ImmunoTox Letter Digest

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Report on the 29th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT 2022)

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School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Japan

The 29th Annual Meeting of the Japanese Society of Immunotoxicology was held in Sapporo for two days, September 12th (Mon) and 13th (Tue), 2022. Since the spread of coronavirus infections decreased, this meeting was fortunately held on-site after two years' web-meetings. The final number of abstract submissions was more than those in the web-meetings, and was similar to those in the on-site meetings before pandemic of coronavirus. We are really grateful that we were able to hold the annual meeting successfully with the cooperation and support of many people.

At this meeting, symposium, special lectures and educational lectures were planned with the main theme of "Immunotoxicity and Diseases - Leave our footmark". Symposium was titled "Environment, Immunity and Diseases". Dr. Takehiro Suzuki (National Institute for Environmental Studies) gave a lecture on "Multigenerational effects and epigenetic modification changes by gestational inorganic arsenic exposure", Dr. Naoki Takemura (Graduate School of Pharmaceutical Sciences, Osaka University) gave a lecture on "Understanding and controlling environmental particle-induced inflammatory diseases", Dr. Chikako Shimokawa (National Institute of Infectious Diseases) gave a lecture on "Suppressive mechanisms on autoimmune diseases by helminth infection", Dr. Hiroto Hatakeyama (Graduate School of Pharmaceutical Sciences, Chiba University) gave a lecture on "Exacerbation of an immune checkpoint inhibitor-induced anaphylaxis by tumor-associated myeloid cells in tumor-bearing mice models", Dr. Michihiko Aoyama (National Institute of Health Sciences) gave a lecture on "Off-target cytotoxicity of antibody-drug conjugates", and Dr. Shunsuke Ito (Chugai Pharmaceutical Co., Ltd.) gave a lecture on "Involvement of Fc receptors in the immunotoxicity of biopharmaceuticals".

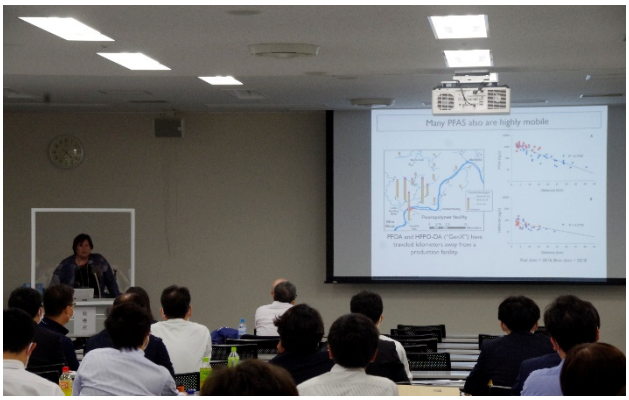
Special lectures of the annual meeting were "Indigo treatment targeting the mucosal healing of ulcerative colitis; Yin and yang" by Dr. Takanori Kanai (Keio University School of Medicine) and "Protecting public health from per- and polyfluoroalkyl substances : Focus on immunotoxicity" by Dr. Jamie C. DeWitt (East Carolina University), who was sent by SOT-ITSS. Educational lectures of the annual meeting were "Understanding efficacy, effectiveness and safety

of vaccines: from an epidemiological perspective" by Dr. Wakaba Fukushima (Osaka Metropolitan University Graduate School of Medicine) and "How far can cancer be prevented?" by Dr. Masahiro Asaka (Health Sciences University of Hokkaido).

Workshop on the test methods was titled "Challenges of immunotoxicity assessment for diverse pharmaceutical modalities", Drs. Yoshihiro Takahashi (Shin Nippon Biomedical Laboratories, Ltd.), Tetsuo Aida (Daiichi Sankyo Co., LTD.), Chiyomi Kubo (Chugai Pharmaceutical Co., Ltd.), and Shogo Matsumura (Astellas Pharma Inc.) presented on several issues of *in vivo* and *in vitro* test methods for immunotoxicity evaluation of biopharmaceuticals and so on. The comprehensive discussion had a precious time to improve our understanding.

General session included 7 oral presentations and 19 posters, and Young Scientist Session included 13 presentations which were to be presented as oral presentations and/or posters. In general session, fifteen minutes was given for each talk to ensure enough discussion time as previous annual meetings. As a result, Dr. Toshinobu Kuroishi (Graduate School of Dentistry, Tohoku University) received the Best Presenter Award. In addition, Mr. Issei Omori (Graduate School of Engineering, Kyoto University) and Mr. Shin-Ichiro Yamaguchi (Graduate School of Pharmaceutical Sciences, Ritsumeikan University) received the best Student and Young Scientist Presentation Award.

