Phospholipid Presentation for Auto-Antibody Binding

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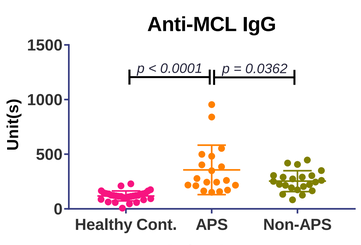
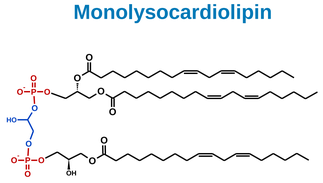
Phospholipid Context & Antibody Binding

- Autoantibodies that bind self lipids are common
- Sometimes, but not always, these autoantibodies cause or exacerbate autoimmune disease(s)
- Antiphospholipid syndrome (APS) presents as recurrent miscarriage or generalized thrombosis and is diagnosed by prolonged clotting time (reversed by phospholipids) and persistent elevated autoantibodies against phospholipids and phospholipid binding proteins
- Cardiolipin (CL) is the canonical lipid in APS, but many additional phospholipids and proteins have been implicated
- Traditional ELISAs for diagnosing APS use CL and other lipids & proteins adsorbed to microtiter polystyrene plates
- How closely does this mimic in vivo?

ELISAs For Rare APS Phospholipids



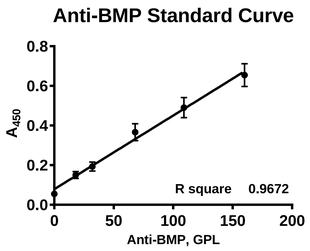


Monolysocardiolipin (MCL) is a metabolite of CL remodeling typically found in the outer mitochondrial membrane and ER

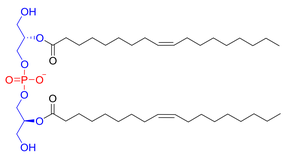
ASSERT Clinical Samples

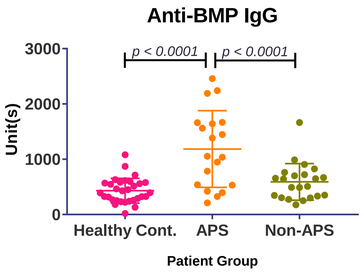
Patient Type	n =
Healthy Control	31
APS	19
Non-APS	22

* Non-APS samples are a combination of recurrent pregnancy loss and venous thromboembolism patients



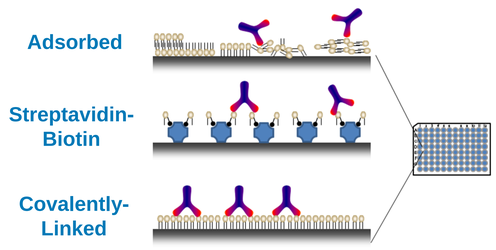
Bis(monoacylglyceryo)phosphate

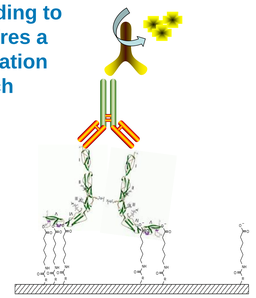




Bis(monoacylglyceryo)phosphate (BMP) or lysobisphosphatidic acid (LBPA) is an important intracellular lipid localized to late endosomes involved in cholesterol transport and virus trafficking

In Vitro Assays For Auto-Antibody Binding



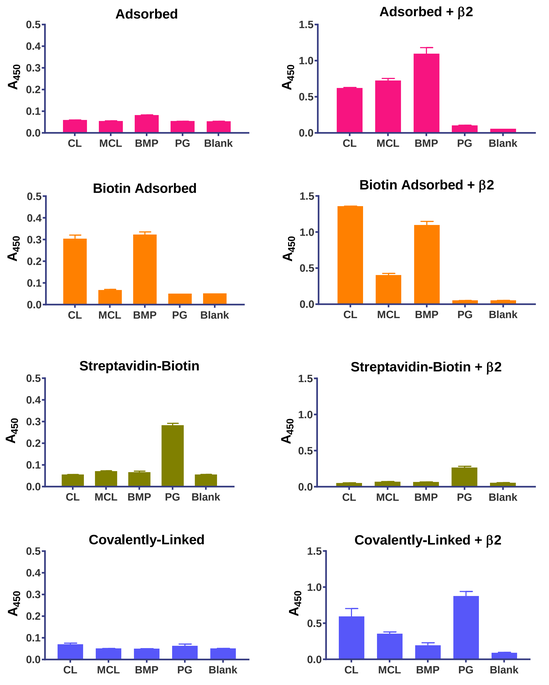


- Lipids and lipid analogs have modified structures which can impact auto-antibody binding
- Lipids can be presented in multiple ways also effecting auto-antibody binding
- Biological membranes are complex compared to in vitro systems, so one needs to be cautious in translating in vitro antibody-lipid interaction results to real life

