GCOE Program, The University of Tokyo

Center of Education and Research for the Advanced Genome-Based Medicine
For personalized medicine and the control of worldwide infectious diseases

IMSUT & RCAST Global COE Program
Symposium on Mucosal Immunity

Monday December 7th, 2009　13 : 00 - 17 : 00

Venue: Auditorium, Institute of Medical Science, The University of Tokyo (IMSUT)
Free Admission (No advance registration required)

13:00 – 13:05　Opening Remarks

13:05 – 13:25　**Takahiro Nagatake (Graduate Student, Division of Mucosal Immunology, IMSUT)**
Tear duct–associated lymphoid tissue organogenesis in ocular immunity

13:25 – 14:10　**Reinhard Pabst (Professor, Institute of Functional and Applied Anatomy, Medical School Hannover, Germany)**
Compartmentalisation of lymphocytes in the lung and its functional relevance

14:10 – 14:30　**Taishin Akiyama (Associate Professor, Division of Cellular and Molecular Biology, IMSUT)**
Requirement for TRAF6 signaling in development of naturally occurring regulatory T cells

14:30 – 15:15　**Andreas Diefenbach (Professor, Institute of Medical Microbiology & Hygiene (IMMH), University of Freiburg, Germany)**
Genetic Lineage tracing reveals developmentally and functionally distinct subpopulations of NK cell receptor-expressing cells

15:15 – 15:30　Coffee Break

15:30 – 15:50　**Shinobu Saijo (Assistant Professor, Center for Experimental Medicine, IMSUT)**
The roles of C-type lectins in the host defense against fungal infection

15:50 – 16:35　**David Artis (Assistant Professor, Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, USA)**
Regulation of innate and adaptive immunity in the intestine

16:35 – 16:55　**Ryutaro Fukui (Postdoctoral Fellow, Division of Infectious Genetics, IMSUT)**
Unc93 homolog B1 regulates the balance of toll-like receptor 7 and toll-like receptor 9 responses reciprocally

16:55 – 17:00　Concluding Remarks

Organizers: Jun Kunisawa, Lecturer, and Hiroshi Kiyono, Professor,
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Direction to the venue: the Auditorium of the first building, Shirokane Campus
Shirokanedai Station (Subway Namboku Line & Mita Line), Exit 2; about 3 minutes walk
http://www.ims.u-tokyo.ac.jp/imsut/en/access/access/

Tear duct–associated lymphoid tissue organogenesis in ocular immunity

Takahiro Nagatake (Division of Mucosal Immunology)

The eye is protected by the ocular immunosurveillance system. Here we show that tear duct–associated lymphoid tissue (TALT) is located in the murine lacrimal sac and shares immunological characteristics with mucosa-associated lymphoid tissues (MALTs), including the presence of M cells and immunocompetent cells for antigen uptake and subsequent generation of mucosal immune responses against ocularly encountered antigens. Initiation of TALT genesis began postnatally; it occurred even in germfree conditions and was independent of signaling through organogenesis regulators, including inhibitor of DNA binding/differentiation (Id)2, retinoic acid-related orphan receptor (ROR)γt, lymphotoxin (LT)α1β2-LTβR, and lymphoid chemokines (CCL19, CCL21, and CXCL13). Indeed, $CD3^{-}CD4+CD45+$ cells identified at TALT anlagen (TALT–inducer cells: TALTi) displayed different gene expressions pattern from canonical lymphoid tissue–inducer cells (LTi). Thus, TALT shares immunological features with MALT but has a distinct tissue genesis mechanism and plays a key role in ocular immunity.

Compartmentalisation of lymphocytes in the lung and its functional relevance

Reinhard Pabst (Institute of Functional and Applied Anatomy, Medical School Hannover, Germany)

Lymphocytes are localised in different compartments of the lung as there is the air conducting part with intraepithelial and lamina propria lymphocytes, which differ in their subset composition and the bronchus-associated lymphoid tissue (BALT) which can be called a tertiary lymphoid organ. BALT shows great species differences and seems to develop only after external microbial or other stimuli (like cigarette smoke). However, it might be possible to stimulate and activate it purposely to use it as an entry site of vaccines against pulmonary infections. Further often overlooked compartments are the intravascular and the interstitial lymphocyte pool. A unique only partially understood compartment is the area around the branchus of the pulmonary artery (periarterial space). The most often clinically studied compartment is the broncho-alveolar space (BAL) which only partially represents the lympho cytes of the lung.