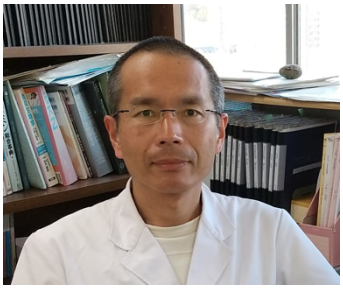
We would like to express sincere appreciation for attendants who actively discuss both oral and poster presentations. Totally over 110 people attended this on-site meeting. After all, I felt a lot of advantages of on-site meeting, the so-called "face to face". We wish the further development of the Society.



Photo, Situation of presentation by Dr. Jamie C. DeWitt at the meeting room.

The Best Presentation Award

Ni-binding protein CXCL4 and vitamin D3 analogue augment sublingual immunotherapy for Ni allergy



Toshinobu Kuroishi
Division of Oral Immunology
Tohoku University Graduate School of Dentistry

Metal allergens induce Th1-dependent type-IV allergies. The most frequent metal allergen is nickel (Ni). By taking advantage of pro-tolerogenic features of the oral mucosa, allergen-specific sublingual immunotherapy (SLIT) has become a promising non-invasive, safe, and effective therapy for allergies. Our laboratory previously reported the identification of antigen-presenting cells in the oral mucosa and the mechanisms by which SLIT induced allergen-specific regulatory T (Treg) cells. In this study, we investigated the preventive and therapeutic effects of SLIT on an Ni allergy mouse model.

An Ni allergy mouse model was constructed as reported previously. Briefly, mice were sensitized with an intraperitoneal injection of solution containing Ni and lipopolysaccharide. Two weeks after sensitization, mice were challenged with Ni using an i.d. injection into both pinnae. Ear swelling was measured 48 h after the challenge. SLIT was performed by applying an antigen solution under the tongue of anesthetized mice.

First, we analyzed whether SLIT can induce immunotolerance to Ni ions. Naïve mice were treated using SLIT, then Ni allergy was induced. We used an Ni-binding protein CXCL4 as an additive to SLIT. SLIT with [Ni + CXCL4], but not Ni alone, significantly inhibited ear swelling after Ni challenge, indicating that immunotolerance to Ni was induced by SLIT with [Ni + CXCL4].

To further analyze the induction of Ni-tolerance by SLIT with [Ni + CXCL4], adaptive transfer experiments were performed. Mice were treated using SLIT followed by Ni-sensitization. Non-Treg $CD4^+$ cells were sorted from the spleen and transferred to recipient mice. The recipient mice were sensitized and challenged with Ni. Mice receiving non-Treg $CD4^+$ cells from control mice

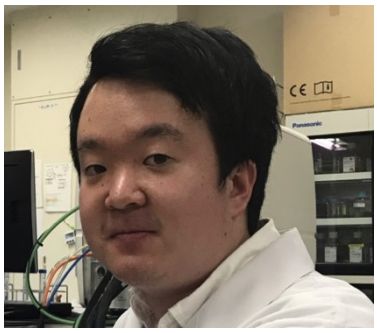
or mice treated using Ni-SLIT showed ear swelling after Ni challenge. On the other hand, no significant ear swelling was induced in recipient mice receiving non-Treg $CD4^+$ cells from mice treated using [Ni + CXCL4]-SLIT. These results indicated that Ni-specific $CD4^+$ cells were not induced in mice treated using [Ni + CXCL4]-SLIT. Treg transfer experiments revealed that Treg cells induced in the draining mandibular lymph nodes were indispensable in SLIT-induced immune tolerance to Ni.

To investigate the therapeutic effects, SLIT was applied after the induction of Ni allergy. No therapeutic effects were detected in mice treated using [Ni + CXCL4]-SLIT. However, the addition of calcipotriol, a vitamin D3 analogue, to [Ni + CXCL4]-SLIT showed significant therapeutic effects on Ni allergy. These results indicated that calcipotriol augments the therapeutic effects of SLIT. Moreover, these therapeutic effects were detected even after 6 months, suggesting that the therapeutic effects were not temporary desensitization, but permanent tolerance.

Our findings suggested that SLIT with metal-binding proteins and a vitamin D3 analogue is promising as an effective therapy for metal allergies.

