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Keynote Lecture 4

Hypothesis-Generating Studies and Allergy

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Disclosure of potential conflict of interest

In the past three years, Dr. Hirohisa Saito has received research support and travel support from the Ministry of Health, Labour and Welfare; is employed by the National Center for Child Health and Development; has received research support from the Japan Society for the Promotion of Science (21390303 & 23390262); has received payment for lectures from Teijin Pharma, Shiseido, Merck Sharp and Dohme K.K., Taiho Pharmaceutical, Nippon Boehringer-Ingelheim, Ono Pharmaceutical, GlaxoSmithKline K.K., Pfizer Japan, Novartis Pharma K.K., Kyowa Hakko Kirin, Kyorin Pharmaceutical, and Daiichi Sankyo; has received payment for manuscript preparation from Taiho Pharmaceutical; has received payment for educational presentations from Shimane University and Toho University; and has received travel support from the Shimane University Japanese Society of Allergology and the Japanese Society of Pediatric Allergy & Clinical Immunology; and Pfizer Japan.

The above mentioned payments were made with respect to activities unrelated to this presentation.

Learning Objective (Summary)

Large scale hypothesis-generating study

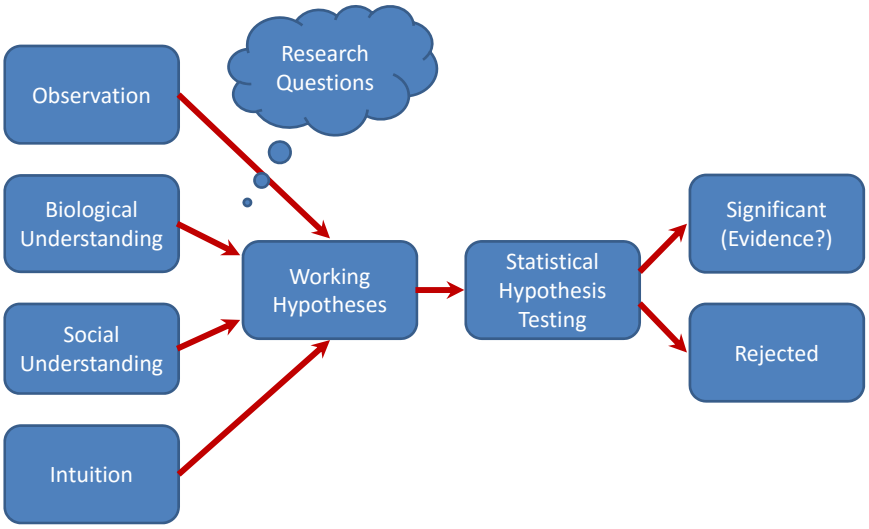
It can not provide clinical evidences.

Its newly-generated hypothesis should be verified.

It can provide more probable hypothesis than your intuition can.

It can bring a new paradigm which we can not imagine.

Working Hypothesis and Hypothesis Testing

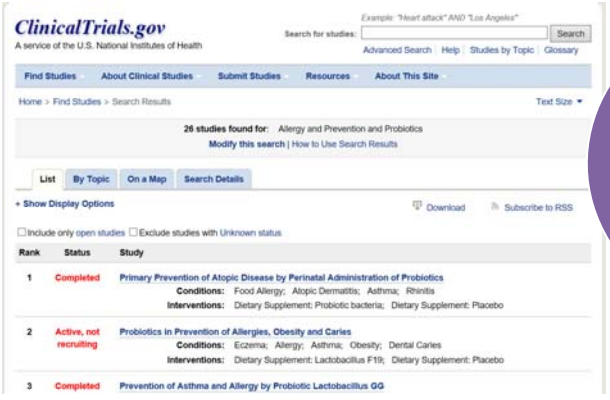


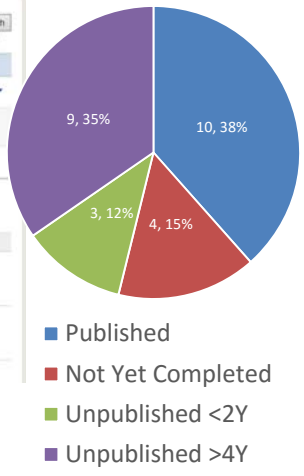
Hypothesis-Testing and Hypothesis-Generating Studies

