

# MINI-SYMPOSIUM FOR MUCOSAL IMMUNOLOGY AND ALLERGY THERAPEUTICS

## DECEMBER 16, 2015

INOHANA ALUMNI HOUSE (あのはな同窓会館)

### PROGRAM

#### 13:00-13:05 Opening Remarks

Takeshi Tokuhisa (President, Chiba University)

#### 13:05-13:20 "International Center of Excellence in Mucosal Immunology and Innovative Allergy Therapeutics at Chiba University"

Toshinori Nakayama (Dean, Graduate School of Medicine, Vice President, Chiba University)

#### 13:20-14:40 Symposium 1 : Mucosal Immunology [Chair: Satoshi Uematsu & Kiyoshi Hirahara]

- Satoshi Uematsu "Roles of innate immunity in radiation-induced intestinal injury"  
Shinobu Saijo "Mechanisms of anti-fungal immunity"  
Yoshiyuki Goto "Group 3 innate lymphoid cells regulate intestinal epithelial fucosylation"  
Yosuke Kurashima "Elucidation and Regulation of Disease-specific Mast Cell Activations"

#### 14:40-15:00 Coffee Break

#### 15:00-16:20 Symposium 2 : Allergy Therapeutics [Chair: Satoshi Uematsu & Kiyoshi Hirahara]

- Kiyoshi Hirahara "Thy1<sup>+</sup>IL-7<sup>+</sup> lymphatic endothelial cells in iBALT are potential therapeutic targets for allergic airway inflammation"  
Mark Bix "Constraint of whipworm expulsion associated with Cryptdin repression"  
Yuumi Matsuoka-Nakamura "Cooperation of IL-1 and IL-36 plays a pivotal role in epicutaneous *S.aureus*-induced skin inflammation via IL-17 production"  
Hiroshi Nakajima "Roles of C-type lectin receptors in HDM-induced allergic airway inflammation"

#### 16:20-16:40 Coffee Break

#### 16:40-17:25 Plenary Lecture

[Chair: Hiroshi Kiyono]

**Peter Ernst** (Department of Pathology, University California San Diego)

"The role of purine metabolism in mucosal immune homeostasis: Adenosine regulates Th cell plasticity and the control of Th cell-mediated enterocolitis"

#### 17:25-17:30 Closing Remarks

Hiroshi Kiyono

#### 17:30- Get Together Party "See-Saw"

入場無料



Organized by Future Medicine Education and Research Organization at Chiba University

Co-Organized by Leading Graduate School at Chiba University

Inquiries

Future Medicine Education and Research Organization  
Administrative Office

TEL : 043-226-2832 / FAX : 043-226-2489 / E-mail : inohana-kaikaku@chiba-u.jp

# International Center of Excellence in Mucosal Immunology and Innovative Allergy Therapeutics at Chiba University

Tosihnoru Nakayama

*Dean, Graduate School of Medicine,  
Vice President, Chiba University*



## **Roles of innate immunity in radiation-induced intestinal injury**

Satoshi Uematsu, Naoki Takemura

*Department of Mucosal Immunology, Graduate School of Medicine, Chiba University, Japan*



Radiation-induced intestinal fibrosis (RIF) is a serious complication after abdominal radiotherapy. Here we show RIF is mediated by eosinophil interactions with  $\alpha$ -smooth muscle actin ( $\alpha$ -SMA)<sup>+</sup> stromal cells. Abdominal irradiation induced fibrosis of the submucosa (SM) of small intestine associated with the excessive accumulation and degranulation of eosinophils, even in the absence of lymphocytes. Eosinophil-deficiency markedly ameliorated RIF, suggesting their importance. Chronic crypt necrosis post-irradiation elevated extracellular adenosine triphosphate levels, which induced C-C motif chemokine 11 (CCL11) expression by pericryptal  $\alpha$ -SMA<sup>+</sup> cells to recruit eosinophils to the SM via CCR3. Unexpectedly, type 2 innate lymphoid cells were also dispensable for eosinophil activation in RIF. For activation of eosinophils, activated  $\alpha$ -SMA<sup>+</sup> cells expressed granulocyte macrophage colony-stimulating factor (GM-CSF), which induced expression of transforming growth factor- $\beta$ 1 from intestinal eosinophils, leading to the promotion of collagen expression by  $\alpha$ -SMA<sup>+</sup> cells. In addition, eosinophils released cytotoxic granule proteins upon co-stimulation with GM-CSF and CCL11. Thus, radiation-induced crypt injury triggers mutual activation of eosinophils and  $\alpha$ -SMA<sup>+</sup> cells, leading to a progressive fibrogenesis. Our findings provide an immunopathological mechanism of RIF and clues for the development of therapeutic strategies for RIF.

