Report on "Radiation Disaster Recovery Studies"

Course: Radiation Disaster Medicine
Name: Ho Minh Van

1. Regarding "Radiation Disaster Recovery Studies"

(Describe your thoughts, the process you engaged in and your research progress regarding Recovery from Radiation Disaster.)

On outline of radiation disaster, Japan is the only country in the world experienced with two radiation disasters (atomic bomb in 1945 and nuclear power plant accident in 2011). For 7 years pasted from triple disaster in Fukushima, Japan still face with complex scenarios on the way of recovery from radiation disaster. From the level of government to localized citizen, many efforts put on renaissance at Fukushima were done with hope to build up new life at exposure areas. In fact, many projects as such environmental cleaning, health surveys, and social troubleshooting were going and achieved successful effectives on soil decontamination, continuity health management, encourage people returning home, etc. However, recently remaining many social issues with anxiety, post-traumatic stress disorder (PTSD) (Maeda and Oe 2017), discrimination, unbalance population due to difficult-to-return (Hasegawa et al. 2016), etc., that require actions of Japanese experts and international roles in process of resilience from radiation disaster at Fukushima (Tanigawa et al. 2017).

Evacuees were living in temporary house or moved-place for a long time, they have built a new life with job, education, etc., hence refused to return for building life again. Moreover, lacking job opportunity at return-zone blocks the back of young citizen. Consequently, leading to unbalance population structure and more overs. On the other hand, people do have high level of worries about healthy as cancer developments or any radiation-associated disorders. Regarding on those concerns, Fukushima Health Survey Project (Yasumura and Abe 2017) has conducted right after the disaster. Its goals were to manage population health in term of general exam (physical and labs exam) for all citizen at the contaminated zone. This project has contributed low level of radiation-based evidences of health troubles. The data was open to public, as well as citizen via home visit. Pooling with this trend, we are now looking for biomarkers, that support to detect the suppression of immune system in radiation-exposure people.

The study considers on early detection of the paths, that radiation directs immune disorders. Radiation victims might category into two groups as moderate and over

exposure to radiation (in cases of radiation accident) and mild exposure to radiation (experienced with radiation at low dose). For moderate and over exposure patients, the disease profiles character with multiple organ dysfunction, infection, and death. Typically, sepsis with immunoparalysis is the common scenario of patient upon severe radiation exposure. This scenario is a complicated medical trouble with many co-mortality factors and poorly outcomes. However, the understanding of how immune system tolerated with infection under irradiation remain unclear. Otherwise, mild exposure or lightly experienced to radiation in environment (low dose exposure), the long-term effects and bio predictors are currently medical issues. Among these, biomarkers derived by radiation, that can predict the early development of immune disorders are placed on hot topics of radiation-related research. In this threshold, our research conducts on relationship of innate immune responses to danger-associated molecular patterns (DAMPs), that were released by cell deaths upon irradiation to human serum. We expect that the DAMPs activities in serum might reveal evidence of immune disorder development upon irradiation.

Our project planned as two steps such 1) attempting to assess the responses of innate immune cells to DAMPs activities in serum from common clinical settings (healthy, trauma, and sepsis) with expectation of identifying the comparable baseline of those patient profiles; 2) attempting to assess the responses of innate immune cells to serum from radiation victims with expectation of finding valuable biomarker of radiation-associated disorder. At the time, we are anchored on the first step of plan. Further research related to radiation victims is required.

References:

Hasegawa, A., Ohira, T., Maeda, M., Yasumura, S. &Tanigawa, K. (2016) Emergency Responses and Health Consequences after the Fukushima Accident; Evacuation and Relocation. Clinical Oncology. ***28****, 237-244.*

Maeda, M. &Oe, M. (2017) Mental Health Consequences and Social Issues After the Fukushima Disaster. Asia Pacific Journal of Public Health. ***29****, 36S-46S.*

Tanigawa, K., Lochard, J., Abdel-Wahab, M. &Crick, M.J. (2017) Roles and Activities of International Organizations After the Fukushima Accident. Asia Pacific Journal of Public Health. ***29****, 90S-98S.*

Yasumura, S. &Abe, M. (2017) Fukushima Health Management Survey and Related Issues. Asia Pacific Journal of Public Health. ***29****, 29S-35S.*

2. Title of doctoral thesis

Sera from Septic Patients Contain the Inhibiting Activity of the Extracellular ATP-Dependent Inflammasome Pathway

(敗血症患者の血清には細胞外 ATP 依存性インフラマソーム経路の活性抑制物質が含まれる)

3. Summary of doctoral thesis

(Describe so as to be easily understood, by relating it to "Radiation Disaster Recovery Studies")

Sepsis, a systemic inflammatory response to infection, causes multi-organ failure, severe morbidity, and death; endothelial dysfunction, metabolic changes, and overwhelming or hyperinflammatory innate immune responses to pathogen-associated molecular patterns have been proposed to be the main underlying mechanisms of this condition. Sepsis-induced immunoparalysis, in which both innate and adaptive immunity are suppressed, is also an active focus of research.

