ImmunoTox Letter Digest

June 2025

The 32nd Annual Meeting of the Japanese Society of Immunotoxicology (JSIT2025)

1. Date
September 4–5th, 2025

2. Venues
Gifu City Culture Center
(https://gifu-culture.info/accesss/)

3. President
Tsuyoshi Nakanishi
(Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University, Japan.)

4. Main theme of the meeting
Aiming to contribute to the creation of innovations in immunotoxicity research.

5. Meeting secretariat
Laboratory of Hygienic Chemistry and Molecular Toxicology, Gifu Pharmaceutical University.
E-mail: JSIT2025@gifu-pu.ac.jp
URL:
https://www.japanimmunotox.org/jsit2025/

6. Program (tentative)
Special lecture
1) "Immunotoxicity Regulation Through Gut Microbiota-Based Strategies."
Jun Kunisawa (Microbial Research Center for Health and Medicine, National Institutes of Biomedical Innovation, Health and Nutrition (NIBN))
2) <TBA>
Marie-Soleil Piche（Charles River）

Educational lecture
"Basics of mass spectrometry imaging and its applications."
Shuichi Shimma (The University of Osaka)

Symposium
Immune toxicity involvement in brain dysfunction
1) "Oligodendrocyte Toxicology -Beyond Microglia-."
Yasuhiro Ishihara (Hiroshima University)
2) Assessment of microglial morphology and function using in vivo whole-brain imaging of larval zebrafish
Yuhei Nishimura (Mie University)
3) <TBA>
Gi-Wook Hwang (Tohoku Medical and Pharmaceutical University)
4) "Mechanistic insights into brain function modulation driven by immune system activation."
Michio Miyajima (The University of Tokyo)

Special lecture of the recipient of the 15th JSIT award
"Immunotoxicological mechanisms and integrated health risk assessment of allergy and inflammatory immune dysregulation induced by heavy metals, chemicals, and nutrients."
Hiroyuki Yanagisawa (The Jikei University School of Medicine)

Special lectures of the recipients of the 15th JSIT prize for encouragement
"Investigation of unclarified risk factors regulating the onset of HLA-mediated drug-induced immunotoxicity."
Takeshi Susukida (University of Toyama)

Workshop: Trends in the development of respiratory sensitization tests and domestic initiatives

1) "The feature and detection methods of respiratory sensitization induced by chemicals including pesticides."
   Tomoki Fukuyama (Azabu University)
2) "Development of the detailed review paper at OECD and activities of JaCVAM respiratory sensitization test editorial committee."
   Eiko Koike (National Institute for Environmental Studies)
3) "Utilization of reconstructed Human bronchial epithelium in *in vitro* evaluation of respiratory sensitization potential of chemicals."
   Shinkichi Ishikawa (Japan Tobacco Inc.)
4) "Development of the 3D coculture systems (DCsens and DC/Tsens) capable of accurately predicting chemical respiratory sensitizing potential."
   Izuru Mizoguchi (Tokyo Medical Univerity)
5) Comprehensive discussion

Oral sessions of young scientist
Oral presentations
Poster presentations
Luncheon seminars

7. Social gathering

Hotel Grandvert Gizan
September 4, 18:30–20:30 (tentative)
https://grandvert.com/

The 14th Japanese Society of Immunotoxicology Award
(The 2024 JSIT Award)

Immunotoxicology; the origins of medicine that protects lives in interaction with environment. -From segmented research to research with an integrated perspective-

Takahiko Yoshida
Asahikawa Medical University

The importance of an appropriate environment and good lifestyle habits in maintaining human health advocated by Hippocrates was a key concept in early medicine. With the invention of the microscope in the late 16th century, bacteria and microorganisms were discovered in 1976, but at that time there was no idea that they could cause infectious diseases. However, the concept of infection control spread, with the idea that washing and disinfecting hands and instruments during childbirth or wound treatment could prevent infectious diseases. The emergence of the microbial etiology theory linking pathogenic microorganisms to infectious diseases had to wait until the invention of pasteurization by L. Pasteur (1862) and the proposal of Koch's postulates by R. Koch and his proof that anthrax was caused by bacterial infection (1876). Therefore, maintaining a proper environment and lifestyle is considered the basis of practical medicine in terms of preventing health problems.

