ImmunoTox Letter Digest

June, 2022

The 29th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT2022)

1. Date
September 12-13th, 2022

2. Venue
ACU Sapporo,
"http://www.jsit2022.jp"

3. President
Hiroyuki Kojima Ph.D.
(Professor, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido, Ishikari-Tobetsu, Hokkaido, Japan)

4. Main theme of the meeting
Immunotoxicity and Diseases - Leave our footmark-

5. Meeting Secretariat
Department of Health and Environmental Sciences, School of Pharmaceutical Sciences, Health Sciences University of Hokkaido.
E-mail: jsit2022@ml.hoku-iryo-u.ac.jp
URL: http://www.jsit2022.jp

6. Program (tentative)

Special lecture

1) "Maintenance and failure of immune homeostasis by gut microbiota"
Takanori Kanai
(Professor, Division of Gastroenterology and Hepatology, Department of Internal Medicine, Keio University School of Medicine)

2) "Protecting public health from per- and polyfluoroalkyl substances
: Focus on immunotoxicity"
Dr. Jamie C. DeWitt
(Professor, Department of Pharmacology & Toxicology, East Carolina University)

Symposium: Environment, Immunity and Diseases

1) "Multigenerational effects and epigenetic modification changes by gestational inorganic arsenic exposure"
Takehiro Suzuki
(Health and Environmental Risk Division, National Institute for Environmental Studies)
2) "Understanding and controlling environmental particle-induced inflammatory diseases"
Naoki Takemura
(Laboratory of Bioresponse Regulation, Graduate School of Pharmaceutical Sciences, Osaka University)
3) "Suppressive mechanisms on autoimmune diseases by helminth infection"
Chikako Shimokawa
(Department of Parasitology, National Institute of Infectious Diseases (NIID))
4) "Exacerbation of an immune checkpoint inhibitor-induced anaphylaxis by tumor-associated myeloid cells in tumor-bearing mice models"
Hiroto Hatakeyama
(Laboratory of DDS Design and Drug Disposition, Graduate School of Pharmaceutical Sciences, Chiba University)
5) "Off-target cytotoxicity of antibody-drug conjugates"
Michihiko Aoyama
(National Institute of Health Sciences)
6) "Involvement of Fc receptors in the immunotoxicity of biopharmaceuticals"
Shunsuke Ito
(Translational Research Division, Chugai Pharmaceutical Co., Ltd.)

Educational lecture

1) "Understanding efficacy, effectiveness and safety of vaccines: from an epidemiological perspective"
Wakaba Fukushima
(Professor, Department of Public Health, Osaka Metropolitan University Graduate School of Medicine)
2) "How far can cancer be prevented?"
Masahiro Asaka
(President, Health Sciences University of Hokkaido)

Special lecture of the recipient of the 12th JSIT award

"Involvement of immunological dysregulations in arsenic-induced diseases"
Seiichiro Himeno
(Division of Health Chemistry, School of Pharmacy, Showa University)

Special lectures of the recipients of the 12th JSIT prize for encouragement

1) "Preclinical immunotoxicity and immunogenicity studies aimed at improving safety predictions of biopharmaceuticals"
Chiyomi Kubo
(Translational Research Division, Chugai Pharmaceutical Co., Ltd.)
2) "Study on immunotoxicity through mRNA stability control mechanism"
Ryuta Muromoto
(Department of Immunology, Graduate School of Pharmaceutical Sciences, Hokkaido University)

Workshop: Challenges of immunotoxicity assessment for diverse pharmaceutical modalities

1) "Recent *in vivo* immunotoxicology studies using cynomolgus moneys at SNBL"
Yoshihiro Takahashi
(Shin Nippon Biomedical Laboratories, Ltd.)
2) "Issues and considerations for evaluation of therapeutic antibodies using cytokine release assay"
Shiho Ito
(Medicinal Safety Research Laboratories, Daiichi Sankyo Co., LTD.)
3) "Considerations in immunotoxicity assessment of biopharmaceuticals"
Chiyomi Kubo
(Translational Research Division, Chugai Pharmaceutical Co., Ltd.)
4) "Challenges in immunotoxicity evaluation for nucleic acid and gene therapy drugs"
Shogo Matsumura
(Non-Clinical Biomedical Science, Applied Research & Operations, Astellas Pharma Inc.)
5) Comprehensive discussion

Oral session of young scientist
Oral presentation
Poster presentation

The 11th Japanese Society of Immunotoxicology Award
(The 2021 JSIT Award)

Immunotoxicity of dioxins and their mechanisms

Keiko Nohara
National Institute for Environmental Studies

I am very honored to receive the JSIT Award for 2021. I would like to express my sincere thanks to the members of the award committee, as well as all my collaborators and colleagues, and all the JSIT members.

