Center for Experimental Medicine and Systems Biology

Laboratory of Innate Immunity
自然免疫研究分野

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Pathogen sensors, such as Toll-like receptor (TLR), play sentinel roles in detecting pathogenic ligands during infection and induce both innate and acquired immune responses. Meanwhile, excessive TLR responses are strongly associated with fatal diseases such as septic shock and autoimmune diseases. For this reason, immune system must strictly control TLR responses to avoid disruption of homeostasis. However, molecular mechanisms involved in TLR regulation are not fully elucidated. We have previously shown that TLRs are regulated by various TLR associating molecules including MD-2, PRAT4A and Unc93B1. Our goal is to uncover unknown molecular mechanism which is indispensable for appropriate TLR responses using genetically engineered mice.

1. Roles of PRAT4A (PRotein Associated with Toll-like receptor 4) in immune modulation.

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TLRs induce complex inflammatory responses that functions to protect the host from invading pathogens. But, in excessive or persistent inflammatory responses, hosts suffer the disadvantage of septic shock, delayed tissue repair and autoimmune diseases. To control the extent and dilated inflammation by TLRs, hosts evolved the multiple regulatory mechanisms, called tolerance.

We have previously reported that a novel TLR associating protein 'PRotein Associated with TLR4 (PRAT4A)', an endoplasmic reticulum resident protein, controls maturation and intracellular trafficking of multiple TLRs. PRAT4A deficient Macrophages/Dendritic cells (DCs), with abnormal TLR distribution, showed impaired immune responses to TLR2/4/5/7/9 ligands, except to TLR3 ligand. As mRNA level of PRAT4A significantly decreased after stimulation by various TLR ligands in physiological state, PRAT4A deficiency presumably mimicked the tolerant state. These facts raised the possibility that PRAT4A play a central role in tolerance suppressing excessive inflammation by TLRs. Overexpression studies may be helpful in evaluating the tolerant effect of PRAT4A.

Furthermore, using established anti-PRAT4A monoclonal antibodies, we found secreted PRAT4A which was detected in the serum for wild mice and the culture supernatant of Macrophages. There is another possibility that PRAT4A is not only a TLR associating chaperone but also another immune modulator.

To further address PRAT4A function, we constructed knock-in transgenic mice using ROSA26 locus which enables us to analyze cell type-specific overexpression studies both *in vitro* and *in vivo*. Now we continue to analyze PRAT4A transgenic mice.

2. The meaning of intracellular localization of TLRs.

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Toll-like receptor 5 (TLR5), a sensor for bacterial flagellin, mounts innate and adaptive immune responses, and has been implicated in infectious diseases, colitis and metabolic syndromes. Although TLR5 is believed to belong to cell surface TLRs, cell surface expression has never been verified. Moreover, it has remained unclear which types of immune cells express TLR5 and contribute to flagellin-dependent responses. We established an anti-mouse /human TLR5 monoclonal antibody and studied cell surface expression of TLR5 on various immune cells. A macrophage cell line J774 expressed endogenous TLR5 on the cell surface and produced IL-6 and G-CSF in response to flagellin. Cell surface expression of TLR5 and flagellin-induced responses were completely abolished by silencing ER proteins, PRotein Associated with TLR4 A (PRAT4A) and Unc93B1. In *in vivo*, cell surface TLR5 was mainly found on neutrophils and $CD11b^{hi}Ly6C^{hi}$ classical monocytes in the bone marrow, circulation, spleen and an inflammatory lesion. $Ly6C^{hi}$ classical monocytes, but not neutrophils, produced cytokines in response to flagellin. Splenic $CD8^{-}CD4^{+}$ conventional dendritic cells and $CD11c^{hi}CD11b^{hi}$ lamina propria DCs, also clearly expressed cell surface TLR5. Collectively, cell surface expression of TLR5 is dependent on Unc93B1/PRAT4A and restricted to neutrophils, classical monocytes and specific DC subsets.

3. Finding a novel tetraspanin protein involved in the negative regulation of multiple TLR responses.

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Multiple TLRs work in concert to sense a pathogen and mount defense responses. Little is known, however, about a mechanism coordinating multiple TLRs responses.

Under the screening of TLR2 regulating molecules, we found a novel tetraspanin protein which co-precipitated with TLR2 in immunoprecipitation assay.

