IMSUT International Joint Usage/Research Center

International Project-completion Report (FY2022 ver.)

Date of submission: 5/12/2023

Principal Investigator	Position, Institution: Professor, Univ. Bonn Name: Eicke Latz
IMSUT Host Researcher	Division: Innate Immunity Name: Kensuke MIYAKE
Project Title	The impact of DNases on alveolar hemorrhage
Duration	From 4/1/2022 to 3/31/2023

Project Members

Name	Position, Institution
Takuma Shibata	Assistant Professor, Institute of Medical Science, University of Tokyo
Ryota Sato	Assistant Professor, Institute of Medical Science, University of Tokyo
Tatjana Reuter	PhD student, University of Bonn
Tsuneyasu Kaisho	Professor, Wakayama Medical University

Project-completion Report

Tissue damage causes DNA release from the cells undergoing necrosis or pyroptosis. Released DNA activates a variety of immune responses ranging from cell death to cytokine production. Released DNAs are sensed by DNA sensors such as Toll-like receptor 9 (TLR9) in the endosomal compartment, cyclic GMP-AMP synthase (cGAS) in the cytoplasm, and cytoplasmic AIM2. TLR9 responds to single-stranded DNAs, whereas cGAS and AIM2 respond to double-stranded DNAs. TLR9 and cGAS activate inflammatory responses, whereas AIM2 initiates inflammasome formation, leading to release of IL-1β and IL-18 or pyroptosis, an immunogenic cell death program. These DNA sensor's responses to self-derived DNAs are under the control of DNases: DNase 1, DNase 1 like 1, DNase 1 like 2, and DNase 1 like 3 in the extracellular space; and DNase II in the endosomal compartment. Little is known, however, about the role of each DNase in the disease state. In this proposal, we will study the role of extracellular DNases in pristane-induced diffuse alveolar hemorrhages (DAH). We have already generated mice lacking DNase I, DNase I like 1, DNase I like 2, or DNase I like 3. We intraperitoneally administrated them with pristane to cause DAH.

Among the mice above, *Dnase1*$^{-/-}$ mice are partially resistant to pristane-induced DAH. Other mutant mice suffered from DAH as much as wild-type mice. We, therefore, focused on *Dnase1*$^{-/-}$ mice. FACS analyses demonstrated that much smaller numbers of lymphocytes, dendritic cells, and macrophages infiltrated lung in *Dnase1*$^{-/-}$ mice that survived pristane treatment than those in wild-type mice. Alveolar macrophages disappeared in wild-type mice treated with pristane, but not in *Dnase1*$^{-/-}$ mice that survived pristane treatment, suggesting that alveolar macrophages undergo cell death upon pristane treatment.

We hypothesized that self-derived DNAs released from dead cells act on DNA sensors in alveolar macrophages, leading to their cell deaths. Alveolar macrophages were cultured with thymus-derived DNAs for a few days. FACS analyses, however, filed to detect cell death of alveolar macrophages.

To understand the role of DNA sensors in pristane-induced DAH, we intraperitoneally administered mice lacking TLR9 with pristane. TLR9-deficiency, however, failed to ameliorate pristane-induced DAH. Mice lacking AIM2 and STING are to be studied.

Facilities to be Used

FACS core-laboratory,
Animal facility
Imaging core-laboratory

Research Results from the Project during FY2022

<Publications>

<Patent Applications>

Days of visits to IMSUT during FY2022

*Please include visits without travel allowances.

*If the project members could not visit IMSUT due to the pandemic of COVID-19 during FY2022, please present how many days in total your project has held online meetings, discussions via e-mail or communication tools such as Slack, etc. among your project members since April 1st, 2022.

*For the "Sex" and "Age" sections, the information shall be used only for statistical purposes.

*Please select the age range based on the age at the end of FY2022.

Name	Position, Institution	Sex	Age	Visits to IMSUT (Days)
Eicke Latz	Prof., Univ Bonn	Male	**40 or older**	2
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	

Name	Position, Institution	Sex	Age	Online Meetings (Days)
Eicke Latz	Prof., Univ Bonn	Male	**40 or older**	2

Tatjana Reuter	PhD students, Univ Bonn	Female	**35 or younger**	1
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	
Name	**Position, Institution**	**Sex**	**Age**	**Discussions via E-mail, Slack, etc. (Days)**
Eicke Latz	Prof., Univ Bonn	Male	**40 or older**	2
Tatjana Reuter	PhD students, Univ Bonn	Female	**35 or younger**	2
		Pull-down▼	Pull-down▼	
		Pull-down▼	Pull-down▼	

Usage of Facilities/Equipment during FY2022

*Please enter '0' or 'N/A' if you have not used any facilities.

*For this fiscal year only, if the project members could not visit IMSUT due to the pandemic of COVID-19, please include the uses by IMSUT faculty members to conduct this joint research project.

Name of Facility	Equipment	Number of Use (Times)	Usage time (Hours)
FACS Core Laboratory	FACS Aria (BD)	10	20
Medical Proteomics Laboratory	e.g.) Orbitrap QSTAR Elite	0	0
Imaging Core Laboratory	Zeiss Multiphoton Microscopy （LSM710NLO)	5	10
Gene Manipulated Mouse Section	Creation and cryopreservation embryo of Knockout mouse	0	0
Human Genome Center	Supercomputer	0	0
Amami Laboratory of Injurious Animals	Experimental lab	0	0
Other		0	0

Usage of Scientific Resources *Please enter'0' or 'N/A' if you have not used any.

Name of Scientific Resource	Number of Samples/Lines
Serum (BioBank Japan)	0
DNA (BioBank Japan)	0

Knockout mouse	0
Pathogenic bacteria	0
Other	0
Usage of Database *Please enter'0' or 'N/A' if you have not used any.	
Name of Database	**Number of Use (Times)**
	0
	0