ImmunoTox Letter Digest

December 2020

Report on the 27th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT 2020)

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The 27th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT 2020) was held as the virtual meeting from September 26th to 27th, 2020. It was originally planned to be held with the main theme of the annual meeting as "Past, Present and Future of Immunotoxicology" at the Prism Tower of Kitasato University, Tokyo. However, because of the pandemic of coronavirus disease 2019 (COVID-19), under the decision of Board Meeting of the Society, it was held as the virtual meeting.

After the decision of the Board Meeting, the organized committee of the JSIT 2020 faced on the problem of high cost in case that the meeting was held on the virtual meeting. At that time, Dr. Shigeru Hisada, one of Board members, offered to use the zoom webinar system of ASKA Pharmaceutical Co. and the virtual meeting became possible. Dr. Hisada contributed greatly in many ways such as the notice how to participate the virtual meeting and the management of the virtual meeting on the days of the meeting.

Finally, the symposium "Immunotoxicology, past, present and future", "The 10th JSIT Award" and "The 10th JSIT prize for encouragement" lectures, the workshop "Development of the latest technology for immunotoxicity evaluation" and Student and Young Scientist Session were held as live via the zoom webinar. In addition, the poster presentation was held on the website of the annual meeting with the abstract book.

The number of posters was 19, and the number of the presentation of Student and Young Scientist Session was 8. The 130 people were registered to participate in the meeting, and the actual number of the participant who attended to the meeting at least a day was 115.

On the first day, at the symposium "Immunotoxicology, past, present and future" which is same as the main theme of the annual meeting, Professor Ikuo Tsunoda, Department of Microbiology, Kindai University Faculty of Medicine, with the coauthor Dr. Shigematsu Toriyama gave a lecture titled with "Role of gut microbiota in Theiler's virus model for multiple sclerosis: Max Theiler and Hideyo Noguchi". Professor Yasuo Yoshioka, Institute for Open and Transdisciplinary Research Initiatives, Osaka University, gave a lecture "Vaccine Safety: Current

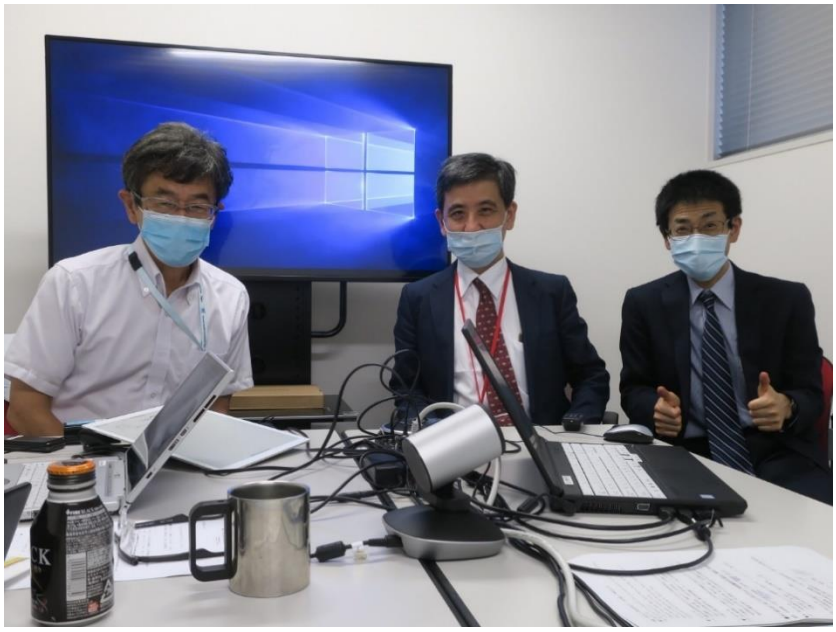
status and challenges in the development of safe potent vaccines". Professor Masashi Takano gave a lecture "Carcinogenesis of ovarian clear cell carcinoma through toxic mechanism".

