PROGRESS REPORT
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Division of Immunology and Allergy

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Lymphocytes including T cells, B cells and natural killer (NK) cells play essential roles in the immune system. These cells are generated from hematopoietic stem cells (HSCs), which reside and are maintained in the bone marrow (BM) throughout life. HSCs differentiate into T cells in the thymus and B and NK cells in BM through successive lineage decision processes. Transcription factors (TFs) act in concert with epigenetic modifiers to regulate gene expression programs that determine lymphocyte cell fates. Dysregulation of these key regulators—through either loss or gain of function—can lead to hematological malignancies such as leukemia and lymphoma.

We have previously established experimental systems to examine gene regulatory networks during lymphoid lineage specification from HSCs. In one system, we overexpressed Id3 protein fused to ERT2 (an estrogen receptor) protein, whose nuclear translocation is induced by 4-hydroxytamoxifen (4-OHT), in hematopoietic progenitors and cultured them under B cell differentiation conditions. B cell differentiation of Id3-transduced cells was blocked at an early developmental stage; however, these cells exhibited extensive proliferation and maintained multipotency in the presence of 4-OHT (patented; Ikawa et al. Stem Cell Reports, 2015). We termed these multipotent progenitors induced leukocyte stem (iLS) cells.

In another system, we generated T/NK progenitors that predominantly retain differentiation potential toward T and NK lineages by culturing HSCs on OP9 stromal cells overexpressing the Notch ligand, delta-like-1 (OP9/DLL1) in the presence of high concentration of cytokines (Ikawa et al. Science, 2010).

These novel systems enabled comprehensive analyses of the regulatory molecules controlling the generation of T, B and NK cells. Using these approaches, we elucidated the transcriptional network underlying B-lineage commitment (Miyai et al. Genes Dev. 2018). Furthermore, our systems can be applied to the *ex vivo* expansion of human hematopoietic stem/progenitor cells, which is of great importance for immune cell therapy and HSC transplantation.

The aims of our study are therefore as follows:

1. **Basic science:** to elucidate the molecular mechanisms orchestrating cell fate specification, commitment and differentiation during normal and neoplastic development of lymphocytes
2. **Clinical application:** to establish innovative methods for generating genetically engineered lymphocytes–such as chimeric antigen receptor (CAR)-T cells, CAR-NK cells, or TCR-T cells–from self-renewing hematopoietic progenitors.

Ultimately, these studies will pave the way for groundbreaking advances in future cancer immunotherapies.

Molecular mechanisms of B-precursor acute lymphoblastic leukemia (B-ALL) development

B-precursor acute lymphoblastic leukemia (B-ALL) is most common childhood tumors and the leading cause of cancer-related death in children and young adults. Although recent therapeutic advances have markedly improved

the prognosis of ALL, certain translocation subtypes still exhibit poor outcomes. For these refractory and relapsed forms of ALL, novel therapeutic strategies based on a deeper understanding of their pathogenic mechanisms are urgently needed.

Among these, the t(17;19)(q22;p13) translocation subtype—also known as *TCF3::HLF*-positive ALL—is relatively rare, comprising approximately 0.5% of B-ALL cases, but is associated with an extremely poor diagnosis. Thus, the development of effective therapies for this subtype is urgent clinical challenge.

Recently, we found that introduction of *TCF3::HLF* into iLS cells via retroviral transduction induced the onset of B-ALL. When these *TCF3::HLF*-transduced iLS cells were transplanted into sublethally irradiated B6 mice, all recipients developed B-ALL within three to four months. Remarkably, similar to human patients, osteolytic lesions were observed in these mice. Using this mouse model, we comprehensively analyzed genes specifically expressed in *TCF3::HLF*-positive B-ALL and identified strong upregulation of inflammatory cytokines such as IL-1β and IFNγ. Knockdown of IL1B in the human *TCF3::HLF*-positive B-ALL cell line YCUB2 markedly suppressed cell proliferation. Moreover, treatment of YCUB2 with either an anti-IL1β neutralizing antibody or the cytokine release inhibitor JTE-607 significantly inhibited their growth, suggesting that IL-1 blockade may be a promising therapeutic strategy for this leukemia subtype (Japanese Patent Application No. 2023-082464).

