ImmunoTox Letter Digest

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# Report from the 23th annual meeting of the Japanese Society of Immunotoxicology (JSIT2016)

Yasuo Morimoto

University of Occupational and Environmental Health, Japan.

**The 23th annual meeting of the Japanese Society of Immunotoxicology (JSIT 2016) was held at Kitakyushu International Conference Center, from 5th to 7th September, 2016. The main theme of this meeting was "Immunotoxicology for Social Practice". The meeting consists of 1 special lecture, 3 educational lectures, 1 symposium, 1 workshop, 2 luncheon seminars, JSIT Research Award lectures, 10 oral presentations, 14 poster presentations, and 2 student and young scientist presentations.**

**Special lecture**

**Inhaled Nanoparticles: Consequences of Exposure and Approaches for Hazard Identification**

**(Victor J. Johnson, Burleson Research Technologies, Inc., USA)**

**Master's Lecture**

**1. Autoimmunity induced by adjuvants**

**(Minoru Satoh, Department of Clinical Nursing, School of Health Sciences, University of Occupational and Environmental Health, Japan)**

**2. Toxicity and side effects of immunosuppressive drugs for treatment in rheumatic diseases**

**(Kazuyoshi Saito, The First Department of Internal Medicine, School of Medicine, University of Occupational and Environmental Health, Japan)**

**3. Clinical characteristics and mechanisms of drug-induced pulmonary toxicity.**

**(Kazuhiro Yatera, Department of Respiratory Medicine, University of Occupational and Environmental Health, Japan)**

**Symposium**

**"Estimation of pulmonary toxicity by fine and ultrafine particles and its social practice"**

**1. Development of hazard screening methods for industrial nanomaterials: intratracheal instillation study.**

**(Hiroto Izumi, Department of Occupational Pneumology, Institute of Industrial Ecological Sciences, University of Occupational and Environmental Health, Japan)**

**2. Lung inflammation and allergy induced by fine particles**

**(Etsushi Kuroda, Laboratory of Vaccine Science, Immunology Frontier Research Center, Osaka University)**

**3. Development of devices to screen asbestos exposure and mesothelioma based on immunological analysis**

**(Yasumitsu Nishimura, Department of Hygiene, Kawasaki Medical School, Kurashiki, Japan)**

**4. From databases and bioinformatics to pulmonary diseases**

**(Kenji Mizuguchi, National Institutes of Biomedical Innovation, Health and Nutrition, Japan)**

**Workshop**

**"Adverse outcome pathway (AOP) and immunotoxicity research"**

**1. The Concept of development for AOP in OECD**
**(Hajime Kojima, National Institute of Health Sciences (NIHS))**

**2. The Adverse Outcome Pathway on immunotoxicity of chemicals that dysregulates the induction of Th subsets through affecting IL-2 transcriptional activity.**
**(Yutaka Kimura, Department of Dermatology, Tohoku University Graduate School of Medicine, Japan)**

**3. The Adverse Outcome Pathway on immunosuppression caused by formation of FKBP12-FK506 complex**
**(Shiho Ito, AOP Working Group, Testing Methodology Committee, The Japanese Society of Immunotoxicology)**

**4. Point to note learned from the experience of AOP development**
**(Kiyoshi Kushima, AOP Working Group, Testing Methodology Committee, The Japanese Society of Immunotoxicology)**

**JSIT Research Award**

**Immunotoxic effects of agro-chemicals**
**(Tadashi Kosaka, Study Management Division, The Institute of Environmental Toxicology)**

**JSIT prize for encouragement**

**Effects of environmental chemicals on immune response via nuclear receptors.**
**(Hiroyuki Kojima, Department of Environmental and Health Sciences, Hokkaido Institute of Public Health, Sapporo)**

**The Best Presentation Award**

**LPS-rich urban particulate matter 10 suppresses immune responses in splenocytes**
**(Yasuhiro Yoshida, Department of Immunology and Parasitology, University of Occupational and Environmental Health, Japan)**

**The Student and Young Scientists Award**

**Construction of an in vivo Model for Evaluating the Immune-Mediated Idiosyncratic Drug Toxicity Using Chimeric HLA Transgenic Mice**
**(Takeshi Susukida, Laboratory of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Chiba University, Japan)**