On the second day, "The 10th JSIT Award" lecture was given by Dr. Tomoaki Inoue with the title "Investigation of novel *in vivo* evaluation methods for prediction of *in vitro* immunotoxicity". "The 10th JSIT prize for encouragement" lectures were given by Dr. Shigeki Aoki with the title "Investigation of idiosyncratic drug toxicity using HLA transgenic mice" and Dr. Takamasa Kido with the title "Immune dysfunction derived from zinc deficiency exacerbates the Th2 cell-M2 macrophage pathway.

At the workshop "Development of the latest technology for immunotoxicity evaluation", Dr. Yoshitaka Shirasaki, Dr. Etsuhi Kuroda, Dr. Yoh-ichi Tagawa and Dr. Chiyomi Kubo gave lectures.

Among the poster presenters, Dr. Izumi Sasaki with his presentation "The roles of unfold protein responses in cholera toxin B-induced interleukin-1β production" as the best poster of the annual meeting. Among the Student and Young Scientist Session, Dr. Yuuto Murata with his presentation "Immunotoxicity of G-CSF through myeloid-derived suppressor cells in tumor-bearing mice" was awarded as the best presenter among the session.

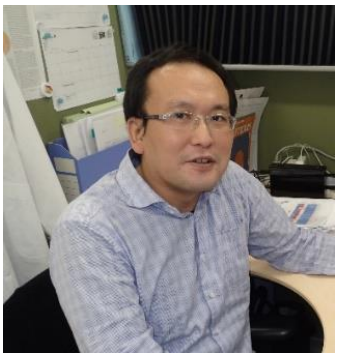
The annual meeting in this year was exceptionally held as the virtual meeting, however, without serious trouble, and with many good discussions. I think it was held successfully. I would like to express my gratitude for many people who contributed this meeting on this report.



Photo, Dr. Shigeru Hisada, Masashi Tsunoda and Professor Masashi Takano
at the meeting room of ASUKA Pharmaceutical Co. on September 26, 2020

The Best Presentation Award

The roles of unfold protein responses in cholera toxin B-induced interleukin-1β production



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Cholera toxin B (CTB) functions as an immune adjuvant. The response of CTB is dependent on binding with membrane glycolipids, ganglioside GM1 (hereafter GM1). This binding facilitates internalization of CTB. However, the underling mechanisms of CTB-induced immune adjuvant effects are poorly understood. We have found that CTB, incorporated through GM1, can induce IL-1β production from resident peritoneal macrophages (RPMs) prestimulated with lipopolysaccharides (LPS) through the pyrin inflammasome as well as the NLRP3 inflammasome (Int Immunol, 2019). However, it remains unclear how CTB activates the inflammasomes or induces IL-1β production. In this study, we investigated the molecular mechanisms of CTB-induced IL-1β production.

First, we performed transcriptome analysis in CTB- and/or LPS-stimulated RPMs. In the stimulation of CTB+LPS, the expression of 510 genes was more than 2-fold upregulated compared with LPS alone stimulation. Pathway analysis of these genes showed enrichment for the unfold protein response (UPR) genes. Endoplasmic reticulum (ER) stress is triggered by accumulation of unfold proteins in the ER lumen. These unfold proteins are recognized by UPR sensors which contains inositol-requiring enzyme 1-alpha (IRE1α), Protein kinase R (PKR)-like endoplasmic reticulum kinase (PERK) and Activating transcription factor 6 (ATF6). Then, these sensors are activated and produce several transcription factors. IRE1α activation induces X-box binding protein 1 (XBP1) mRNA splicing and generate transcription factor XBP1. Activation of PERK induces the expression of transcription factor C/EBP homologous protein (CHOP). Activation of ATF6 induces autoprocessing to its mature form. Subsequently, mature ATF6 can function as transcription factor. These transcription factors induce the expression of ER stress-

