ImmunoTox Letter Digest

December 2023

Report on the 30th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT2023)

Ryosuke Nakamura
President of the 30th Annual Meeting of the JSIT
National Institute of Health Sciences, Japan

The 30th JSIT Annual Meeting unfolded in Kawasaki, Japan, from September 11 to 13, 2023, at the Tonomachi district of the KING skyfront. Hosted at the Shimadzu Tokyo Innovation Plaza and the Kawasaki LiSE, supported by Shimadzu Corporation and the city of Kawasaki. On behalf of the Annual Meeting of the Japanese Society of Immunotoxicology, I would like to express our sincere gratitude to them for providing us with such comfortable and relaxing venues.

This year's theme, "New Immunotoxicology Research Required by Society," was chosen to highlight the growing societal demand for innovative drugs with diverse structures and modes of action, such as mRNA vaccines. These drugs have the potential to revolutionize medicine, but they also raise new concerns about immunotoxicity. This is a pivotal issue that the Japanese Society of Immunotoxicology, a consortium of immunotoxicology experts, aims to address.

Aligned with the theme, Dr. Junichi Koga delivered a special lecture on vaccine development at SCARDA on Day 1. The symposium "Immunotoxicology in the R&D of New Modality Drugs and Vaccines" explored challenges and opportunities, featuring insights from experts like Dr. Akiko Ishii, Dr. Shogo Matsumura, Dr. Yukari Fujiwara, Dr. Yuko Nagayama, and Dr. Yoshimasa Takahashi.

Day 2 shifted focus to methodology. Dr. Kristina E. Howard's insightful online special lecture on humanized mice models for immunotoxicity assessment sparked lively discussions. The workshop "Animal Models and the Alternatives Reflect Human Immune Responses: Current Status and Future Prospects" provided insights from Dr. Shigeki Aoki, Dr. Eita Sasaki, Dr. Takeshi Watanabe, and Dr. Yasumitsu Nishimura. Professor Riichiro Abe's educational lecture on the mechanism of severe drug rash enhanced our understanding of this critical adverse reaction.

The last day's open symposium, "Immunotoxicity Risk Assessment for Chemicals in the Environment," underscored the societal importance of environmental chemicals, featuring perspectives from Dr. Yasunobu Aoki, Dr. Reiko Teshima, Dr. Kiwako Yamamoto, and Dr. Eiko Koike.

Marking the 30th anniversary, past presidents Professors Takahiko Yoshida and Kazuichi

Nakamura delivered a captivating lecture titled "Immunotoxicology: Honor the Past and Build the Future" on Day 1. Their historical journey, culminating in the field's role in ICH S8 and highlighting future challenges, resonated deeply, urging us to learn from the past and construct a brighter future for immunotoxicology.

Award lectures by Professor Kazuichi Nakamura and Dr. Toshinobu Kuroishi were held on the Day 2 afternoon. Drs. Takeshi Susukida and Hideki Hara shared the annual meeting award, tying for first place. Mr. Takayuki Sakai received the best Student and Young Scientist Presentation Award.

We would like to express our sincere gratitude to all the participants, sponsors, exhibitors, and volunteers for their active discussions and presentations. We are especially grateful for the participation of over 160 researchers from all over Japan. Your contributions helped to make this meeting a truly successful event.

We look forward to seeing you all again at the 31st annual meeting in 2024 in Amagasaki!

The Best Presentation Award

Glycolysis in CD8$^+$ T cells plays an important role in the onset of HLA-mediated idiosyncratic drug toxicity

Takeshi Susukida
Graduate School of Medicine and Pharmaceutical Sciences
University of Toyama

I am very honored to receive The Best Presentation Award at the 30th Annual Meeting of Japanese Society of Immunotoxicology. I would like to sincerely appreciate the support from the selection committee.