Finally the lung draining bronchial lymph nodes have to be included in studies on lymphocyte dynamics in the lung.

Requirement for TRAF6 signaling in development of naturally occurring regulatory T cells

Taishin Akiyama (Division of Cellular and Molecular Biology)

Naturally occurring regulatory T cells (Tregs) are crucial for self-tolerance. However, the molecular mechanisms underlying Treg development are largely unknown. In this symposium, I describe that TNF receptor-associated factor 6 (TRAF6) is essential to the commitment of thymocytes to Treg lineage. We found that although TRAF6 is required for the development of medullary thymic epithelial cells (mTECs), the absence of TRAF6 in thymic stroma does not largely affect Treg development. Instead, mixed fetal liver transfer experiments revealed a T-cell autonomous requirement for TRAF6 in thymic Treg development. Interestingly, the *in vitro* induction of $Foxp3^+$ cells from naive T cells was not impaired by the lack of TRAF6, suggesting that the requirement of TRAF6 for Treg development is limited to the thymus. We showed that activations of classical NF-kB and JNK induced by TCR ligation were severely impaired by *Traf6*$^{-/-}$ thymocytes. On the other hand, activations of Akt and ERK were rather slightly enhanced by the lack of TRAF6. Overall, our data strongly suggest that TRAF6 controls TCR-signals in order to commit thymocytes to Treg lineage.

Genetic lineage tracing reveals developmentally and functionally distinct subpopulations of NK cell receptor-expressing cells

Andreas Diefenbach (Institute of Medical Microbiology & Hygiene (IMMH), University of Freiburg, Freiburg, Germany)

Our laboratory studies the development, function and tolerance of natural killer (NK) cells. We are striving to understand the molecular basis of how NK cells recognize tumor cells and virally infected cells. A focus has been the characterization of stimulatory NK cell recognition receptors and their specificities. Our studies have led to the identification of a family of class I MHC-related molecules as ligands for one activating NK cell receptor, NKG2D. We found that these ligands are not expressed by most normal cells but upregulated following various forms of cellular stress (including tumor transformation and infection). An important problem is the question of the molecular programs regulating NKG2D ligand expression. Elucidation of these mechanisms will help us to understand how normal protective immune responses differ from inappropriate ones that result in inflammation and autoimmunity.

Our studies on mucosal NK cells have led to the identification of a novel lymphocyte population that co-expresses NK cell receptors and the orphan nuclear receptor RORγt. Interestingly, these cells are distinct from authentic NK cells in that they develop independently of IL-15 but require RORγt for their development. These NK marker-expressing RORγt+ cells are related to NK cell marker-negative RORγt-expressing cells that induce differentiation of secondary lymphoid organs in the fetus and of microbiota-dependent intestinal tertiary lymphoid follicles in the adult (called lymphoid tissue inducer cells, LTic). We are currently investigating the lineage relationship between these NK-like cells and LTic.

We found that the RORγt-expressing NK-like cells differentiate under the influence of the commensal microflora and are an important source of the cytokine IL-22. The IL-22 receptor is exclusively expressed by epithelial cells and IL-22R signalling leads to the upregulation of molecules involved in maintaining epithelial homeostasis. We are currently identifying the molecular targets of IL-22 in epithelial cells. These studies will allow us to identify target genes involved in the protection against inflammatory bowel diseases (ulcerative colitis and Crohn’s disease).

The roles of C-type lectins in the host defense against fungal infection

Shinobu Saijo (Center for Experimental Medicine)

The C-type lectins are a group of proteins that have a Ca^{2+}-dependent- carbohydrate-recognition domain (CRD) in their extracellular carboxyterminal domains. Some C-type lectin family members recognize the carbohydrate structures of microbes as pathogen-associated molecular patterns. Dectin-1 was first reported as a dendritic cell (DC)-specific C-type lectin receptor, and is also highly expressed on macrophages and neutrophils. Dectin-1 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 β-1, 3-linked and/or β-1, 6-linked glucans (β-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 decttin-2-deficient mice to determine the roles of these molecules in the defense against pathogenic fungi. *In vitro*, β-glucan-induced cytokine production from wild-type DCs and macrophages was abolished in dectin-1-deficient cells, and a-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 infection, and dectin-2-deficient mice were more susceptible to candida infection. Thus, dectin-1 and dectin-2 are required for the immune responses to some fungal infections as a protective immunity.

