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# Report on the 26th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT 2019)

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The 26th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT) was held on Sept 8 – Sept.10, 2019 at Kitakyushu International Conference Center. This was the second Annual Meeting of JSIT in Kyushu since the 23rd meeting three years ago. I truly feel grateful for all your help and support that made this meeting successful.

Majority of members of the Japanese Society of Immunotoxicology is basic scientists and clinicians are only a few, however, collaboration with clinical medicine is crucial in studying the immunotoxicity in human. The main theme of the annual meeting was “Immunotoxicology, from basic science to clinical medicine” with the idea that this annual meeting would be an opportunity to help application of immunotoxicology as basic science to clinical medicine through communication and discussion of basic scientists with clinicians.

Lecture Open to the Public, prior to the annual meeting focused on immunotherapy of cancer, which has been attracting much attention following the Nobel Prize in Physiology or Medicine given on this topic. Experts gave lectures on the basis of immunology and immunotherapy of cancer, lung cancer and renal cancer, two main types of cancer where immune checkpoint inhibitors have been used. Many inquiries and applications to participate following the announcement of the Lecture Open to the Public let us know the high interest from general public. More than 70 people attended the lecture and active questions and answers session.

Educational lectures of the annual meeting were “Environmental factors and respiratory diseases” by Dr. Kazuhiro Yatera (UOEH) and “Pros and cons of biological agents in rheumatic diseases” by Dr. Yoshiya Tanaka (UOEH). Dr. James C. Bonner (North Carolina State University) who was sent by SOT ITSS gave a special lecture. His talk titled “Immunotoxicology of inhaled nanoparticles and the implications for lung disease susceptibility” focused on the effects of nanoparticles on human body, which is one of a major area of interests in immunotoxicology.

Symposium was titled “Inflammation and pathogenesis from the viewpoint of immunotoxicology”. Drs. Yasuhiro Yoshida (UOEH), Yasuo Morimoto (UOEH), Yasumitsu Nishimura (Kawasaki Medical College), and Maiko Hajime (UOEH) gave lectures and active discussion to better understand the inflammation and pathogenesis of the disease.

Workshop on test methods was titled “Adverse Outcome Pathway (AOP) of immunotoxicity and its goal”. Drs. Hajime Kojima (National Institute of Health Sciences), Shigeru Hisada (ASKA Pharmaceutical Co., Ltd.), Takumi Ohishi (BoZo Research Center), Shogo Matsumura (Astellas Pharma Inc.,), Setsuya Aiba (Tohoku University), and Takao Ashikaga (National Institute of Health Sciences) presented on the current status of AOP and AOP cases under development.

General session included 9 oral presentations and 18 posters. Fifteen minutes was given for each talk to ensure enough discussion time as previous annual meetings. Thirteen abstracts were submitted to the Students and Young Scientist Session, which forced us to limit time for each presentation to 5 minute combined with poster presentation. Poster presentation was on both dates due to its large number.

Dr. Tatsuya Ikuno (Chugai Pharmaceutical Co. Ltd.) received the Best Presenter Award and Mr. Kyosuke Yokozeki (Kao Corporation) and Ms. Minzeng Zhang (UOEH) received the best Student and Young Scientist Presentation Award.

JSIT Award lecture by Dr. Koichi Ueno (Chiba University) and JSIT Young Investigator Award lectures by Drs. Eita Sasaki (National Institute of Infectious Diseases) and Tomoko Fukuyama (Azabu University) were on the second day.

During the reception in the evening, a symposium scientist and laboratory technicians working on immunotoxicology research also did wonderful music performance.

We would like to express sincere appreciation for attendants who traveled long distance and adjusted their schedule due to typhoon in Kanto and Tokai areas. Despite the fact that no new attendants arrived from outside of Kitakyushu on the second day, 120 people attended the meeting. We really feel that the success of the meeting was thanks to your understanding and cooperation, as well as support from organizations and companies. We wish you continued success to close this report.