This study aimed to examine whether intracellular metabolism change in CD8$^+$ T cell contributes to the susceptibility to immune-mediated idiosyncratic drug toxicity (IDT), using our established transgenic (Tg) mice carrying chimeric human leukocyte antigen (HLA). Although genome-wide association studies revealed that the occurrence of IDT strongly associates with particular HLA allotype, it does not occur in all subjects of a given HLA model population, suggesting the difficulties to predict the IDT risk without considering additional factors. Focusing on HLA-B*57:01 and abacavir (ABC; an anti-HIV drug)-induced immune-mediated IDT (skin rash), we have generated a Tg mouse line carrying HLA-B*57:01 (B*57:01-Tg) and successfully reproduced abacavir (ABC)-induced skin toxicity with increase of IFN-γ-secreting effector CD8$^+$ T cells (CD44^{high}CD62L^{low}) and CD8$^+$ T cell dermal infiltration in B*57:01-Tg mice [1-2]. Moreover, we found that serum level of pyruvate, which is a metabolite formed by glycolysis, was substantially increased in ABC-treated B*57:01-Tg mice, suggesting the importance of metabolic regulation for CD8$^+$ T cell immunity in the susceptibility to HLA-mediated ABC-induced IDT. In this study, we examined whether intracellular metabolism change in CD8$^+$ T cells contributes to the susceptibility to ABC-induced IDT in the B*57:01-Tg mouse model, by comparing control and calorie restricted (CR) group.

As expected, glycolytic rate in CD8$^+$ T cells was significantly increased by ABC treatment

in B*57:01-Tg mice, whereas this tendency was abrogated in the CR diet-fed group. In CR condition, ABC treatment failed to increase the proportion of effector IFN-γ-secreting $CD8^+$ T cells, thereby abrogating plasma thymus and activation-regulated chemokine (TARC) level elevation and $CD8^+$ T cell dermal infiltration (i.e., skin toxicity). Treatment of a glycolysis inhibitor, 2-Deoxy-D-glucose (2-DG), also attenuated the $CD8^+$ T cell activation and ABC-induced skin toxicity in normal diet-fed B*57:01-Tg mice. These results suggested that glycolysis in $CD8^+$ T cells may play a major role in the onset of ABC-induced IDT in B*57:01-Tg mice. This finding lays a foundation to identify unclarified onset risk factors in HLA-related IDT through further investigation on the molecular regulation of glycolysis. We also believe that our study provides a valuable insight to reduce the IDT onset risk by considering individual differences in nutritional status in humans.

I would like to express my gratitude to my supervisors; Prof. Yoshihiro Hayakawa (Univ. of Toyama), Prof. Kousei Ito (Chiba University) and Assoc. Prof. Shigeki Aoki (Chiba Univ.) for beneficial advisement on this project. I also thank to Dr. Yuchen Sun (National Institute of Health Sciences) for his valuable technical support on this project.

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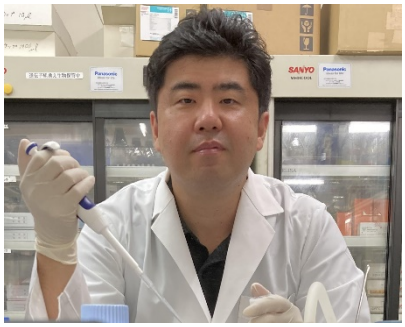
The Best Presentation Award

Exacerbation mechanism of infectious diseases through inflammasome activation



Hideki Hara
Department of Infectious Diseases, Division of Microbiology and Immunochemistry,
Asahikawa Medical University

It is my great honor to be selected for the 30th Annual Meeting Award of the Japanese Society of Immunotoxicology in 2023. I would like to express my sincere gratitude to Dr. Ryosuke Nakamura, President of the Annual Meeting of the Japanese Society of Immunotoxicology, and other members of the selection committee.

When pathogens such as bacteria infect us, our bodies attempt to eliminate them by activating innate immunity. Bacterial ligands are recognized through receptors expressing on the cell surface and inside cells. It has been known that some intracellular receptors induce activation of inflammasome, an immune system that induces inflammatory responses. The inflammasome is an intracellular protein complex composed mainly of intracellular receptors such as Nod-like receptors and AIM2-like receptors, adapter molecule ASC, and caspase-1 precursor, which activates caspase-1 to induce maturation and secretion of their substrates, IL-1β and IL-18. Gasdermin D has also been reported to be a substrate of caspase-1, and it has been shown to trigger pyroptosis, a programmed cell death associated with inflammation. In general, immune responses are thought to contribute to host protection, and inflammasomes were previously reported to be protective in some infectious diseases. However, we have found that when inflammasome-deficient mice are infected with pathogens such as *Listeria monocytogenes* and *Staphylococcus aureus*, the number of bacteria in their organs is lower than that of wild-type mice and they show higher resistance to infection. These results indicate that pathogens exacerbate the infectious diseases by activating inflammasome responses. We also investigated the mechanism of inflammasome activation in infectious diseases, and found that *Listeria* delivers bacterial