Reanalysis of published chromatin immunoprecipitation (ChIP)-seq and ATAC-seq data from human *TCF3::HLF*-positive B-ALL cells (Hang Y et al., 2019) revealed that *TCF3::HLF* directly binds to two putative enhancer regions (E1 and E2) within the *IL1B* locus. Interestingly, while E2 was also present in the acute monocytic leukemia cell line THP-1, E1 was absent, suggesting that E1 represents a B-ALL–specific enhancer. Consistent with this, ChIP-qPCR using YCUB2 cells expressing 3×FLAG–*TCF3::HLF*–IRES–hNGFR confirmed direct binding of *TCF3::HLF* to E1. Furthermore, CRISPR-Cas9–mediated mutagenesis of E1 resulted in a significant reduction of *IL1B* expression, indicating that *TCF3::HLF* directly regulates *IL1B* transcription through a B-ALL–specific enhancer.

To further clarify the role of IL-1β in human *TCF3::HLF*-positive B-ALL, we performed single-cell RNA sequencing (scRNA-seq) analyses using bone marrow samples from patients and healthy donors. While *IL1B* expression was almost undetectable in healthy controls, it was markedly upregulated in B-ALL patients. Notably, the frequency of *IL1B*-expressing cells was even higher at relapse compared to initial diagnosis. In addition to *IL1B*, genes involved in inflammatory responses—such as *NFKB1*, *NLRP1*, and *IFNGR1*—were also upregulated in ALL samples. These findings suggest that *IL1B* plays a crucial role in disease progression and relapse of *TCF3::HLF*-positive ALL.

Collaborators:

Aisa Suzuki, Daisuke Sato, Tsukasa Shigehiro, Masatoshi Takagi (Institute for Science Tokyo), Kazuo Okamoto (Kanazawa Univ.), and Takeshi Inukai (Yamanashi Univ.)

Development of a novel immunotherapy using T/NK progenitor cells

Adoptive immunotherapy using chimeric antigen receptor (CAR)–engineered lymphocytes, such as CAR-T or CAR-NK cells, has emerged as a promising new strategy for cancer treatment. Infusion of CAR-T cells targeting CD19 has demonstrated remarkable efficacy against relapsed or refractory B-cell malignancies. However, therapeutic effects against other hematologic malignancies and solid tumors remain limited, and severe adverse events such as

cytokine release syndrome (CRS) and neurotoxicity continue to pose major clinical challenges.

Compared to CAR-T cells, CAR-NK cells offer several advantages:

(1) a lower risk of graft-versus-host disease (GVHD) and thus higher safety
(2) intrinsic antitumor cytotoxicity of NK cells
(3) feasibility of allogeneic transplantation

Indeed, promising clinical outcomes of CAR-NK therapy have been reported for glioblastoma and chronic lymphocytic leukemia (Burger et al., *Frontiers in Immunology*, 2019; Liu et al., *New England Journal of Medicine*, 2020). However, current CAR-NK cell manufacturing relies primarily on NK cells derived from human umbilical cord blood (hCB) or peripheral blood (PB), which limits scalability due to the small number of source cells and progressive senescence or exhaustion during long-term expansion.

We previously reported that $CD4^{-}CD8^{-}$ double-negative (DN) thymocytes, which are at an intermediate stage of T-cell differentiation, retain the potential to differentiate into both T and NK lineages and can be massively expanded under specific culture conditions while maintaining an undifferentiated state (Ikawa et al., *JEM*, 1999; Ikawa et al., *PNAS*, 2001; Ikawa et al., *Science*, 2010; Ikawa et al., *Stem Cell Reports*, 2015). These T/NK progenitor cells can be generated by culturing HSPCs on OP9/DLL1 stromal cells with defined cytokines for approximately two weeks, and can be induced to differentiate into mature T or NK cells within only seven days.

In the previous fiscal year, we successfully optimized the culture conditions to expand human T/NK progenitor cells by approximately 1,000-fold within three weeks. Using this system, it is possible to generate approximately 5×10^{11} NK cells from 1×10^{5} hCB-derived HSPCs. We are currently developing large-scale production methods for CAR-NK cells derived from hCB HSPCs using this culture platform.