The activities of two great researcher in medical history who tackled the cholera epidemic from different etiological theories – M.J. von Pettenkoffer, the founder of hygiene who inherited exploring hygienic environments to protect health and R. Koch, the founder of bacteriology who discovered the pathogenicity of the cholera bacterium - led to advances in infectious disease control and the development of immunology. Here, there is an intersection between hygiene and immunology, and environmental factors are the factors that connect them. This highlights the importance of immunotoxicology, the study of the effects of external factors on the immune system.

From the mid-1960s to the 1970s, articles appeared on the environmental chemicals and the increase in infectious diseases, and in the 1970s, articles increased also in carcinogenesis. This led to the establishment of the concept of compromised host in the

mid-1970s and 1980s, and simultaneously established the field of immunotoxicology. Due to the complexity of the pathogenesis of immunotoxicity caused by exogenous agents, it is not easy to identify chemical contaminants as the cause of health effects observed epidemiologically in the environment of human populations.

Review article on the immunotoxicity of environmental chemicals was published in 1978 (F.W. Sunderman Jr., "Carcinogenic effects of metals"), and followed by studies on metals (Pb, Cd, Hg, As, etc.) in the early 1980s and continue to this day. From the mid-1980s, reviews on organochlorine environmental chemicals appeared, and are on the rise. The target chemicals and immunotoxicity change depending on what is of interest at the time. Recent epidemiological reports have shown that air pollution exacerbates the severity of infections caused by SARS-Cov-1 and SARS-Cov-2 and that AhR is involved in this mechanism. Some reports showed that exposure to organophosphate compounds exacerbates the viral infection by inhibiting antiviral immune response through oxidative stress.

Advances in molecular biological technology have made it possible to observe detailed structures and to analyze biological phenomena through molecular dynamics and signal transduction even in immunotoxicity. On the other hand, overlap between immune responses and biological responses as a general toxicity caused by exposure to environmental chemicals has also been elucidated. It is as if environmental pollutants and pathogenic microorganisms work together to threaten human health. Now is the time for immunotoxicology to address these phenomena. To "protect human health" in relation to the environment, there are high expectations for immunotoxicology, which takes a comprehensive perspective that combines the micro and macro aspects.

The 14th Japanese Society of Immunotoxicology Prize for Encouragement

Molecular mechanisms of pathogen sensors-mediated cytokine production from innate immune cells

Izumi Sasaki
Department of Immunology
Institute of Advanced Medicine
Wakayama Medical University

It is my great pleasure and honor to be awarded the 14th JSIT prize for Encouragement for the year 2024. I would like to sincerely express my appreciation to the selection committee members and to Dr. Shigeki Aoki and Prof. Yasuo Yoshioka who recommended me. I deeply appreciated to our Wakayama medical university Department Immunology Lab members including Prof. Tsuneyasu Kaisho, and all collaborators.

Cholera toxin (CT) is a bacterial exotoxin comprised of one A and five B subunits. CTB binds the cell-surface ganglioside GM1, enabling internalization of CT and subsequent dissociation of CTA to elicit diarrheal disease called by cholera. On the other hand, CT acts as a potent immune adjuvant that activates T cells, B cell and induces production of pro-inflammatory cytokines. Previously, we have found that CT can induce production of interleukin-1β (IL-1β), a proinflammatory cytokine, in synergy with a lipopolysaccharide (LPS), from murine resident peritoneal macrophages (RPMs) in a GM1-dependent manner. IL-1β production depends on an inflammasome complex, which comprises a sensor, a protease precursor, procaspase-1, and an adaptor, apoptosis-associated speck-like protein containing CARD (ASC). The sensors include nucleotide-binding oligomerization domain-like receptors (NLRs) such as NLRP3, absent in melanoma 2 (AIM2), or pyrin. We have then investigated which inflammasome is involved in CT-induced IL-1β production by using inflammasome component-deficient mice and clarified that not only the NLRP3 inflammasome but also the pyrin inflammasome were involved in CT-induced IL-1β production. However, how CT activates these inflammasomes in the macrophages has been unclear.