**Analysis of Mechanism of Idiosyncratic Drug toxicities Using Keratinocyte Derived From HLA Tg Mice**
**(Sota Fujimori, Laboratory of Biopharmaceutics, Graduate School of Pharmaceutical Sciences, Chiba University, Japan)**

## The 5th Japanese Society of Immunotoxicology Prize for Encouragement

### Crosstalk between immunotoxicology and adjuvant innovation

Etsushi Kuroda

Immunology Frontier Research Center,

Osaka University

**I'm very honored to receive this award, the 5th Japanese Society of Immunotoxicology Prize for Encouragement. First of all, I will introduce my work and interest in immunotoxicology. I earned this award as the study of particulate-induced immune responses. So far, many scientists have investigated how particulate stimulate immune cells, however detailed mechanisms are still unknown. In general, immune systems are initially activated by pathogens through unique receptors, referred to as pattern recognition receptors (PRRs). These PRRs were mainly expressed on innate immune cells such as macrophage and dendritic cells, and these cells are at first activated. Next, for the activation of acquired immunity, signals from innate cells are required. Given that particulates activate acquired immune responses, many scientists believe that particulates directly activate innate immunity through known or unknown receptor(s). However, recent studies including our work revealed that particulate-induced cell death and released damage-associated molecular patterns (DAMPs) trigger immune responses. These DAMPs are mainly released from dying macrophage that engulfed particulate, and we found that lipid mediator, prostaglandin E2 (PGE2), plays an important role for the adjuvant activity of particulate alum and silica. PGE2 is one of DAMPs and mainly released from alum- or silica-stimulated macrophages. We also observed that PGE2 regulates antigen-specific antibody production. Other research group reported that host DNA release from dying cells by alum function as adjuvant to induce immune responses, suggesting that many kinds of DAMPs are involved in particulate-induced immune responses.**

**Recently, we focus on particulate-induced lung inflammation. In this case, DAMPs are also important for the activation of lung immune responses. In addition, in the case of the lung, we observed that several unique responses are elicited, such as the formation of tertiary lymphoid organ mediated by unique alveolar macrophage functions. Thus, DAMPs might be important factors for particulate-induced immune activation, and further investigation of DAMPs or mechanisms of cell death induced by particulate might open for the novel adjuvant innovation.**

## The Best Presentation Award

### LPS-rich urban particulate matter 10 suppresses immune responses in splenocytes

Yasuhiro Yoshida[1], Yuan Song[1],

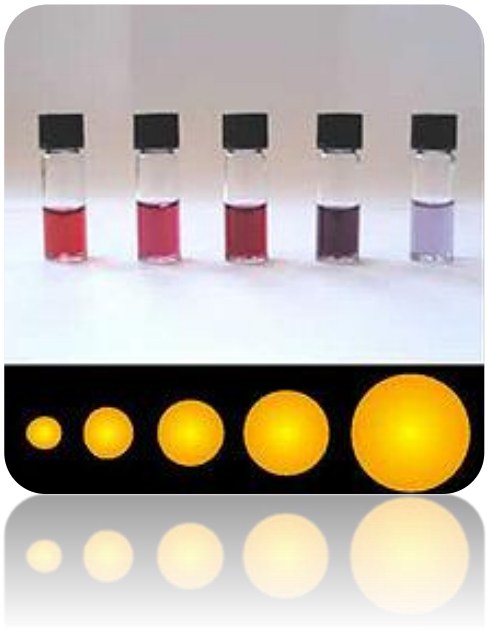
Takamiti Ichinose[2]

[1]Department of Immunology and Parasitology, University of Occupational and Environmental Health, Japan

[2]Department of Health Sciences, Oita University of Nursing and Health Sciences

**Exposure to elevated concentrations of particulate matter (PM) is known to have adverse health impacts. Lelieveld et al. reported that outdoor air pollution causes 3.3 million premature deaths per year worldwide, predominantly in Asia (Nature, 2015, 525, 367), based on data on the global burden of disease for 2010 (Lancet,**