Inflammatory responses mediated by innate immunity generally promote healing and microbial clearance, although the massive production of pro- and anti-inflammatory cytokines by macrophages might also lead to pathological inflammation. Primary inflammatory cytokines include tumor necrosis factor-α, interleukin-1β (IL-1β), and IL-6, which mediate acute inflammation but also cause cell injury and cell death. In turn, injured tissues and dying cells generate damage-associated molecular patterns (DAMPs), which further engage various inflammasomes to promote or suppress inflammation. Although the underlying mechanisms are unknown, damage-associated molecular pattern molecules (DAMPs) from septic tissues might be involved. DAMPs stimulate nucleotide oligomerization domain-like receptor (NLR) inflammasomes to activate caspase-1, which then cleaves pro-IL-1β to generate mature, bioactive IL-1β. One such NLRP3 inflammasome is particularly sensitive to a wide variety of DAMPs including adenosine triphosphate (ATP), crystalline substances, nucleic acids, and hyaluronan. In this study, we screened sera from septic patients for the ability to alter innate immune responses to DAMPs and inflammasomes, with the goal of defining mechanisms that drive immunoparalysis during sepsis.

Macrophages derived from THP-1 human acute monocytic leukemia cells are one of key points in innate immune response. THP-1-mediated macrophages were incubated with each DAMP, in the presence or absence of sera that were collected from critically ill patients. Sera from critically-ill patients diagnosed with sepsis or trauma were collected at the time of admission to Emergency and Critical Care Center. Secreted cytokines in supernatant were then quantified, and treated-cell lysates were assayed for relevant intracellular signaling mediators.

The results indicated that sera collected from septic patients at the time of admission tended to suppress the in vitro production of IL-1β by macrophages; however, sera from trauma patients or healthy volunteers did not. This result suggests that septic sera, collected at the time of patient admission, are likely predominantly anti-inflammatory, despite enhanced levels of both pro- and anti-inflammatory cytokines. Strikingly, sera from septic patients who ultimately did not survive significantly suppressed IL-1β production in

response to extracellular adenosine triphosphate (ATP) only. This effect was most pronounced with sera collected on day 3, and persisted with sera collected on day 7. However, this effect was not observed when THP-1 macrophages were treated with sera from survivors of sepsis. Therefore, we hypothesize that sera from septic patients have inherent anti-inflammatory functions and might contain factor(s) that suppress the response to extracellular ATP by possibly mechanisms as ATP hydrolysis by ATPases in sera from septic patients, inhibition of P2X7, or inhibition of NLRP3 inflammasome.

The further experiments were setting up for identifying mechanisms of the observed loss of ATP-induced IL-1β production. The results suggest that ATPase activity in sera from septic patients is negligible, and septic sera from non-survivors enhance P2X7 activity. Then cell lysates were subjected to the detection of cytosolic mediators associated with the ATP-dependent inflammasome pathway. Results shown that septic sera collected at the time of admission (day 1) also diminished intracellular levels of inositol 1,4,5-triphosphate and cytosolic calcium ($P < 0.01$), both of which are essential for ATP signaling. Finally, activated caspase-1 was significantly diminished in cells exposed to sera collected on day 7 ($P < 0.05$). Taken together, these data suggest that activation of NLRP3 and caspase-1 in response to extracellular ATP is suppressed by factors that are present in sera from septic patients. This suppressive effect is clearly associated with prolonged immunoparalysis and poor clinical outcomes since it was found to be more pronounced in sera collected from patients who did not survive.

Our data indicated that sera from septic patients suppress IL-1β production in macrophages stimulated with extracellular ATP, likely by antagonizing upstream NLRP3 or pro-inflammatory pathways. This suppressive effect peaked on day 3 after admission and persisted until day 7 among patients who did not survive, but was lost by day 7 among patients who survived. These observations suggest a new mechanism through which sepsis mediates immunoparalysis, and partially support the prior hypothesis that persistent suppression of innate and adaptive immunity can cause late-stage death in septic patients. In conclusion, factors present in the sera of septic patients that persistently suppress the immune response to extracellular ATP might trigger adverse clinical outcomes.

4. Other thesis published in academic research journals

- Title of thesis: Memorable experiences of U.S long-term internship, supported by Phoenix Leader Education Program
- Joint authors: Van Minh Ho, Akira Nishisaki, Nobuyuki Hirohashi, Nobuaki Shime
- Journal: Anaesthesia and Resuscitation, 53(4):81-84 · March 2018