In the current fiscal year, we analyzed the properties of NK cells differentiated from T/NK progenitors. When compared with NK cells derived from hCB or PB, T/NK progenitor–derived NK cells exhibited comparable proliferation capacity. Cytotoxicity assays using K562 target cells revealed that T/NK progenitor–derived NK cells showed similar killing activity to that of hCB- or PB-derived NK cells. Expression levels of effector molecules such as TNF-α, IFN-γ, and granzyme B were also equivalent among these NK cell populations.

Flow cytometric analysis of surface marker expression demonstrated that T/NK progenitor–derived NK cells highly expressed activation markers such as NKG2D, NKp44, and NKp46, while expression of inhibitory receptors (KIR2DL2/L3) and exhaustion markers (TIGIT) was low, indicating that these cells exhibited a relatively immature NK cell phenotype.

Furthermore, by introducing antigen-specific CAR constructs into T/NK progenitor cells, we successfully generated CD19 CAR-NK cells targeting hematologic malignancies, HER2 CAR-NK cells targeting breast and gastric cancers, and GD2/GPC2 CAR-NK cells targeting neuroblastoma (Japanese Patent Application No. 2023-127747; PCT/JP2024/27781). Interestingly, HER2 CAR-NK cells derived from T/NK progenitors exhibited significantly higher cytotoxic activity against target cells compared to CAR-NK cells derived from hCB or PB.

We are currently evaluating the *in vivo* antitumor efficacy of T/NK progenitor–derived CAR-NK cells using xenograft mouse models. Our ultimate goal is to establish a robust and scalable platform for the mass production of CAR-T and CAR-NK cells from T/NK progenitors, which will contribute to the next generation of cellular immunotherapies.

Collaborators:

Hiroyuki Kadota, Jia Han, Karin Noma, Dilnaze Karahan, Ryuki Ueda and Tsukasa Shigehiro

Regulation of dendritic cell differentiation by Polycomb Group Proteins

Polycomb group (PcG) proteins are essential epigenetic regulators involved in stem cell differentiation and maintenance of cell fate. They function as part of two major complexes: Polycomb Repressive Complex 1 (PRC1) and PRC2. Multiple variant forms of PRC1 have been identified (Gao et al., *Molecular Cell*, 2012). While the canonical PRC1 complexes contain PCGF2 (MEL18) or PCGF4 (BMI1), non-canonical PRC1 complexes incorporate other PCGF members (PCGF1, 3, 5, or 6). However, the *in vivo* functions of these PRC1 subtypes remain largely unclear.

We previously demonstrated that RING1A/B, the core components of PRC1, are indispensable for maintaining the lineage commitment of T-cell progenitors (Ikawa et al., *Genes & Development*, 2016). In collaboration with Prof. Atsushi Iwama at the University of Tokyo, we also reported that PCGF4 (BMI1) suppresses the expression of B cell–specific transcription factors EBF1 and PAX5, thereby contributing to the maintenance of HSCs (Oguro et al., *Cell Stem Cell*, 2010). More recently, we revealed that PCGF1 preserves normal nucleosome formation during DNA replication and maintains PcG-mediated transcriptional repression, thereby regulating the lineage commitment of hematopoietic stem/progenitor cells (HSPCs) toward the B-cell fate (Takano, Ikawa et al., *Nature Communications*, 2022).

Although these studies indicate that PcG proteins play crucial roles in HSC maintenance and differentiation, it remains poorly understood how individual PcG members coordinate lineage specification from HSCs into distinct hematopoietic lineages. To address this, we are currently investigating the roles of PCGF1–6 in hematopoiesis using various conditional knockout mouse models. In the present fiscal year, we focused on elucidating the function of PCGF2/4 (canonical PRC1 components) in the differentiation of HSCs into dendritic cells (DCs).