**2012, 380, 2224). We previously reported that Asian sand dust (ASD), which contains PM less than 10 μm in diameter (PM10), induced subacute inflammation in splenocytes (Environ Toxicol, 2015, 30, 549). However, it was unclear whether the PM itself or compounds attached to its surface induced the inflammation. Here we characterized the role of organic substances adsorbed to the PM10 surface in triggering inflammation by comparing the effect on splenocyte activation of PM10 from urban areas (urPM10), which is rich in lipopolysaccharide (LPS) as compared to ASD, with that of heated PM10 (H-PM). BALB/c mice were intratracheally administered urPM10 or H-PM with or without LPS (1 ng and 10 ng) four times at 2-week intervals, and splenocytes were prepared at 24 h after the final administration to assay the immune responses. To clarify whether urPM10 affects splenocytes, we examined the activation status of splenocytes obtained from mice treated intratracheally with urPM10. Lower activity in the splenocytes of mice treated with urPM10 than in those of control mice was observed. To investigate any involvement of components adsorbed to the urPM10 surface (microbiological materials, sulfate, etc.), the urPM10 was heated to remove these substances. In contrast to urPM10, H-PM induced activation of splenocytes and enhanced mitogen-induced immune responses. To clarify the involvement of microbiological materials in the suppressive effect of urPM10 on splenocytes, the concentrations of LPS in urPM10 and H-PM were measured. urPM10 had 0.326 ng LPS/mg PM, while no LPS was detected in H-PM. This finding indicated that urPM10 contains a substantial amount of endotoxin as compared to flying PM10 (ASD). ASD contained 0.0614 ng LPS/mg PM. As expected, LPS neutralization by polymyxin B rescued urPM10-induced immune suppression. Co-administration of LPS with H-PM clearly suppressed mitogen-induced immune responses in the spleen. Consistent with these results, H-PM induced the phosphorylation of nuclear factor κB (NF-κB) p65 and I kappa B kinase (IKK), which was inhibited by co-administration of LPS. In mice deficient in the LPS signal transducer MyD88, splenocyte activation after LPS or H-PM treatment in vivo was comparable to that in the control. Taken together, our results indicate that PM10 itself could activate NF-κB though the MyD88 pathway, which was modulated by the amount of LPS attached. In conclusion, exposure to endotoxin-rich particles generates immune-suppressive phenomena. Exposure to environmental pollutants induces lung inflammation, which can be tackled by the immune system. However, chronic pollution-induced inflammation reactions cannot be overcome by an immune system that is suppressed by the action of endotoxin-rich pollutants. In turn, constitutive inflammatory events cause severe inflammation that can be linked to certain organ-specific cancers. Future studies are warranted to investigate these potential links further.**



The Student and Young Scientists Award

## Construction of an *in vivo* Model for Evaluating the Immune-Mediated Idiosyncratic Drug Toxicity Using Chimeric HLA Transgenic Mice

Takeshi Susukida

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

**I am very honored to receive the Student & Young Scientists Award at the 23th Annual Meeting of Japanese Society of Immunotoxicology. I would like to sincerely appreciate the support from the selection committee.**

**This study aimed to construct transgenic mice carrying chimeric human leukocyte antigen (HLA) and consider the usefulness of chimeric HLA transgenic mice for evaluating the immune-mediated idiosyncratic drug toxicity (IDT). Although genome-wide association studies revealed that the occurrence of IDT strongly associates with particular HLA allotype, little is known about the underlying mechanisms of these associations by specific drugs and their development in specific organs. Therefore, in this study, we focused on HLA-B*57:01 and abacavir (an anti-HIV drug)-induced immune-mediated IDT (skin rash), and evaluated the immune toxicity in transgenic mice carrying chimeric HLA-B*57:01 (B*57:01-Tg) after abacavir exposure. As a result, abacavir exposure elevated immune response, such as the increase of lymph node weight and the proliferation of lymphocytes, in B*57:01-Tg mice; while this tendency was not observed in littermate wild type mice and B*57:03-Tg mice (negative control differing by 2 amino acids from HLA-B*57:01). These results suggested that chimeric HLA transgenic mice might be useful model for evaluating the immune-mediated IDT. Since this mouse model could potentially evaluate IDT prospectively, it may be possible to elucidate the underlying detailed mechanism of abacavir-induced IDT. Furthermore, this mouse model will probably provide an answer to the question of the organ specificity of IDT, given that chimeric HLA protein was expressed ubiquitously.**

