



December,  
2017

Report on the 24th Annual Meeting of the  
Japanese Society of Immunotoxicology  
(JSIT2017)

Kazuichi Nakamura

President of the 24th JSIT Annual Meeting

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The 24th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT) was held on September 4th - 5th, 2017 at the School of Veterinary Medicine, Kitasato University in Towada, Aomori. “New perspective of immunoenhancement and immunosuppression” was set as the main theme of the meeting by myself. Autoimmunity or allergy is not necessarily initiated by “immunoenhancement”, but can be caused by even immunosuppression or normal immunity simply recognizing altered-self antigen or MHC. I intended to discuss and to disseminate this new aspect of immunotoxicity in this meeting. In addition, I thought that we should consider the physiological (normal) state of “immunosuppression”. In this connection, a symposium titled “Reproductive immunotoxicology: Immunological mechanism of pregnancy and its breakdown” was presented. On September 3rd, one day before the meeting, Dr. Fujio Kayama (Jichi Medical University) gave a lecture to Towada citizens about “Safety of rice as a staple food: Findings derived from nationwide epidemiological study in cadmium intake” receiving keen questions from them.

Dr. Jack H. Dean (University of Arizona) gave a keynote lecture through a video over the internet from Eminent Toxicologist Lecture Series “Immunotoxicology: A historical perspective”. It was a great honor and privilege for me to invite him to the JSIT meeting. His lecture gave

very impressive messages to audiences. I am so pleased that his name has been inscribed in the history of our JSIT.

I was also lucky and honored to be the meeting president because I could invite Dr. Danuta Herzyk (Merck & Co.) as a part of the researcher exchange program between the ITSS and JSIT. I really appreciate Dr. Mitch Cohen for his efforts on this project. Her lecture “Immunotoxicity Assessment of Biopharmaceuticals” received great attention from many audiences because it was well prepared being based on the data and experiences in her immunotoxicological research. Another special lecture was presented by Dr. Tsuyoshi Yokoi (Nagoya University), who is now a distinguished researcher on the immunology of drug-induced liver injury (DILI). He reported his works and clearly explained involvement of Th17 cells and TLR4 of Kupffer cells in the liver injury. Dr. Takeharu Minamitani (National Institute of Biomedical Innovation, Health and Nutrition) gave an educational lecture on autoimmune diseases. In his current study, he demonstrated the molecular pathogenesis by which Epstein-Barr virus infection induces autoimmune diseases.

The symposium “Reproductive immunotoxicity: Immunological mechanism of pregnancy and its breakdown” discussed the immunological tolerance and placenta formation during pregnancy. The symposium focused on Th1/Th2 balance, the roles of Treg, gamma/delta T cells and uNK cells during pregnancy. The immunotoxicological effects of nano-silica on pregnancy was also presented. The workshop dealt with safety evaluation method of biopharmaceuticals (protein formulation) with the perspective of regulatory agency and industry. Expectation of in vitro studies to support in vivo studies and immunogenicity assessment of antibody/antibody-drug conjugate therapeutics were main topics in the workshop. Interpretation of ICH S6 (R1) guideline was also mentioned by the PMDA.

Among 11 oral and 22 poster presentations, the Best Presentation Award went to Dr. Masashi Tachibana (Osaka University) presenting “Regulation of myeloid-derived suppressor cells by glutamate signaling” and the Students and Young Scientists Award to Dr. Toshiyuki Ohtake (Chemicals Evaluation and Research Institute) presenting “Comparison of skin sensitization potential of isocyanates by a local lymph node assay and an integrated testing strategy with an in silico, in chemico and in vitro assays”, respectively.

The meeting held a ceremony to honor Dr. Hidekazu Fujimaki (National Institute of Environmental Studies) for the JSIT Research Award and Dr. Katsumori Yamaura (Keio University) for the Prize for Encouragement 2017. They gave an award lecture “Differential immunologic responses in mice exposed to low levels of volatile organic compounds” and “The role of skin immunogenicity induced by topical steroid therapy in chronic pruritic skin disorders” respectively.