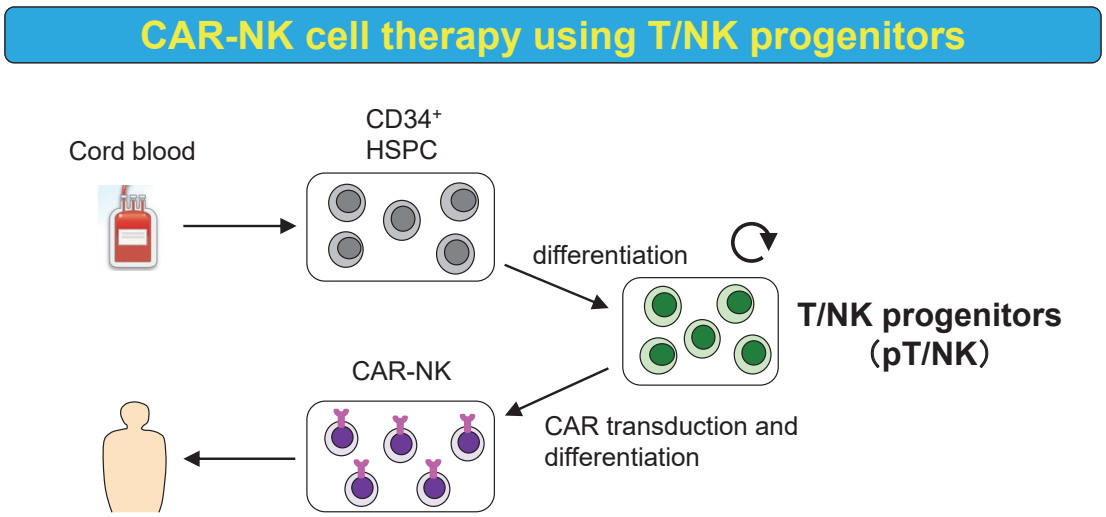
In our previous study, using *ERT2-Cre PCGF2/4 flox* mice, we showed that PCGF2/4 are essential for early B-cell and erythroid differentiation. We also observed a marked reduction in the proportion of type 2 conventional dendritic cells (cDC2) in hematopoietic-specific *PCGF2/4* double knockout (DKO) mice. This year, we investigated the specific role of PCGF2/4 in DC differentiation.

To determine whether DC differentiation defects in *PCGF2/4* DKO mice could be rescued by deletion of *CDKN2A*, a known PCGF4 target gene, we generated *PCGF2/4, CDKN2A* triple knockout (TKO) mice. Similar to the DKO mice, we produced hematopoietic chimeras to assess hematopoietic-specific effects. Interestingly, the proportion and total number of DCs were not restored in TKO mice, suggesting that genes other than *CDKN2A* contribute to the DC differentiation defect. To further delineate the role of PCGF2/4 in DC development, we cultured bone marrow cells from *PCGF2 KO*, *PCGF4 KO*, and *DKO* mice under in vitro DC-inducing conditions. Compared to controls, all DC subsets—including plasmacytoid DCs (pDCs), type 1 conventional DCs (cDC1s), and cDC2s—were markedly reduced. Notably, DC differentiation was more severely impaired in *DKO* mice than in either single knockout, indicating cooperative functions of PCGF2 and PCGF4 in this process.

Collectively, these findings suggest that PCGF2 and PCGF4 act synergistically to promote differentiation from HSCs to DCs. We are currently generating DC-specific *PCGF2/4* conditional knockout mice, developing *in vitro* differentiation systems that enable generation of all DC subsets (cDC1, cDC2, and pDC) from HSPCs, and searching for target genes to elucidate the downstream regulatory networks controlled by PCGF2/4 during DC differentiation.

Collaborators:

Kai Ishigami, Yutaro Ohashi, and Mayumi Hirakawa



✓ **Mass production of CAR-NK cells from T/NK progenitors**

✓ **High antitumor activity, stability and safety**

✓ **Off-the-shelf**

Publications

Original articles

1. Aoyama Y, Yamazaki H, Nishimura K, Nomura M, Suzuki T, Zang W, Tatara Y, Shigehiro T, Ito H, Hayashi Y, Koike Y, Fukumoto M, Tanaka A, Zhang Y, Saika W, Kasai S, Sakamoto T, Kong Y, Minakuchi Y, Itoh K, Yamamoto M, Toyokuni S, Toyoda A, **Ikawa T**, Takaori-Kondo A, Inoue D. Selenoprotein-mediated redox regulation shapes the cell fate of HSCs and mature lineages. ***Blood***. 145:1149-1163, 2025
2. Miyatake Y, **Ikawa T**, Yanagiya R, Kotaki R, Kameda K, Koyama-Nasu R, Okuyama K, Hirano K, Hosokawa H, Hozumi K, Ohtsuka M, Kishikawa T, Shibata C, Otsuka M, Maruyama R, Ando K, Kurosaki T, Kawamoto H, Kotani A. A single microRNA miR-195 rescues the arrested B cell development induced by EBF1 deficiency. ***eLife***. https://doi.org/10.7554/eLife.101510.1, 2024
3. Xiao M, Kondo S, Nomura M, Kato S, Zang W, Akashi T, Viny A, Fukumoto M, Shigehiro T, **Ikawa T**, Yamazaki H, Tanaka A, Zhang Y, Nishimura K, Hayashi Y, Koike Y, Aoyama Y, Nishikawa H, Kitamura T, Kanai A, Yokoyama A, Fujiwara T, Noguchi H, Lee SC, Toyoda A, Hinohara K, Abdel-Wahab O and Inoue D. BRD9 determines the cell fate of hematopoietic stem cells by regulating chromatin looping. ***Nat Commun***. 14: 8372, 2024
4. Uehata T, Yamada S, Ori D, Vandenbon A, Giladi A, Jelinski A, Murakawa Y, Watanabe H, Takeuchi K, Toratani K, Mino T, Kiryu H, Standley DM, Tsujimura T, **Ikawa T**, Kondoh G, Landthaler M, Kawamoto H, Rodewald HR, Amit I, Yamamoto R, Miyazaki M and Takeuchi O. Regulation of lymphoid-myeloid lineage bias through Regnase-1/3-mediated control of Nfkbiz. ***Blood***. 143: 243-257, 2024

Division of Immunology and Allergy

Yasutaka Motomura, Ph.D.

We aim to achieve a comprehensive understanding and eventual control of allergic diseases by elucidating the molecular regulatory mechanisms of allergic pathology centered on type 2 cytokines. The type 2 cytokines IL-4 and IL-13 are key mediators of allergic responses, and understanding how their expression is regulated is essential for dissecting disease pathogenesis. We first focused on type 2 helper T (Th2) cells, the major IL-4–producing population, and demonstrated that binding of GATA3 to the HS2 enhancer in intron 2 of the *Il4* gene induces epigenetic remodeling and endows Th2 cells with IL-4–producing capacity (Nat Immunol 2011). Furthermore, we showed that Th1 cells, which do not normally produce IL-4/IL-13, begin to produce IL-13 under chronic antigen stimulation, and that this plastic IL-13 induction strictly depends on the transcription factor E4BP4, thereby contributing to our understanding of the mechanisms underlying chronic allergic inflammation (Nat Immunol 2011). E4BP4 induces not only IL-13 but also IL-10, and functions as a key regulator of type 2 cytokine expression plasticity across T cell subsets. These studies preceded and supported the subsequent establishment of the widely accepted concept of "T cell plasticity."

Expression of IL-4/IL-13 is not restricted to T cells. We therefore extended the analysis to innate immune cells, identifying cell type–specific enhancers in basophils and mast cells and generating mice lacking these regulatory regions. Functional analysis revealed that loss of basophil-derived IL-4 markedly attenuates eosinophilic inflammation. Further investigation identified group 2 innate lymphoid cells (ILC2s) as the main IL-4–responsive population, and demonstrated that basophils promote allergic pathology by activating ILC2s (Immunity 2014). These findings clearly established that innate immunity, particularly ILC2s, plays an indispensable role in allergic disease, which had previously been discussed primarily from the perspective of adaptive immunity, and thereby served as a turning point in the conceptual framework of allergy research.

ILC2s are a novel lymphocyte subset identified in 2010 that do not require antigen recognition; instead, they respond to a wide range of endogenous factors, including cytokines, lipids, neuropeptides and hormones, to drive allergic responses. The discovery of ILC2s challenged the traditional view that "allergic diseases are induced by allergens" and provided important clues to pathologies that cannot be fully explained by adaptive immunity alone. Working within the laboratory that discovered ILC2s, we led research on this new cell population over a 10-year period, demonstrating the central role of ILC2s in allergic pathology (Immunity 2014, J Exp Med 2021) and their contribution to a variety of conditions, including pulmonary fibrosis (Nat Commun 2023).

