Lysophospholipids as mediators of lung inflammation and potential tuberculosis biomarkers

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1. Introduction

Lipids play a key role in the development of a number of diseases through regulation of chronic inflammation. Lysophosphatidylcholine (LPC) is a lipid mediator that is mainly derived from the hydrolysis of phosphatidylcholine (PC) in the cell membrane by phospholipase A_2 (PLA_2). Lysophosphatidylcholine acyltransferase (LPCAT) catalyses the conversion of LPC to PC, which is rapidly recycled in the Lands Cycle. Our recently published data has shown accumulation of LPC in TB granulomas, suggesting a role of the lipid in disease pathogenesis (1).

AIM: To investigate the role of LPC as a potential mediator of lung inflammation during TB progression

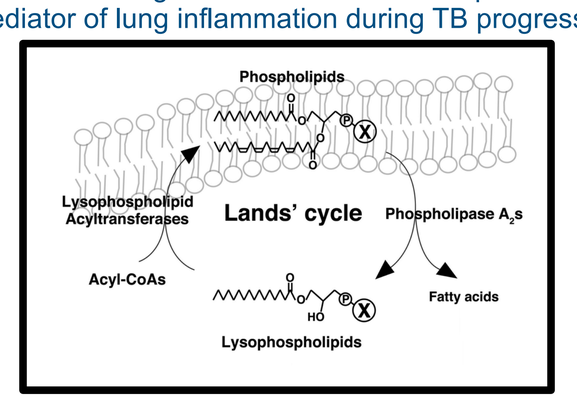

Fig 1 Lands Cycle. Adapted from "Discovery of a lysophospholipid acyltranferase family essential for membrane asymmetry and diversity" by D Hishikawa, 2008, Proceedings of the National Academy of Sciences of the United States of America

2. Methods

Blood and tissue samples were collected from 3 patient groups

Healthy n= 20 | **TB** n= 30 | **LTBI** n= 20

- **plasma**: Plasma was isolated from blood to investigate levels of LPC and PC using ELISA. A multiplex ELISA was used to investigate any correlation with pro-inflammatory cytokines
- **RNA**: The gene expression of the enzymes involved in the production of LPC was investigated by synthesizing cDNA from RNA and running RT-qPCR
- **Lung tissue**: Mass spectrometry was used to investigate spatial compartmentalization of LPC and PC in TB granulomas
- **PBMCs**: PBMCs were isolated from blood and were used to investigate pharmaceutical inhibition of the enzyme involved in LPC production

Fig 2 Schematic showing the experimental process that was followed

3. LPC associates with disease severity

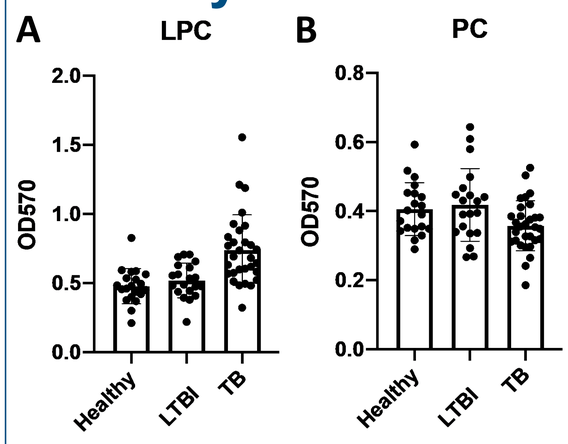

Fig 3 Levels of LPC and PC detected by ELISA in patients with active TB, latent TB (LTBI) & healthy individuals. A. LPC was significantly more abundant in patients with active TB when compared to the healthy group. B. Conversely, we found that PC levels were increased in the healthy group when compared to those with active TB

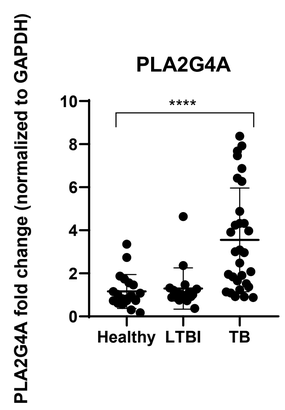

Fig 4 Gene expression of Pla2G4A which encodes phospholipase A2 which is involved in the production of LPC. A. Pla2G4A expression was significantly upregulated in the TB group compared to the healthy group

4. LPC is abundant in the caseum of human TB granulomas

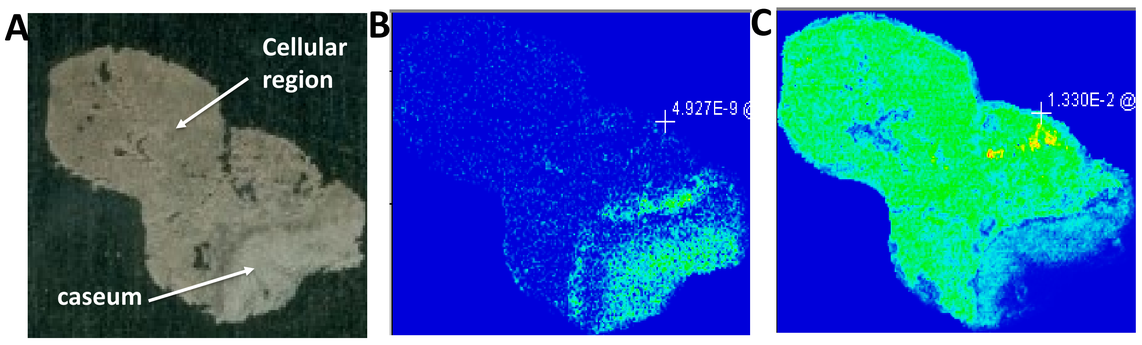

Fig 5 A. MALDI mass spec imaging of a section of human TB lung granuloma. B. LPC is shown to accumulate in the caseous region of the granuloma. C. PC is highly enriched in the cellular regions

