No.	K23-3086	
研究課題名	The role of lysosomal RNase T2 in RNA-sensing Toll-like receptor responses	
研究代表者	Latz Eicke (Deutsches Rheuma-Forschungszentrum Berlin (DRFZ)・教授)	
研究組織	受入教員	三宅 健介 (東京大学医科学研究所・教授)
	分担者	Kensuke Miyake (Div Innate Immunity, Dep Microbiol. Immunol.・Professor)
	分担者	Takuma Shibata (Div Innate Immunity, Dep Microbiol. Immunol.・assistant professor)
	分担者	Ryota Sato (Div Innate Immunity, Dep Microbiol. Immunol.・assistant professor)
	分担者	Tsuneyasu Kaisho (Wakayama Medical University・Professor)

(Form 2)

Principal Investigator
Eicke Latz

IMSUT International Joint Usage/Research Center Project <International>

Joint Research Report (Annual/Project Completion)

Project Completion Report

Report

Introduction

Nucleic acids (NAs) released from damaged cells are engulfed by macrophages. Engulfed NAs are degraded in the endosomal compartment by a set of enzymes including DNases and RNases. If NA metabolism in the endosomal compartments is impaired, NA metabolites accumulate in the endosomal compartment and cause a variety of pathologies. In this proposal, we will study the impact of the endosomal RNase, RNase T2, on RNA sensor responses that reside in endosomes. In the previous studies by us and others, RNase T2 is shown to be required for mouse TLR7 and human TLR8 responses, whereas RNase T2 negatively regulates mouse TLR3 responses. We established *Rnaset2*$^{-/-}$ mice, in which splenomegaly and hepatomegaly developed due to monocyte/macrophage accumulation, suggesting that an endosomal RNA sensor is activated in macrophages and drive their proliferation. We here study molecular and cellular mechanisms underlying innate immune responses in monocyte/macrophage from *Rnaset2*$^{-/-}$ mice.

Results and Discussion

We conducted FACS analyses and found that both Ly6C^{low} and Ly6C^{hi} macrophages accumulated in the spleen and the liver. By immunohistochemistry analyses, we found that F4/80-positive macrophages accumulated in the kidney, lung, and brain.

To identify the RNA sensor that drives monocyte/macrophage expansion in *Rnaset2*$^{-/-}$ mice, these mice were crossed with mice lacking Unc93B1, the chaperon required for all the endosomal TLR responses. Macrophages did not accumulate in the spleen and liver in the absence of Unc93B1, suggesting that an RNA-sensing TLR drives macrophage accumulation in *Rnaset2*$^{-/-}$ mice. We next crossed *Rnaset2*$^{-/-}$ mice with mice lacking RNA-sensing TLRs such as TLR3, TLR7, and TLR13. We found that splenomegaly and hepatomegaly did not develop in the absence of TLR13, the sensor for bacterial 23S ribosomal RNAs.

TLR13 drove emergency myelopoiesis, proliferation of macrophages, and accumulation of IL-10-producing Ly6C^{low} macrophages in the spleen. In contrast, TLR13 caused accumulation of monocyte-derived Kupffer cells (moKCs), in which transcription factors such as LXR and MafB were TLR13-dependently activated. Target genes of LXR and MafB included tissue clearance genes such as CD5L, MerTK, and AXL. Accumulation of moKCs turned *Rnaset2*$^{-/-}$ mice resistant to acetaminophen (APAP)-induced liver injury. APAP induced hepatocyte damage in both wild-type and *Rnaset2*$^{-/-}$ mice, but resultant inflammatory responses were observed only in wild-type mice. TLR13 impaired TLR-dependent production of proinflammatory cytokines and upregulated tissue clearance genes such as CD5L, MerTK, and AXL in moKCs, suggesting that TLR13 drove tissue protective responses in moKCs by inhibiting inflammatory responses and promoting tissue clearance upon tissue damage.

These results suggest that TLR13 drives tissue protective responses in moKCs in the liver and that RNase T2 negatively regulates TLR13 responses in moKCs.