double-stranded DNA, a ligand of AIM2, into the cytoplasm, and the inflammasome activation is promoted by listeriolysin O, the *Listeria* major virulence factor. More recent studies have revealed that lipoteichoic acid, a cell wall component, activates NLRP6 inflammasome. Lipoteichoic acids is widely expressed in Gram-positive bacteria, and we found that activation of the NLRP6 inflammasome also exacerbate *Staphylococcus aureus* infection. Finally, we clarified that inflammasome-dependent production of IL-18 is detrimental to the host during infection with *Listeria monocytogenes* and *Staphylococcus aureus*. The future progress of research on the mechanism by which IL-18 exacerbates infectious diseases and the establishment of novel treatment for infection targeting IL-18 are highly anticipated. I am looking forward to the 31st Annual Meeting to be held at Hyogo Medical University where IL-18 was discovered.

References

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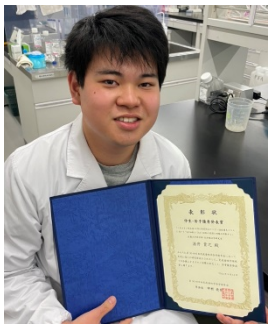
The Student and Young Scientists Award

A CCR4 inhibitor suppresses atopic dermatitis-like skin inflammation by inhibiting the recruitment and expansion of Th2 cells and Th17 cells

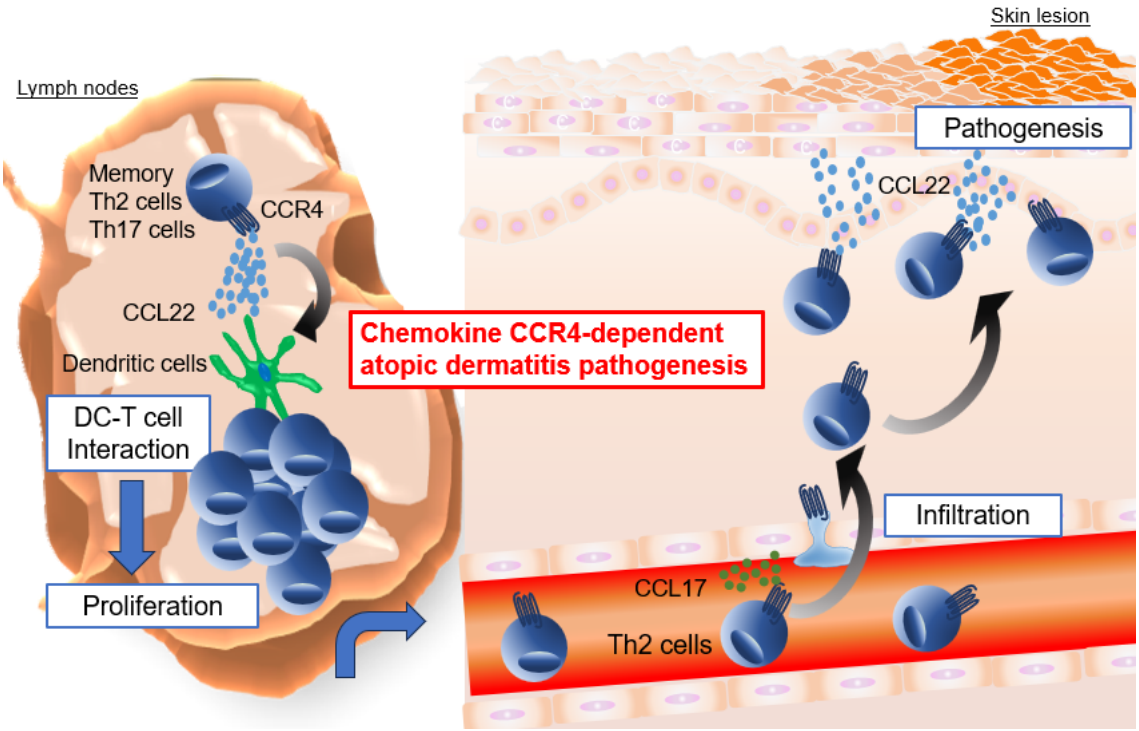


Takayuki Sakai
Kindai University Faculty of Pharmacy

I'm deeply honored to receive the Student and Young Scientists Award at the 30th Annual Meeting of the Japanese Society of Immunotoxicology. I would like to express my sincere appreciation to the organizing committee and all those who have contributed to this remarkable event.