Here, we first characterized various inflammasome responses such as pro-IL-1β processing, procaspase-1 cleavage, gasdermin D (GSDMD) cleavage, pyroptosis, or ASC

speck formation induced by CT. For comparison, adenosine triphosphate (ATP) and Clostridium difficile toxin B (TcdB), which activate NLRP3 and pyrin inflammasomes, respectively, were utilized. We found that CT mainly induces secretion of mature IL-1β with mild levels of procaspase-1 cleavage, without induction of gasdermin D (GSDMD) cleavage, pyroptosis, or ASC speck formation. Analysis of NLRP3 specific inhibitor MCC950 revealed that NLRP3 inflammasome is partially involved in CT-induced procaspase-1 cleavage and IL-1β production. Then, pyrin inflammasome is required for CT-induced procaspase-1 cleavage and IL-1β production by analyzing pyrin-deficient mice.

Next, we performed comprehensive analysis of CT-induced genes in LPS-primed RPMs. In the stimulation of CT+LPS, the expression of 853 genes was more than 2-fold upregulated with significance compared with LPS alone stimulation. Pathway analysis of these genes showed enrichment for the endoplasmic reticulum (ER) stress-related genes. The ER stress sensors include inositol-requiring enzyme-1α (IRE1α) and protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK). IRE1α activation leads to X-box binding protein 1 (XBP1) mRNA splicing and generate transcription factor XBP1s. We found that CT induces XBP1 mRNA splicing and the generation of XBP1s through GM1 and IRE1α-dependent manner. IRE1α inhibitor decreased not only XBP1 mRNA splicing but also IL-1β production in RPMs stimulated with CT+LPS. CT is incorporated into ER, depending on GM1. IRE1α-deficient RPMs showed a significant impairment of CT- or ATP- or TcdB-induced IL-1β production, indicating that IRE1α was required for NLRP3 or pyrin inflammasome-mediated IL-1β production from RPMs. These findings should contribute not only to the understanding of the molecular mechanisms on CT-induced immune adjuvant effects but also to clarification of novel roles of IRE1α in immune responses and pathogenesis of inflammatory disease involving NLRP3 or pyrin inflammasome activation.

Encouraged by this award, I'm going to continue my research to elucidate the molecular mechanisms involved in pathogen sensors-mediated cytokine production from innate immune cells.

The Best Presentation Award (JSIT 2024)

Self-assembling peptide CK2 contributes to the induction of antigen-specific cytotoxic T lymphocyte as a carrier of adjuvant and antigen.

Dr. Yasuda (left) and Prof. Kuroda (right)

Koubun Yasuda
Hyogo Medical University, Japan

I am deeply honored to receive The Best Presentation Award at the 31st Annual Meeting of Japanese Society of Immunotoxicology. I would like to sincerely appreciate the support from the selection committee. This was a big surprise to me because I am a faculty member of the laboratory of Professor Kuroda, the president of this meeting, and I was working as a stuff member during the meeting.

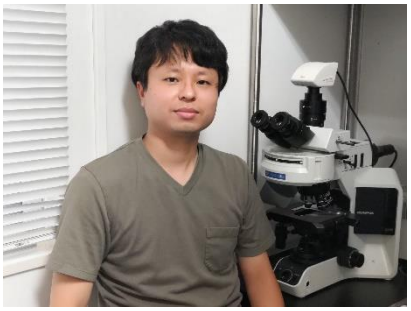
For a potent induction of immunity, an adjuvant is required in addition to the antigen, but most adjuvants have side-effect problems. In this study, we investigated the potential of self-assembling peptide CK2, which is safe and retains antigen in vivo for a long period of time and induces antibody production, as a new carrier. CK2 by itself is not particularly reactive to the body, but when mixed with antigen and administered subcutaneously, it shows robust induction of Ovalbumin (OVA)-specific antibody production, as do aluminum hydroxide and AddaVax. To induce a Th1-type/cytotoxic T cell (CTL)-dominated immune response that reduces allergy-causing IgE and is effective against viral and bacterial infections, we added a nucleic acid adjuvant CpG-DNA(K3), which induces IL-12 expression in dendritic cells, to CK2 and immunized with the antigen. Mixing K3 with CK2 resulted in no detectable production of OVA-specific IgE, a slight increase in IgG1, and a marked induction of IgG2c production compared to CK2 alone. Furthermore, intranasal OVA administration significantly increased H-2Kb-OVA (SIINFEKL)-tetramer-positive CD8-positive cells in the lungs of K3/CK2/OVA-immunized mice. Stimulation of these lung cells with OVA peptide, increased IFNγ production in both CD4- and CD8-positive T cells, indicating that mixing K3 with CK2

