens such as pathogens and allergens, but also establishes tolerance that does not react excessively to beneficial antigens such as food-derived proteins and commensal microorganisms. Our laboratory's mission is to elucidate and understand the uniqueness of the mucosal immune system which controls the immunological balancing act between the elimination of and commensalism with harmful and beneficial antigens, respectively, and aim to develop the basic platform for creating the novel strategies of prevention and treatment of various infectious and immunological diseases by the fusion science with agriculture, engineering and plant biology.

1. Critical role of the CCR5–CCL5 interaction for preferential migration of HSV-2-specific effector cells to the vaginal mucosa upon nasal immunization.

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Our current study focused on elucidating the role of specific chemokine－receptor interactions in antigen (Ag)-specific immune cell migration from nasal to genital mucosal tissues. This cellular migration is critical to induce effective Ag-specific immune responses against sexually transmitted infection in the genital tract. In this study, nasal immunization with live attenuated HSV-2 TK－ induced the upregulation of CCR5 expression in effector immune cells, including CD4＋ T cells, in Ag-priming sites and vaginal tissue. The CCR5 ligands CCL3, CCL4, and CCL5 all showed upregulated expression in vaginal tissue; in particular, CCL5 expression was highly enhanced in the stromal cells of vaginal tissue after nasal immunization. Intra-vaginal blockade of CCL5 by using neutralizing antibody diminished the number of HSV-2-specific effector cells in the vagina. Furthermore, loss of CCR5, a receptor of

CCL5, impaired the migration of nasally primed Ag-specific effector cells from the airway to vagina. Effector cells adoptively transferred from CCR5-deficient mice failed to migrate into vaginal tissue, consequently increasing recipient mice's susceptibility to HSV-2 vaginal infection. These results indicate that the CCR5–CCL5 chemokine pathway is required for the migration and retention of nasally primed Ag-specific effector cells in vagina for providing protective immunity against HSV-2 infection.

2. Lymphoid tissue-resident *Alcaligenes* LPS induces IgA production and has an adjuvanticity without excessive inflammatory responses via weak TLR4 agonist activity.

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Intestinal commensal bacteria affect the development and function of the host immune system, including the production of secretory IgA (SIgA) and the development of intraepithelial T lymphocytes. Accumulating evidence has revealed that particular kinds of commensal bacteria control the differentiation of specific T cell populations. For example, segmented filamentous bacteria induce the differentiation of Th17 cells and clostridial strains can induce regulatory T cells. Although these studies mainly focused on the commensal bacteria in the intestinal lumen or mucus layers, genome-based bacterial analysis using intestinal tissue allowed us to identify *Alcaligenes* as symbiotic resident bacteria of Peyer's patches (PPs), a major gut-associated lymphoid tissue in the small intestine. However, how *Alcaligenes* create and maintain their homeostatic environment, without inducing an excessive inflammatory response remained unclear. We show here that *Alcaligenes*-derived lipopolysaccharide (*Alcaligenes* LPS) acts as a weak agonist of toll-like receptor 4 and promotes IL-6 production from dendritic cells, which consequently enhances IgA production. The inflammatory activity of *Alcaligenes* LPS was weaker than that of E. coli-derived LPS and therefore no excessive inflammation was induced by *Alcaligenes* LPS in vitro or in vivo. As an application of *Alcaligenes* LPS, we found that *Alcaligenes* LPS has an adjuvanticity. *Alcaligenes* LPS induced antigen-specific humoral immune responses as well as Th1, Th2, and Th17 cells without excessive inflammation. These findings reveal the presence of commensal bacteria mediated homeostatic inflammatory conditions within Peyer's patches that produce optimal IgA induction without causing pathogenic inflammation and suggest that *Alcaligenes* LPS could be a safe and potent adjuvant.

3. Epithelial α(1,2)-fucosylation in the large intestine.

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Fucosylated carbohydrates are expressed on intestinal epithelial cells. They are involved in the creation of an environmental niche for commensal bacteria. Fucosyltransferase 2 (Fut2) is a key enzyme regulating intestinal epithelial α(1,2)-fucosylation, and the polymorphism of the *FUT2* gene is reported to be associated with Crohn's disease. Our previous research showed that α(1,2)-fucosylation in the small intestine is upregulated by IL-22, type 3 innate lymphoid cells and by microbial stimuli.

