PROGRESS REPORT
Division of Molecular Pathology

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T cells play a central role in the effector and regulatory functions of the immunological surveillance system. Cytokines are critical factors in transmitting information from the membrane receptor to the nucleus and establishing communication networks among cells. When the immune system encounters a potential threat like invading a pathogen, antigen-presenting cells deliver information about the pathogen to naive T cells, leading to the differentiation of functional helper T (Th) cells subsets, Th1, Th2, Th17, and follicular helper T (T_{FH}). Our long-term goal is to understand how type II cytokines (IL-4 and IL-13) and T_{FH}-derived cytokines contribute to T and B cell immunity, significantly impacting several pathogenic situations. In this context, we have mainly focused on infectious virus immunity and innate and acquired immunity in allergic responses, including atopic asthma and dermatitis.

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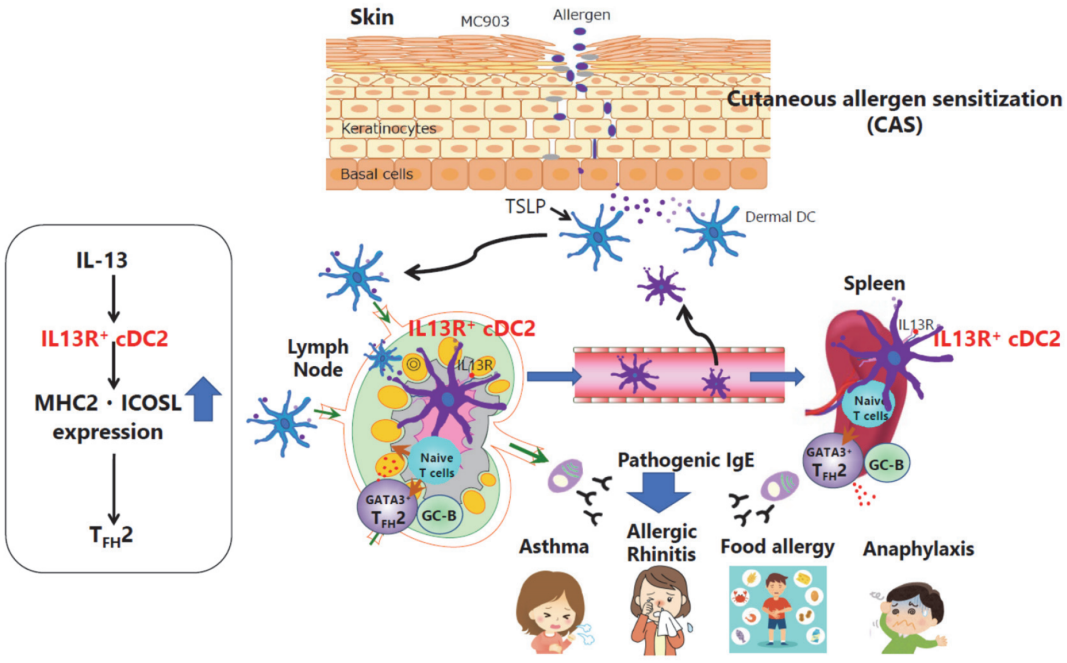
1) Cutaneous allergen sensitization is critical for allergic humoral responses controlled by IL-13 receptor-expressing conventional dendritic cells.

Allergy is a complex disease that combines innate and adaptive immunity, and host genetic polymorphisms, and environmental triggers influence allergy susceptibility. Skin barrier dysfunction in the setting of atopic dermatitis (AD) leads to cutaneous allergen sensitization (CAS). The atopic march hypothesis proposes that CAS leads to the subsequent development of



Migration of IL-13R^{+}cDC2 into second lymphoid organs controls allergy march

other allergic disorders such as asthma and food allergy. The latter is associated with IgE-mediated anaphylaxis[6]. However, the precise mechanisms by which CAS promotes IgE-mediated allergic responses remain unclear. We thus established a CAS model of anaphylaxis and identified a unique role of the Interleukin (IL)-13 signal in DCs to generate anaphylactic IgE responses. Single-cell RNA-seq revealed the emergence of IL-13 receptor alpha 1 chain (IL-13Rα1)-expressing type 2 conventional dendritic cells (cDC2s) in CAS. Similar DC populations were identified in allergic rhinitis humans with cedar pollan-specific IgE antibodies. Lineage-specific disruption of IL-13 signaling resulted in a marked reduction in cDC2s that highly expressed MHC class II: these cells controlled the germinal center entry of T_{FH} cells required for IgE responses. These data reveal a unique role for IL-13 in controlling the expansion of $MHCII^{hi}$ cDC2s and associated T_{FH} cell responses in the setting of allergy.