Hypothesis-testing studies are predominate in
Established fields,
Interventional studies,
Prospective studies,
Longitudinal studies

Hypothesis-generating studies are often found in
Less developed fields,
Observational studies,
Retrospective studies,
Cross-sectional studies

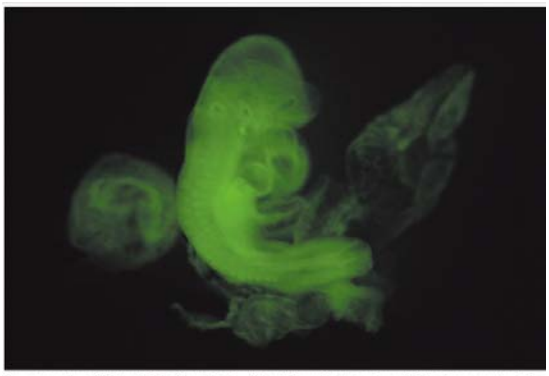
The fact is not so beautiful as the theory. Hypothesis-testing studies often fail.





26 studies found for: Allergy and Prevention and Probiotics in ClinicalTrial.gov

Sometimes, the fact is even ugly when the theory is too beautiful.

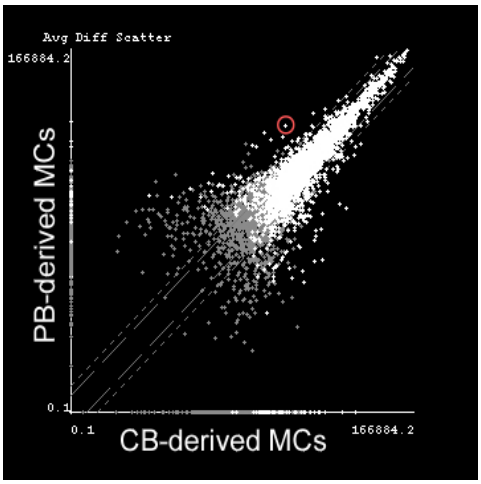


A mouse embryo injected with cells made pluripotent through stress, tagged with a fluorescent protein.

REGENERATIVE MEDICINE

Acid bath offers easy path to stem cells

We started a large-scale hypothesis-generating study by using microarray in 2000.



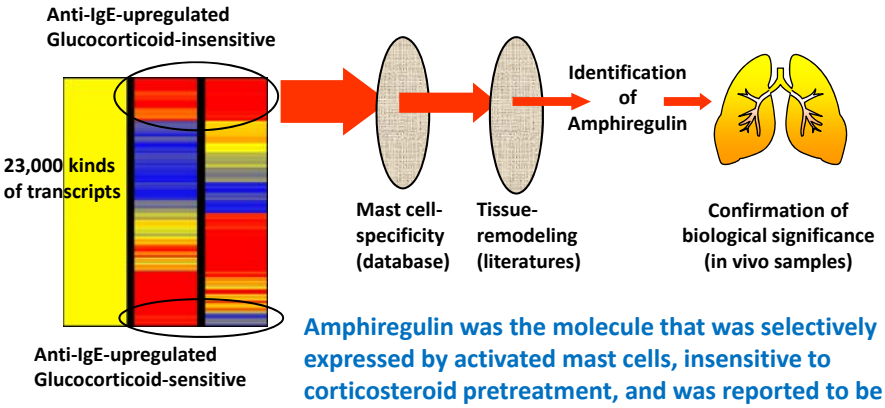
Working Hypothesis for this hypothesis-generating study:

We might find differentially regulated genes between neonatal and adult hematopoietic progenitor derived mast cells.

The mRNA expression of FcεRIα in neonatal progenitor-derived cultured mast cells is selectively down-regulated compared to adult progenitor-derived mast cells, even though they were cultivated in the same condition.

