ImmunoTox Letter Digest

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Report on the 25th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT2018)

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The 25th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT) was held on September 18th and 19th, 2018 at International Congress Center in Tsukuba. As the main theme of this meeting "Discussing and deepening our insight into the interaction between the immune system and the environment" calls for, enthusiastic discussions were carried among participants on a variety of immune reactions themselves and in relation to environments inside and outside bodies.

The three topics featured in this meeting were "gut microbiota", "cancer immunotherapy" and "epigenetics". The first two topics were desired by many members and epigenetics was one I proposed. I appreciate all the outstanding speakers who kindly accepted our request taking time out of their busy schedule. All the sessions and talks were very impressive and instructive.

On the first day in Educational Lecture, Dr Akihiko Yoshimura (Keio University School of Medicine) gave a talk titled "Immune modulation by epigenetic modification of regulatory Tregs".

In the Symposium session "Gut microbiota and immune diseases - A new perspective on immunotoxicology", four talks were given: "Association between gut microbiota and environmental chemicals" by Dr Rie Yanagisawa (National Institute for Environmental Studies), "Relation between gut microbiome and allergic diseases in childhood: clinical perspective" by Dr Naoki Shimojo (Graduate School of Medicine, Chiba University), "Involvement of epithelial indigenous flora in allergy and inflammation" by Dr Akira Shibuya (TARA, Tsukuba University) and "Microbiota in immune disorders" by Dr Kiyoshi Takeda (Immunology Frontier Research Center, Osaka University).

On the second day, Special lecture was given by Dr B Page Lawrence (University of Rochester School of Medicine and Dentistry, USA). She was a representative of the researcher exchange program between the ITSS, SOT and JSIT. Her talk "Developmental exposure alters cellular processes critical for T cell functions, and affects some T cell properties across generations" was very new for many audience and exciting.

We were also provided and enjoyed video messages from ITSS executives, President Dr Jamie Dewitt (East Carolina University), Postdoctoral representative Dr Alessandro Venosa (University

of Pennsylvania), and Student representative Dr Alexa Murray (The State University of New Jersey).

In the workshop “Development of cancer immunotherapy and safety assessment of immune checkpoint inhibitors”, three speakers gave talks: Dr Hiroshi Shiku (Department of Immuno-Gene Therapy/Personalized Cancer Immunotherapy, Mie University) on “Guidances for Development of Cancer Immunotherapy”, Dr Kazuhiko Taguchi (Bristol-Myers Squibb K.K.) on “Nonclinical assessment of CTLA-4 and PD-1 inhibitors for predicting adverse events in clinical studies”, and Dr Minoru Satoh (Department of Clinical Nursing, University of Occupational and Environmental Health) on “Autoantibodies as biomarkers for predicting risk to develop autoimmune diseases following treatment with immune checkpoint inhibitors”. After their talks, the issues were further discussed as a panel discussion.

As a new program, we had International session, in which Dr Khaled Hossain (University of Rajshahi, Bangladesh）and Dr Myint Myint Nyein (University of Medicine 1, Myanmar) presented human data obtained in their countries.

On the second day, we had Award ceremony to honor Dr Reiko Teshima (Okayama University of Science) for the JSIT Research Award and Dr Kiyoshi Kushima (Astellas Pharma Inc, Astellas Research Institute of America) for the prize of Encouragement 2018. Dr Teshima gave award lecture titled “Immunotoxicological evaluation of food allergens”. Dr Kushima talked on “Promotion of Developmental ImmunoToxicology (DIT) assessment and Adverse Outcome Pathway (AOP)”.

On the night after the first day of meeting, we had a social gathering.

Young executives of JSIT performed a dance to a famous J-pop number “Choo Choo TRAIN”, which was a fun surprise.

The meeting gathered more than 130 participants. I would like to sincerely appreciate all who supported our meeting. Thank you very much.