These studies raised a central question: why does the same ILC2 population give rise to distinct immune responses and disease phenotypes in different settings? We hypothesized that ILC2 heterogeneity (functional diversity) is the key. Canonically, ILC2s produce IL-5 and IL-13 in response to IL-33 to induce eosinophilic inflammation; however, we found that under specific conditions ILC2s can produce IL-4 and thereby exacerbate IgE-dependent pathology, or

produce amphiregulin (Areg) and contribute to airway fibrosis and remodeling. In addition, alternative inflammatory pathways involving IL-9 and IL-17 have been reported, indicating that ILC2s can switch function in a context-dependent manner. This view is aligned with the "plasticity" concept we previously established in T cells (Nat Immunol 2010), and has led us to propose that ILC2 heterogeneity constitutes a cellular basis for the diversity of allergic diseases.

On this basis, we aim to obtain a comprehensive understanding of the molecular basis of ILC2 heterogeneity and the tissue-derived signals that regulate it. By doing so, we seek to define a shared pathological foundation linking allergic disease, fibrosis, metabolic disorders and chronic inflammatory diseases, and to develop novel diagnostic and therapeutic strategies targeting ILC2s. This project is expected to extend the limits of current medical practice, open new avenues in personalized and preventive medicine, and provide an innovative basis for overcoming intractable diseases.

Mechanisms underlying ILC2-mediated exacerbation of allergic inflammation

Antigen-independent allergic inflammation driven by ILC2s is distinct from classical adaptive immune–dependent allergy and can be defined as "innate allergy." We found that, in B cell responses traditionally attributed primarily to adaptive immunity, ILC2s can also function as important regulatory elements. We therefore investigated how ILC2s control B cell responses. Using an asthma model induced by intranasal administration of IL-33, we performed single-cell RNA sequencing (scRNAseq) of lung tissue and observed a pronounced accumulation of ILC2s, which were identified as the major source of IL-4, IL-5 and IL-13 in the lung. Notably, $CD19^+$ B cell clusters underwent marked changes in response to IL-33 even in the absence of exogenous antigen. Because B cells do not express the IL-33 receptor (*Il1rl1*), these findings suggested that B cell responses are induced indirectly via ILC2s.

Analysis of antibody production revealed that serum IgE levels were robustly elevated following IL-33 administration, indicating that ILC2s may regulate IgE production in the presence of IL-33. To assess the contribution of ILC2s to IL-33–induced IgE, we examined ILC2-deficient mice and found that IgE induction was completely abolished, demonstrating that ILC2s are essential for this process. In lungs from IL-33–treated mice, we observed not only an increase in B cell numbers but also upregulation of IgE post-switch and germline transcripts, suggesting that ILC2s promote IgE class switching in peripheral tissue B cells. Moreover, IL-33–induced IgE production was entirely suppressed in IL-4–deficient mice, indicating that IL-4 is indispensable for IgE induction. Given that ILC2s are the major source of IL-4 in IL-33–driven allergic responses, these findings demonstrate that ILC2s control IgE class switching via IL-4.

We further found that IL-4 production by ILC2s is induced not only by IL-33 but also by lipid stimuli, indicating that ILC2s can drive IgE production in an antigen-independent manner and thereby contribute to local allergic inflammation. Collectively, these results define a novel pathway of IgE induction and show that ILC2s, alongside T cells, play a critical role in the regulation of IgE production.

Understanding regulatory mechanisms of ILC2s specialized in tissue repair

In our body, tissues and organs are continuously exposed to the risk of injury. Examples include muscle damage caused by physical activity and epithelial injury in the lung associated with infection or inflammation. As a result, the body is subjected to constant micro-injury from internal and external stimuli. In response, tissue repair mechanisms are rapidly

activated to restore damage and maintain homeostasis. While such repair is essential for survival, excessive or prolonged activation can lead to sustained fibroblast activation and excessive extracellular matrix deposition, ultimately causing fibrotic disease. Intractable diseases such as pulmonary fibrosis, liver cirrhosis and cardiac fibrosis can thus be viewed as consequences of "runaway repair responses."