## Mechanisms of anti-fungal immunity

Mari Iwasawa<sup>1</sup>, Yuumi Matsuoka-Nakamura<sup>1</sup> and Shinobu Saijo<sup>2</sup>



<sup>1</sup>*Dermatology, Chiba University, Chiba Japan*

<sup>2</sup>*Medical Mycology Research Center, Chiba University, Chiba Japan*

Myeloid cells are key player in anti-microbial responses. Members of C-type lectin receptors (CLRs) surpass among the specialized receptors that expressed in these cells to orchestrate immune responses these responses. They are characterized by the extracellular domain called CRD (carbohydrate binding domain). Although they had been suggested to be members of pattern recognition receptors (PRRs), their functions have long been remained unclear. Therefore, we aimed to clarify the molecular mechanisms of C-type lectins in the host defense against microbial infections. Dectin-1 was first reported as a dendritic cell (DC)-specific C-type lectin receptor, and is also highly expressed in macrophages and neutrophils. It has a CRD in its extracellular carboxyl terminus and an immunoreceptor tyrosine-based activation motif (ITAM) in its intracellular amino terminus, and is suggested to be the receptor for  $\beta$ -1, 3-linked and/or  $\beta$ -1, 6-linked glucans ( $\beta$ -glucans) found in the cell walls of fungi. Dectin-2 is also expressed in DCs and macrophages, and has a CRD, but has no known signaling motif in its intracellular domain. We have generated Dectin-1 and Dectin-2-deficient mice to determine the roles of these molecules in the defense against pathogenic fungi. *In vitro*,  $\beta$ -glucan-induced cytokine production from wild-type DCs and macrophages was abolished in Dectin-1-deficient cells, and  $\alpha$ -mannan-induced cytokine production was abolished in Dectin-2-deficient cells. *In vivo*, Dectin-1-deficient mice were more susceptible than wild-type mice to *Pneumocystis carinii* infection, and Dectin-2-deficient mice were more susceptible to *Candida albicans* (*C. albicans*) infection. Interestingly, candida preferentially induced Th17 cell differentiation, which was markedly reduced by Dectin-2 deficiency. Because IL-17A-deficient mice were more susceptible to candida infection, it was suggested that Dectin-2 signaling preferentially induces Th17 cell differentiation and Th17 cell-derived IL-17A is important for the host defense against *C. albicans*. Thus, Dectin-1 and Dectin-2 play important roles for the defense against fungal infections as a protective innate and acquired immunity. In this mini symposium, I would like to share my recent findings.

## Group 3 innate lymphoid cells regulate intestinal epithelial fucosylation

Yoshiyuki Goto

*Division of Molecular Immunology, Medical Mycology  
Research Center, Chiba University*



Intestine is a unique organ which is constitutively exposed by external antigens including commensal and pathogenic bacteria. Intestinal epithelial cells and immune cells create barrier system against harmful antigens. At the same time, host provide peaceful symbiotic environment for commensal bacteria.  $\alpha$ 1, 2-fucose expressed on intestinal epithelial cells, catalyzed by fucosyltransferase 2 (Fut2), is one of major carbohydrate moiety which mediates host–microbiota symbiosis. Since specific symbionts such as *Bacteroides* utilize epithelial  $\alpha$ 1, 2-fucose as a dietary carbohydrate, dysfunction of Fut2 leads to dysbiosis of gut microflora and predisposes to the development of host diseases. Indeed, human FUT2 non-sense polymorphisms are associated with various inflammatory diseases such as Crohn’s disease, type I diabetes and primary sclerosing cholangitis. Therefore, it is important to identify the mechanism of the induction of epithelial Fut2 and  $\alpha$ 1, 2-fucose (fucosylation). Here, we show that segmented filamentous bacteria (SFB), one of commensal bacteria specifically colonized at ileal epithelium, have a potential to induce epithelial fucosylation under physiological condition. Furthermore, group 3 innate lymphoid cells (ILC3) are also necessary for the induction of intestinal epithelial fucosylation. Interleukin-22 and lymphotoxin produced from ILC3 are required for the induction of epithelial fucosylation in a commensal bacteria–dependent and –independent manner, respectively. Mice lacking epithelial fucosylation are susceptible to infection by *Salmonella typhimurium*. Taken together, these data uncovered the novel function of commensals-ILC3-epithelial cell network in the induction of epithelial glycosylation, creation of gut barrier system against pathogenic bacterial infection, and regulation of intestinal homeostasis.