Conditional knock out and Conditional transgenic mice using ROSA26 locus of this novel tetraspanin protein have been generated to allow rigorous assessment of its function. Further study is under way to reveal a role of this gene in immune responses.

4. Characterization of cleaved forms of TLR7 and TLR9

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Vertebrates have TLR3, 7, 8 and 9 as sensors of microbial nucleic acids, however it is suggested that TLR7/9 responses strongly associate with autoimmune diseases owing to inappropriate recognition of self nucleic acid. In endolysosome, TLR7 and TLR9 recognize a single-stranded RNA and an unmethylated CpG motif in microbial DNA, respectively. TLR7/9 ordinarily reside in Endoplasmic Reticulum, and ligand stimulation enhance the trafficking of TLR7/9 to endolysosome. Such strict regulation of TLR7/9 subcellular localization seems to have a role for blocking self nucleic acid recognition. Previous reports indicated novel posttranscriptional modification in TLR7/9 that ectodomains of TLR7/9 were cleaved in endolysosome. It seemed that ectodomain cleavage in TLR7/9 represents another strategy to restrict excessive TLR7/9 activation. Recently, we established new monoclonal anti-TLR7 and anti-TLR9 to detect endogenous or non-tagged TLRs and used these antibodies to clarify the mechanism of TLRs cleavage.

Our data showed that cleaved TLR7 N-terminal binds to C-terminal by its disulfide bond and the cysteines are important for response of TLR7 (Kanno et al., *Int. Immunol*. in press). We focused on four cysteines of TLR7 (C98, C445, C475, and C722) and made serine mutants of these cysteines for analysis of cleavage pattern and the effect on response. As results, C98 and C475 were required for binding of TLR7 N-terminal to C-terminal, and proteolytic cleavage of TLR7. These cysteine mutants did not respond to TLR7 ligands, and also no response was observed in deletion mutants of TLR7

cleavage site or truncated form of TLR7 C-terminal alone. From these data, it is suggested that proteolytic cleavage of TLR7 and binding of N-terminal to C-terminal are essential for TLR7 response, and dependent on its cysteines.

In the case of TLR9, we found no effect of disulfide bond but the binding of N-terminal fragment to C-terminal fragment is important for TLR9 response. Now we are trying to confirm the relation between proteolytic cleavage of TLR9 and its response.

Furthermore, we generated the mice having mutation at cleavage sites of TLR7 or TLR9. To understand physiologic meanings of proteolytic cleavage in nucleic acid sensing-TLRs, these mice will be analyzed.

5. Roles for Unc93 homolog B1-dependent TLR 7/9 balance in vivo

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Nucleic acid sensing Toll-like receptor 7 (TLR7) and TLR9 recognize microbial RNA and DNA, respectively. These TLRs potentially recognize self-derived nucleic acid and have been shown to have a role in autoimmune diseases. For maintenance of homeostasis, it is important to keep the responsiveness of the nucleic acid-sensing TLRs under the tight control.

Unc93 homolog B1 (Unc93B1) is reported to be indispensable for TLR7/9 responses. We have previously found that the alanine substitution for the 34th aspartic acid (D34A) of Unc93B1 enhanced TLR7 response but downregulated TLR9 response. These results suggest that TLR7 and TLR9 are reciprocally linked by Unc93B1, and the TLR7/TLR9 balance is biased towards TLR9 in the steady state.

To further clarify a role for Unc93B1-dependent TLR7/TLR9 balance *in vivo*, we started to generate knock-in mice harboring various types of mutations around D34 locus in the Unc93B1 gene which showed more accelerated phenotypes than D34A mutant mice. And also, we are generating the mutant mice lucking glycosylation sites in Unc93B1. Several mutant mice were succeeded in germ line transmission, thus further analysis is now on going.

6. Identification of regulatory molecules for TLR responses and constructing genetically engineered mice.

Takuma Shibata[1,2], Yuji Motoi[1], Atsuo Kanno[1], Natsuko Yamakawa[1], Mabel Chan[1], Xiaobing Li[1], Yusuke Murakami[1], Hirotsugu Hatai[1], Ryutaroh Fukui[1], Shin-ichiroh Saitoh[1], Nobuaki Yoshida[2] and Kensuke Miyake[1,2]: [1]Division of Infectious Genetics, Department of Microbiology and Immunology, [2]Laboratory of Innate Immunity, Center for Experimental Medicine and Systems Biology, The Institute of Medical Science, The University of Tokyo, 4-6-1 Shirokanedai, Minatoku, TOKYO1208-8639, Japan.

