ImmunoTox Letter Digest

June, 2021

The 28th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT2021)

1. Date

September 6-7th, 2021

2. Venue

The meeting will be held on the web site "http://www.jsit2021.jp".

3. President

Reiko Teshima Ph.D.
(Professor, Faculty of Veterinary Medicine, Okayama University of Science, Imabari, Ehime, Japan)

4. Main theme of the meeting

The relationship between innate immunity and acquired immunity and immunotoxicity

5. Meeting Secretariat

Division of Food Safety, Faculty of Veterinary Medicine, Okayama University of Science (OUS).
E-mail: 28th-info@jsit2021.jp
URL: http://www.jsit2021.jp

6. Program (tentative)

Special lecture

1) "New immunotherapy using dendric cells"
Shin-ichiro Fujii (RIKEN Center for Integrative Medical Science)
2) "Immunogenicity-related toxicity"
Dr. Jeanine Bussiere
(Scientific Executive Director of Toxicology, Amgen com.)

Symposium: About the development status of various vaccine and their safety evaluation

1)"MicroRNA and inflammation during vaccination"
Hiroyuki Oshiumi
(Faculty of Life Sciences, Kumamoto University)
2)"Safety assessment of nasal vaccine"
Yoshikazu Yuki
(Hana Vax inc.)
3)"Safety assessment of vaccines using humanized mice"
Eita Sasaki
(Department of Safety Research on Blood and Biological Products, National Istitute of Infectious Diseases)

Educational lecture

1) " Significance of allergic diseases from the perspective of evolution (including the role of innate lymphoid cells (ILC-2))"
Kenji Matsumoto
(Department of Allergy and Clinical Immunology, National Center for Child Health and Development)
2)"About the new corona vaccine"
Yasuhiro Yoshikawa
(Dean, Faculty of Veterinary Medicine, Okayama University of Science)

Special lecture of the recipient of the 11th JSIT award

" Immunotoxic effects of dioxins and their mechanisms"
Keiko Nohara
(Health and Environmental Risk Division, National Institute of Environmental Studies)

Special lectures of the recipients of the 11th JSIT prize for encouragement

"Study on the expression of immunotoxicity through bone marrow-derived immunosuppressive cells"
Masashi Tachibana
(Project for Vaccine and Immune Regulation, Graduate School of Pharmaceutical Sciences, Osaka University)

Workshop: Toward the development and guidance of in vitro immunotoxicity test methods
Organizer: Kiyoshi Kushima (Astellas)
Presenters: Setsuya Aiba (Department of Dermatology, Tohoku University School of Medicine), Masahiro Fujita (Safety evaluation Center, Fujifilm Holdings co.), Takao Ashikaga (National Institute of Health Sciences)

Oral session of young scientist
Oral presentation
Poster presentation

The 10th Japanese Society of Immunotoxicology Award (The 2020 JSIT Award)

Investigation of novel *in vitro* evaluation methods for prediction of *in vivo* immunotoxicity

Tomoaki Inoue
Former-Chugai Pharmaceutical Co., Ltd.

Non-clinical immunotoxicity evaluation of pharmaceuticals is performed by *in vivo* studies in experimental animals and *in vitro* studies using immune cells. An object of the *in vitro* evaluation is prediction of *in vivo* toxicity using cells involved in the toxicity. In addition, *in vitro* evaluation needs small amount of test chemicals, and is useful for rapid evaluation of many chemicals in early stages of drug discovery. Animal species of the cells need to be the same with the *in vivo* evaluation. Therefore, human cells are used for prediction of clinical safety, especially in the case of biologics which have human specific activities. However, in the case of pharmaceuticals with new action mechanisms, it is difficult to judge *in vitro – in vivo* relationship, because the clinical safety is unknown. To establish predictive *in vitro* systems, investigation of new culture forms which maintain functions of organs such as organotypic cultures or microphysiological systems would be expected.