Iida M, et al. *Blood* 2001

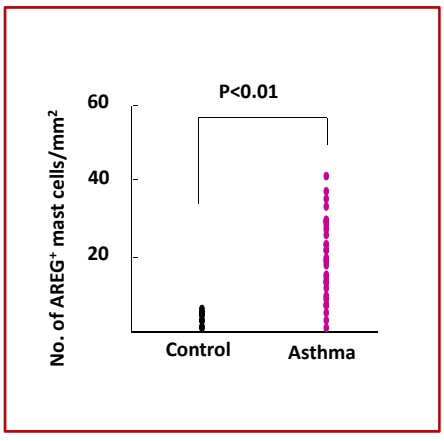
Hypothesis: A druggable and steroid-resistant molecule in relation to airway remodeling in asthma may be found in the whole transcriptome of IgE-dependently activated human mast cells, which had been treated with glucocorticoid.



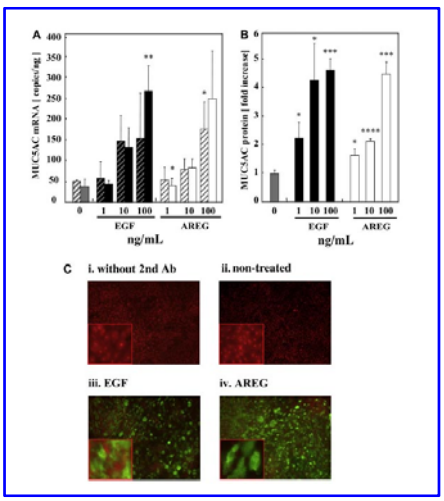
Amphiregulin was the molecule that was selectively expressed by activated mast cells, insensitive to corticosteroid pretreatment, and was reported to be involved in the tissue remodeling.

Okumura S, et al. *J Allergy Clin Immunol* 2005; 115: 272

- **Amphiregulin was increasingly expressed by mast cells in bronchial biopsy samples only of asthmatics.**
- **Amphiregulin promoted mucin production in bronchial epithelial cells.**



Okumura S, et al. *J Allergy Clin Immunol* 2005; 115: 272



Enomoto Y, et al. *J Allergy Clin Immunol* 2009; 124: 913

Hypothesis: Genetic variation associated with asthma onset in the whole human genome may be identified.

Results: IL-33 and IL-33R were constantly identified as asthma (and eosinophilia)-associated genes in these 3 independent large scale studie.

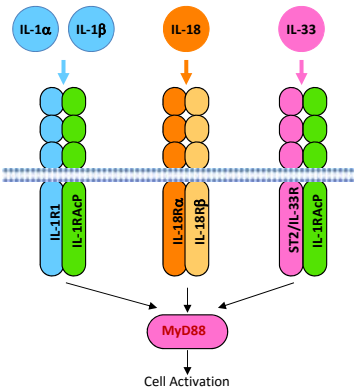
Gudbjartsson DF, Bjornsdottir US, Halapi E, et al. Sequence variants affecting eosinophil numbers associate with asthma and myocardial infarction. Nat Genet 2009; 41: 342-47.
IL18/IL33R, (IL33, TSLP/WDR36, MYB)
Meta analysis of 7996 adult asthma patients of 10 different regions

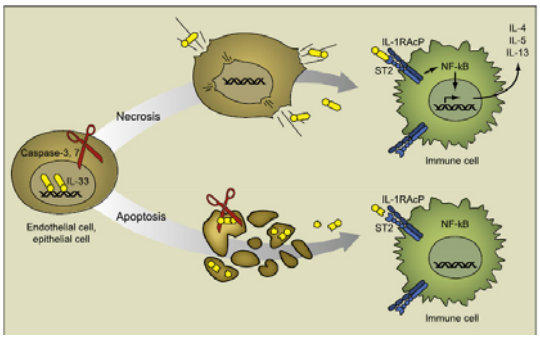
Moffatt MF, Gut IG, Demenais F, et al. A large-scale, consortium-based genome-wide association study of asthma. N Engl J Med 2010; 363: 1211–21.
IL18/IL33R, IL33, 17q21 (ORMDL3/GSDMB), HLA-DQ, SMAD3, IL2RB
Meta analysis of 10365 European adult asthma patients

Torgerson DG, Ampleford EJ, Chiu GY, et al. Meta-analysis of genome-wide association studies of asthma in ethnically diverse North American populations. Nat Genet. 2011; 43: 887-92.
IL18/IL33R, IL33, TSLP/WDR36, 17q21 (ORMDL3/GSDMB), PYHIN1
Meta analysis of 5416 North American asthma patients of various races

*P<0.05/1,000,000 SNPs=$5x10^{-8}$

New Questions: What is IL-33? How does it work?



