ImmunoTox Letter Digest

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Report on the 28th Annual Meeting of the Japanese Society of Immunotoxicology (JSIT 2021)

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The 28th Annual Meeting of the Japanese Society of Immunotoxicology was held in the form of a web conference for two days, September 6th (Sat) and 7th (Sun), 2021. Initially, it was planned to be held at the lecture building of the Imabari Campus, Faculty of Veterinary Medicine, Okayama University of Science, but since the spread of coronavirus infections continued to be uncontrollable, the regular holding was abandoned in early June after discussions. We have decided to hold it as a web society. This is the second year since last year, and as with last year, posters are delivered on demand and oral presentations are delivered live. The final number of abstract submissions was not so different from last year due to the fact that it was held on the web, and we are truly grateful that we were able to hold the annual meeting successfully with the cooperation and support of many people.

At this meeting, symposium, special lectures and educational lectures were planned with the main theme of "relationship between innate immunity and acquired immunity and immunotoxicity".

Symposium was titled “The development status of various vaccine and their safety evaluation“. Dr. Hiroyuki Oshiumi (Kumamoto University) gave a lecture on "Micro RNAs associated with adverse reactions of vaccine”, Dr. Yoshikazu Yuki (HanaVax Co., Ltd.) gave a lecture on "Safety assessment of nasal vaccine", and Dr. Eita Sasaki (National Institute of Infectious Diseases) gave a lecture on "Screening for a safe and effective novel vaccine adjuvant using genomics technology”.

Special lectures of the annual meeting were “Dendritic cell- based new immunotherapy" by Dr. Shinichiro Fujii (RIKEN Center for Integrative Medical Sciences) and “Immunogeniity-related toxicity” by Dr.Jeanine Bussiere (Amgen Inc.), who was sent by SOT-ITSS, with a video distribution.

Educational lectures of the annual meeting were “The significance of allergy and innate lymphocytes (ICL2) from the evolutionary perspectives” by Kenji Matsumoto (National Research Institute for Child Health and Development) and “New Corona Vaccine” by Dr. Yasuhiro Yoshikawa (Okayama University of Sciences).

Workshop on the test methods was titled “Toward the development and guidance of *in vitro* immunotoxicity test method”, Drs. Setsuya Aiba (Tohoku University), Masaharu Fujita (Fujifilm Safety Evaluation Center), and Takao Ashikaga (National Institute of Health Sciences) presented on the development of *in vitro* immunotoxicology test methods for an immunosuppression or skin sensitization and the status of inclusion in the OECD test guidelines or guidance.

General session included 7 oral presentations and 15 posters, and Young Scientist Session included 8 presentations which were to be presented as posters as well as oral presentations. Fifteen minutes was given for each talk to ensure enough discussion time as previous annual meetings.

Dr.Michihiko Aoyama (National Institute of Health Sciences) received the Best Presenter Award and Mr.Takashi Kato (Wakayama Medical University) received the best Student and Young Scientist Presentation Award.

We would like to express sincere appreciation for attendants who actively discuss both oral and poster presentations. Totally 96 people attended the meeting.

We really feel the success of the meeting was thanks to your understanding and cooperation, as well as support from organizations and companies. We wish the further development of the Society.

The Best Presentation Award

Fcγ receptor-dependent internalization and off-target cytotoxicity of antibody-drug conjugate aggregates

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Antibody-drug conjugates (ADCs), which are monoclonal antibodies (mAbs) conjugated to highly toxic small molecules (payloads) via linkers, are one of the fastest growing classes of next-generation therapeutic mAbs. ADCs combine the advantages of the target-specificity of mAbs and the high tumor-killing efficacy of payloads, and achieve wider therapeutic windows than traditional chemotherapy. On the other hand, conjugation of payloads often increases the hydrophobicity of mAbs, resulting in reduced stability and increased aggregation. It is known that mAb aggregates have a potential risk for immunogenicity, because they can activate Fcγ receptors (FcγRs) on immune cells, and are internalized via FcγRs. However, the impacts of aggregation on the off-target toxicity of ADCs have not been well studied. In this study, we investigated the impacts of aggregation of ADCs on off-target cytotoxicity.

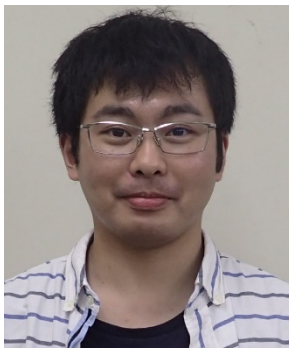
We generated aggregates of two commercial anti-HER2 mAb (trastuzumab)-based ADCs (T-DM1 and T-DXd) by stirring stress (600 rpm, 20 h) or thermal stress (90ºC, 3 min). A qLD size distribution analysis showed that aggregates between 0.1 and 50 μm were generated by the stress treatment, and these aggregates were removed after filtration of ADC aggregates with a 0.45 μm filter. We evaluated the cytotoxicity of ADC aggregates in HER2-positive cells and -negative cells. In several HER2-negative cell lines, the aggregation of ADCs enhanced the off-target cytotoxicity compared with non-stressed ADCs, whereas the target-dependent cytotoxicity was reduced by aggregation of ADCs. The enhancement of off-target toxicity was suppressed by filtrating the samples with a 0.45 μm filter. These results strongly suggested that the aggregates, which were removed by the 0.45 μm filter, were responsible for the enhancement of off-target cytotoxicity.