Collaborators:

Takahiro Matsuyama and Hiromasa Inoue (Kagoshima University), Takashi Watanabe (RIKEN IMS), Brian S. Kim (Washington University School of Medicine), Hideki Ueno (Kyoto University), Peter D. Burrows (University of Alabama at Birmingham)

3) Role of TFH and IL-4 signal in Boost-vaccination with SARS-CoV-2 spike protein.

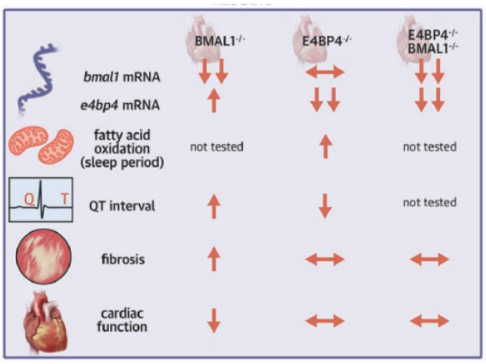
Vaccines have proven effective in eliciting an immune response capable of providing protective immunity in healthy individuals. However, the effect of immunocompromised conditions on SARS-CoV-2 vaccination is poorly understood. This study employed recombinant Spike-based vaccination and mice with deficiencies in germinal center (GC) related cytokines. Through quantitative and qualitative assessment of antibody responses in immunocompromised mice, we observed impaired antibody responses following prime and booster immunizations. The results indicated a striking impairment in virus-specific antibody responses after primary immunization due to defects in IL-4 and IL-21 signals, critical for GC-dependent antibody responses. Furthermore, while the defective primary responses improved after boosting for vaccinated strains, the defected mice of IL-4 signal in B cells, but not of T_{FH} cells, failed to generate broadly protective antibodies against omicron variants in memory responses. These findings indicated the crucial role of T_{FH}-independent IL-4 in memory responses. Therefore, the IL-4 signal significantly impacts the breadth of antibody responses against SARS-CoV-2 variants and contributes to long-term protective immunity.

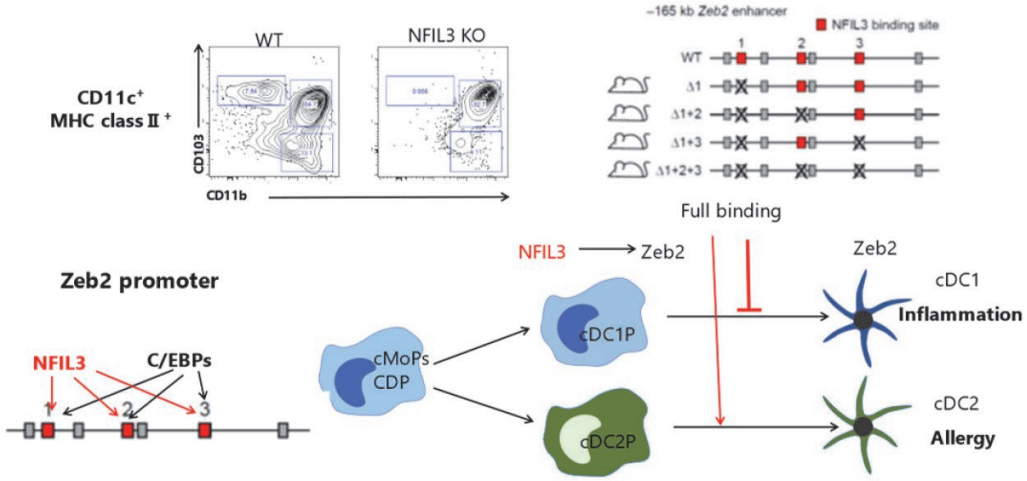
Collaborators:

Kosuke Miyauchi (National Institute of Infectious Diseases), Yuichiro Yamamoto and Kohji Noguchi (Tokyo University of Science), Rina Hashimoto and Kazuo Takayama (Kyoto University)

4) Role of E4BP4/NF-IL-3 in Circadian clocks and immune cells.

Circadian clocks temporally orchestrate biological processes critical for cellular/organ function. Our group has reported the independent role of E4BP4/NF-IL-3 in different tissue and immune cells.





In the correlation with Dr. Martin E. Young group at the University of Alabama at Birmingham, we report that genetic disruption of the cardiomyocyte clock results in chronic induction of the transcriptional repressor E4BP4/NF-IL-3. Importantly, E4BP4/NF-IL-3 deletion prevents age-onset cardiomyopathy following clock disruption. These results also indicate that E4BP4/NF-IL-3 regulates both cardiac metabolism (eg, fatty acid oxidation) and electrophysiology (eg, QT interval). These studies reveal that E4BP4/NF-IL-3 is a novel cardiac physiology and pathophysiology regulator.