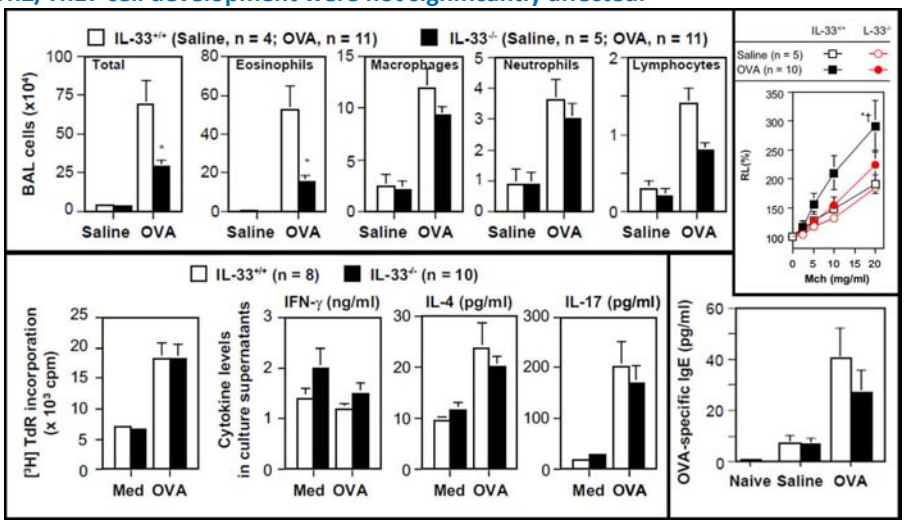


IL-33 is one of IL-1 family cytokines and shares its common receptor, IL-1RAcP, with IL-1. Unlike IL-1 beta and IL-18, however, without caspase-mediated proteolytic cleavage, IL-33 released from necrotizing cells can act on inflammatory cells to release cytokines.

Lamkanfi M, et al. *Immunity* 2009; 31: 84-98 (Editorial)
Luthi AM, et al. *Immunity* 2009; 31: 84-98 (Original)

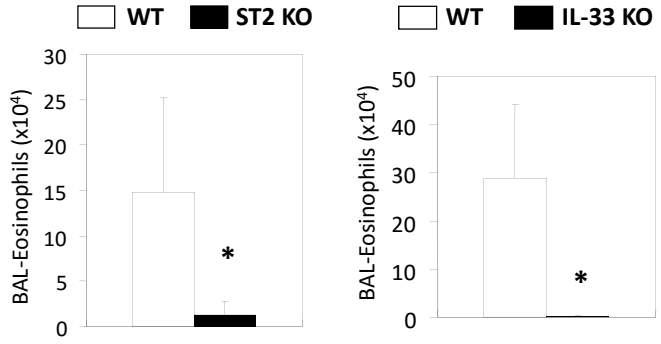
We conducted a new hypothesis-generating studies by producing IL-33 gene-deficient mice.→

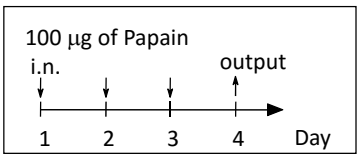
Allergen-induced eosinophilic inflammation and airway hypersensitivity were markedly diminished in the IL-33 deficient mice, while IgE production and Th2/Th17 cell development were not significantly affected.



Oboki K, et al. *Proc Natl Acad Sci USA* 2010; 107: 18581

IL-33 and its receptor were essential for papain-induced innate airway eosinophilic inflammation.