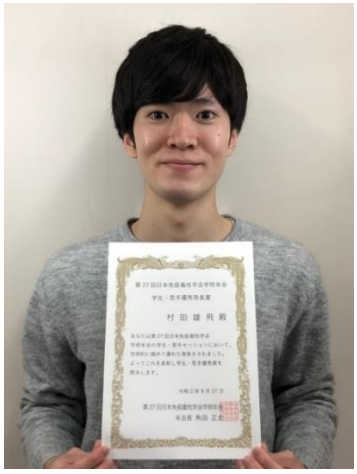
related genes such as chaperone proteins or apoptosis signaling molecules. These responses are collectively termed as ER stress response or UPR. In RPMs, XBP1 splicing and the expression of CHOP were induced in response to CTB+LPS. These inductions were abolished in GM1-deficient RPMs. These results indicate that CTB could induce UPR in a GM1-dependent manner.

CTB is translocated from the cell surface through Golgi to the ER. This series of event is generally called as retrograde transport. However, the details of retrograde transport of CTB in RPMs are not fully characterized. Then, we investigated whether CTB is translocated to the ER. In WT RPMs, FITC-labeled CTB was co-localized with IRE1α. The co-localization did not occur in GM1-deficient RPMs. The result suggests that CT is incorporated into the ER in a GM1-dependent manner.

Finally, we analyzed the effects of inhibitors for UPR sensors. Inhibition of IRE1α, but not PERK and ATF6, decreased not only XBP1 mRNA splicing but also IL-1β production in RPMs stimulated with CTB+LPS. In conclusion, our findings indicate that CTB is a novel ER stress inducer and IRE1α is involved in CTB-induced IL-1β production from RPMs.

The Student and Young Scientists Award

Immunotoxicity of G-CSF through myeloid-derived suppressor cells in tumor-bearing mice



Yuuto Murata
Project for Vaccine and Immune Regulation,
Graduate School of Pharmaceutical Sciences,
Osaka University

It is honor to win The Student and Young Scientists Award at the 27th Annual Meeting of Japanese Society of Immunotoxicology. I would like to sincerely appreciate the selection committee and the president Dr. Tsunoda.

Myeloid-derived suppressor cells (MDSCs) are immunosuppressive cells that promote tumor progression by inhibiting anti-tumor immunity. Granulocyte-colony stimulating factor (G-CSF) is a preventive agent for neutropenia, which is the major adverse reaction following chemotherapy. On the other hand, some reports have mentioned G-CSF immunotoxicity through MDSCs. Thus, we decided to elucidate this mechanism.

In order to investigate whether G-CSF enhanced the immunosuppressive activity of MDSCs, we induced MDSCs in the presence of G-CSF and then co-cultured with CD4+ T cells. It was revealed that these MDSCs demonstrated stronger suppression on the proliferation of T cells than those induced without G-CSF. Next, we identified gamma-glutamyltranspeptidase (GGT) as a candidate for the enhanced immunosuppressive function of MDSCs by RNA-seq analysis. GGT is the only hydrolase against extracellular glutathione. We confirmed that G-CSF enhanced the GGT activity of MDSCs. In addition, the expression levels of Arginase-1 and iNOS, which are the immunosuppressive factors of MDSCs, were also increased by G-CSF. Furthermore, these increases were thoroughly canceled by a GGT inhibitor. Consistent with this, the administration of this inhibitor into 3LL-tumor bearing mice resulted in the inhibition of tumor progression.

These results suggest that the enhancement of immunosuppressive function by G-CSF is mediated by GGT. We previously reported that glutamate enhanced the immnosuppressive function of MDSCs (the 24th Annual Meeting, *Biol Pharm Bull*, 2018). Therefore, it is considered that the production of glutamate by degrading glutathione via GGT might be nature of G-CSF immunotoxicity.

In the end, I would like to express my greatest appreciation for Dr. Tachibana and all the people who supported and advised me for my research.

The 9th Japanese Society of
Immunotoxicology Prize for Encouragement

Construction of adjuvant safety evaluation method using human peripheral blood mononuclear cells (PBMC) and novel human peripheral blood mouse and application to vaccine development

Eita Sasaki
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National Institute of Infectious Diseases

It is my great pleasure and honor to be awarded the JSIT prize for encouragement. I would like to express my sincere thanks to all of the members of the awarding committee.