The Best Presentation Award

IL-17-induced mRNA stabilization dictates the expression level of IκB-ζ in keratinocytes

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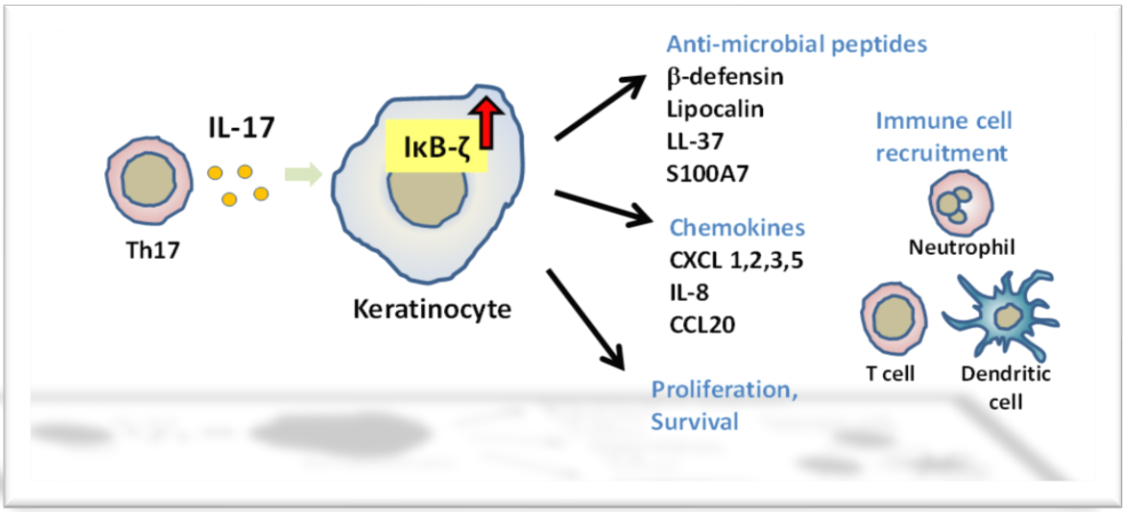
Interleukin-17A (IL-17) is an immune cell-derived cytokine that acts on various types of cells, including epidermal keratinocytes, and induces production of antimicrobial peptides and chemokines to induce antibacterial and antifungal defense responses. An excess of this IL-17 action leads to inflammatory skin disease such as psoriasis. Recently, we demonstrated that IκB-ζ, a nuclear IκB protein that modulates NF-κB-dependent transcription, is IL-17-inducible and mediates IL-17-induced responses. However, the mechanism controlling IκB-ζ expression in IL-17-stimulated cells remained unclear. It has been reported that the 3'-untranslated region (3'UTR) downstream of the protein coding sequence of IκB-ζ mRNA has an influence on the stability of IκB-ζ mRNA. In this study, we investigated whether and how the posttranscriptional regulation of mRNA stability is involved in the IL-17-mediated induction of IκB-ζ. A reporter plasmid (LUC-IκB-ζ 3'UTR), in which a 3'UTR sequence derived from murine IκB-ζ mRNA was ligated downstream of a luciferase, was used to examine the effects of IL-17 stimulation. The IL-17 stimulation showed a significant enhancement of the activity of 3'UTR-regulated reporter. On the other hand, IL-17 could not activate the activity of a luciferase reporter plasmid that is expressed under the control of the promoter region upstream of the transcription start site of the IκB-ζ gene. These data suggested that IL-17 enhances the stability of the IκB-ζ mRNA post-transcriptionally. In addition, our flow cytometric assay using fluorescent protein (Venus)-based reporter fused to the truncated IκB-ζ 3'UTR (1 - 150 nt from stop codon) showed that this portion of IκB-ζ 3'UTR can respond sufficiently to IL-17 stimulation. This portion has two characteristic stem-loop structures that are conserved between species and required for the recognition and degradation by a ribonuclease, Regnase-1/MCPIP1 (encoded by ZC3H12A). We mutated the two stem-loops in Venus-IκB-ζ 3'UTR reporter to disrupt these secondary structures and found that the loop-mutant reporter shows enhanced expression than the control intact reporter under unstimulated condition while it does not respond to IL-17. Knockdown of endogenous Regnase-1 in HaCaT cells by siRNA resulted in an accumulation of basal levels of IκB-ζ mRNA. These results suggest that Regnase-1-mediated degradation of IκB-ζ mRNA occurs constitutively. An IL-17 signaling adaptor protein ACT1 has been shown to mediate IL-17-induced upregulation of IκB-ζ in