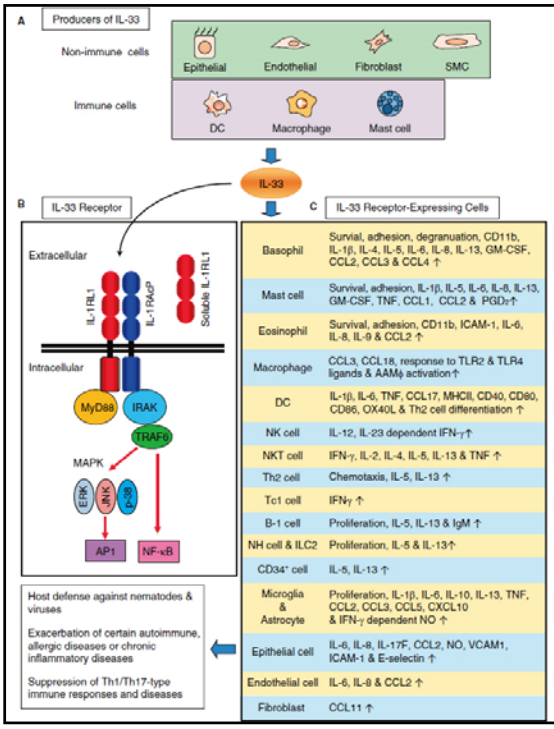




Papain is a cysteine protease (EC 3.4.22.2) enzyme, present in papaya or pineapple, used as a meat tenderizer, and having a high structural homology with major mite group 1 allergens (Der f1 and Der p1). Mol Immunol. 1992;29:257.

Oboki K, et al. *Proc Natl Acad Sci USA*. 2010;107:18581

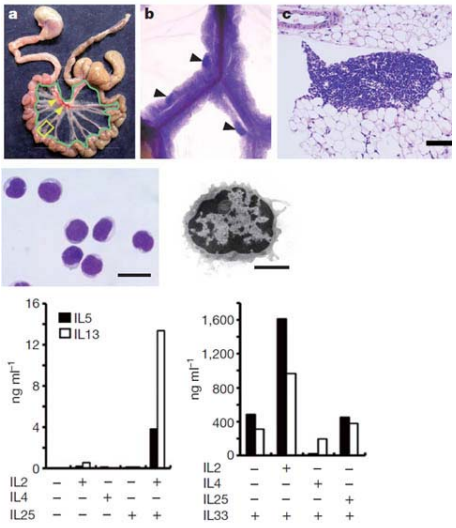
→A New Hypothesis: Innate immune cell types probably induce more inflammation in response to IL-33.



Hypothesis:
Several cell types can release IL-33 and a variety of cell types express IL-33 receptors, innate cell types related to allergic inflammation such as mast cells and basophils may be more important.

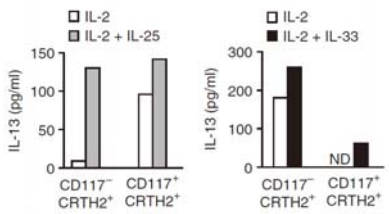
Nakae S, et al. *Allergol Int.* 2013;62:13-20

A Hypothesis: Innate immune cell types would induce more inflammation in response to IL-33.
→Discovery of natural helper cells by Moro & Koyasu.



Mouse natural helper cells can produce >1μg IL-13 or IL-5 per 10^5 cells in response to IL-33.

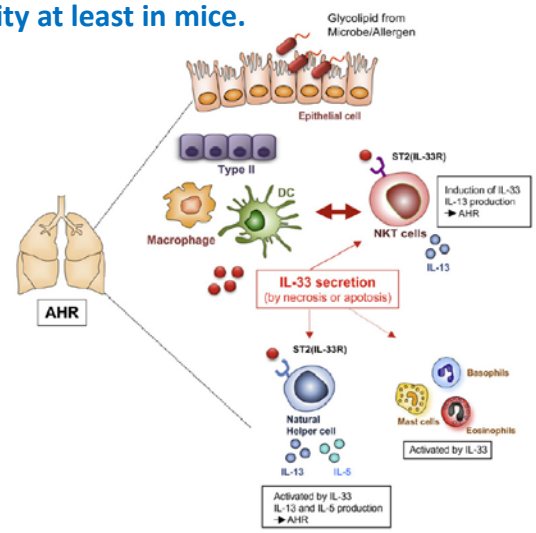
Moro K, et al. *Nature* 2010; 463: 540-4.