We try functional cloning and co-immunoprecipitation assay to comprehensively identify regulatory molecules associating with TLR responses. After simple screenings *in vitro*, we constructed conditional knock-out mice or knock-in transgenic mice using ROSA26 locus to reveal the physiological function of novel molecules *in vivo*. So far, we have found more than 20 candidate genes, and continue to construct genetically engineered mice.

Publications

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Center for Experimental Medicine and Systems Biology

Laboratory of Developmental Genetics
システム疾患モデル研究センター 発生工学研究分野

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Gene targeting technology has revealed many aspects of gene functions in vivo. Knock out mice offer the opportunities of not only analyzing the complex gene functions in vivo, but also presenting various human disease models, where new therapeutic approaches can be explored. To allow more detailed dissection of gene function, we introduce a point mutation or disrupt genes in certain lineages (or stages) using Cre-loxP system, a method of conditional gene targeting. In the process of analyzing knock out mice, we have isolated spontaneous mutant mice which develop chylous ascites and edematous limbs. In order to understand the mechanism of lymphatic development and functions in more detail, we are also generating various knock-out/knock-in mouse lines including a conditional knock out mouse. In addition, we focus on analysis of neural development, aiming to understand the molecular mechanism of the maintenance of stemness and neural differentiation and to advance towards cell therapy of the damaged or degenerating nervous system. For this purpose, we are generating several conditional knock out mouse lines.

1. Functional analysis of PTB/nPTB using conditional knockout mice.

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We focus on analysis of neural development, aiming to understand the molecular mechanism of the maintenance of stemness and neural differentiation and to advance towards cell therapy of the damaged or degenerating nervous system.

PTB is a member of the heterogeneous nuclear ribonucleoprotein (hnRNP) family that binds specifically to pyrimidine-rich sequences of RNAs. PTB is a multifunctional protein involved in RNA processing and internal ribosome entry site (IRES)-dependent translation, and preferentially expressed in neural stem cells (NSCs) in the central nervous system. Although RBPs are indispensable for the normal functions and cell migration of neurons, little is known about the role of RBPs in neural stem cells (NSCs). In vitro functional analysis of PTB in neuronal cell have revealed that PTB has important roles on many alternative splicing events during neural cell differentiation. But it is still unknown whether self-renew and/or cell fate of NSCs are regulated by PTB in vivo. To explore the role of PTB in the early development of mouse brain, we inactivated the gene by employing a Nestin pro-

moter-driven Cre-mediated conditional gene targeting system. We found that most mutant mice die by 10 weeks and almost all mutant mice developed a characteristic dome-like appearance of their heads. Histological analyses of PTB mutant brains revealed that these mice develop severe hydrocephalus. The cell polarity and adherens junction (AJ) of the apical ventricular surface in dorsal cortex were lost in a pacthy distribuion at E15.5. By E 16.5, Tbr2-positive neural progenitor cells and neurons were observed in ventricular zone (VZ) of all AJ-negative spots, which leads depletion of VZ by E18.5. Thus postnatal maturation of ependymal cells with ciliary tufts from radial glia cells (RGCs) was disturbed, which may compromises cerebrospinal fluid dynamics and results in hydrocephalus. Our findings suggest that PTB is important for self-renew and/or cell fate of RGCs, through involving in maintenance of cell polarity and AJ in the dorsal neuroepithelium of lateral ventricles.

Neural polypyrimidine-tract-binding protein (nPTB), which is identified as a homologue of PTB. It is common knowledge that many genes are regulated by alternative splicing during neural development, and previous studies suggested that alternative splicing could contribute to the gene expression and functional diversity of isoforms. nPTB is expressed predominantly in the nervous system, muscle and testis. During neural development, expression of PTB is decreased along with differentiation of neural stem cells into neurons. In contrast, expression level of nPTB that was repressed by PTB is accordingly increased. Thus, tissue-specific RNA binding protein, nPTB may play an important role in neural development. But, functional differences between PTB/nPTB and meaning of developmental stage associated expression change from PTB to nPTB remain to be elucidated. In our study, we are generating nPTB conditional knockout mice to shed light on a role of nPTB in vivo. And now we are also analyzing the function of PTB/nPTB in vitro by using knockdown experiment via a formation of embryoid body (EB) from ES cells.

