2016Joint Usage and Research Report

Title of Research Project		Role of AHR-inducible ADP-ribosyltransferase TIPARP in the innate defense system
Applicant	Institution	University of Oslo
	Job title and name	Professor Jason Matthews
Visiting researcher	Name	Professor Jason Matthews
Purpose of the Research Project (approx. 250 words)		The AHR is a ligand-activated transcription factor that is activated by many environmental xenobiotics, dietary products and endogenous metabolites. Despite its notoriety as a mediator of toxicological responses, the AHR has emerged as an important regulator of numerous physiological processes, including the immune system and in T cell differentiation. However, its role in the innate immune response after viral infection is incompletely understood. We reported that TIPARP, an AHR target gene and ADP-ribosyltransferase, is a critical regulator of many aspects of AHR activity. TIPARP has also been reported to play a role in viral replication and inflammation. Together with Dr. Takaoka and colleagues, we recently reported that TIPARP is the key regulator of AHR-dependent innate immune suppression. Moreover, we found that TIPARP specifically ADP-ribosylates TBK1 reducing its ability to activate type I interferon responses. This suggests that pharmacological inhibition of TIPARP could be a beneficial anti-viral therapy. This work was published in Yamada et al. Nature Immunology 2016. We now plan to further determine the importance of TIPARP in the innate immune response and its potential role in T cell function. These future projects will include comprehensive studies of Tiparp knockout mice and T-cell specific Tiparp knockout mice that will be generated using floxed Tiparp (Tiparpfl/fl) mice. We will also use mass spectrometry to identify proteins that are modified by TIPARP and map the modified sites.

Development of the Research Project and Results (approx.. 850 words)	The research project has continued as outlined in our initial proposal. We want to build on our findings from our first manuscript that was published last year. My lab has used mass spectrometry to identify ADP-ribosylated peptides in TIPARP and AHR. In collaboration with Dr. Takaoka, we will use the same approach to map the ADP-ribosylated sites in TBK1. We have received protein as well as expression plasmids from Dr. Takaoka's lab to pursue this research. We have struggled with protein expression and optimization of the ADP-ribosylation and mass spectrometry for TBK1. However, many of the initial problems we have been having, such as poor yield, have been solved. Dr. Takaoka has also found that many virus' induce the expression of TIPARP in a manner that is independent of AHR. This intriguing finding potentially uncovers a novel feedback regulation involving viral infection, pathogen associated molecular patterns and TIPARP. We are working together, to try to uncover this mechanism behind this interplay by using a variety of molecular approaches. To further understand the role of the AHR-TIPARP axis in innate immunity, I have provided Dr. Takaoka's lab with Tiparp knockout mice as well as Tiparp fl/fl mice. These mice are presently being rederived from frozen embryos. We anticipate being able to use the mice in experiments later this spring or early summer. The knockout mice will be exposed to various viruses and a number of relevant endpoints determined. The Tiparpfl/fl mice will be used to make tissue or cell type specific deletions of Tiparp to provide more insight into its role in innate immunity. My lab has recently generated a catalytic mutant Tiparp mouse using CRISPR-Cas9. We have isolated mouse embryonic fibroblasts and plan to share these cells as well as the mice with Dr. Takaoka to further enhance the quality of the data generated in this project. Preliminary data on these mice, suggest that they exhibit similar phenotypes following AHR activation as Tiparp knockout mice. This suggests that Tiparp's role in AHR signaling and physiology is dependent on its catalytic activity. This unique mouse model will be instrumental in demonstrating the importance of Tiparp catalytic activity in mediating its role in innate immunity.

Publication *Enter the names of conference or journal and its vol. No. where the above work was presented.	【Conference, symposium, workshop etc.】
	【Journals】 1. Yamada, T., Horimoto, H., Hayakawa, S., Kameyama, T., Yamato, H., Dazai, M., Takada A., Kida, H., Asaka, M., Bott, D., Hutin, D., Zhou, A., and Watts, T., Matthews, J. and Takaoka, A. (2016) TIPARP ADP-ribosyltransferase Links AHR Signaling to Innate Immune Suppression. Nature Immunology 17:687-694.