Human natural helper cells can produce 1ng IL-13 per 10^5 cells in response to IL-33 or IL-25.

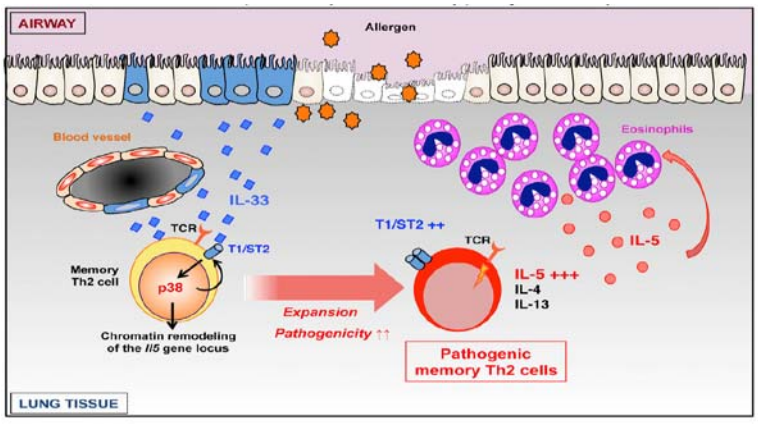
Mjösberg JM, et al. *Nat Immunol* 2011;12:1055-62.

A New Hypothesis: Natural helper cells/ILC2s capable of producing more IL-13 and IL-5 in response to IL-33 may be important.
→Natural helper cells were essential to induce airway hypersensitivity at least in mice.



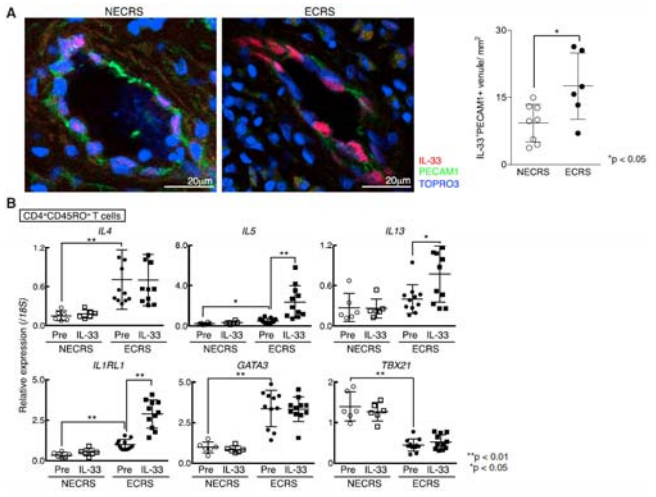
Kim HY, et al. *J Allergy Clin Immunol* 2012;129:216.

Another question raised by GWAS: Why does the eosinophil number increase in IL-33R gene-variant individuals?
IL-33 plays a substantial role in increasing the eosinophil number by inducing the development of pathogenic memory Th2 cells, which can produce a massive amount of IL-5.



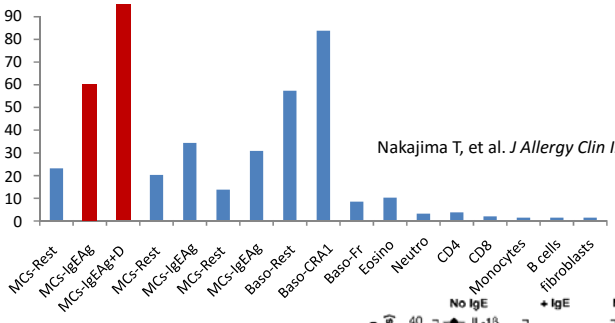
Endo Y, et al. *Immunity.* 2015;42:294-308.

- **IL-33 expression was increased in the endothelial cell nuclei of nasal polyps derived from eosinophilic chronic rhinosinusitis (CRS) compared to non-eosinophilic CRS.**
- **The addition of IL-33 to the nasal polyp tissue increased the levels of IL-5, IL-13 and IL-33R only in eosinophilic CRS where pathogenic Th2 cell number was significantly high.**



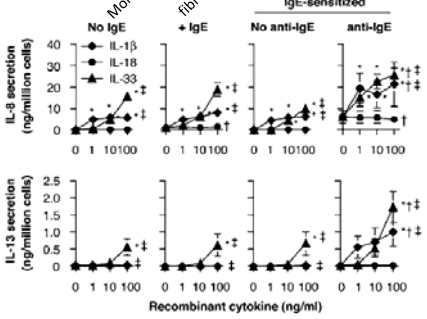
Endo Y, et al. *Immunity.* 2015;42:294-308.