To address the function of PTB/nPTB, we generated and are going to analyze PTB/nPTB double knockout mouse.

2. Role of PTB on spermatogenesis

Takashi Takijiri, Manabu Ozawa, Reiko Sakamoto and Nobuaki Yoshida

Polypyrimidine tract-binding protein (PTB/PTBP 1/hnRNP I) is an RNA-binding protein that binds specifically to pyrimidine-rich sequences of RNAs and plays multiple roles including RNA alternative splicing. Testis is an organ where alternative splicing occurs frequently, and that is believed to be important for sustainable and normal spermatogenesis. In Drosophila, PTB is necessary for spermatid individualization. Since PTB is expressed higher in testis in mammals, it would be hypothesized that PTB acts for homeostasis of spermatogenesis/spermiogenesis. Our previous study and others showed that gene knockout of PTB shows embryonic lethal. To escape the limitation, we have established Cre-loxP mediated germ cell specific PTB knockout model using PTB-floxed mice crossbred with Nanos3Cre mouse of which Cre transgene expresses only in germ cells. PTB conditional knockout (cKO) mice is fertile at younger age (~15-weeks-of-age), whereas have apparently small testes since 3-week-of age or older. Immunohistochemical analysis revealed that, although a few seminiferous tubules showed normal progress of spermatogenesis from spermatogonia to elongated spermatid in the testis from cKO mature male (>8-week-of age), majority of seminiferous tubules contained only Sertoli cells and no germ cells were observed. Number of sperms in epididymis in cKO mice also drastically reduced in the cKO mice. Interestingly, cells at first meiosis are observed in many seminiferous tubules at 4 to 5-week-of-age, indicating that first asymmetric division of spermatogonial stem cells for differentiation occurs, whereas self-renewal activity to maintain stem cell population is severely ruined by lacking of PTB. Further and more detail determination how the abnormality in spermatogonial stem cell occurs by the lacking of PTB is now under estimated.

3. Ras- and Ets-mediated modulation of *Vegfr3* gene expression in lymphatic endothelial cels

Taeko Ichise, Nobuaki Yoshida and Hirotake Ichise

Modulation of VEGFR-3 expression is important for altering lymphatic endothelial cell (LEC) characteristics during the lymphangiogenic processes that occur under developmental, physiological, and pathological conditions. However, the mechanisms underlying the modulation of *Vegfr3* gene expression remain largely unknown. Using genetically engineered mice and LECs, we demonstrated previously that Ras signaling is involved not only in VEGFR-3-induced signal transduction but also in *Vegfr3* gene expression. Here, we investigated the roles of the transcription factor Ets and the histone acetyltransferase p300 in LECs in Ras-mediated transcriptional regulation of *Vegfr3*. Ras activates Ets proteins via MAPK-induced phosphorylation. Ets knockdown, similar to Ras knockdown, resulted in a decrease in both *Vegfr3* transcript levels and acetylated histone H3 on the *Vegfr3* gene. *Vegfr3* knockdown results in altered LEC phenotypes, such as aberrant cell proliferation and network formation, and Ets knockdown led to milder but similar

phenotypic changes. We identified evolutionarily conserved, non-coding regulatory elements within the *Vegfr3* gene that harbor Ets-binding motifs and have enhancer activities in LECs. Chromatin immunoprecipitation (ChIP) assays revealed that acetylated histone H3 on the regulatory elements of the *Vegfr3* gene was decreased following Ras and Ets knockdown, and that activated Ets proteins, together with p300, were associated with these regulatory elements, consistent with a reduction in *Vegfr3* gene expression in p300-knockdown LECs. Our findings demonstrate a link between Ras signaling and Ets- and p300-mediated transcriptional regulation of *Vegfr3*, and provide a potential mechanism by which VEGFR-3 expression levels may be modulated during lymphangiogenesis.