Regulation of innate and adaptive immunity in the intestine

David Artis (Department of Pathobiology, University of Pennsylvania School of Veterinary Medicine, USA)

Intestinal epithelial cells (IECs) were recently shown to play a critical role in maintaining the balance of tolerance, immunity, and inflammation in the gastrointestinal tract. Based on these findings, there are three major research areas in the lab. First, we are employing inducible deletion or overexpression of genes in IECs to interrogate how they regulate intestinal dendritic cell and $CD4^+$ T cell function. The long term goal of these studies is to improve oral vaccination against enteric bacterial infection and prevent inflammation associated with IBD. Second, we are employing gnotobiotic mice to examine the influence of commensal bacteria on intestinal and peripheral immune cell function. Our preliminary findings indicate commensal bacteria have amajor regulatory influence on $CD4^+$ T cell function and susceptibility to multiple inflammatory diseases. To determine if the immune system reciprocally regulates the acquisition and/or composition of commensal communities, we are undertaking high-throughput metagenomic analyses of commensal bacteria in murine models of health and disease. Third, we are investigating how IECs regulate immunity to intestinal nematode parasites. Secretion of IEC-derived cytokines including IL-25, IL-33, and thymic stromal lymphopoietin (TSLP) appear to be important early events in influencing dendritic cell and $CD4^+$ T cell responses required for immunity to infection and prevention of intestinal inflammation. Our recent studies suggest that IECs also govern granulocyte responses that have a critical role in the development of Th2 cytokine responses. It is hoped that the results of these studies will advance understanding the pathophysiology of multiple mucosal inflammatory diseases, including asthma, allergy and inflammatory bowel diseases and provide a framework to test the therapeutic potential of manipulating IEC responses in these disease states.

Unc93 homolog B1 regulates the balance of toll-like receptor 7 and toll-like receptor 9 responses reciprocally

Ryutaro Fukui (Division of Infectious Genetics)

Toll-like receptors (TLRs) recognize components of pathogens and activate immune systems. Some molecules are known as the accessory molecules for TLR activation, for example, TLR4/MD-2 complex. We performed functional cloning for detection of brand-new accessory molecules that complement TLR7 response, and found Unc93 homolog B1 (Unc93B1) as a candidate. It is reported that Unc93B1 binds to TLR3, 7, 9 and complements their functions by stimulant dependent intracellular trafficking (Kim et al., *Nature* 2008). Our functional cloning revealed that amino acid D34 (aspartic acid, thirty fourth from N-terminus) regulates TLR7 and TLR9 responses reciprocally (Fukui et al., *J. Exp. Med.* 2009). We constructed the D34 alanine-mutant (D34A) Unc93B1 and expressed the mutant and wild type Unc93B1 into dendritic cells (DCs) taken from Unc93B1 deficient mice, called “3d” mice. As reported previously, (Tabeta et al., *Nat. Immunol*. 2006), the responses to TLR3, 7, 9 ligands are recovered by wild type Unc93B1. Surprisingly, in the DCs D34A mutant expressed, the response of TLR7 was up-regulated and response of TLR9 was down-regulated. TLR3 response was not changed by the mutation. We hypothesized that this reciprocal regulation was based on the binding activity of Unc93B1 to TLRs and following intracellular trafficking of TLRs. As a result, more TLR7 and less TLR9 were co-precipitated with Unc93B1-D34A mutant than Unc93B1-wildtype. Furthermore, this binding activity was reflected on the intracellular trafficking of TLR7 and TLR9. According to the result, it is implicated that Unc93B1 controls the balance of nucleotide-sensing TLRs responses and keeps it TLR9-dominant in steady state. Based on this finding, we generated the Unc93B1-D34A knock-in mice because it is suggested that over-response of nucleotide-sensing TLRs link to autoimmune disease. We are now proceeding to analyze the phenotype of Unc93B1-D34A knock-in mice to reveal the significance of the reciprocal TLR-balancing system *in vivo*.