In 1999, Dr Chiharu Tohyama, the Director of our department, started the new dioxins research project covering immune, nervous, and endocrine systems and their risk assessment. Among dioxins, 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) is the most toxic congener, and this congener is commonly referred to as dioxin. Majority of the toxicities of TCDD and other dioxins are caused by activation of the transcription factor, arylhydrocarbon receptor (AhR) and TCDD is the most potent ligand. By the time the project started, TCDD was known to induce thymus involution and suppression of primary antibody production. Our immunotoxicity group aimed to determine the target cell type in which AhR activation is critical in each TCDD toxicity and the events following AhR activation.

Which cell type is the target of dioxin-induced thymus involution, AhR in the thymic stromal cells or thymocytes?

Thymus involution by TCDD is characterized by decrease in cell number and skewing of thymocyte differentiation toward CD8 single-positive (SP) T cells. There was controversy about the direct target cells of TCDD: a study using fetal thymus reaggregation culture reported the thymic stromal cells as the targets, on the other hand, a study utilizing chimeric mice having AhR-deficient hemopoietic cells or stromal cells reported the thymocytes or their precursor cells are responsible for the TCDD-induced thymus involution. To address this issue as well as analyze AhR function in T cells, we generated transgenic (Tg) mice expressing a constitutively active mutant of AhR (CA-AhR) specifically in T-lineage cells[1)]. This mice model mimics the situation where only T lineages are exposed to TCDD.

In the T cell specific CA-AhR Tg mice, the number of thymocytes were decreased and the percentage of CD8 single-positive thymocytes were increased. These results clearly show that AhR activation in the T-lineage cells is crucial in thymocyte involution.

Exploring the molecules involved in the thymus involution following AhR activation

To explore the molecular pathways leading to reduction of T cell numbers by AhR activation, we investigated the effects of transfection of CA-AhR in AhR-null Jurkat T cells. The results suggested that activated AhR in T cells causes both apoptosis and cell cycle arrest through expression changes of related genes[2].

The apoptosis-related gene Fas was included in the list of candidate genes whose alteration by activated AhR may lead to T cell loss[2]. To assess the involvement of Fas/Fas ligand (FasL)-dependent apoptosis in dioxin-induced thymus involution, we crossed the T cell specific CA-AhR Tg mice with Faslpr or FasLgld mice, which are both defective in Fas/FasL signaling[3]. The results showed that only AhR activation in thymocytes induces thymus involution in the absence of Fas/FasL signaling. Although we could not identify the down-stream signaling pathway leading to thymus involution by this study[3], our experimental model might be useful to examine the contribution of other candidate genes to thymus involution by dioxins.

The suppressive effects of dioxin exposure on antibody production and the mechanism

We also conducted several studies regarding the effects of TCDD on antibody production. We found that TCDD exposure inhibits generation of high-affinity antibody forming cells (AFCs) and high-affinity antibody production during primary humoral immune response. These alterations were suggested to be caused by the suppression of antigen-responding B-cell proliferation by TCDD during GC formation[4]. We also found that TCDD exposure suppresses IgM and IgG1 antibodies in serum and production of Th2 type cytokines, including IL-4, IL-5 and IL-6, in C57BL/6 mice and suppresses IgE production as well in Nc/Nga mice, a mouse model for atopic dermatitis, after secondary immunization[5, 6].

When we performed these studies in early 2000s, Th2 cells were thought to play a critical part in regulation of antibody production. After that, follicular helper T cells (Tfh) were identified as the responsible cells regulating antibody production. Thus, the IL-4 suppression that we detected might have represented suppression of Tfh function.

In the T cell specific CA-AhR Tg mice, splenocyte proliferation after immunization was inhibited, however, Th2 cytokine production or antibody production was not

suppressed[7]. On the other hand, IFN-γ production was increased after immunization in the Tg mice[7] as observed in TCDD-exposed and immunized wild type mice[5), 6)]. These results suggested that AhR activation in other cell types are collaborating in the antibody suppression.

Here I just presented a part of studies we performed on TCDD immunotoxicity. I would like to thank all my research team members again.

After 2005 or so, the new fundamental functions of AhR in immune system have been discovered, such as involvement in differentiation of Treg cells and Th17 cells and regulation of macrophage function[8]. As AhR is activated by various environmental factors not only by dioxins, I look forward to learn the new findings by JSIT members and researchers in the world regarding immunotoxicity of environmental factors through disruption of fundamental AhR function.