The Best Presentation Award

## Human induced pluripotent stem cell-derived mast cells useful for *in vitro* mast cell activation assay exhibiting phenotypes and morphological characteristics of human mast cells

Tatsuya Ikuno
Research Division, Chugai Pharmaceutical

Mast cells are key players in the inflammatory response with an important role in allergic reactions. Mast cells have many granules rich in histamine and heparin, and express high affinity-IgE receptors (FcεRI) on their cell surfaces which mediate their immunological activity. When antigens bind to IgE molecules on the surface of mast cells, linkages between IgE receptors are formed and granules or chemical mediators are released. This mechanism is fundamental to immediate-type allergic reactions and asthma. However, mast cells are difficult to isolate due to their low abundance and wide distribution in a variety of tissues. Because it is so difficult to obtain human mast cells with high purity in sufficient numbers, a lot of studies rely on rodent mast cells such as rat peritoneal mast cells or mouse bone marrow-derived cultured mast cells, human cord blood-derived mast cells, mouse and human embryonic stem (ES) cell-derived mast cells, and mouse induced pluripotent stem (miPS) cell-derived mast cells. However, in most cases, there are some issues, including species differences, low sensitivity, and so on. To overcome this, we generated and characterized mast cell-like cells derived from human induced pluripotent stem (hiPS) cells. These hiPS cell-derived mast cells (hiPS-MCs) were generated using recombinant human bone morphogenetic protein 4 (BMP4), vascular endothelial growth factor 165 (VEGF), stem cell factor (SCF), interleukin-4 (IL-4), interleukin-6 (IL-6), and interleukin-9 (IL-9) in a StemPro-34 medium. The hiPS-MCs exhibited the morphological characteristics of human mast cells, expressing FcεRI and mast cell markers such as tryptase, chymase, and CD117. In addition, FcεRI stimulation with agonistic anti-IgE functionally increased the expression of activation markers CD63 and CD203c, as well as the amount of released histamine. Our results show these hiPS-MCs exhibited not only the morphology and phenotypes of mast cells, but also their functional characteristics.

In conclusion, The hiPS-MCs generated in this study shared many of the same characteristics as human mast cells—especially in activation by FcεRI stimulation—and were more sensitive than mast cells from routine sources. We think the hiPS-MCs generated in this study will be useful for assessing the pharmacology and toxicity of anti-allergy medicines.

The Student and Young Scientists Award

## The importance of dose metrics in the forecasting onset risk rate of immediate-type hypersensitivity

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Safety Science Research Laboratories,
Kao Corporation

In order to accurately assess human health risks, it is necessary to appreciate the dose metrics that define the incidence of toxicity. Although the incidence of delayed-type hypersensitivity (skin sensitization) correlates with the antigen dose per unit area, the dose metric which correlates with incidence of percutaneous immediate-type hypersensitivity (antigenicity) remains unclear. Therefore, we investigated the dose metrics for accurately predicting the incidence of percutaneous immediate-type hypersensitivity.

Papain, which causes immediate-type hypersensitivity via percutaneous sensitization in humans, was used as the antigen. During the sensitization phase, the total dose or dose per unit area was adjusted to clarify the conditions that contribute to the incidence of immediate-type hypersensitivity. Then, in the elicitation phase, we performed passive cutaneous anaphylaxis (PCA) and ELISA for papain specific IgG1. The PCA test revealed that positive PCA reaction rates did not correlate with dose per unit area but with total dose. Papain-specific IgG1 levels also correlated with total dose. To elucidate the underlying mechanism that contributes to the dose metrics for predicting immediate-type hypersensitivity, Alexa488-labeled papain was applied in varying total doses or doses per unit area. The number of antigen-bearing B cells ($Alexa488^{+}B220^{+}$) in the draining lymph nodes was calculated using flow cytometry. FACS analysis showed that the number of antigen-bearing B cells in the draining lymph nodes correlated with total dose.

Taken together, these results clarify that the incidence of percutaneous immediate-type hypersensitivity correlates with the amount of total dosage, unlike the incidence of delayed-type hypersensitivity, which is defined by that of those per unit area. Although the process by which antigens activate dendritic cells in the skin is important for delayed-type hypersensitivity, the process by which antigens reach draining lymph nodes to activate B cells is important for percutaneous immediate-type hypersensitivity.