The Student and Young Scientists Award

Macrophage responses to single-walled carbon nanotubes and multi-walled carbon nanotubes



Shin-Ichiro Yamaguchi
Laboratory of Immunology and Microbiology,
Graduate School of Pharmacy, Ritsumeikan University

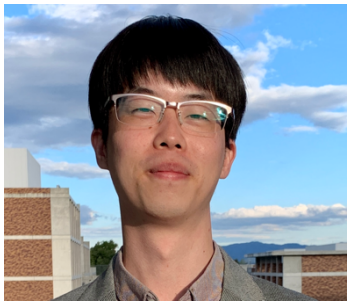
Multi-walled carbon nanotubes (MWCNTs), the highly representative products of nanotechnology, induce macrophage cell death and NLRP3 inflammasome activation leading to granuloma and mesothelioma in rodents[1),2)]. Single-walled CNTs (SWCNTs) are considered to be less toxic[3)]. SWCNTs and MWCNTs show different toxicity; however, the molecular mechanisms remain unknown. Recently we identified T cell immunoglobulin mucin（Tim）4 and Tim1 as CNT receptors in mice[4)]. Here we identify a Tim4-like CNT receptor CNT-R1. The gain-of-function assay using THP-1 cells ectopically expressing CNT-R1 showed that CNT-R1 mediated phagocytosis both of SWCNTs and MWCNTs by THP-1 cells; however, these THP-1 inflammatory responses were different. MWCNTs but not SWCNTs caused lysosomal damages and NLRP3 inflammasome activation, resulting in IL-1b secretion. On the other hand, both MWNCTs and SWCNTs induced IL-8 secretion. Taken together, these results suggest that SWCNTs induce only CNT-R1-signal whereas MWCNTs induce both CNT-R1 signal and lysosomal damages, which may explain the difference in toxicity between SWCNTs and MWCNTs.

References

1) De Volder et al., *Science*, vol. 339, pp535-539, 2013.
2) Poland et al., *Nat. Nanotechnol*., vol. 3, pp423-428, 2008.
3) Genady et al., *ACS Appl. Nano Mater.*, vol. 3, pp11819-11824, 2020.
4) Omori et al., *Cell Rep.*, vol. 34, pp108734-108754, 2021.

The Student and Young Scientists Award

Effects of particulate matters on the expression and function of COVID-19-related proteins



Issei Omori
Graduate School of Engineering,
Kyoto University

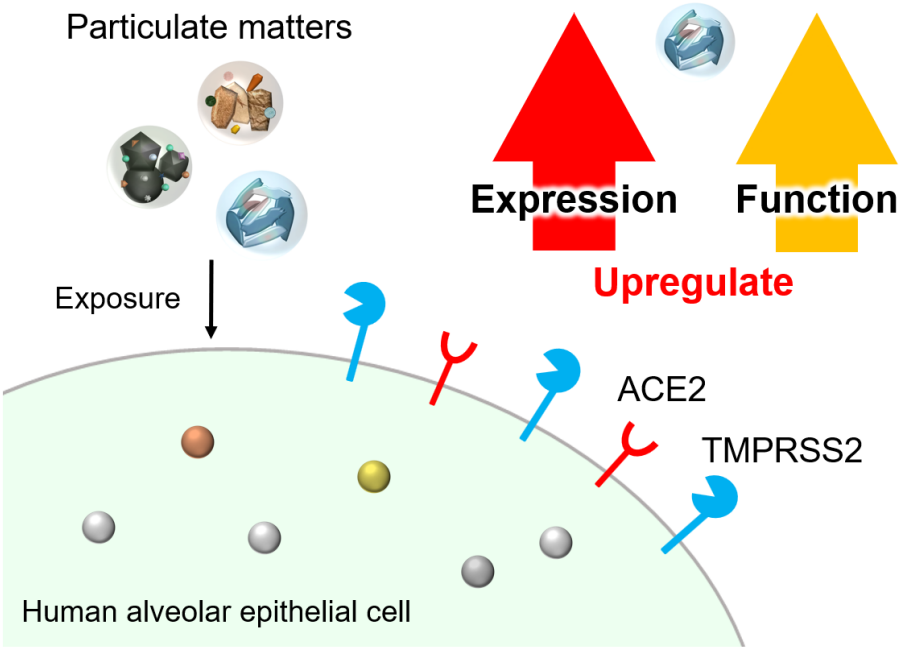
It was my honor to receive the Student and Young Scientists Award at the 29th annual meeting of the Japanese Society of Immunotoxicology. I would like to appreciate president Dr. Kojima and the selection committee.