It should be noted that Dr. Victor J. Johnson (Burlerson Research Technologies, Inc.) who gave a lecture at the last year’s JSIT meeting kindly sent me video messages from himself and his young colleagues Dr. Angela Groves (University of Rochester) and Jaijun (Brian) Zhou (Michigan State University) to be shown in the evening banquet. They introduced ITSS current activities. Very big cheers rose from the banquet participants.

The meeting gathered about 100 audiences. Since the JSIT encourages enthusiastic participation of researchers especially from the next generation and the JSIT members know each other well, the discussion was very active in very friendly atmosphere. I would like to express my sincere gratitude for your support. Thank you very much.

### The Best Presentation Award

Glutamate signaling regulates the immunosuppressive function of myeloid-derived suppressor cells.

Masashi Tachibana

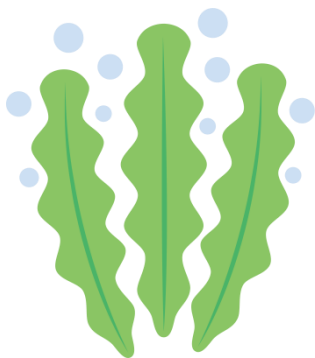
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Myeloid-derived suppressor cells (MDSCs) are an immunosuppressive population of immature myeloid cells that accumulate in tumor bearer. MDSCs suppress anti-tumor immunity, the activation of CD4<sup>+</sup> T cells, CD8<sup>+</sup> T cells, and natural killer (NK) cells, resulting in the promotion of tumor growth. The relationship between nutrition and cancer has been reported by several research groups. It has been reported that the levels of most amino acids, including glutamate, in tumor tissues obtained from patients with colon and stomach cancers are significantly higher than those in the normal colon and stomach tissues of the patients. It has also been reported that high mobility group box 1, consistently overexpressed in tumor bearer, leads to an increase in glutamate levels in the serum. These findings imply that glutamate could be a biomarker and modulator of cancer. However, how glutamate regulates tumor progression remains unclear.

MDSCs suppress immune reactions owing to the exhaustion of arginine by arginase and by iNOS, which uses arginine to produce nitric oxide. Thus, arginine metabolism is important for the immunosuppressive function of MDSCs. ATP production from the tricarboxylic acid cycle is decreased in tumor cells, and tumor cells often rely on elevated glutaminolysis. Therefore, in tumor-bearing hosts, tumor cells require glutamine for their energy production. Once imported into the cells, glutamine is converted to glutamate, followed by ATP

production. Furthermore, high glutaminase activity and low glutamine synthase activity have been observed in several types of tumor cells, suggesting an elevated glutamate level in tumor-bearing hosts; elevated glutamate levels in plasma and tumor tissues have been reported in tumor-bearing animals. Therefore, it is speculated that MDSCs are exposed to high glutamate concentrations. We hypothesized that glutamate enhances tumor progression by inducing MDSCs or enhancing the immunosuppressive function of MDSCs.

In this study, we revealed that the metabotropic glutamate receptor (mGluR) 2/3 are expressed on MDSCs. LY341495, an mGluR2/3 antagonist, attenuated the immunosuppressive activity of MDSCs *in vitro* and MDSCs from B16-F10 melanoma-bearing mice *in vivo*. Furthermore, we observed that LY341495 treatment inhibited the growth of B16-F10 melanoma *in vivo*. Additionally, the frequency of NK cells increased, and CD4<sup>+</sup> and CD8<sup>+</sup> T cells were activated in the spleen. Taken together, these data suggest that LY341495 would enhance the anti-tumor immunity through the inhibition of MDSCs in B16-F10 melanoma-bearing mice. These data suggest that glutamate signaling plays the role to promote tumor growth by increasing the potency of immune suppression. Furthermore, our findings suggest that targeting MDSCs would enhance the efficiency of immune checkpoint blockade therapy that uses the anti-PD-1 antibody