**I would also like to express my gratitude to my research professor Prof. Kousei Ito, Res. Assoc. Shigeki Aoki, and other colleagues in Laboratory of Biopharmaceutics including Mr. Sota Fujimori and Mr. Kotaro Kogo for supporting throughout the progress.**

The Student and Young Scientists Award

## Analysis of Mechanism of Idiosyncratic Drug toxicities Using Keratinocyte Derived From HLA Tg Mice

Sota Fujimori

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

**I am very happy and honored to be the best Young presenter Award at the 23th Annual Meeting of JSIT 2016. I would like to sincerely thank you for the committee.**

**Genome-wide association studies indicated that idiosyncratic adverse drug reactions (IADR) are related with human leukocyte antigen (HLA) alleles. HLA-associated IADR include many cutaneous adverse reactions. For example, abacavir (ABC)-hypersensitivity is associated with HLA-B*57:01 allele, and patients carrying the allele develop skin rash. However, little is known about the reason why the IADRs are caused selectively in skin. Therefore, we have originally constructed HLA transgenic mice (HLA-B*57:01-Tg and HLA-B*57:03-Tg) and**

**investigated immune reactions by keratinocytes (KC) in ABC-hypersensitivity using KC derived from these HLA-Tg (ABC is not presented by B*57:03).**

**In KC derived from HLA-B*57:01-Tg , mRNA expression of proinflammatory cytokines (IFN-γ and IL-1β) and cell surface expression of HLA protein were both increased by ABC exposure. These phenomena were not observed in KC from littermate controls or HLA-B*57:03-Tg.**

**By contrast, in HeLa cells transfected with HLA-B*57:01, increased cell surface expression of HLA protein were not detected. Cytokines secretion or increased drug presentation in KC might stimulate cutaneous dendritic cells, T cells, or other immune cells, therefore such characteristic reactions might determine skin-selective IADR. I intend to understand the mechanism of immune reactions caused by ABC-HLA-B*57:01 interaction specific to KC and establish the prediction method of HLA-associated IADR.**

## Words from New councilors

Akiko Ishii-Watabe

National Institute of Health Sciences

**I would like to express my sincere appreciation to the Board of Directors and the members of the JSIT for accepting me as a councilor. It is my great pleasure to contribute the activity of JSIT with this responsibility.**

**Currently, I am engaged in the regulatory science research for biopharmaceuticals in National Institute of Health Sciences. Since the active ingredients of biopharmaceuticals are proteins of high molecular weight, they potentially have safety issues related to the unwanted immunogenicity. Emergence of anti-drug antibodies may lead to hypersensitivity reactions or other adverse events caused by the immune complex. Elucidating the risk factors related to these unwanted immunogenicity and establishing the risk mitigating strategies are important issues for immunotoxicological researches.**

**There are accumulated experiences about the unwanted immunogenicity of biopharmaceuticals overseas and regulatory guidelines/guidances have been released from EMA and FDA. On the other hand, the regulatory requirements for immunogenicity assessment have not been established in Japan. Since newly developed biopharmaceuticals and biosimilars are increasing, there is an emergent need to establish the fundamental issues in immunogenicity assessment. Elucidating the mechanisms and establishing the evaluation method of other immunological adverse events such as cytokine release syndrome is also important. I would like to contribute to the JSIT through promoting these immunotoxicological researches related to the safety issues of biopharmaceuticals.**

## Words from New councilors

Tetsuya Sakairi
Safety Research Laboratories
Sohyaku. Innovative Research Division
Mitsubishi Tanabe Pharma Corporation

**First of all, I would like to take this opportunity to express my gratitude to members of the Board of Directors, Japanese Society of Immunotoxicology for recommending and accepting me as a councilor. I am greatly honored to be approved as a councilor of this Society.**