6. LPC correlates with inflammatory cytokines

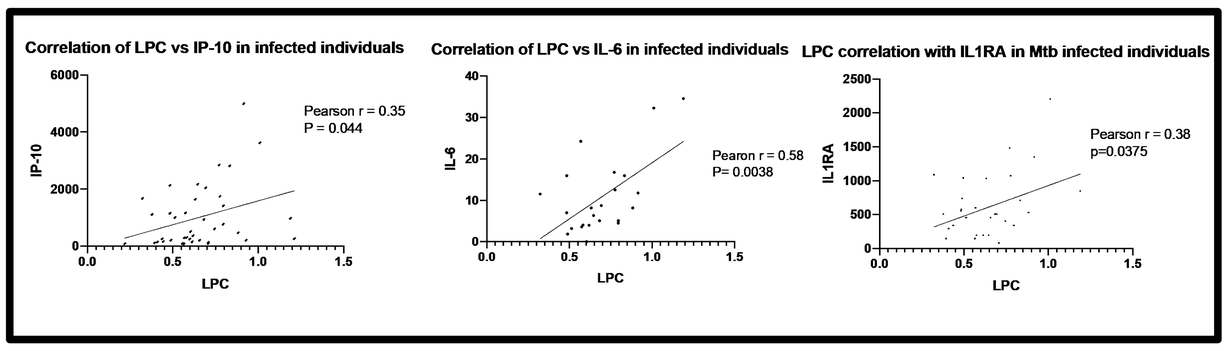

Fig 6. Correlation of plasma LPC with circulatory inflammatory markers IP-10, IL-6 & IL-1RA in infected individuals (LTBI and TB). Pearson correlation was used for analysis.

5. Pharmaceutical inhibition of phospholipase A2 results in reduction of inflammatory cytokines

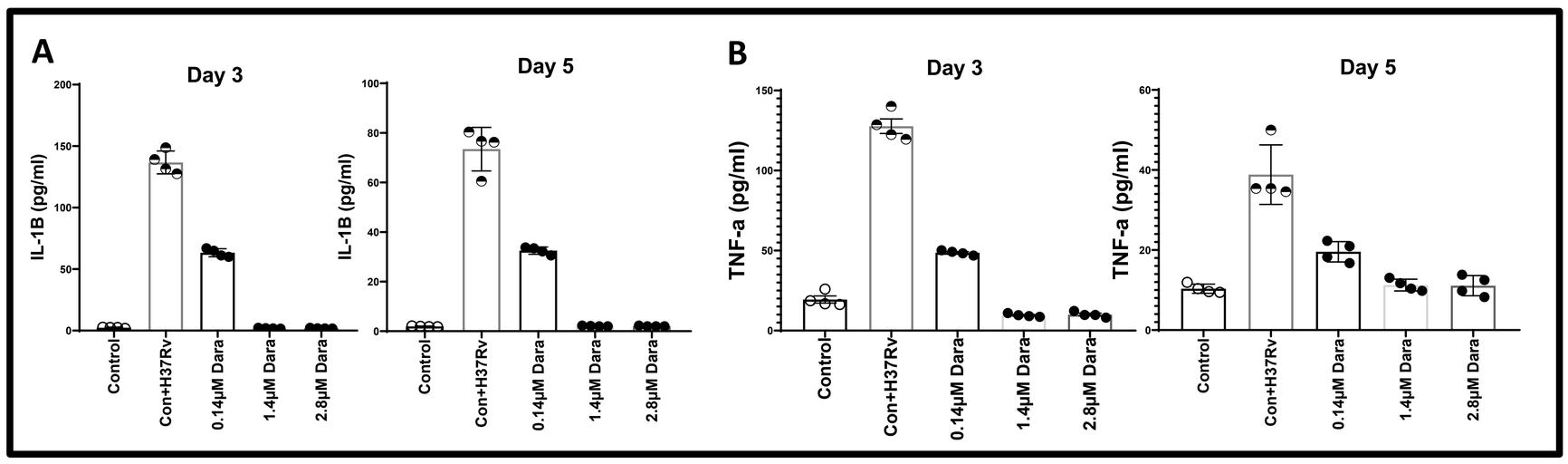

Fig 7 Interception of LPC production through pharmaceutical inhibition of phospholipase A2 in PBMCs using darapladib. A. IL-1β expression is significantly decreased in H37RV infected PBMCs when treated with different concentrations of darapladib in both day 3 & 5. B. TNF-α production is significantly decreased in H37RV infected PBMCs when treated with different concentrations of darapladib in both day 3 & 5. Similar results were seen in the expression of IFN-γ, IP-10, IL-10 & IL-6 not shown here.

7. Conclusion

1. Our data shows that the LPC:PC ratio strongly predicts active TB and has potential as a diagnostic biomarker.
2. LPC accumulates in the caseous regions of human TB granulomas which suggests a role in lung immunopathology
3. Our data further shows that LPC may drive disease progression in TB by inducing inflammation

Future work

- Further testing of LPC as a potential marker of disease severity
- Targeting Lands' cycle for host directed therapy development

Reference:

1. Marakalala, M.J., Raju, R.M., Sharma, K., Zhang, Y.J., Eugenin, E.A., Prideaux, B., Daudelin, I.B., Chen, P.Y., Booty, M.G., Kim, J.H. and Eum, S.Y., 2016. Inflammatory signaling in human tuberculosis granulomas is spatially organized. Nature medicine, 22(5), pp.531-538.

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