For the cell sources used in the *in vitro* evaluation, human peripheral blood cells are often used, because the culturing methods and the evaluation methods of immune functions in these cells are well established in the immunology research. However, there are genetic variations in individual donors, and it is difficult to obtain required genotypes. Recently, many types of immune cells can be differentiated from human iPS cells. Human iPS cells can be expanded with characteristics of pluripotency. Human iPS cells would be a source to obtain large amount of characterized human cells. The differentiation methods of human iPS cells to functional mature cells have been investigated, but are complicated. The authors established differentiation methods to derive human dendritic cells and mast cells. The derived dendritic cells presented antigen peptides associated with the HLA molecules expressed on the cells. The derived mast cells expressed mast cell makers such as FcεR, CD117, and activation and degranulation were observed by crosslinking of IgE receptors. In general, human iPS cell-derived differentiated cells show fetus or new bone-like characteristics, and express limited functions as mature cells. The expression of the target function of the evaluation should be confirmed before using as the *in vitro* assay. Even if the target function is confirmed, other functions should be confirmed, if needed for the evaluation.

Test systems of acquired immune responses are often necessary as a target for evaluation of immunotoxicities. Antigen-presenting cells, T-cells and B-cells are involved in the acquired immune responses. For cell sources of these cells in *in vitro* systems, peripheral blood cells include these all cell types, but immune responses in the peripheral blood are suppressed. Removal or inactivation of the suppressor cells such as CD8+ T-cells would be needed to obtain detectable immune reactions. In addition, effective detection of reacting cells would be considered because of low frequency of the reacting cells. Lymph node cells and spleen cells include all these cell types, and the acquired immune responses are observed in the *in vitro* culture system. These cells are obtained only in experimental animals but not in humans. Stem cells such as human iPS cells have advantages to derive large amount of human immune cells from the same donor, but the differentiation methods are not completely established. In addition, the culture forms to maintain cell functions as an organ and inducing effective acquired immune responses would also need to be established.

The 10th Japanese Society of Immunotoxicology Prize for Encouragement

Investigation of idiosyncratic drug toxicities using human leukocyte antigen (HLA)-transgenic mice

Shigeki Aoki
Laboratory of Biopharmaceutics,
Graduate School of Pharmaceutical Sciences,
Chiba University

It is my great pleasure and honor to be awarded the JSIT prize for encouragement. I would like to sincerely express my gratitude to all of the members of the awarding committee and to Prof. Yoshioka who recommended me.

Idiosyncratic drug toxicity (IDT) is an unpredictable and potentially life-threatening adverse event because it is impossible to generate prospective mechanistic studies in humans. Recent genome-wide association studies have indicated that IDT is strongly associated with specific polymorphisms in genes encoding human leukocyte antigens (HLAs). However, the pathogenic mechanisms governing such reactions remain unclear. This is partly due to a lack of suitable experimental animal models, which assess IDT.

To augment this gap in literature, we engineered a transgenic mouse model, the HLA-Tg, by partially substituting the mouse HLA sequence by the corresponding human sequence to trigger an HLA-dependent immune response in mice. Here, we demonstrated our work on the specific allele and drug combination of HLA-B*57:01 and abacavir. Local abacavir exposure was found to trigger a significant immune response in an HLA-dependent manner that especially involved activating $CD8^+$ T cells and infiltrating lymphocytes in the exposure area. In addition, we revealed the importance of immune tolerance for IDT onset, using $CD4^+$ T cell-depleted programmed death-1 receptor (PD-1)-deficient HLA-B*57:01 transgenic mice. We believe that an individual difference in the immune tolerance system affects susceptibility to HLA-mediated IDT in humans. Additionally, we developed a technique for evaluating structural alterations in HLA complexes that result from drug exposure based on phage display to ensure specificity. Such *in vitro* systems can be applied in the pre-clinical stage of drug development and are sure

to help prevent harmful adverse events.

Elucidation of the mechanism(s) underlying drug-induced immune reactions using the HLA-Tg model, as well as enhanced methods for predicting adverse event incidence are anticipated to help resolve issues surrounding HLA-associated drug hypersensitivity.

Again, I would like to express my deepest appreciation to all the members who supported and guided me in my research.