**I started my career as a pathologist at the Laboratory of Veterinary Pathology, the University of Tokyo, and have been in charge of pathological examination after joining to Mitsubishi Tanabe Pharma Corporation. During my Ph.D. thesis, I worked on the characterization of chemically-induced hepatoblastomas in mice by pathological, immunohistochemical and molecular biological approach to focus on their pathogenesis. At the same time, I have been engaged in non-clinical safety evaluation including immunotoxicity risk assessments of candidate compounds for drugs. In the toxicity studies, pathological examination plays an important role in detecting immunotoxic effects of a test compound and in making a beginning for a systematic investigation into the pathogenesis; however, broad knowledge of immunology and immunotoxicology is also indispensable to clarify the mode of toxicity and relevance to humans.**

**I will do my best to make meaningful contributions to the Japanese Society of Immunotoxicology through developing expertise in pathology as well as in immunotoxicology. I hope you will give me your further guidance and encouragement.**

## Workshop on immunotoxicity testing methods: Adverse Outcome Pathway and Immunotoxicity

**Introduction**

**At the request of the Japanese Center for Validation of Alternative Methods (JaCVAM), the Japanese Society of Immunotoxicology (JSIT) Committee on Immunotoxicity Testing Methods has established an Adverse Outcome Pathway working group, whose members are predominantly junior JSIT members, to develop Adverse Outcome Pathways (AOP) for immunotoxicity.**

**This AOP working group held a workshop during the recent JSIT Annual Meeting to introduce the concept of AOP and explain AOP of immunotoxicity that are now under development.**

**The concept of an AOP and the Organization for Economic Co-operation and Development project**

**Dr. Hajime Kojima of the National Institute of Health Sciences (NIHS) spoke about this topic.**

**The AOP concept**

**An AOP describes a sequential chain of causally linked events at different levels of biological organization, which lead to an adverse health or ecotoxicological effect. It starts with a molecular initiating event (MIE) at a target molecule and connects key events (KE) at each different level to reach an adverse outcome (AO). KEs are identified as the most important events at each level of the molecular, cellular, organ, or organism response. This can include whole populations when ecotoxicological effects are also considered. Information about KEs includes key event relationships (KER)—such as mechanisms, relevance, and species differences—as well as measuring methods and validity.**

**Chemicals oftentimes interact with multiple toxicological target molecules. Thus an AOP induced by such a chemical could include a complex of AOPs with their respective MIEs. If a common KE exists in the set of AOPs, the downstream pathways might link to a single pathway and, in some cases, another pathway might join together at the common KE to form an AOP network.**

**The OECD AOP Knowledge Base**

**The Organization for Economic Co-operation and Development (OECD) is currently building an AOP knowledge base to be used in regulatory decision-making. AOPs are available via the Internet on the AOP Wiki, which contains a total of 167 AOP as of December, 2016.**

**Clarification of the key events through the development of AOP is also expected to lead to the development of new in silico, in chemico, and in vitro methods to predict these risks. Furthermore, as the knowledge obtained through the development of AOP becomes better known, it will be integrated into comprehensive approaches to risk assessment, known as Integrated Approach to Testing and Assessment (IATA). An ultimate goal of the AOP project is to enable the application of IATA to regulatory decision making.**