keratinocytes, although it has not yet been examined whether the IκB-ζ 3'UTR-mediated mRNA stabilization is affected by ACT1 in IL-17-treated cells. We observed that IL-17-induced expression of Venus-IκB-ζ-3'UTR reporter was abrogated by ACT1 knockdown. In addition, overexpression of ACT1 counteracted the suppressing effect of Regnase-1 on the expression of Venus-IκB-ζ 3'UTR reporter. These results suggest that IL-17/ACT1 signaling counteracts the constitutively occurring Regnase-1-mediated degradation of IκB-ζ mRNA. These findings would improve our understanding of the molecular mechanisms of IL-17 action in immunity and chronic inflammation.



The Student and Young Scientists Award

nSP50-inducible hepatic damages would worsen via acquired immune system

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It's my honor to win the best presentation award among the young scientists at the 25th Annual Meeting of the Japanese Society of Immunotoxicology. I would like to appreciate the selection committee and the researchers, who gave me lots of advices, from the bottom of my heart. Now, I'll introduce the awarded research topic.

In recent years, the use of nanoparticles has been expanding in various industries such as medicine, foods, and cosmetics because of their innovative functions. On the other hand, their unexpected biological effects resulted from the refinement of the particle size have been concerned. From this viewpoint, safety analysis of nanoparticles has been studied intensively. And we revealed that silver nanoparticles could trigger metal allergy-like symptoms by sensitization of themselves, which is not induced by silver ion sensitization (T. Hirai et al., Nat. Nanotechnol., 2016). Given that the hazard induced by multiple exposures may be different from one by single exposure, hazard analysis focused on the multiple exposures-induced acquired immune systems is required. Thus, we attempted to assess the effect of amorphous silica nanoparticles (nSPs) on acquired immune responses. As a result, we showed that pretreatments of nSPs could worsen nSPs-inducible hepatic damage though there were no differences in immunodeficient mice. Moreover, this exacerbation disappeared by depletion of T cells. These results suggest that the pretreatment of nSPs would worsen nSPs-inducible hepatic damages through the acquired immune system, especially the cell-mediated immunity. Considering that we are exposed to nanoparticles many times on a daily basis, this exacerbation of acute toxicity has crucial meaning, that is, pre-exposure to nanoparticles has potential to trigger the transition of NOAEL (No Observable Adverse Effect Level) to lower doses. Now, we try to elucidate the mechanism of this exacerbation for developing optimal design of nanoparticles. We consider that our researches would contribute not only to the sustainable use of nanoparticles but also to the advancement of immunotoxicology.

The Student and Young Scientists Award

Facilitated antigen sensitization on the skin by triacylglycerol in an FITC-induced contact hypersensitivity mouse model.

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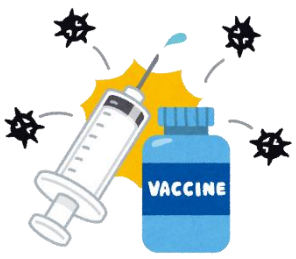
Aim: Triacyl glycerol (TAG) is glycerol ester with fatty acids. TAG is used in cosmetics and we have chances to contact with it on the skin. However, effects of TAG on cutaneous immunity are not well established. We investigated the effects of TAG on skin sensitization in an FITC-induced contact hypersensitivity mouse model. This model has been employed to reveal adjuvant effect of chemicals, such as dibutyl phthalate (DBP).