The Student and Young Scientists Award

**Role of methionine in human B cell differentiation and the relevance to pathological processes of SLE**

Mingzeng Zhang
The First Department of Internal Medicine,
School of Medicine,
University of Occupational & Environmental Health

It is my great pleasure and honor to be Student and Young Scientists Award at the 26th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT 2019). I would like to appreciate the selection committee and the researchers, who gave me lots of advices. Now, I'll introduce the awarded research topic.

Our research started when I was entered the University of Occupational and Environmental Health, Japan (UOEH). However, the First Department of Internal Medicine has focused immunotoxicology research for long time. Since the role of amino acid metabolism in the regulation of human B cell function remains elusive in terms of immunotoxicology. We studied amino acid metabolism in human B cell differentiation and relevance to the pathogenesis of SLE. In the in vitro arm of the study, purified $CD19^+$ B cells from healthy donors were cultured with TLR7/9 ligand (LOX or CpG), IFN-α and B cell receptor (BCR) cross-linking, in the presence or absence of amino acids. We determined 1) the types of amino acids that are important for PB differentiation, 2) the main signaling pathway(s) involved in the presence of amino acids, 3) the transcriptional factors used in the presence of amino acids. In the clinical arm of the study, peripheral blood mononuclear cells (PBMCs) were obtained from 24 patients with RA, 35 patients with SLE, and 28 age-matched healthy controls, and subjected to flow cytometric analysis to determine the expression of amino acids-related markers.

Stimulation with the combination of BCR, IFN-α and TLR7/9 ligand induced plasmablast differentiation accompanied by uptake of amino acids. Plasmablast differentiation was abrogated in the absence of essential amino acid methionine, and to a lesser extent leucine, but not in non-essential amino acid cystine. Previous studies reported that amino acids were perceived by the sensor, leading to mTORC1 phosphorylation. However, the mechanism by which amino acids activate other intracellular signaling pathways in B cells remains elusive. We found that methionine facilitated both the BCR and mTORC1 signals. In addition, the two signals

synergistically induced EZH2 expression, which is well known as a transcriptional factor for histone modification via induction of H3K27me3, in the presence of methionine. Methionine induced EZH2 expression, leading to suppression of BACH2, induction of BLIMP1, XBP1 and plasmablast differentiation. These results indicate that EZH2 is a critical factor for plasmablast differentiation in the presence of methionine. EZH2 expression in $CD19^{+}$ cells correlated with markers of disease activity and autoantibody production in SLE. The results indicate that methionine is important for plasmablast differentiation through the induction of methyltransferase EZH2, which is closely related to the pathogenesis of SLE.

In the end, I would like to express my deepest appreciation to Prof. Tanaka, Prof. Satoh, Dr. Iwata, and all of the people who involved, supported and advised me for my research.

Immunotoxicological Research

**TNF-α-stimulated macrophages undergo necroptosis-like death and secret 14-3-3η**

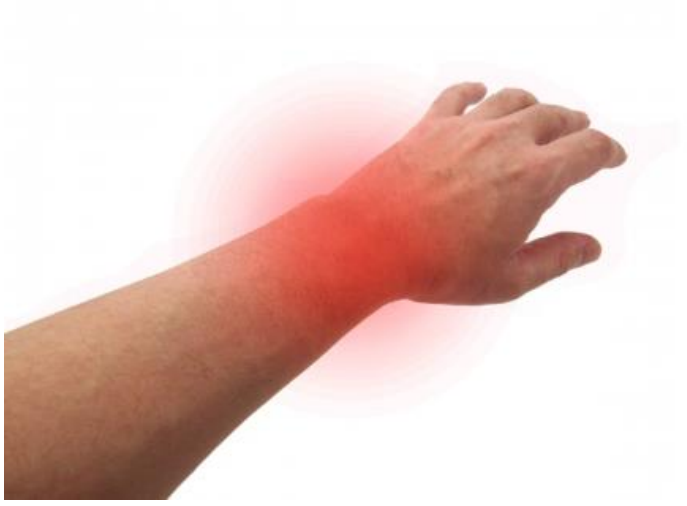
First Department of Internal Medicine,
University of Occupational and Environmental Health

I am honored to receive an invitation to write an article in ImmunoTox Letter. Many thanks for the great experience of participating and discussing our research work at the 26th Annual Meeting of the JSIT.