After the outbreak of coronavirus disease 2019 (COVID-19), many epidemiological studies were conducted to determine the association between COVID-19 and air pollution across the world. They showed that short and long-term exposure to PM2.5 or PM10 is associated with COVID-19 onset, severity, and mortality. However, the mechanism by which particulate matters (PMs) affect COVID-19 aggravation is not clear.

Our research group focused on the intracellular entry mechanism of the SARS-CoV-2 virus. Viral spike proteins bind to ACE2 receptors on the surface of the host cells. After that, the spike proteins are cleaved by the TMPRSS2 on the host cells, resulting in viral genome invasion into the cells. Thus, ACE2 and TMPRSS2 play an important role in the intracellular entry of SARS-CoV-2. We have revealed that exposure to ambient PMs upregulates ACE2 and TMPRSS2 in the murine lung (*Environ. Res.*, **195**, 110722, 2021). However, the component of ambient PMs that affects ACE2 and TMPRSS2 expression levels and the variation of ACE2 and TMPRSS2 functional activity were still unclear.

In the study, human alveolar epithelial cells were exposed to titanium dioxide (TiO_2), diesel exhaust particle (DEP), and Asian sand dust (ASD). TiO_2 nanoparticles have a wide range of applications such as personal care products, food additives, and paints. DEP and ASD are major ambient PM constituents and they contain many kinds of chemical and biological components such as sulfates, nitrates, metals, and microorganisms. Expression and function assay of ACE2

and TMPRSS2 showed that some of these PMs can promote the intracellular entry of SARS-CoV-2. Thus, we revealed one possible mechanism by which PMs affect COVID-19 aggravation.



Particulate matters promote intracellular entry of SARS-CoV-2

Real Voices of International Immunotoxicologists

Jamie DeWitt

Immunotoxicology is a boundary field, and so it's important to exchange some information obtained from one's experience each other. Additionally, young immunotoxicologists and students may hope to know a hint: what they should do now and in future? In particular, we have less chance to know actual experience of international and senior immunotoxicologists, that're "**Real Voices**". This time, we interviewed Dr. Jamie DeWitt from East Carolina University, USA. Let's go to listen to his voice. What will you feel and learn?

(Check the message on the board behind Jamie with her smile, very nice!)

Jamie DeWitt, PhD, DABT
Professor
Department of Pharmacology and Toxicology
Brody School of Medicine
East Carolina University
Greenville, NC, USA

Q1. What was the most impressive event for you in your trip to Japan this time?

This was my first ever trip to Japan so it all was impressive! However, I think that what impressed me the most was how welcoming and friendly everyone was to me and my husband, who traveled with me. Professor Kojima was the most wonderful host who took care of each and every detail so that my husband and I could enjoy the science as well as the experience of being in Hokkaido

for the first time. The scientists who we met at the JSIT meeting and at Hokkaido University shared not only their amazing scientific work but their love of immunotoxicology and their kindness toward us as visitors. I really enjoyed my first dinner in Hokkaido as I was able to try many different delicious foods that I wouldn't normally find in the U.S. I loved everything that I tried!

Q2. What is the most exciting thing in your career to date?

My work over the past decade has focused on the immunotoxicological effects of per- and polyfluoroalkyl substances (PFAS) and we have been working to understand why T cell-dependent antibody responses (TDAR) are suppressed after exposure to PFAS. We have received funding to try to uncover some of the molecular mechanisms underlying this suppression and we've been focusing on the immunometabolic processes that PFAS might be disturbing as B cells transition from naïve B cells to antibody secreting plasma cells. It's been exhilarating to develop protocols to evaluate mitochondrial function within B cells! As a result of this work, we've also obtained funding to develop a cell-based assay for the TDAR based on the B cell metabolic "fingerprint." I'm excited to take our research in a more biotechnology-focused direction that I think will add to the immunotoxicological toolbox for more rapidly screening chemicals of potential immunotoxicological concern.

Q3. What are the things you are doing energetically, right now?

Again, most of our work focuses on the immunotoxicological effects of PFAS. We spend most of our time and energy trying to describe the effects of understudied PFAS that have been found in drinking water sources here in North Carolina in the US. Some of these PFAS have been measured in the blood of people drinking the contaminated water and there is little to no toxicological information available for these PFAS! People living in communities impacted by PFAS contamination are concerned about the effects of PFAS exposure on their health and many are scared because of knowledge gaps that exist for some of these PFAS. We are working very hard to generate high quality data that can inform decision-making so that our state leaders can decide if they need to move forward with measures to reduce exposures to these understudied PFAS or not. We also are working with many of different scientists to ensure that our research on PFAS addresses many unanswered questions and so that the pool of scientific data for PFAS is available to many different groups of stakeholders.