Also, IL-10-prodicing CD4+ T cells negatively regulate it. However, the mechanism of α(1,2)-fucosylation in the large intestine is still unknown. In current study, we examined α(1,2)-fucosylation in the large intestine. Fucosylated goblet cells were observed in wild type mice, while those were not found in Fut2 deficient mice. In these regions, α(1,2)-fucosylation was Fut2-dependent. Next we evaluated the extent of α(1,2)-fucosylation focused on IL-22. The α(1,2)-fucosylation in the large intestine was not downregulated in IL-22 deficient mice, compare to those in the wild type mice. Moreover, *FUT2* gene expression in the large intestinal epithelial cell organoid cultures, did not require recombinant IL-22. From these results, the mechanism of α(1,2)-fucosylation in the large intestine is regulated by other molecular than IL-22. It is expected that to reveal the mechanism of large intestinal α(1,2)-fucosylation lead to understanding of intestinal homeostasis.

4. Innate and adaptive immune cells regulate Paneth cell granule formation and α-defensin secretion

Mariko Kamioka[1,2], Yoshiyuki Goto[2,3], Kiminori Nakamura[4], Shintaro Sato[2,5], Jun Kunisawa[2,6], Yu Takahashi[7], Yosuke Kurashima[1,2,8,12], Steven E. Domino[9], Jean-Christophe Renauld[10], Tokiyoshi Ayabe[4] and Hiroshi Kiyono[1,2,11,12]: [1]Department of Mucosal Immunology, IMSUT Distinguished Professor Unit, The Institute of Medical Science, The University of Tokyo, [2]International Research and Development Center for Mucosal Vaccines, The Institute of Medical Science, The University of Tokyo, [3]Division of Molecular Immunology, Medical Mycology Research Center, Chiba University, [4]Department of Cell Biological Science, Graduate School of Life Science, Faculty of Advanced Life Science, Hokkaido University, [5]Mucosal Vaccine Project, BIKEN Innovative Vaccine Research Alliance Laboratories, Research Institute for Microbial Diseases, Osaka University, [6]Laboratory of Vaccine Materials and Laboratory of Gut Environmental System, National Institutes of Biomedical Innovation, Health and Nutrition (NIBIOHN), [7]Food Biochemistry Laboratory, Department of Applied Biological Chemistry, Graduate School of Agricultural and Life Sciences, The University of Tokyo, [8]Department of Innovative Medicine, Graduate School of Medicine, Chiba University, [9]Department of Obstetrics and Gynecology, Cellular and Molecular Biology Program, University of Michigan Medical Center, [10]Ludwig Institute for Cancer Research and Université Catholique de Louvain, [11]Mucosal Immunology and Allergy Therapeutics, Institute for Global Prominent Research, Graduate School of Medicine, Chiba University, [12]Division of Gastroenterology, Department of Medicine, School of Medicine, CU-UCSD Center for Mucosal Immunology, Allergy and Vaccines, University of California, San Diego.

The gastrointestinal tract is constantly exposed to numerous foreign antigens. Intestinal epithelial cell layer acts as a first line of defense and is divided into villi and crypt regions. In the crypts, epithelial stem cells and Paneth cells are preferentially located. Paneth cells release granules containing a variety of antimicrobial peptides as a major part of the host innate immune system. α-defensin is most abundant and highly bactericidal peptide specifically produced by Paneth cells.

It has been known that crypts are surrounded by immune cells. Type3 innate lymphoid cells located beneath of crypts preferentially produce Interleukin 22 (IL-22) known as innate immune signaling. We found that IL-22 promotes the differentiation of Paneth cells with matured granules containing α-defensin. We further found that granule release of Paneth cells is regulated by acquired immune signaling via membrane trafficking system.

Our results indicate that the cell fate and function of Paneth cells are dually regulated by innate and adaptive immune cells for the production and secretion of α-defensin in gastrointestinal tract. α-defensin plays a crucial role for the creation and maintenance of intestinal homeostasis, thus we concluded that the mutual interaction of Paneth cells and immune cells provide healthy intestinal environment.

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