With the start of my PhD course at University of Occupational and Environmental Health, Japan in 2015, I began to study the intracellular chaperone 14-3-3eta. Elevated levels of serum 14-3-3eta have strong correlation with rheumatoid factor and anti-citrullinated protein autoantibodies (ACPA), established biomarkers of rheumatoid arthritis (RA), and disease activity. Thus, testing 14-3-3eta helps doctors detect RA at early stage and predict the outcome of treatment. 14-3-3eta belongs to 14-3-3 proteins family involved in various biological events of the cells such as cell cycle progression, intracellular protein trafficking, apoptosis, and DNA repair. 14-

3-3eta can be found in the extracellular space exclusively during disease condition; in the brain and cerebrospinal fluid in Alzheimer's disease and schizophrenia, in the articular fluid and serum in patients with RA. However, the source of 14-3-3η and the mechanism of its release into the extracellular space remain unclear. Our data showed that the RA synovial tissue had dense and widespread staining for 14-3-3eta protein. 14-3-3eta was abundantly expressed by synovial $CD68^+$ macrophages and co-localized with peptidylarginine deiminase 4 (PAD4), an enzyme inducing citrullination of proteins, which becomes a target for ACPA. Also, TNF-α but not any other cytokines promoted necroptosis of $CD68^+$ macrophages and secretion of 14-3-3eta. TNF-α is highly expressed in joints of patients with RA and activates PAD4, which may trigger 14-3-3eta citrullination with consequent production of autoantibodies to 14-3-3eta. Based on its involvement in the pathogenesis of RA, 14-3-3eta can be a promising target for the treatment of RA. Taken together, our data defined the secretion mechanism of 14-3-3eta and suggested its important role in the pathogenesis of RA.

I would like to express my gratitude to all my teachers for the opportunity to learn, gain new knowledge and people who was involved, supported and advised me for my research. Being in Japan, I get in touch with the unique culture of the Japanese people, which will remain in me and in the soul of every foreign student who has ever been to Japan. Thanks to Japan for the knowledge, kind warmth and care.



## Real Voices of International Immunotoxicologists

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. James C. Bonner from North Carolina State University, USA. Let's go to listen to his voice. What will you feel and learn?

James C. Bonner, Ph.D.
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**Q1. What was the most impressive event for you in your trip to Japan this time?**

There were so many impressive experiences during my first trip to Japan, both scientifically and culturally. Having the opportunity to serve as the guest speaker for the 26th annual Japanese Society of Immunotoxicology (JSIT) conference in Kitakyushu was a great honor. It was also very exciting to be an invited delegate for the International Exchange program between JSIT and the Society of Toxicology's Immunotoxicology Specialty Section (ITSS). This JSIT-ITSS program, which was found in 2005 by Professors Kazuichi Nakamura and Mitchell Cohen, has been fantastic for promoting opportunities for young scientists and students to network with international colleagues. My wife Anna was able to join me on this journey and we were made to feel incredibly welcome in Kitakyushu. Professor Minoru Satoh was a most gracious host for our visit and made us feel very welcome. The kindness shown to us by everyone at the JSIT conference was very special to us. The conference banquet and award ceremony was perhaps the most impressive event for me at the JSIT conference, since I was able to meet and have many

conversations with my new colleagues and friends. It was a lively event, with music, singing, wonderful food and drink, and laughter. Anna and I were so impressed by the incredibly talented entertainers. We enjoyed the performance by the musicians who played stringed instruments such as mandolins and guitars, and really liked hearing the beautiful singing voice of a performer who wore beautiful costumes including that of a white swan for her final song. This banquet was a fun, happy and friendly celebration that impressed me beyond words. I will always remember this experience and will always value this opportunity to meet new friends and colleagues. Anna and I also were enchanted by the city of Kitakyushu and had time to explore the vast street markets and visited Kokura Castle, which was spectacular. After departing Kitakyushu, we traveled by Shinkansen to Tokyo, where we joined Anna's mother, Shizuko Tsuruya Melton, who grew up in Tokyo before moving to North Carolina and marrying Anna's father Terry Melton. We were greeted in Tokyo by Shizuko's family (siblings, nieces and nephews) and had a wonderful dinner with all of the family which was a truly an impressive event. We also had a tour of Tokyo that included learning the Japanese tea ceremony and visiting the inspiring Buddhist temple Sensō-ji. This was the first time that Anna met her Japanese family and seeing her happiness in meeting her relatives will always be a treasured memory for me.