Q4. What is required for breakthrough in immunotoxicology research in the future, do you think?

New approaches to reduce in vivo experiments and to increases the speed at which different chemicals can be evaluated for potential immunotoxicity. Developing new approaches is especially important for developmental immunotoxicity (DIT) as we know that the developing immune system is very sensitive to perturbations. Right now, immunotoxicity testing is not required under many testing paradigms for industrial chemicals or pharmacological agents. Although there is a harmonized test guideline for evaluating immunotoxicity in adult organisms and DIT testing can be included in a harmonized test guideline to reproductive and developmental toxicity testing, these are not generally conducted unless a "red flag" is raised in other types of tests. For example, changes in lymphoid organ weights might trigger an evaluation of the TDAR. However, changes in lymphoid organ weights generally occur at higher administered doses than those that affect the TDAR. This means that we may be failing to recognize the immunotoxic potential of many different agents. If we had more rapid cell-based approaches for evaluating DIT and/or immunotoxicity, then immunotoxicity testing may become a required approach.

Q5. Any other comments?

When can I come back to Hokkaido? I thoroughly enjoyed my time there and want to say a very special thank you to Professor Kojima, Assistant Professor Kubota, and graduate student Wataru Murase for taking my husband and I on a very special trip around Hokkaido. We enjoyed talking about science, traveling, laughing, eating, drinking, and bathing (in the mineral baths) with our wonderful hosts!

Comment from a Special Honorary Member of JSIT

Jack H. Dean

It was a great pleasure to learn that I had been elected a Special Honorary Member of the Japanese Society of Immunotoxicology (JSIT). I will start by thanking Professor K. Nakamura for my nomination and this honor. For the past 25 years I have watched and been impressed with the growth, direction, and development of the JSIT from a small research group into an important national society that helped shape the direction of Immunotoxicology both in Japan and Internationally. I am grateful for both Professors K. Nakamura and T. Yoshida and others JSIT leaders for their continued cooperation with the Immunotoxicology Specialty Section (ITSS) of the Society of Toxicology (SOT). This cooperation encouraged joint symposium and exchange of researcher at both groups' national meetings.

As a founding member and first President of the ITSS (1988) it has been a great pleasure to watch the maturation of Immunotoxicology from an observational science describing chemical and drugs that produced immunotoxicity into a science defining molecular pathways targeted by these agents. These studies have also focused on the relevance of these observations in animals for man. The JSIT has been a significant contributor to this maturation and molecular focus in Immunotoxicology. Thank you again for this special honor.

Jack H. Dean, Ph.D., Sc.D. (Hon.), Diplo. ABT, Fellow ATS

Professor Jack Dean and wife Suellen on our patio in Arizona with the family dog

Comment from a Special Honorary Member of JSIT

Michael I. Luster

To my Japanese colleagues:

I am humbled and deeply grateful for your invitation to be an honorary member of the Japanese Society of Immunotoxicology. I look with great fondness when recollecting upon the development of the ITSS in the U.S., which only had a few members in the 1970s and was tenaciously spearheaded by my friend, Dr. Jack Dean and then later the formation of the JSIT in 1994 and the hard work of your first president, Dr. Hiroshi Nagura. Both societies not only have thrived but have interacted well over the years and have been instrumental in the continued development of Immunotoxicology as an important subdiscipline in toxicology. Over the past 25 years the JSIT has made many outstanding international contributions in both basic and applied research to help protect human health and the environment and I am sure will continue to do so. Between the JSIT and ITSS there are now well over 500 members.

I am also very grateful to the many Japanese scientists that I had the honor to work with in the laboratories at NIEHS/NIH and then later at NIOSH/CDC including Drs. Tadaki Sugawara, Wataru Toriumi and Yasuhide Kouchi. I particularly want to thank my good friends and long-time colleagues Drs. Takahiko Yoshida and Fujio Kayama for the many excellent scientific collaborations and friendly dinners we had over many years. I would also like to pay tribute to Dr. Motoyasu Ohsawa, who I first met in the 1980s when he was working on cadmium immunotoxicity and I was working with dioxins. Over the years we shared the stage at numerous conferences and had many wonderful conversations.

Again, thank you very much for this honor

ありがとうございました

Mike Luster