Although the balance between tissue repair and fibrosis is tightly regulated, the underlying molecular mechanisms, particularly those involving the immune system, remain insufficiently understood. ILC2s are innate immune cells that lack antigen receptors and produce type 2 cytokines such as IL-5 and IL-13 to modulate immune responses, while also producing amphiregulin (Areg) to directly promote tissue repair. ILC2s are rapidly activated in damaged tissues by the alarmin IL-33, which is released upon injury. In the course of analyzing mice lacking negative regulatory mechanisms of ILC2s, we discovered that hyperactivation of ILC2s downstream of IL-33 induces spontaneous pulmonary fibrosis (Nat Commun 2023), thereby demonstrating that ILC2-mediated tissue repair is normally subject to stringent control. Thus, while IL-33–induced Areg production by ILC2s promotes tissue repair, the same axis can also contribute to the development of fibrosis. Both "tissue repair" and "fibrosis" are regulated by the IL-33–ILC2 axis; however, the mechanisms that determine the divergence between these outcomes remain unclear and constitute an important unresolved issue.

To resolve this, we focused on the "tissue adaptation" of ILC2s. Increasing evidence indicates that ILC2s acquire distinct properties depending on the tissue environment in which they reside. For example, lung ILC2s highly express the IL-33 receptor and respond strongly to IL-33, whereas intestinal ILC2s express the IL-25 receptor and respond to IL-25. This tissue-dependent functional plasticity of ILC2s supports the maintenance of homeostasis in each organ, but may also contribute to disease progression under pathological conditions. In scRNAseq analyses of ILC2s from a pneumonia model, we identified a distinct ILC2 population that produced high levels of Areg. This population had largely lost the capacity to produce IL-5 and IL-13 and was instead specialized for Areg production. These findings suggest that ILC2s can differentiate into subsets with a particular capacity to drive fibrosis under specific environmental conditions.

Furthermore, under type 1 immune conditions such as viral infection, we observed enhanced Areg production by ILC2s accompanied by suppression of IL-5 and IL-13 production. These results indicate that, under type 1 immune responses, ILC2s undergo functional reprogramming toward a tissue repair–specialized phenotype. ATAC-seq analysis of ILC2s under type 1 immune conditions revealed increased chromatin accessibility at the promoter region of the *Areg* locus. Therefore, in the context of type 1 immunity, ILC2s were found to enhance the transcriptional activity of the *Areg* gene, thereby augmenting their tissue-repair capacity. Moreover, disruption of the regulatory mechanisms is expected to drive this pathway into an excessive state, triggering fibrotic responses and functionally converting ILC2s into "fibrotic ILC2s."

Collaborators:

Nozomi Hanawa, Kazuyo Moro (University of Osaka)

Understanding of 'tissue inflammatory memory' that drives chronic allergic inflammation

This study addresses the fundamental unresolved question of why allergic inflammation becomes chronic by investigating its basis from the perspective of "tissue inflammatory memory mediated by ILC2s." Clinically, allergic

inflammation is characterized by repeated exacerbations even after the acute trigger has resolved, but the upstream mechanisms that sustain this chronicity remain largely unknown. To explore this issue, we performed scRNAseq on lungs from an asthma model. This analysis revealed a marked accumulation of ILC2s, which play a central role in allergic inflammation, and identified epithelial stem cells. basal cells, as upstream regulators of ILC2 activation.

The scRNAseq data showed that cells with high expression of the ILC2-activating cytokines IL-33 and TSLP were restricted to basal cells expressing KRT15 and TP63. Highly proliferative MKI67^{+} basal cells expressed IL-33 and TSLP, as well as the neuropeptide neuromedin U (NMU), a potent activator of ILC2s. In addition, basal cells expressed receptors for IL-13 produced by ILC2s, suggesting the presence of a bidirectional activation loop between basal cells and ILC2s. These findings indicate that basal cells function as an "ILC2-activating niche" and may serve as a driving source of chronic allergic inflammation. As epithelial stem cells, basal cells possess self-renewal capacity and are constitutively present in tissues. ILC2s also exhibit tissue residency and tend to expand and persist under chronic inflammatory conditions. A structure in which these two cell types reside in close proximity and mutually activate one another is therefore likely to constitute the material basis of "tissue inflammatory memory" that maintains inflammation even after the initial stimulus has disappeared.