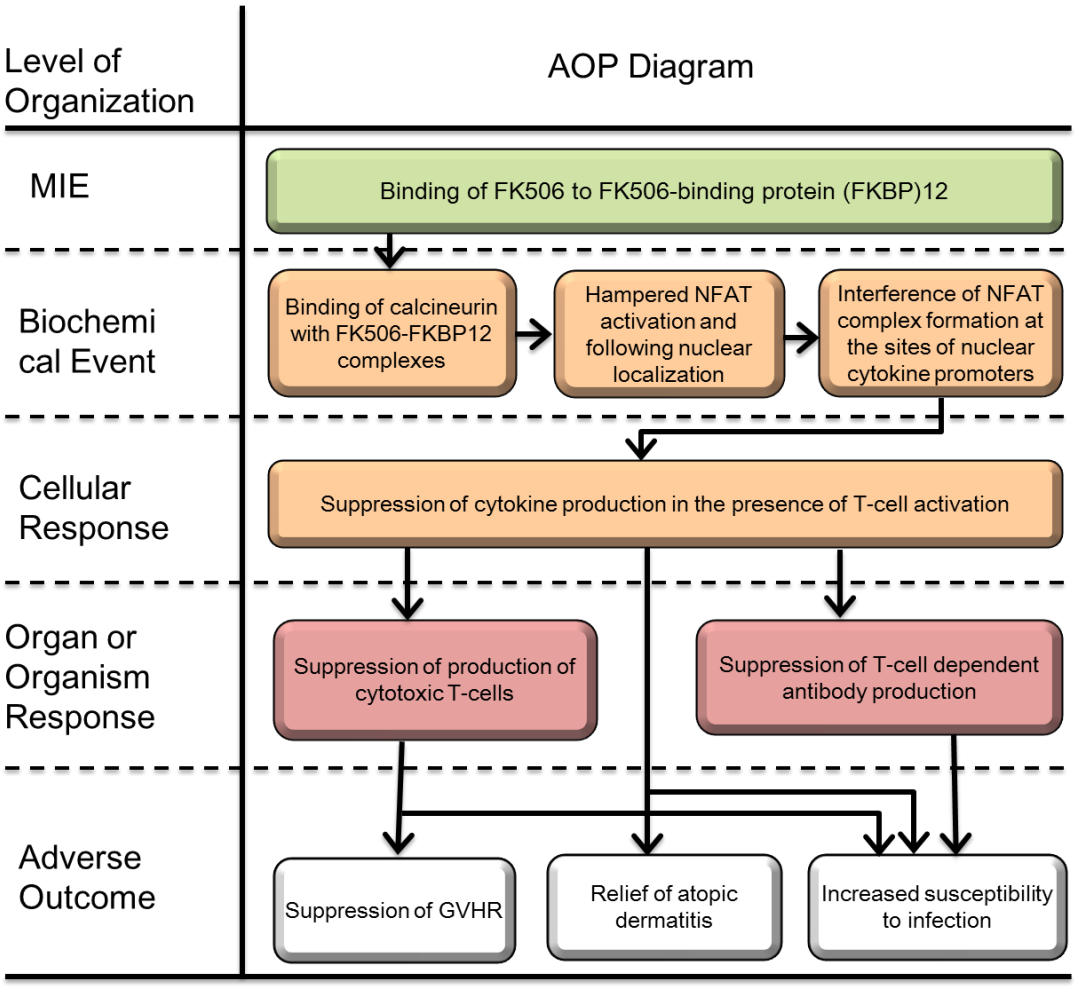



Fig. 1 AOP for immunosuppression induced by FKBP12-FK506 complex formation

**AOP of immunotoxicity**

**[AOP of immunosuppression induced by FKBP12-FK506 complex formation]**

**Drs. Shiho Ito and Kiyoshi Kushima from the JSIT AOP working group presented an outline for an AOP of immunosuppression and described their experience in AOP development.**

**In its efforts to further development of AOP and IATA of immunosuppression, the working group has created potential AOP for several types of immunosuppressants with differing modes of action (MOA).**

**The first AOP the working group focused on was for immunosuppression related to calcineurin inhibition induced by FK506 (Tacrolimus), for which they collected information on the FK506-related immunosuppressive events irrespective of pharmacology or adverse effects based on a review of recent literature and then identified KEs at each level of the biological organization and evaluated the relevant KERs.**

**As a result, despite a diverse variety of FK506-related events noted in different types of immune cells, there was only one pathway found connecting the KEs from MIE to AO. The pathway initiates at the MIE of FK506-binding protein12 (FKBP12)-FK506 complex formation, followed by calcineurin inactivation, suppressed nuclear translocation of nuclear transcription factor of activated T cells (NFAT), and inhibition of Th1 and Th2 type cytokine production by T cells. Suppression of GVHR (graft-versus-host reaction), relief of atopic dermatitis, and increased susceptibility to infection are recognized as AOs in this AOP. (Fig.1)**

**The working group continues to revise the AOP through a series of internal and external reviews per the process defined by the OECD AOP project. Once this AOP is finalized, the working group will use the experience gained to proceed with the development of other new AOPs for different classes of immunosuppressive effects.**