IL-33R gene is preferentially upregulated in human mast cells.



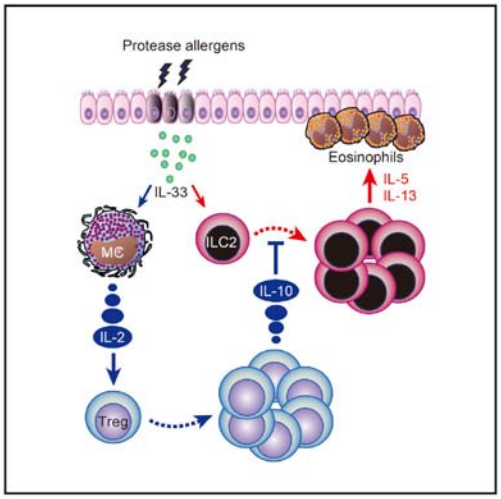
Nakajima T, et al. *J Allergy Clin Immunol* 2004

IL-33 was the only cytokine that can stimulate IL-13 release by human and mouse mast cells in the absence of IgE-dependent signals without causing degranulation.



Iikura M, et al. *Lab Invest* 2007
Ho LH, et al. *J Leukoc Biol* 2007

A New Question: What is the role of mast cells in IL-33-mediated reaction?

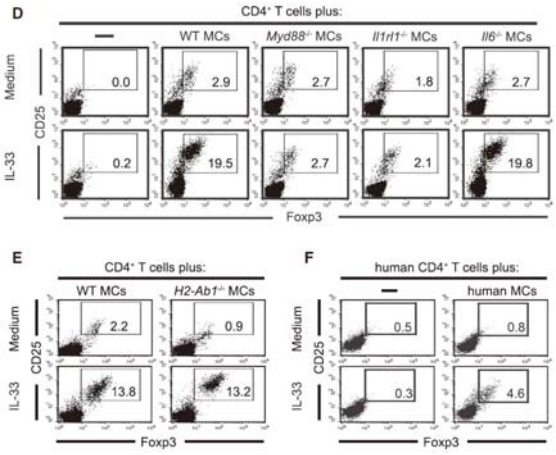


A New Hypothesis: Mast cells would promote IL-33-mediated inflammation.

Results: Mast cells inhibited protease allergen-induced and IL-33-mediated eosinophilic inflammation by promoting the polyclonal expansion of regulatory T cells. The hypothesis was rejected.

On the other hand, natural helper cells enhance such inflammation by producing a large amount of IL-5 and IL-13. Morita H, et al. *Immunity.* 2015;43:175-186.

A New Question: Do human mast cells promote polyclonal Treg expansion like mouse mast cells do?→Yes, they do.



Morita H, et al. *Immunity.* 2015;43:175-186.

A pressing research question for next working hypotheses: What are the real roles of IL-33 in human diseases?

Summary

Large scale hypothesis-generating study

It can not provide clinical evidences.

Its newly-generated hypothesis should be verified.

It can provide more probable hypothesis than your intuition can.

It can bring a new paradigm which we can not imagine.

Collaborators of the topics which I mentioned today

National Research Institute for Child Health & Development
Susumu Nakae (University of Tokyo), Hideaki Morita (Swiss Institute of Allergy and Asthma Research), Toshiharu Nakajima (Yokohama College of Pharmacy), Atsushi Kato (Northwestern University), Mokoto Iida (Jikei University), Keisuke Oboki (Tokyo Metropolitan Institute of Medical Science), Ichiro Nomura, Kenji Matsumoto

RIKEN Research Center for Allergy & Immunology
Shigeru Okumura (Biolegend), Jun-ich Kashiwakura (RIKEN), Yoshimichi Okayama (Nihon University)