## **Elucidation and Regulation of Disease-specific Mast Cell Activations**

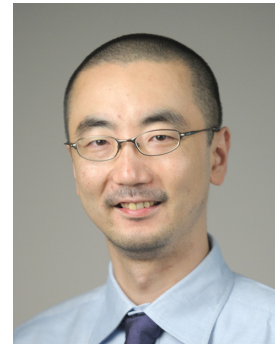
Yosuke Kurashima

*Division of Mucosal Immunology, Department of Microbiology and Immunology, The Institute of Medical Science, The University of Tokyo, Tokyo*



Since the discovery of mast cells in 1878, and IgE 50 years ago, the important roles of mast cells in both allergic diseases and parasitic infections have been extensively recognized. A few years after the discovery of IgE, it was reported that histamine is released from mast cells upon the stimulation of extracellular ATP; however, the diseases related to the extracellular ATP-mediated mast cell activations are issued relatively recently. In this symposium, we would like to introduce our recent findings, indicating the ATP-mast cell pathway as a new drug target against inflammatory bowel diseases. In addition, a unique skin-barrier homeostatic network operating through retinoic acid-metabolism-mediated inhibition of ATP-dependent MC activation by fibroblasts. At the end of the presentation, I would like to share the results obtained recently indicating the important roles of mast cells in both development and inhibition of food allergy.

## **Thy1<sup>+</sup>IL-7<sup>+</sup> lymphatic endothelial cells in iBALT are potential therapeutic targets for allergic airway inflammation**



Kiyoshi Hirahara<sup>1</sup>, Kenta Shinoda<sup>2</sup>, Toshinori Nakayama<sup>2</sup>

1. *Department of Advanced Allergology of the Airway, Graduate School of Medicine, Chiba University, Chiba, Japan.*
2. *Department of Immunology, Graduate School of Medicine, Chiba University, Chiba, Japan.*

Memory CD4<sup>+</sup> T helper (Th) cells are crucial to long-term protection against pathogens. But they can also be pathogenic and drive chronic inflammatory disorders including allergic airway inflammation such as asthma. In 2011, we identified that IL-5-producing memory Th2 cell subsets are critical for the pathology of allergic inflammation, and function as “memory type pathogenic Th2 (Tpath2) cells (Endo et al. *Immunity*, 2011). Very recently, we found that Interleukin-33 (IL-33)-ST2 signals as a key pathway for the induction of memory-type Tpath2 cells during the eosinophilic airway inflammation in both mice and human systems (Endo et al. *Immunity*, 2015).

But It has been still unclear how memory type pathogenic Th2 (Tpath2) are maintained, particularly at the site of local inflammation. We found that ectopic lymphoid-like structures called inducible bronchus-associated lymphoid tissue (iBALT) are formed during chronic allergic inflammation in the lung, and that memory-type Tpath2 cells are maintained within the iBALT structures. Maintenance of the Tpath2 cells within iBALT is supported by novel Thy1<sup>+</sup>IL-7-producing lymphatic endothelial cells (LECs) within the lung. Moreover, ectopic lymphoid structures consisting of memory CD4<sup>+</sup> T cells were found in nasal polyps of eosinophilic chronic rhinosinusitis patients, indicating that the persistence of inflammation is controlled by these structures. Therefore, the cell components that organize and contribute to iBALT formation and the functional molecules that maintain pathogenic inflammatory memory Th2 cells are likely to be excellent targets for the treatment of chronic allergic airway inflammation.

## **Constraint of whipworm expulsion associated with Cryptdin repression**

Mark Bix

*Department of Innovative Therapeutics, School of Medicine,  
Chiba University, Japan*



Cryptdins (also known as alpha-defensins) are a major class of anti-microbial peptides secreted by Paneth cells located at the base of intestinal Crypts of Lieberkuhn. Cryptdins exhibit potent anti-microbial activity toward viruses, bacteria, fungi and protozoa. However, activity toward helminths has not been described. I will present evidence that under inflammatory conditions the JmjC-family epigenetic regulatory protein Mina represses Cryptdin expression. Further, during intestinal whipworm infection of Mina KO mice, where Cryptdin expression is de-repressed, helminth expulsion is accelerated, raising the possibility that Cryptdins may possess anti-helminthic activity.