In correlation with Dr. Jiyeon Park and Suk-Jo Kang, we report a new mitotic transitional basophil precursor population (transitional basophils, tBasos) expressing the FcεRI alpha chain at higher levels than mature basophils. tBasos are less responsive to IgE-linked degranulation but produce more cytokines in response to IL-3, IL-33, or IgE cross-linking than mature basophils. In particular, we found that the expression of E4BP4/NF-IL-3 gradually rises as cells mature from the basophil progenitor (BaP) stage. Basophil-specific deletion of E4BP4/NF-IL-3 reduces the expression of genes necessary for basophil function and impairs IgE-receptor signaling, cytokine secretion, and degranulation in murine AD. Therefore, we conculed the "tBasos", a novel late-stage mitotic basophil precursor cell population between BaPs and post-mitotic mature basophils. We demonstrated that E4BP4/NF-IL-3 augments the IgE-mediated functions of basophils, pointing to a potential therapeutic regulator for allergic diseases.

In correlation with Dr. Kenneth M. Murphy's group, we report the transcriptional basis of CDP divergence and describe the first requirements for pre-cDC2 specification. Genetic epistasis analysis7 suggested that *Nfil3* acts upstream of *Id2, Batf3*, and *Zeb2* in cDC1 development but did not reveal its mechanism or targets. Analysis of newly generated NFIL3 reporter mice showed extremely transient E4BP4/NF-IL-3 expression during cDC1 specification. CUT&RUN and ChIP-seq analysis identified endogenous E4BP4/NF-IL-3 binding in the –165 kb *Zeb2* enhancer at three sites that bind CCAAT enhancer-binding proteins C/EBPα and C/EBPβ. *In vivo* mutational analysis using CRISPR/Cas9 targeting showed that the E4BP4/NF-IL-3+C/EBP sites are functionally redundant, with C/EBPs supporting and E4BP4/NF-IL-3 repressing *Zeb2* expression at these sites, respectively. A triple mutation of all three E4BP4/NF-IL-3/C/EBP sites ablated *Zeb2* expression in myeloid but not lymphoid progenitors, causing complete loss of pre-cDC2 specification and mature cDC2 development *in vivo*. These mice failed to generate TH2 responses against *H. polygyrus* infection, consistent with cDC2 supporting TH2 responses to helminths. Thus, CDP divergence into cDC1 or cDC2 is controlled by competition between NFIL3 and C/EBPs at the –165 kb *Zeb2* enhancer.

Collaborators:

Sobuj Mia and Martin E. Young (University of Alabama at Birmingham), Jiyeon Park and Suk-Jo Kang (Korea Advanced Institute of Science and Technology), Tian-Tian Liu, Takeshi Egawa, Theresa L. Murphy, and Kenneth M. Murphy (Washington University in St. Louis)

Publications

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1. Harada, Y., Sasaki, T., Wibisana, J-N., Okada-Hatakeyama, M., Ueno, H., Burrows, P. D., and **Kubo, M.**: Type 2 helper T cells convert to Interleukin-1 13 expressing follicular helper T cells after antigen repriming. ***Translat Regulat Sci.*** Vol. 5 No. 1: 1-12, Advanced Epub 2023 Feb 28, doi: 10.33611/trs.2022-010
2. Okuyama, K., Tanaka, H., Halley, C., Chihara, R., Harada, Y., Muroi, S., **Kubo, M.**, and Taniuchi, I.: The Majority of the Serine/Threonine Phosphorylation Sites in Bcl11b Protein Are Dispensable for the Differentiation of T Cells. ***J. Immunol.*** 210 (11): 1728–1739, 2023 Apr 19, DOI: 10.4049/jimmunol.2200101
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5. Tang, J., Yang, L., Guan, F., Miller, H., Camara, N. O. S., James, L. K., Benlagha, K., **Kubo, M.**, Heegaard, S., Lee, P. P., Lei, J., Zeng, H., He, C., Zhai, Z., Liu, C. : The Role of Raptor in Lymphocytes Differentiation and Function, ***Front. Immunol.*** Vol.14, 2023 May 22, doi: 10.3389/fimmu.2023.1146628
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7. Takamura, S., Nomura, A., **Kubo, M.**：BCL6 fine-tunes long-term tumor control ***Sci. Immunology,*** Vol.8, 88, 2023, DOI: 10.1126/sciimmunol.adj6724.
8. Park, J., Cho, Y., Yang, D., Yang, H., Lee, D., **Kubo, M.**, and Kang, S-J.: The transcription factor NFIL3/E4BP4 regulates the developmental stage-specific acquisition of basophil function. ***J. Allergy Clin. Immunol.*** S0091-6749(23)01210-1, 2023 Oct 1, DOI: 10.1016/j.jaci.2023.09.029
9. Ochiai, S., Takahashi, S., Takahashi, N., Jin, J., Ishigam, H., Kabashima, K., **Kubo, M.**, Nakayama, M., Shiroguchi, K., and Okada, T.: Sensory neuronal STAT3 is critical for IL-31 receptor expression and inflammatory itch. ***Cell Report***. In press.