My research focused on the role of chemokine receptor CCR4 in atopic dermatitis (AD). The chemokine receptor CCR4 is a major trafficking receptor expressed by T-helper (Th) 2 cells and Th17 cells and is considered as a potential therapeutic target for AD. The CCR4 ligand CCL17 and CCL22 have been reported to be upregulated in the skin lesions of AD patients. In this study, we investigated the role of CCR4 in an AD mouse model induced by MC903. Topical application of MC903 to ear skin induced increased expression of not only TSLP but also CCL17, CCL22, IL-4 (Th2 cytokine), and IL-17A (Th17 cytokine). Compound22, a CCR4 inhibitor suppressed Th2 cells, Th17 cell, and ILC2 which expressed CCR4. We further confirmed that compound22 diminished the expansion of Th2 cells and Th17 cells in the regional lymph nodes. In addition, compound22 ameliorated AD-like skin lesions. Collectively, CCR4 inhibitors is involved in the pathogenesis of AD by recruitment and expansion of Th2 cells and Th17 cells.

This award is not just a personal achievement but a testament to the collective efforts of my dedicated team and the invaluable support I have received from mentors and peers.


Skin lesion
Lymph nodes
Memory
Th2 cells
Th17 cells
CCR4
CCL22
Dendritic cells
DC-T cell
Interaction
Proliferation
Chemokine CCR4-dependent
atopic dermatitis pathogenesis
Pathogenesis
CCL22
Infiltration
CCL17
Th2 cells

The 12th Japanese Society of Immunotoxicology Prize for Encouragement

Preclinical immunotoxicity and immunogenicity studies aimed at improving safety predictions of biopharmaceuticals

Chiyomi Kubo
on behalf of non-clinical immunosafety researchers
Translational Research Division
CHUGAI PHARMACEUTICAL CO., LTD.

I am deeply honored to have received the 12th Japanese Society of Immunotoxicology Prize for Encouragement. I would like to express my sincere gratitude to all who nominated me for the award, to the members of the selection committee, and to all who have enlightened and lead me over the years. I would like to share the happiness of receiving this award with my colleagues who have overcome many challenges together.

I started immunotoxicity and immunogenicity research when I joined this pharmaceutical company. In the immunotoxicity research, our purpose is to support non-clinical safety assessment for effective prediction of toxicity for patients. Firstly, we have prepared several assays (e.g., T-cell dependent antibody response (TDAR), NK assay, Immunophenotyping) in rodents and monkeys in line with the ICH S8 guideline (Immunotoxicology Studies for Human Pharmaceuticals) and our drug discovery pipeline. And we also developed human *in vitro* assays such as a cytokine release assay. On the other hand, in the immunogenicity research, our purpose is to support molecular designs and engineering technologies for the development of biotherapeutics with no problematic immunogenicity. Therefore, we developed human *in vitro* T-cell assays to evaluate the immunogenicity potential, mechanism tools (e.g., MHC-associated peptide proteomics (MAPPs), binding/uptake assays, APC functional assays), and de-immunization strategy. We have been assessing discovery candidates and approaching the mechanism based de-immunization in all biology projects since a humanized bispecific antibody

to coagulation factors IXa and X with a factor VIIIa-cofactor activity, Emicizumab, project.

Lately, we have been addressing analysis of species differences and mechanisms in immune-related adverse effects of immunotherapy drugs which are often hard to predict in the animal testing. In addition, we have been analyzing the mechanism of high immunogenic investigational and marketed drugs and sorting factors that contribute to the immunogenicity.

We will continue this research with changes in line with advances in antibody engineering technologies, diversification of molecular structures and modes of action, and expansion of evaluation systems. I would like to make every effort to contribute to the drug safety, the innovative drug development, and the scientific progress through this research.