Hypothesis-Generating Studies and Allergy

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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

Observation
Biological Understanding
Social Understanding
Intuition

Research Questions

Working Hypotheses

Statistical Hypothesis Testing

Rejected
Significant (Evidence?)

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.

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.

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.

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.


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

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.


IL18/IL33R, (IL33, TSLP/WDR36, MYB)

Meta analysis of 7996 adult asthma patients of 10 different regions


IL18/IL33R, IL33, 17q21 (ORMDL3/GSDMB), HLA-DQ, SMAD3, IL2RB

Meta analysis of 10365 European adult asthma patients


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.


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.

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

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.

Mouse natural helper cells can produce >1μg IL-13 or IL-5 per 10^6 cells in response to IL-33.


Human natural helper cells can produce 1ng IL-13 per 10^6 cells in response to IL-33 or IL-25.

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.

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.
• 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.

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

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.

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.


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

A pressing research question for next working hypotheses:
What are the real roles of IL-33 in human diseases?
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Collaborators of the topics which I mentioned today

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