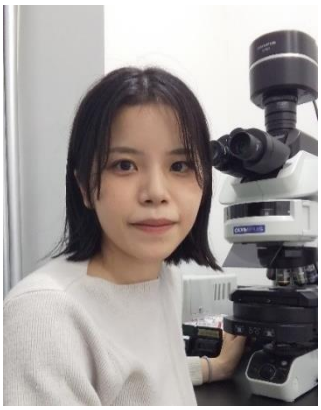
High safety is required in vaccines because the vaccine is given to humans in a wide range of age groups, from children to the elderly. I have been conducting research with the idea that it will be possible to obtain information from immunogenicity to toxicity by applying genomics analysis. Such methods have already been applied to synthetic drugs, etc., but in biological products such as vaccines, they have been limited to assesse antibody production, body weight changes and pathological analysis.

Since many adjuvants act on innate immunity, I suspect that early genomics data within 24 hours after vaccination may contain information on the efficacy and toxicity of vaccine adjuvants. I focused on inactivated influenza vaccines and developed safety evaluation system for adjuvanted influenza vaccines using biomarker genes in lung.

On the other hand, these data were experiments in mice. So I wondered if this test method reflects effects in humans? I first performed analysis the changes of biomarker genes expression patterns by vaccine addition in human peripheral blood mononuclear cells (PBMC) culture. I found that in PBMC, biomarker genes expression fluctuations related to type 1 interferon (IFN) were similar to those obtained in mouse in vivo. Although some cytokines and chemokines do not cross in humans and mice, by using NOD/Shi-scid-*IL2rγ*^{null} (NOG) mice receiving human peripheral blood mononuclear cells (hu-PBL-NOG mice), it has been revealed that some biomarker genes expression changes in human PBMC by vaccinations are occurred due to interaction between human PBMC and lung parenchyma cell. Although the humanized mouse models are not easy to apply to safety tests due to instability of the model and cost issue, it has been found to be an excellent tool for predicting extrapolation to humans.

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

The Student and Young Scientists Award



Association between non-alcholic steatohepatitis and immunologic factor

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Non-alcoholic steatohepatitis (NASH) is a lifestyle related disease that has developed fatty liver, inflammation and fibrosis despite absence of the alcohol drinking career. NASH has a risk factor of the development cirrhosis and liver cancer. Therefore, it needs to be managed before the condition worsens.

We examined the liver lesions over time from early stage to serious hepatic lesion in Fischer 344 rats fed choline- deficient, methionine- lowered, amino acid-defined (CDAA) diet, the feeding periods were set for 3 days, 2, 4, 13, 26 and 52 weeks. CDAA diet caused oxidative stress, inflammation and fibrosis from the early period, and these lesions have progressed over time.

“Liver-Gut Axis” contribute to the initiation and promotion of hepatic inflammation and fibrosis. The second bile acid, LPS and inflammatory cytokines flow into the liver from intestinal tract, and these factors affect hepatic stellate cell (HSC) and Kupffer cell though the toll like receptors (TLR), which induced senescence of HSC and production of inflammatory cytokines (Seki E, *et al.*, Nat Med, 2007). Failure of intestinal barrier system and lipid metabolism causes influx of the related factors including such as bile acid and LPS, etc. from intestinal tract. These changes were also observed in rats fed the CDAA diet, which also revealed that the enterohepatic axis is involved in NASH in the experimental model.

CD68- positive macrophage and Th1 type cytokines were increased in the same model, and M1 macrophage is classified as pro-inflammatory macrophage and activated by Th1 type cytokines and LPS. CD44 is a hyaluronic acid receptor, deletion of CD44 enhanced the polarization of the M2 macrophage, CD44 has also been reported that the major player in the role of NASH. (Stephanie Patouraux, *et al.*, Hepatology, 2017). From these information, I would like to perform the detailed analysis about relationship polarization of macrophage and CD44 based on the study of immunology and toxicology.