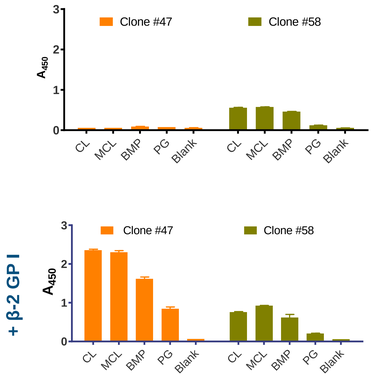
In Vitro Binding of Monoclonal Anti-PL Autoantibodies

HCAL is a mouse/human chimera recombinant APS mAb IgG standard

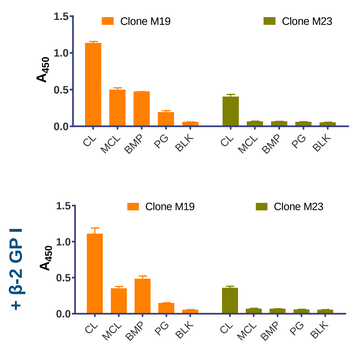
Protein Cofactor β-2 Glycoprotein I



Mouse "APS" Monoclonal



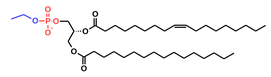
Human "APS" Monoclonal

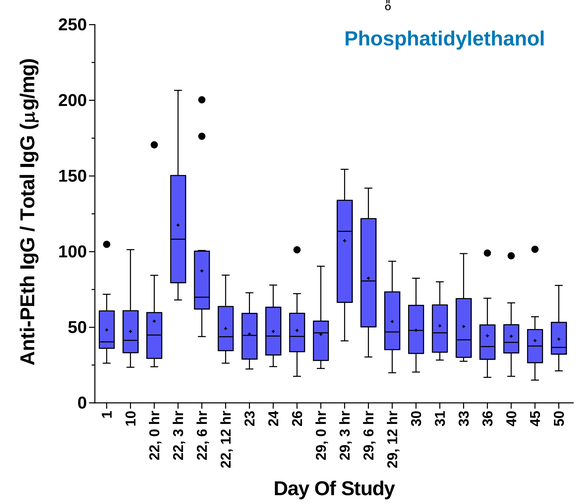


- Monoclonal human and mouse anti-phospholipid antibodies are impacted by lipid presentation and protein cofactors for in vitro binding assays
- These auto-Ab/phospholipid interactions were modified by serum, purified β2 glycoprotein I, and presentation of lipids either adsorbed to plates or bound as biotinylated or covalently-coupled analogs

Cell-Bound Autologous Anti-Lipid IgG

- PEth (a promising biomarker for long-term detection of alcohol ingestion) is a lipid formed in the presence of EtOH by transphosphatidylation reaction of PC by PLD
- Autoantibodies to PEth were first described by Nissinen et al. (Addict Biol. 2012;17(6):1057-67)
- We measured total plasma IgG & anti-PEth in 15 subjects enrolled in a binge drinking study





- We measured elevated ***plasma*** levels of anti-PEth at 3 & 6 hours post-binge for two drinking episodes which corresponds to elevated blood alcohol levels
- Working Hypothesis: the partial ligand ethanol competes with PEth for binding to auto anti-PEth IgG, displacing it from the surface of red blood cells. Anti-PEth auto-antibodies reattach to RBCs after blood alcohol levels subside.

Conclusions, Future, & Acknowledgements

- Traditional adsorption of phospholipids to polystyrene does not mimic in vivo presentation
- ELISAs for anti-MCL and anti-BMP IgG were as good or better than traditional anti-CL tests for detecting & diagnosing APS
- Anti-PEth IgG levels *in plasma* remain elevated 6 hours after binge alcohol ingestion returning to basal levels as EtOH is eliminated
- Together these data suggest that lipid presentation and context is important for binding of autologous antibodies to their phospholipid targets both in vitro and in vivo.
- Better presentation of phospholipids is needed in a biological context (fluid membrane & protein) to enable more accurate next-generation diagnostics

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