The 10th Japanese Society of
Immunotoxicology Prize for Encouragement

Inflammatory response under zinc deficiency is exacerbated by dysfunction of the Th2 lymphocyte – M2 macrophage pathway

Takamasa Kido
Department of Public Health and Environmental Medicine,
The Jikei University School of Medicine

I am great pleasure and honored to be the Encouragement Award at the JSIT. I would like to sincerely thank you for the committee.

In this study, inflammatory response under zinc deficiency is exacerbated by dysfunction of the Th2 lymphocyte – M2 macrophage pathway. Nutritional zinc deficiency leads to immune dysfunction and aggravates inflammation. However, the underlying mechanism remains unknown. In this study, the relationship between macrophage subtypes (M1 and M2) or helper T lymphocytes (Th1 and Th2) were investigated using the spleen from rats fed zinc-deficient or standard diet. In experiment I, five-week-old male Sprague-Dawley rats were fed zinc-deficient diet (without zinc additives) or standard diet (containing 0.01% zinc) for 6 weeks. In experiment II, the rats were divided into four groups: one group was fed a standard diet for 6 weeks; two groups were fed zinc-deficient diet and were injected three times a week with either saline or interleukin (IL)-4 (zinc-deficient/IL-4 i.p.); a fourth group (zinc-deficient/standard) was fed a zinc-deficient diet for 6 weeks followed by a standard diet for 4 weeks. In experiment I; GATA-3 protein level, M2 macrophage, $CD3^+CD8^+$ cell, and IL-4/IL-13-positive cells significantly decreased in the spleens of zinc-deficient group. Additionally, IL-1β and MIP-1α mRNA levels significantly increased in the splenic macrophages of zinc-deficient group. In experiment II; M2 macrophages, $CD3^+CD8^+$ cells, IL-4/IL-13-positive cells, and GATA-3 protein levels signfiicantly increased in the spleens of the zinc-deficient/IL-4 i.p. and zinc-deficient/standard groups. Furthermore, IL-1β and MIP-1α mRNA levels decreased in the splenic macrophages of the zinc-deficient/IL-4 i.p. and zinc-deficient/standard groups. Zinc deficiency-induced aggravated inflammation is related to Th2 lymphocytes and followed by the association with loss of GATA-3, IL-4, and anti-inflammatory M2 macrophages. Importantly, IL-4 injection or zinc supplementation can reverse the effects of zinc deficiency on immune function.

Immunotoxicological Research

Immunotoxicity of palmitic acid on myeloid-derived suppressor cell differentiation

Masashi Tachibana
Project for Vaccine and Immune Regulation,
Graduate School of Pharmaceutical Sciences,
Osaka University

Myeloid-derived suppressor cells (MDSCs) accumulate under the pathological conditions, including cancer and inflammation, and suppress variable immune responses such as T cell proliferation. It has been reported that MDSCs increased in anti-PD-1 therapy resistant tumor-bearing hosts compared with anti-PD-1 therapy sensitive hosts. Therefore, MDSCs are promising target for cancer immunotherapy. Although several inflammatory signals enhance the differentiation and/or function of MDSCs, the molecular mechanism is still unclear.

Since cumulating evidences indicate that fatty acids (FAs) and their metabolism modulate variable immunological processes, it is thought to be important to reveal the effects of FAs on MDSCs. Then, I stimulated bone marrow cells which are cultured in the presence of GM-CSF for the MDSC differentiation in vitro with each fatty acid. I found that palmitic acid (PA) inhibited the differentiation to MDSCs and promoted the differentiation into dendritic cells (DCs). In addition, I found that PA attenuated the immunosuppressive function of MDSCs. Furthermore, it was observed that tumor progression was significantly inhibited by feeding tumor-bearing mice on PA-rich diet. These results suggest that dietary PA inhibits the MDSC differentiation and promotes the differentiation into DCs, leading to activation of anti-cancer immunity. Taken together, PA would show immunotoxicity on MDSC differentiation. Additionally, these results lead to elucidation of the mechanisms of MDSC differentiation and development of novel cancer therapies targeting the metabolic pathways of PA during MDSC differentiation.