4. Functional analysis of histone demethylase Fbxl10 and Fbxl11 on male gamete development

Manabu Ozawa, Tsuyoshi Fukuda, Eri Kawakami, Akinori Tokunaga, Reiko Sakamoto and Nobuaki Yoshida

Histone methylation is one of the important epigenetic modifications of genome to orchestrate appropriate spatiotemporal gene expressions for normal tissue development or differentiation. Fbxl10 and Fbxl11, homolog proteins, are histone dymethylase and catalyzes tri-methylated H3K4 and dimethylated H3K36, either of which modification is believed to repress adjacent gene expressions. Gametogenesis is strictly under control of epigenetic modification, and status of DNA or histone modifications changes drastically during the development. Some previous studies using epigenetic modifier gene-null mice showed infertile phenotypes. These results make us hypothesize that Fbxl10 or Fbxl11 null mice show abnormal gametogenesis. To test the hypothesis, we have determined spermatogenesis in testis of Fbxl10 and Fbxl11 knockout mice (KO mice). Fbxl10 KO mice at younger age ($<$6 month-old) have comparable number of sperms in epididymis as wild type control, whereas aged male ($>$1 year old) has lower number of sperms (almost 4-folds less than control). Abnormal seminiferous tubules containing poor germ cell layers were observed as early as 3-month-old testis. The phenotype becomes severer according to aging and reduction of germ cells occurs almost of all seminiferous tubules in one-year-old null testis. Thus, it is suggested that Fblx10 regulates cellular senescence in spermatogonial stem cells. On the other hand, conventional KO of Fbxl11 shows embryonic lethal phenotype, thus we have developed germ cell specific gene knockout model by crossing Fbxl11 floxed mice with Nanos3Cre mice, of which Cre transgene is expressed only by germ cells. In contrast to the results observed in the Fbxl10 KO mice, cKO of Fbxl11 shows drastic abnormality during the first spermatogenesis, e.g., cells underwent meiosis rarely exist in most seminiferous tubules, and number of sperm at 8-week old decreased less than 10 fold compared with control. Interestingly, abnormal seminiferous tubules have comparable number of PLZF-positive undifferentiated spermatogonia. These results indicate that Fbxl-11 acts critical roles for induction of meiosis. Further analysis to uncover molecular mechanisms of either genes on spermatogenesis is ongoing.

5. Studies on epigenetic gene regulation in neural development

Eri Kawakami, Reiko Sakamoto, Akinori Tokunaga, Nobuaki Yoshida

The polycomb repressive complex (PRC) 1/2 proteins play a critical role in regulation of gene expression through modification of histone and chromatin structure. Histone modification is the important transcription regulatory system that affects mammalian development and cell differentiation. Alterations in epigenetic gene regulation are associated with disease. We aim to determine the mechanisms of epigenetic gene regulation and to understand their roles in neural development, and are focused on the PRC regulatory genes: Fbxl10/11 and RYBP (Ring1A and YY1 binding protein).

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Center for Experimental Medicine and Systems Biology

Laboratory of Systems Biology
システムズバイオロジー研究分野

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Gene-modified mice are considered to be powerful tools for understanding of pathophysiological function of the targeted gene(s) in vivo. Our research focus is the understanding of pathogenesis of rejection and immune disorders such as allergy and autoimmunity using gene-modified mice.

ST2 requires Th2-, but not Th17-, type airway inflammation in epicutaneously antigen-sensitized mice

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IL-33 is known to induce Th2-type cytokine production by various types of cells through its receptors, ST2 and IL-1RAcP. Polymorphism in the ST2 and/or IL-33 genes was found in patients with atopic dermatitis and asthma, implying that the IL-33/ST2 pathway is closely associated with susceptibility to these diseases. Exposure to allergens through damaged skin is suspected to be a trigger for allergen sensitization, resulting in development of such allergic disorders as asthma and atopic dermatitis. To elucidate the role(s) of the IL-33/ST2 pathway in asthma in individuals who had been epicutaneously sensitized to an antigen, wild-type and $ST2^{-/-}$ mice were epicutaneously sensitized with ovalbumin (OVA) and then were intranasally challenged with OVA. We found that the number of eosinophils in BALFs, the levels of Th2 cytokines and chemoattractants in the lungs and OVA-specific IgE in sera from $ST2^{-/-}$ mice were significantly reduced compared with wild-type mice. Although the number of neutrophils in BALFs and the pulmonary levels of IL-17 were comparable in both mice, the levels of MPO activity in BALFs and neutrophil chemoattractants in the lung were reduced in $ST2^{-/-}$ mice. Therefore, the IL-33/ST2 pathway is crucial for Th2-cytokine-mediated eosinophilic, rather than Th17-cytokine-mediated neutrophilic, airway inflammation in mice that had been epicutaneously sensitized with antigens and then challenged with antigen.