Materials and Methods: BALB/c mice were sensitized with FITC in acetone in the presence or absence of a test compound on days 0 and 7. On day 14, mice were challenged with FITC on an ear auricle to assess ear-swelling responses. Migration of FITC positive dendritic cells ($FITC^+DC$) to draining lymph nodes was analyzed by flow cytometer after staining with phycoerythrin (PE)-conjugated anti-CD11c.

Results and Discussion: TAG with short (carbon number C4 and C6) and medium (C8 and C10) chain fatty acids enhanced skin sensitization to FITC. These TAGs promoted $FITC^+DC$ migration to draining lymph node. Short chain TAGs appeared to exhibit stronger effects than the medium chain ones. In contrast, triolein did not enhance skin sensitization. Triolein suppressed the enhancing effect of tributyrin on FITC sensitization. These results suggest that TAG with different types of fatty acids causes different effects on the skin immunity.



Immunotoxicological Research

CpG-ODN contributes to Flucloxacillin-induced acute liver injury through FasL/Fas mediated pathway

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Idiosyncratic drug induced liver injury (IDILI) is often the major cause for drug withdraw which has been reported mostly immune-mediated. Despite of the rarity, IDILI sometimes would induce serious outcomes, even liver injury requiring transplantation or death. However, the pathogenesis of IDILI has not been clearly explained so far, which partially because of the lack of appropriate animal models. Thus, developing valid animal models is essential for better understanding the onset mechanisms.

Flucloxacillin (FLUX) is a Beta-lactam antibiotic of penicillin class which could induce rarely liver injury. Patients may develop liver injury on days 1 to 45 within FLUX treatment. So we tried to co-administrate FLUX and CpG-ODN (toll-like receptor 9 agonist) to C57BL/6J mice to reproduce early phase of FLUX-induced liver injury in animals, and investigate its pathogenesis in liver. Then we detected that co-administration of CpG-ODN with FLUX significantly elevated plasma ALT level in mice, while treatment of FLUX or CpG-ODN alone was insufficient to induce an ALT increase. Moreover, the co-administration also increased expression of Fas ligand on NKT cells and Fas on hepatocytes. When applying FasL-mutated gld/gld mice, no obvious hepatotoxicity was observed despite the co-administration of CpG-ODN with FLUX. Above, we developed an animal model of FLUX-induced acute liver injury by co-treatment of CpG-ODN. These results suggest that CpG-ODN accelerates FLUX-induced liver injury through FasL/Fas-mediated apoptosis pathway between NKT cells and hepatocytes, which plays an important role in this animal model.

Real Voices of International Immunotoxicologists

What the other researchers think about? How can I do more exciting research in the future? Most of the young Japanese immunotoxicologists may hope to listen to some kind of comments from the experts in this field, in particular from the outside of Japan. This time, we interviewed Dr. B. Paige Lawrence from University of Rochester, USA. Let's go to listen to her voice. What will you feel and learn?

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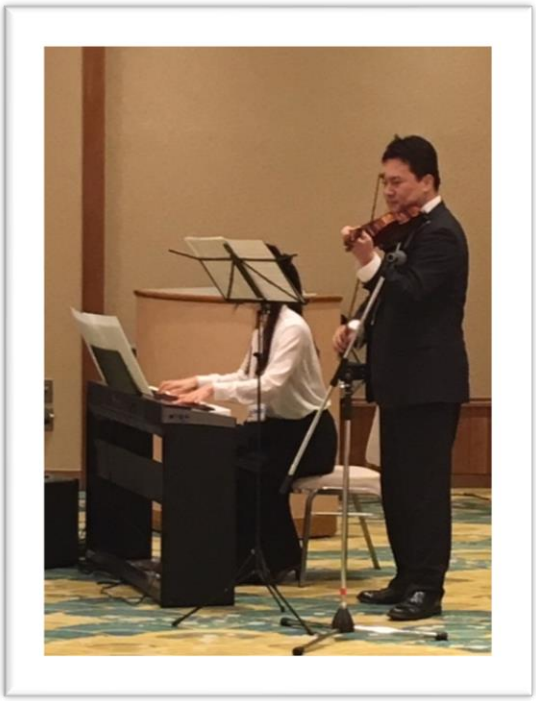
Q1. What was the most impressive event for you in your trip to Japan this time?