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

I have to say that the most exciting and gratifying thing in my career has been to serve a mentor for graduate students in toxicology. As scientists of course we enjoy our own personal journey of discovery, but there is nothing more enjoyable than seeing a student become engaged in their own journey of discovering something new. Their enthusiasm and excitement in learning new things also brings new energy to me and reinforces my own sense of purpose. Being a central part of my student's training as a toxicologist is the most valuable part of being a scientist and it is the most meaningful thing that I can do in my career. I really enjoy advising them on how to think critically and logically to explore the mechanisms through which pollutants cause adverse effects on biological systems. But in a larger sense, being a teacher, or sensei, is to serve as a guide for a student's intellectual development and teach students how to incorporate their research into the larger issue of how toxicants impact human health and the environment. It is gratifying to know that my students will be part of the change that will lead to a better future and a healthier world. In addition to mentoring, I am also very excited about developing new international collaborations to tackle problems in immunotoxicology. Over the past several years, I have collaborated with several European countries on the issue of nanotoxicology and I also enjoyed working on a World Health Organization criteria document on the immunotoxicity of nanomaterials that involved interaction with scientists from many countries, including Japan. Most recently, having the chance to interact with my colleagues at the JSIT conference has been very exciting for me.

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

Right now, and over the past year, I have been giving more thought and energy towards thinking about emerging issues in immunotoxicology that are major challenges for our planet. While studying the toxicology of engineered nanomaterials has been a focus of my lab at North Carolina State University for the past decade or more, there are other issues of importance to the environment and human health that have been gaining headlines in the news and getting my attention, such as the increasing problem of per- and poly-fluoroalkyl substances (PFAS), which are a class of man-made persistent 'forever chemicals' used in many 'non-stick' products. This issue has made an environmental impact close to my home in North Carolina, where industry sources are polluting the water and air with PFAS leading to accumulation in humans, wildlife and the environment. Now there are many scientists at NC State University studying various aspects of PFAS toxicity, including immunotoxicology. I am personally interested in learning how PFAS impact the innate immune function of macrophages in the lungs of mice and how PFAS exposure might result in susceptibility to air pollution particles. This is where some of my energy is now focused. I was also recently invited to join a group of scientists and wildlife biologists on a field trip for sampling PFAS contamination in alligators in eastern North Carolina. Getting out of the lab to join the professional 'alligator wranglers' for this catch and release operation, and having the experience of being allowed to sit atop a live gator to keep the animal still while the team took a blood sample was an energetic thrill! Other emerging issues are also of interest to me, such as the potential immunotoxic effects of micro- and nano-plastics in the environment and whether these pose a threat. All of these issues are energizing me and I hope that my lab or collaborative efforts can contribute in a positive way.

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

I think interdisciplinary research will be required for major breakthroughs in immunotoxicology research in the future and addressing some of the real-world problems related to toxic exposures. It is becoming increasingly clear that issues in immunotoxicology, such as toxicity caused by PFAS or engineered nanomaterials, will need the collaborative efforts of toxicologists, immunologists, environmental chemists, epidemiologists and engineers to work together to solve problems. Such multidisciplinary collaboration will allow immunotoxicologists to be maximally effective and make a real impact on a variety of problems that challenge our future generations. Also, as mentioned already, the value of international collaboration will be increasingly important to these multidisciplinary efforts in immunotoxicology.

**Q5. Any other comments**

I truly hope that the JSIT-ITSS international program will continue into the future. This is such a positive thing for addressing global challenges in immunotoxicology and training the next generation of toxicologists.