The Student and Young  
Scientists Award

Comparison of skin sensitization potential of isocyanates by a Local Lymph Node Assay and an Integrated Testing Strategy with an *in silico*, *in chemico* and *in vitro* assays

Toshiyuki Ohtake

Chemicals Evaluation and Research Institute, Japan (CERI)

It is my great honor to receive the Student & Young Scientists Award at the 24th Annual Meeting of Japanese Society of Immunotoxicology (JSIT). I would like to give a special appreciation to the JSIT committee. Firstly, I would like to introduce my research. The skin sensitization potential of chemicals has been traditionally assessed using regulatory accepted *in vivo* methods, such as guinea pig maximization test or mouse local lymph node assays (LLNA). A huge effort to reduce or replace the use of animals for safety assessments of chemicals because of regulatory requirements and ethical issues is presently underway, and alternative non-animal methods have been greatly developed. However, so far, a few studies have investigated the sensitization potencies of isocyanates which is a group of highly reactive chemicals that are known to be occupational allergens. The present study evaluated nine commonly used isocyanates using an *in vivo* LLNA and assessed the applicability of an Integrated Testing Strategy (ITS) consisting of an *in silico* Derek Nexus prediction, an *in chemico* direct peptide reactivity assay (DPRA), and an *in vitro* human Cell Line Activation Test (h-CLAT) to isocyanates. All nine isocyanates were evaluated as positive using the LLNA, Derek Nexus and DPRA, whereas seven chemicals tested positive using the h-CLAT: hexamethylene diisocyanate tested negative, and 1,5-diisocyanatonaphthalene could not be examined because of a solubility issue. When assessed using the ITS,

the positive/negative outcomes for skin sensitization hazard were consistent with those assessed using the LLNA for all nine chemicals. However, the potency prediction results of the ITS tended to be underestimated, compared with those of the LLNA. The data presented in this work provide insights into the performance of non-animal testing approaches for evaluating the skin sensitization potencies of isocyanates. The paper entitled “Applicability of an Integrated Testing Strategy consisting of in silico, in chemico and in vitro assays for evaluating the skin sensitization potencies of isocyanates” has been accepted in *Toxicology*.

Lastly, I would like to express my great gratitude to my collaborators in CERi for the continuous support for my research, and giving me such a great opportunity to present at this conference.

#### Reference

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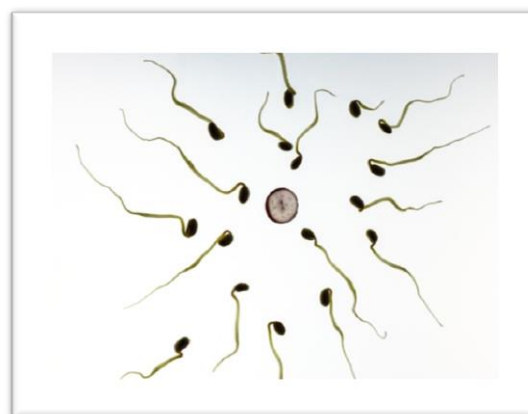
#### Immunotoxicological Research

##### Testicular immune privilege and Reproductive immunotoxicity

Ning Qu, Kou Sakabe

(Department of Anatomy, Division of Basic Medical Science,  
Tokai University School of Medicine)

In generally, the testis is often viewed as one of several immune-privileged organs in the body. Therefore, there is a possibility that xenogeneic Spermatogonia Stem Cells (SSC) transplantation could be also successful even in immunocompetent adult recipient mice. Recently, we tried to transplant rat SSCs into immunocompetent mice after depletion of their own germ cells by means of busulfan, and demonstrated that some transplanted rat SSCs could undergo complete rat spermatogenesis in recipient mouse testes. And then, to determine the effects of busulfan on the immune system and spermatogenesis in immune-competent recipient mice, we detected that after busulfan treatment, the recovery could be seen in the immune system including cell counts and functions of T and B lymphocytes in the spleen, but the spermatogenesis was more compromised. In the present study, we showed some information about therapeutic drug on male infertility after busulfan treatment.



