

Phospholipase A1A deficiency enhances *Cyba* (p22Phox) expression and ROS production in neutrophils and macrophages

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The development of autoimmune diseases, including rheumatoid arthritis, systemic lupus erythematosus, and psoriatic arthritis, results from complex inflammatory signaling. Phosphatidylserine-specific-phospholipase-A1A (PLA1A) and its enzymatic product lysophosphatidylserine have recently been identified as potential mediators in these conditions. We have previously observed elevated PLA1A levels in plasma and synovial fluid of affected patients with variations depending on disease status. This study was designed to investigate the role of PLA1A in autoimmune inflammation using genetic and molecular approaches.

We generated *Pla1a* knockout (KO) mice. In the mouse model of mannan-induced psoriatic arthritis (MIPsA), *Pla1a* KO mice exhibited significantly lower arthritis scores than wildtype (WT) mice on days 6, 8, 10, and 12 ($p < 0.05$), thereby suggesting a role for PLA1A or its product lysophosphatidylserine in the pathophysiology of autoimmune diseases. We performed single-cell RNA sequencing (scRNA-seq) on lymph nodes and inflamed paws of *Pla1a* KO and WT mice on day 12. ScRNA-seq analysis of lymph nodes revealed changes in the frequency of immune cells, including macrophages and gamma/delta T cells. Among differentially expressed genes, *Cyba* (encoding p22_{phox}) and *Cst3* (Cystatin-3) were significantly upregulated in *Pla1a* KO mice. *Cyba* is an essential component of the membrane-associated enzyme phagocyte NADPH-oxidase (NOX). NOX enzymes use NADH or NADPH to reduce oxygen and produce superoxide anion, a reactive oxygen species (ROS). Since NOX2 deficiency exacerbates MIPsA, we evaluated ROS production in vivo and in primary cells (M1/M2 differentiated bone marrow-derived macrophages; and peritoneal macrophages and neutrophils) from *Pla1a* KO and WT mice.

Bioluminescent imaging (IVIS) after i.p. L-012 injection showed increased ROS in *Pla1a* KO mice versus WT controls. Flow cytometry using DHR123 revealed enhanced ROS in mannan-stimulated *Pla1a*-deficient peritoneal macrophages and neutrophils. Although *Pla1a* deficiency did not alter M1/M2 polarization, it increased the percentage of ROS-producing M1 and M2 macrophages versus WT. Luminol-based assays confirmed more sustained ROS production in PMA/ionomycin-stimulated *Pla1a* KO M1/M2 macrophages.

Collectively, *Pla1a* deficiency amplifies ROS production in macrophages and neutrophils primarily via Nox2 activity. This heightened ROS response may alleviate MIPsA development or promote inflammation resolution through undefined mechanisms. Future studies should determine whether enhanced ROS production in *Pla1a* KO mice improves antimicrobial defense.

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