Phospholipid scramblase 1 (Plscr1) is the most studied member of the phospholipid scramblase protein family whose main function is the bidirectional and non-specific translocation of phospholipids between the inner and outer leaflets of the plasma membrane. Activation of Plscr1 allows for the externalization of phosphatidylserine (PS) in the outer membrane, which acts as a dock for many biological processes including coagulation, apoptosis, and activation. Our previous data demonstrated that null mutations of murine Plscr1 augment lung Type 2 immune responses. We hypothesize that Plscr1 is a potent inhibitor of innate immunity, and Type 2 immune responses would be diminished in Plscr1 overexpression animals. The aim of this study is to generate Plscr1 overexpression (Plscr1 OE) mice using a newly developed Rosa26 locus targeted conditional knock-in strategy. Specifically, LysM Cre mice were crossed with Plscr1 OE mice to allow spontaneous overexpression of Plscr1 in myeloid cells. In addition, Cre-ERT2 (estrogen receptor T2) mice were crossed with Plscr1 OE mice, and tamoxifen was administered to induce Plscr1 expression. We characterized Plscr1 expression in lungs and livers of these animals using RT-PCR, Western blots, and Immunohistochemistry. These mice will be used in future studies to determine the role of Plscr1 in Type 2 immune responses.

BACKGROUND & HYPOTHESIS

• Phospholipid scramblase 1 (Plscr1) is the most studied member of the phospholipid scramblase protein family whose main function is the bidirectional and non-specific translocation of phospholipids between the inner and outer leaflets of the plasma membrane1,2,3.
• Our previous data demonstrated that null mutations of murine Plscr1 augment lung Type 2 immune responses.
• Hypothesis: We hypothesize that Plscr1 is a potent inhibitor of innate immunity, and Type 2 immune responses would be diminished in Plscr1 overexpression in animals.
• The aim of this study is to generate Plscr1 overexpression (Plscr1 OE) mice using a newly developed Rosa26 locus targeted conditional knock-in strategy.

METHODS

Injections: Mice were injected with 320uL of Tamoxifen for 5 consecutive days. 48 hours after the final injection, mice were sacrificed.

Immunoblot analysis: Following sacrifice, liver and lungs were lysed with RIPA buffer. Protein content was quantified with the BCA method. Equal amounts of protein were loaded onto 4-20% acrylamide gels and transferred to a PVDF membrane. Membranes were incubated in primary and secondary antibody and developed using an ECL chemiluminescence detection kit.

Histology: Liver and liver tissue samples were loaded into cassettes, stored in 70% ethanol solution and were sent to the Brown University of Pathology and Laboratory Medicine at 70 Ship Street, Providence, R.I. for slide preparation of cross sections for staining.

Immunohistochemistry: Slides were dipped 2X Xylene, 100%, 95%, 80%, and 70% EDI each. Slides were placed in 250mL Antigen Retrieval Buffer 1X. (abcam: ab93678). Slides were stained for paraffin sections following dehydration of samples. DAB Peroxidase Substrate Kit (SK-4100) afterwards to produce proper tissue staining for 1-3 minutes. Subsequently, Vector Nuclear Fast Red Counterstain (H-3405) was performed to obtain optimal stain intensity.

Real Time-PCR: mRNA was isolated from both Liver and Lung tissues using Trizol. cDNA was synthesized from 0.13μg of total RNA using the ScriptRT cDNA synthesis kit (Bio-Rad). i Taq Universal SYBR Green Supermix (Bio-Rad) was mixed with cDNA and gene specific primers at a final volume of 20μL. RT-PCR was performed using a CFX96 real time machine (Bio-Rad). All reactions were performed at least in duplicate and data was normalized to the expression of Rp113A. Fold change values were calculated using the following formula: 2^- ΔΔCt

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