## Cooperation of IL-1 and IL-36 plays a pivotal role in epicutaneous *S.aureus*-induced skin inflammation via IL-17 production

Seitaro Nakagawa<sup>1</sup>, Yuumi Matsuoka-Nakamura<sup>1</sup>,  
Gabriel Nunez<sup>2</sup>, Hiroyuki Matsue<sup>1</sup>



<sup>1</sup>*Dermatology, Chiba University, Chiba, Japan*

<sup>2</sup>*Pathology and Comprehensive Cancer Center, University of Michigan, MI, USA*

Although *S. aureus* is known to colonize the skin of atopic dermatitis patients, little is known about the mechanisms of *S.aureus*-induced eczematous inflammation. MyD88 is known to be an adaptor in TLR- and IL-1 receptor (R)-mediated signaling pathways for innate immunity to microorganisms. *Myd88*<sup>-/-</sup> mice reportedly exhibited more severe skin inflammation than WT mice due to high bacteria burden if *S.aureus* was injected subcutaneously. Contrary to the subcutaneous injection, we found that *Myd88*<sup>-/-</sup> mice showed no skin inflammation, whereas WT mice showed severe eczematous lesions with the similar epicutaneous colonization of *S. aureus*, indicating that MyD88 signaling promoted skin inflammation but not cleared *S. aureus* in our epicutaneous disease model. When applied on keratinocyte (KC)-specific *Myd88* knock-down, *K14-CreMyd88*<sup>-/-</sup> mice, the skin inflammation was also dramatically reduced, indicating the involvement of MyD88 in KC. To explore the upstream signals, we next applied *S. aureus* on *Tlr2/4*<sup>-/-</sup> and *Il1r*<sup>-/-</sup> mice. *Tlr2/4*<sup>-/-</sup> mice showed severe inflammation as WT mice, whereas *Il1r*<sup>-/-</sup> mice showed moderate inflammation, indicating that the inflammation was partially IL-1-mediated. When applied on *Il1r*<sup>-/-</sup> mice with IL-36R-neutralizing antibody (IL-36R Ab), the inflammation was significantly reduced, showing additive IL-36 involvement. Upon the infection, little mRNAs and proteins of IL-17 were detected in *K14-CreMyd88*<sup>-/-</sup>, *Il1r*<sup>-/-</sup> with or without IL-36R Ab mice. In accordance with these, *Il17a*<sup>-/-</sup>-*Il17f*<sup>-/-</sup> mice showed significantly less skin inflammation upon the infection. In conclusion, KC is central for the IL-1-mediated inflammation via IL-17 production and otherwise IL-36 involvement in the skin inflammation of this epicutaneous disease model.

## Roles of C-type lectin receptors in HDM-induced allergic airway inflammation

Hiroshi Nakajima, Takashi Ito, Koichi Hirose

*Department of Allergy and Clinical Immunology, Graduate School of Medicine, Chiba University, Japan*



**Background:** The fact that sensitization against fungi is closely related to the severity of asthma suggests that immune systems recognizing fungi are involved in the pathogenesis of severe asthma. Recently, C-type lectin receptors, Dectin-1 (gene symbol *Clec7a*) and Dectin-2 (*Clec4n*), have been shown to function as not only major pattern recognition receptors for fungi but also receptors for some components of house dust mite (HDM) extract, a major allergen for allergic asthma. However, the roles of Dectin-1 and Dectin-2 in the induction of HDM-induced allergic airway inflammation remain largely unknown.

**Objectives:** To determine the roles of Dectin-1 and Dectin-2 in HDM-induced allergic airway inflammation.

**Methods:** We examined the roles of Dectin-1 and Dectin-2 in the induction of HDM-induced Th2 and Th17 cell differentiation and subsequent allergic airway inflammation by using *Clec7a*-deficient (*Clec7a*<sup>-/-</sup>) mice and *Clec4n*-deficient (*Clec4n*<sup>-/-</sup>) mice. We also investigated Dectin-1- or Dectin-2-expressing cells in the lung and their roles in HDM-induced allergic airway inflammation.

**Results:** Both *Clec7a*<sup>-/-</sup> mice and *Clec4n*<sup>-/-</sup> mice showed significantly attenuated HDM-induced allergic airway inflammation and decreased Th2 and Th17 cell differentiation as compared with wild-type (WT) mice. Both Dectin-1 and Dectin-2 were expressed on CD11b<sup>+</sup> dendritic cells (DCs). CD11b<sup>+</sup> DCs isolated from *Clec7a*<sup>-/-</sup> mice or *Clec4n*<sup>-/-</sup> mice expressed lower levels of proinflammatory cytokines and co-stimulatory molecules which could induce Th2 and Th17 cell differentiation than those from WT mice.

**Conclusion:** Dectin-1 and Dectin-2 were expressed on CD11b<sup>+</sup> DCs and promote HDM-induced allergic airway inflammation.

MEMO

MEMO