In the end, I would also like to thank the wonderful members of SOT and ITSS for their encouragement and kindness.

Baltimore, USA

SOT
Annual Meeting
March 2009,

Tsukuba, Japan

Dr Gary R. Burleson
JSIT Annual Meeting
Sep 2010

Dr B. Paige Lawrence
JSIT Annual Meeting
Sep 2018

Dr Linda S. Birnbaum
NIES International
Advisory Board
Aug 2017

References

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2) Ito T et al. J Biol Chem 279, 25205, 2004.
3) Nagai H et al. Int Immunopharmacol 6, 279, 2006.
4) Inouye K et al. Toxicol Sci 74, 315, 2003.
5) Nohara K et al. Toxicology 172, 49, 2002.
6) Fujimaki H et al. Toxicol Sci 66, 117, 2002.
7) Nohara K et al. Int Immunol 21, 769, 2009.
8) Kimura A et al. J Exp Med 206, 2027, 2009.

The 11th Japanese Society of
Immunotoxicology Prize for Encouragement

Immunotoxicity related to myeloid-derived suppressor cells

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

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

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 immune checkpoint blockade (ICB) therapy resistant tumor-bearing hosts compared with ICB therapy sensitive hosts. Therefore, MDSCs are promising target for cancer immunotherapy.

Granulocyte colony-stimulating factor (G-CSF) is used for the treatment of chemotherapy-induced febrile neutropenia in patients with cancer. However, it has been suggested that G-CSF would promote tumor progression due to activating MDSCs. We showed that MDSCs differentiated in the presence of G-CSF in vitro exhibited the enhanced proliferation and immunosuppressive function compared with those differentiated without G-CSF. In addition, RNA-seq analysis suggested that G-CSF enhanced the immunosuppressive function of MDSCs through the upregulation of gamma-glutamyltransferase (GGT) 1. Moreover, in the neutropenic mouse model, we found that the administration of recombinant G-CSF interfered anti-cancer effect of chemotherapy. We showed that the inhibition of GGT with GGsTop, a GGT selective inhibitor, could prevent tumor progression elicited by G-CSF. These results suggest that the inhibition of GGT1 can eliminate the immunotoxicity of G-CSF in promoting tumor growth due to the attenuation of immunosuppressive function of MDSCs. GGsTop could be an attractive agent when receiving G-CSF treatment for febrile neutropenia in patients with cancer.

Immunotoxicological Research

Establishment of antisense screening method for innate immune activation via TLR9

Misato Tanaka
Astellas Pharma Inc.

Unmethylated CpG in single-stranded DNA is recognized by Toll-like receptor 9 (TLR9) and induces cytokine production. This suggests the potential risk of inducing an undesired immune response in human as an antisense class effect. Therefore, evaluation of immunogenicity in nonclinical studies is considered important for antisense drug discovery.

However, there are species differences in cytokine production. Since monkeys are generally known to be less sensitive than humans, it is considered that general toxicity studies in monkeys are insufficient for the risk assessment of cytokine production in human. Therefore, we established a screening method that can be utilized for the evaluation of the innate immune activation in antisense using human TLR9-expressing cell lines.

Here we used HEK-Blue™ hTLR9 Cells (InvivoGen) that stably co-express the human TLR9 and NF-κB-inducible SEAP (secreted embryonic alkaline phosphatase) reporter gene. We tried several experiments with hTLR9 Cells and the control cell line, HEK-Blue™ Null1 Cells (InvivoGen). The cells were stimulated with various CpG oligos. EC50 and the S/N ratio at each concentration were calculated using the absorbance depending on the amount of CpG oligo-induced SEAP expression. To verify between-day reproducibility, the assay was repeatedly performed 5 times using three types of CpG oligos with different NF-κB reporter activity. It was confirmed that only the combination of hTLR9 Cells and CpG oligo showed NF-κB reporter activity in a concentration-dependent manner. hTLR9 Cells showed low reactivity to class A and C CpG oligos and high reactivity to class B. The five independent tests demonstrated that the order of the activating potential was consistent within each test. These results suggest that the method using hTLR9 Cells is promising screening assay to select candidates of antisense drug with low potential of TLR9-mediated innate immune response.

In the future, we would like to investigate the differences between this screening method and cytokine assays using human PBMC or whole blood. In addition, we will try to clarify the characteristics of this method using our original antisense compounds while performing immunotoxicity research in the field of innate immunity.

Disclosure of conflict of interest : This work was funded by Astellas Pharma Inc. Misato Tanaka is employees of Astellas Pharma Inc.