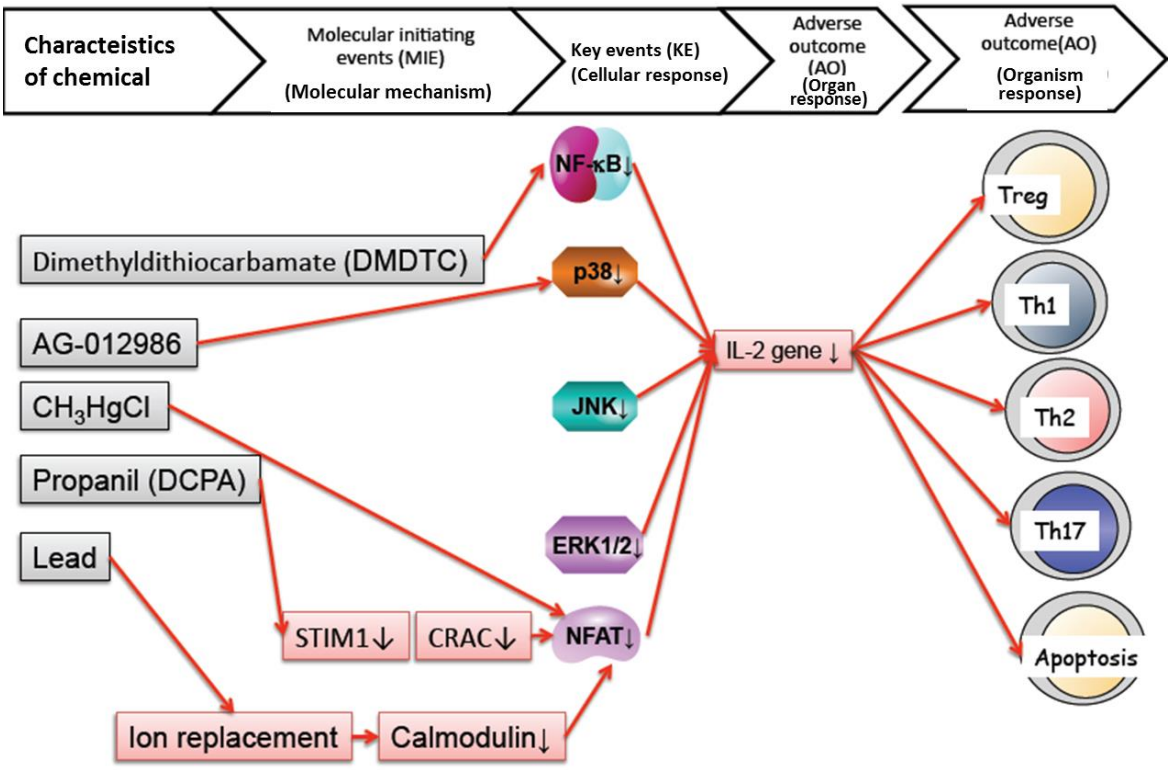



Fig.2 AOP for induction of abnormal T cell differentiation, in which suppressed transcription of the IL-2 gene is a key event

**[AOP of abnormal differentiation of T cells through suppressed transcription of IL-2 gene as a key event]**

**Dr. Yutaka Kimura from Tohoku University School of Medicine introduced a proposed AOP for abnormal differentiation of T-cells through a KE involving the suppression of transcription of IL-2 genes.**

**This AOP has been developed as a rationale for the measurement of IL-2 gene transcription activity in the in vitro Multi-immuno Tox Assay (MITA).**

**During the development of the AOP, the effects of five factors were investigated, namely dimethyldithiocarbamate (DMDTC), AG-012986 (pan-CDK-inhibitor), methymercury (CH3HgCl), propanil (3,4-dichloropropionanilid), and lead (Pb).**

**The AOP shows that each of the five factors suppresses IL-2 gene expression through a different manner of suppression of one of the several transcription factors to alter the function of T-cell subsets such as Treg, Th1, Th2, and Th17 or to induce apoptosis. (Fig.2)**

**Conclusion**

**The working group will continue to develop potential AOP in order to develop IATA of immunosuppression in association with researchers in a broad range of specializations from a number of organizations and enterprises. We look forward to receiving inquiries from JSIT members who have an interest in the development of AOP.**