To examine the mechanism of off-target cytotoxicity, we focused on FcγR-activation properties of ADC aggregates, because ADC aggregates dramatically enhanced the off-target cytotoxicity in FcγR-expressing cell lines (such as THP-1 cells and MEG01-S cells). We evaluated the FcγR-activation properties of ADC aggregates using FcγR-expressing reporter cells. The results showed that the FcγR-activation properties of ADC aggregates varied depending on the kind of mAb/ADCs (trastuzumab, T-DM1, or T-DXd) and the stress treatment (stirring stress or thermal stress), and the FcγR-activation properties of ADC aggregates were related to the internalization and enhanced cytotoxicity of ADC aggregates in FcγR-expressing reporter cells. Additionally, FcγRIIa-blocking reduced the off-target cytotoxicity of ADC aggregates in an FcγRIIa-expressing cell line (MEG01-S), whereas the cytotoxicity of non-stress ADCs or the filtered sample of ADC aggregates was not affected by FcγRIIa-blocking. Fc-engineering for silencing Fc-mediated effector functions also reduced FcγR-mediated off-target cytotoxicity of ADC aggregates. These results indicate that FcγRs play an important role in the off-target toxicity of ADC aggregates.

Our findings indicated that the aggregation of ADCs increases the potential risk for not only immunogenicity but also off-target toxicity of ADCs, and FcγR-dependent internalization of ADC aggregates may be one of the mechanisms underlying the off-target toxicity of ADCs.

The Student and Young Scientists Award

Molecular mechanisms of type I interferonopathy in a novel COPA syndrome model mouse



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COPA syndrome is an autoinflammatory disease with autoimmune disorders, featured by interstitial lung disease, arthritis and glomerulonephritis caused by heterozygous variants in the coatomer subunit alpha (*COPA*) gene. COPA is a subunit of the coatomer protein complex I (COP I), a carrier complex required for retrograde protein trafficking from the Golgi apparatus to the endoplasmic reticulum (ER). In COPA syndrome, expression of interferon (IFN)-stimulated genes (ISGs) was elevated, what we call type I interferonopathy. At present, however, it is not clear how the genetic variant of *COPA* leads to the type I interferonopathy and manifestations of COPA syndrome.

Dr. Matsubayashi of the Seirei Hamamatsu hospital and Dr. Izawa of Kyoto University found a novel COPA variant (c.725T>G, p.Val242Gly) in four patients in one Japanese family. V242 is variant localized in the hotspot of the variants causing COPA syndrome, the WD40 repeat domain, and well conserved beyond species. In order to clarify whether or how the COPA V242G mutant contributes to the patient manifestations, we generated and analyzed the mutant mice.

Copa V242G heterozygous mutant mice manifested interstitial lung disease, which was similar to that of COPA syndrome patients. Furthermore, ISGs expression was elevated in splenocytes from the mutant mice. Dendritic cells from the mutant mice showed elevation of type I IFN production in response to a cytoplasmic DNA sensor, STING. Importantly, phosphorylation of STING and its signaling molecule, TBK1, was enhanced and STING localization was deviated from the ER to the Golgi apparatus in the mutant DCs when stimulated with a STING agonist. These findings suggest that *Copa* V242G heterozygous mutant led to defects in protein

transportation by COP I, thereby causing localization shift of STING from ER to Golgi and hyperactivation of STING-induced type I IFN production. *Copa* V242G mutant mice are novel model mice for COPA syndrome and further study on the mice should contribute to clarification of the pathogenesis, generation of the new therapeutic strategy for COPA syndrome and elucidation of regulatory mechanisms for STING pathway.

The Student and Young Scientists Award

Journey with "Immunotoxicity"

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Inflammatory bowel disease (IBD) is an inflammatory disease of unknown aetiology, and the number of patients in Japan has been increasing. One of the problems of IBD is that the mechanism of pathogenesis is unknown and the curative therapy has not been established. Furthermore, the pressure on medical costs caused by the increase in the number of patients is making it difficult to continue social security, and in 2017, some patients with mild disease were excluded from public subsidies. Against this such background, I started research using clinical big data when I was working in the Pharmaceutical Department, and I continue to do so today. In these analyses, I focused on 5-aminosalicylic acid (5-ASA), a key drug for the treatment of IBD. 5-ASA has been reported to have several mechanisms, including inhibition of reactive oxygen species production, activation of PPARγ, and inhibition of NF-κB. In addition, it has been suggested that 5-ASA induces regulatory T cells (Tregs) via the aryl hydrocarbon receptor (AhR), and we have investigated whether 5-ASA is a direct ligand for AhR using DR-EcoScreen cells, a highly sensitive reporter cell line. In silico docking simulation was used to calculate the affinity of 5-ASA to the ligand binding site. As a result, we recently reported that 5-ASA acts as a ligand

for AhR at high concentrations, and has Treg-inducing ability in a primary spleen culture system (Kubota *et al. Pharmacology*. in press).

Similarly, Elemental diet (ED) therapies, which are formulations of complete gastrointestinal nutrition, have been used empirically to protect the mucosa damaged by inflammation and reduce the burden on intestinal function. Therefore, we focused on the components of ED therapy and its intestinal microflora metabolites and investigated whether they have anti-inflammatory effects using a DSS-induced colitis model. As a result, we found that several metabolites of the components in ED therapy functioned as AhR ligands and induced splenic Tregs in mice treated with ED therapy. Furthermore, we found that ED therapy with enhanced active ingredients significantly suppressed DSS-induced colitis, and we are now focusing on the transport mechanism of the metabolites.

In the future, we would like to establish a method to control immunotoxicity through modification of existing therapies targeting AhR and drug repositioning, and strive to establish basic evidence, which is useful for clinical use.