induces a Th1-type/CTL-dominated immune response. Thus, CK2 has shown potential as an effective carrier to hold not only antigens but also adjuvants at the site of administration and is expected to be a new immunomodulation method in combination with various adjuvants.

At this meeting, we reported on the vaccine effect of the mixture of CK2 and K3. CK2 is a hydrogel developed by Menicon Co., Ltd. The component is a peptide SPG-178 consisting of 13 amino acids, which self-assembles in aqueous solution to form a ß-sheet, which further entangles into fibers to form a gel. Since it is a chemically synthesized peptide, it does not contain any animal-derived components and is colorless, transparent, and neutral. They are highly stable and can be stored at room temperature, and their various safety characteristics (cytotoxicity, sensitization, irritation, acute/subacute systemic toxicity, chronic/subchronic systemic toxicity, genotoxicity, implantation, pyrogenicity, and blood compatibility) have been confirmed. Due to its transparency, stability, and safety, it has received medical device approval (approval number: 30600BZX00110000) as “SEERS”, an ophthalmic field-of-view securing agent used to reduce the inflow of blood into the surgical site during glaucoma ophthalmic surgery to facilitate observation.

Immunotoxicological Research

Evaluation of developmental neurotoxicity using neuronal differentiation reporter mice.



Keishi Ishida
Laboratory of Hygienic Chemistry and Molecular Toxicology
Gifu Pharmaceutical University

Current guideline tests for in vivo developmental neurotoxicity (DNT), such as OECD TG426, are not routinely conducted in chemical risk assessment because they are time-consuming, labor-intensive, and require large numbers of animals. Therefore, new methodologies are needed that can detect and evaluate the DNT potential of chemicals in a simpler, more quantitative, and more objective manner. To this end, we generated neuronal differentiation reporter mice (Syn-Rep mice) that express luciferase (Luc) under the control of the neuronal differentiation marker. Then we evaluated their utility as a tool for detecting chemical-induced DNT.

We evaluated the Luc expression profiles in the developing brain of Syn-Rep mice. Brain Luc expression levels in Syn-Rep mice increased dramatically from just before to after birth, peaked early in the postnatal period, subsequently decreased, and then remained at stable level after weaning. It is thought that this expression profile corresponds to the generally recognized temporal changes in synapse number in the developing mammalian brain. Next, to assess the responsiveness of Luc expression in Syn-Rep mice during DNT induction, we administered valproic acid (VPA), a reference DNT-inducing chemical, to pregnant mice and evaluated its effect on Luc expression in the developing brains of Syn-Rep pups. In vivo luminescence in the brains of VPA-exposed pups was significantly lower than in controls. Moreover, Luc expression in the prefrontal cortexes of 8-week-old VPA-exposed pups was significantly lower than in controls, reflecting the reduced number of neurons in the prefrontal cortex. These results suggest that Syn-Rep mice are expected to be useful tools for the rational detection of chemically induced DNT in the developing mammalian brain.

Recently, epidemiological studies have revealed that maternal immune activation (MIA) during pregnancy increases the risk of neurodevelopmental disorders. There is growing concern that MIA may also be related to chemical-induced DNT; however, the

specific chemicals that trigger MIA and the detailed mechanism underlying MIA-induced DNT remain unknown. To address these issues, we are currently investigating the effects of MIA on brain development using Syn-Rep mice. In the future, we aim to elucidate the mechanisms underlying DNT caused by chemical-induced MIA, and to establish novel and effective endpoints for its detection. Ultimately, we hope that our study will contribute to improving chemical risk assessment for DNT.