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. Victor J. Johnson from Burlleson Research Technologies, Inc., USA. Let's go to listen to his voice. What will you feel and learn?

Danuta Herzyk, Ph.D.

Distinguished Scientist  
Immunology & Oncology Therapeutic Area Leader  
Safety Assessment & Laboratory Animal Resources  
Merck Research Laboratories (Division of Merck Sharp &  
Dohme, a subsidiary of Merck & Co.)  
West Point, Pennsylvania, USA



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

The whole trip was very enjoyable and memorable. The most impressive was the weekend prior to the conference, when I (with my family members) had a chance to tour and stay around beautiful Lake Towada and Aomori region. I am truly grateful to Dr. Kazuichi Nakamura and Dr. R. Kamata for their efforts and time spent on arranging and accompanying us on trips to Oirase Mountain, Hakkoda Mountain, Samurai Castle, Sannai-Maruyama Historical Site, Nebuta Museum, and Asamushi Sakura Kano. In addition to the beauty of the region, the accommodations in traditional Japanese hotels allowed us to experience local culture and customs, including amazing dinners with delicious and beautifully prepared and served food (I only regret that I do not know Japanese language and cannot read menus and names of Japanese dishes).

The second most impressive event was the Conference Banquet. Apart from the beautiful setting at Sun Royal Towada and very tasty food, the wonderful jazz concert prepared and performed by the Student Band from Kitasato University was unforgettable. These supposedly amateur musicians were incredibly good! At the same time, I was pleasantly surprised by remarkably high energy, happy atmosphere and much laughter among the immunotoxicologists attending their annual conference. This was in contrast to my previous perception that Japanese people were quite serious-minded all the time. Sometime it feels good to be proven wrong (big smile here).

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

My background is in immunology and I had to learn toxicology sciences “on the job” after I started working in the pharmaceutical industry, initially at SmithKline Beecham (currently GlaxoSmithKline) and then at Merck. Therefore, I am not a typical immunotoxicologist and have not been exposed to the environmental immunotoxicology field in contrast to my many esteemed colleagues in the US, Japan and other countries. The opportunity to work in the pharmaceutical industry in drug development has been the most exciting thing in my career. A broad spectrum of scientific challenges involving both immunopharmacology and immunotoxicology as two sides of the same coin is fascinating and most interesting to me.

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

I have had the privilege of working in the immuno-oncology area, including the development of the cancer drug, KEYTRUDA®. This is a breakthrough therapy, using anti-PD-1 monoclonal antibody, and is the most important and energizing work I have done in my career. Research related to immune therapies in cancer is growing and evolving, and we are discovering and learning a lot about tumor biology and regulation of immune responses to tumors. At the same time, we face new challenges in the evaluation of safety of these novel anti-cancer therapies that present very different profiles than traditional chemotherapy drugs used to treat cancer diseases for decades. The need to better understand this new therapeutic class and work on new approaches to their safety evaluation stimulates my creative thinking and takes the highest priority in my current work.

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

From my perspective, immunotoxicology research in the future will focus on characterizing relationships between autoimmunity and immune responses to tumors at different stages of the cancer-immunity cycle. In this new era of immune therapies in cancer treatment, the idea of an association between autoimmune toxicity and therapeutic anti-cancer response has become a topic of debate and we need to address this idea as we move forward. With the availability of novel technologies, models and approaches, including humanized immune system mice and genomic and cellular biomarkers of immune interactions, we have new opportunities to move immunotoxicology research to a different level of scientific thinking.