The opportunity to travel, experience new places, and meet new people is one of the many wonderful aspects of being a scientist. My entire visit to Japan was very pleasant and memorable. One thing that particularly stands out is the kindness of strangers. Shortly after I arrived, Dr. Keiko Nohara's daughter, Asuna and her husband Yasunobu, whom I had never met, took me on a wonderful walking tour to see little bit of Tokyo, including the Asakusa Kannon (Sensoji) Temple. They also took me out for a wonderful dinner and I was able to try okonomiyaki (which is delicious!). Also, before I departed for the airport, Dr. Tomoki Fukuyama took me to an ancient Shinto shrine on Mount Tsukuba, and for wonderful lunch at a restaurant nearby. This was a relaxing and very special way to end my trip to Japan, and I am grateful for this excursion. Strangers no more, through their generosity they helped make the world a smaller place.

Another impressive aspect of my visit to Japan was the conference reception and banquet. This was a special meeting for the JSIT, as they were celebrating their 25th year, which is a major milestone. There was a special banquet and award ceremony at the Hotel Grand Shinonome in Tsukuba. Along with celebrating the numerous achievements of JSIT members and welcoming new members, several young investigators received awards. The evening was also filled with lively and warm conversation, but what really impressed me was that throughout the evening several members of the JSIT displayed remarkable talents including dancing, theatrical readings, and musical performances. These were among the highlights, not only because art transcends language, but because the level of encouragement, sharing, and fun that these performances added to the evening was entirely special. The overall ambiance at the reception radiated cheerfulness and camaraderie. It was a wonderful reminder that, no matter how stressful we find our jobs, there is much also happiness, and it is important to take the time to share, to celebrate, and to laugh together.



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

Scientific discovery and figuring something out are very rewarding. However, the most gratifying and exciting thing in my career is the success of the students, postdocs, and the many other types of learners who have spent time in my lab. Mentoring is a privilege and a joy. Mentoring helps me remember to pause and reflect, be a more creative scientist, and a better communicator. It also keeps me invigorated as a scientist, as many ideas and perspectives are always better than one. I believe that it is safe to say I have gained more from the people whom I have had the opportunity to mentor than they have learned from me.

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

I am fortunate to have a fantastic team in my lab, and to work with wonderful collaborators. One of our main undertakings is to understand how signaling through the aryl hydrocarbon receptor (AHR) influences the development and function of the immune system. Much of the field is focusing on teasing out how the AHR regulates the function of the fully mature immune system, and we are interested in this as well. However, we have also extended research in some new directions, such as illuminating how AHR activation during early life changes the function of the offspring's immune system, and influences subsequent generations. By integrating genetic, pharmacological, immunological, and bioinformatic approaches, we are defining specific changes to different immune cell types, and uncovering the causal mechanisms by which they are affected. We also bidirectionally integrate research using model organisms and human populations, discovering associations between early life and longitudinal exposures to persistent organic pollutants and changes in immune function in infants, young children, and adolescents.

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

I think that the future of immunotoxicology research is very exciting. Whether one's passion is therapeutic agents, pollutants, or nutritional factors, the field is poised to start tackling how co-exposures influence the immune system's function and contribute to disease. This will lead to huge breakthroughs because, to date, pharmacology and toxicology research and applications of this research have generally focused on single exposures. This is a very important approach, and should continue. Yet by expanding the complexity of experimental model systems, we will attain a new level of understanding of how exogenous factors influence—for better and for worse—the immune system. The implications for improving health are tremendous, as we will be better able to predict and reduce risks, and also to improve or target new therapies.