In acute allergic responses, ILC2s are rapidly activated and drive inflammation. More recently, however, it has been reported that ILC2s acquire "memory-like" properties, characterized by enhanced responsiveness upon secondary stimulation after a primary challenge. In our analyses, when IL-33–stimulated ILC2s were restimulated after a defined interval, IL-13 production was markedly augmented. We consider this phenomenon to be closely linked to the core pathophysiology of chronic allergic disease. In this project, we will conduct genome-wide analyses of temporal changes in gene expression and epigenetic modifications in ILC2s following initial IL-33 stimulation to elucidate the molecular mechanisms by which ILC2s

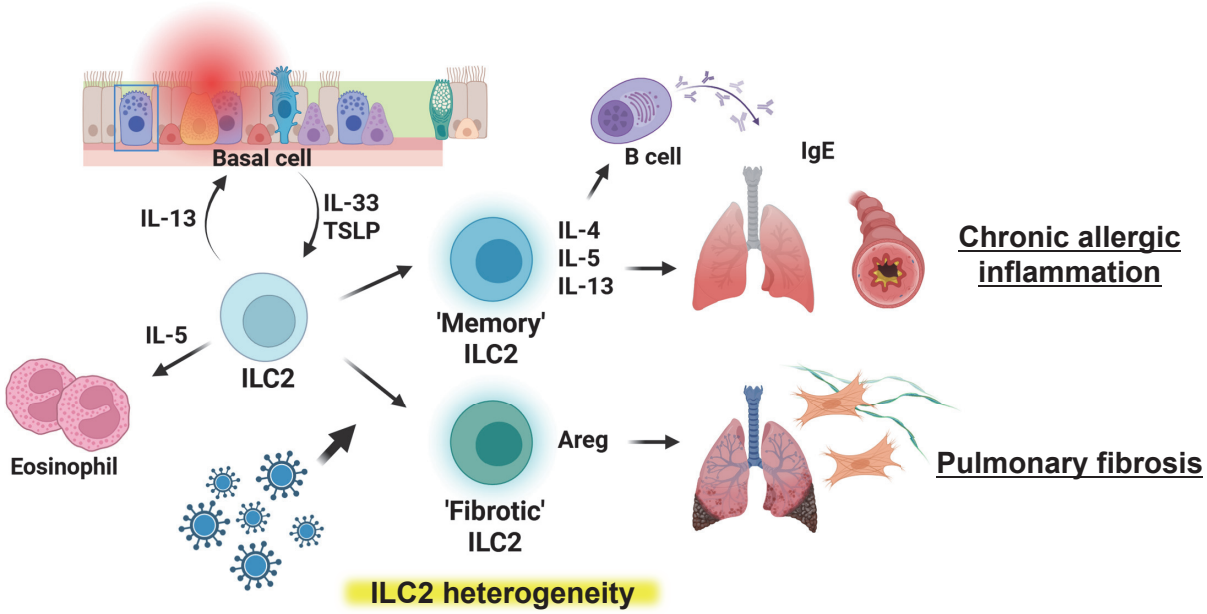


Figure.1 A model proposing that tissue inflammatory memory encoded by ILC2s induces chronic allergic inflammation and pulmonary fibrosis

acquire memory-like properties.

By integrating these lines of investigation, this study aims to define both the self-amplifying loop formed by the bidirectional "basal cell–ILC2" network and the intrinsic memory acquisition mechanisms of ILC2s, and on this basis to propose a new disease concept: "tissue inflammatory memory mediated by ILC2s." This conceptual framework is expected to facilitate the identification of novel therapeutic targets that can control chronic inflammation.

Publications

Original articles

1. Yamagishi, M., Miyata, K., Kamatani, T., Kabata, H., Baba, Rie., Tanaka, Y., Suzuki, N., Matsusaka, M., **Motomura, Y.**, Kiniwa, T., Koga, S., Goda, K., Ohara, O., Funatsu, T., Fukunaga, K., Moro, K., Uematsu, S., Shirasaki, Y. Quantitative live-cell imaging of secretion activity reveals dynamic immune responses, DOI: 10.1016/j.isci.2024.109840 ***iScience*** 27(6), 2024
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