Th17 cell-derived IL-17 is dispensable for B cell antibody production

Akiko Shibui[1], Eri Shimura[2], Aya Nambu[3], Sa-

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IL-17, which is preferentially produced by Th17 cells, is important for host defense against pathogens and is also involved in the development of autoimmune and allergic disorders. Antibody (Ab) production was shown to be impaired in IL-17-deficient mice, suggesting that IL-17 may promote B cell activation and direct secretion of Ab. However, the precise role of IL-17 in Ab production by B cells remains unclear. In the present study, we found constitutive expression of IL-17R in murine splenic B cells. Nevertheless, IL-17, IL-17F or IL-25 alone could not induce Ab production by B cells even in the presence of agonistic anti-CD40 Ab. IL-17 also could not affect IFN-γ-, IL-4- or TGF-1-mediated Ig class-switching. Furthermore, in cocultures of B cells and IL-17$^{-/-}$ CD4^{+} T cells or IL-17$^{-/-}$ Th17 cells, IL-17 deficiency did not influence Ab production by B cells *in vitro*, suggesting that Th17 cell-derived IL-17 was not required for B cell Ab production through T-B cell interaction *in vitro*. Thus, *in vivo*, IL-17 may be indirectly involved in Ab production by enhancing production of B cell activator (s) by other immune cells.

Potential role of γδ T cell-derived IL-17 in acute cardiac allograft rejection

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Although αβ T cells are known to participate in the development of acute cardiac allograft rejection, the role of γδ T cells remains poorly understood. We hypothesized that γδ T cells contribute to acute allograft rejection thru IL-17 production. Donor hearts from FVB mice (H-2^{q}) were heterotopically transplanted into C57BL/6-wild-type (WT) and γδ T cell-deficient (TCRδ$^{-/-}$) recipient mice (H-2^{b}). Graft survival was prolonged in TCRδ$^{-/-}$ recipients compared to WT controls. Graft infiltrating cells, including CD45^{+}, CD4^{+}, CD8^{+}, and Gr1^{+} cells were significantly decreased in TCRδ$^{-/-}$ recipients compared to WT. Donor hearts transplanted into TCRδ$^{-/-}$ recipients had reduced IL-17 and IL-6 mRNA expression. Corroborating the gene expression, intracellular cytokine staining showed decreased IL-17 producing cells in TCRδ$^{-/-}$ recipients. Finally, Vγ1^{+} and Vγ4^{+} T cells did not produce IL-17, although both represent 20-30% total graft infiltrating γδ T cells. Therefore, γδ T cells promote acute cardiac allograft rejection, presumably by producing IL-17. γδ T cell depletion may prove beneficial in prolonging allograft survival by suppressing IL-17 production.

Epithelial cell-derived IL-25, but not Th17 cell-derived IL-17 or IL-17F, is crucial for murine asthma

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IL-17A, IL-17F and IL-25 are ligands for IL-17RA. In the present study, we demonstrated that IL-25-deficient mice—but not IL-17A-, IL-17F-, IL-17A/F-, IL-23p19- or ROR-t-deficient mice—showed significant suppression of (1) the number of eosinophils and the levels of proinflammatory mediators in bronchoalveolar lavage fluids, (2) airway hyperresponsiveness to methacholine and (3) ovalbumin-specific IgG1 and IgE levels in the serum during ovalbumin-induced Th2-type/eosinophilic airway inflammation. The IL-25 deficiency did not affect lung DC migration or antigen-specific memory-Th2-cell expansion during antigen sensitization. Adoptive transfer of either T cells, mast cells or bone marrow cells from IL-25-deficient mice revealed that induction of Th2-type/eosinophilic airway inflammation was dependent on activation of lung epithelial cells and eosinophils by IL-25 produced by airway structural cells such as epithelial cells, but not by such hematopoietic stem-cell-origin immune cells as T cells and mast cells. Therefore, airway structuralcell-derived IL-25—rather than Th17 cell-derived IL-17A and IL-17F—is responsible for induction of local inflammation by promoting activation of lung epithelial cells and eosinophils in the elicitation phase of Th2-type/eosinophilic airway inflammation. It is not required for antigen-specific Th2 cell differentiation in the sensitization phase.

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