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ROLE OF COMPLEMENT AND SPECIFIC ANTIBODIES IN MACROPHAGE INTERNALIZATION OF *ECHINOCOCCUS GRANULOSUS* LAMINATED LAYER PARTICLES

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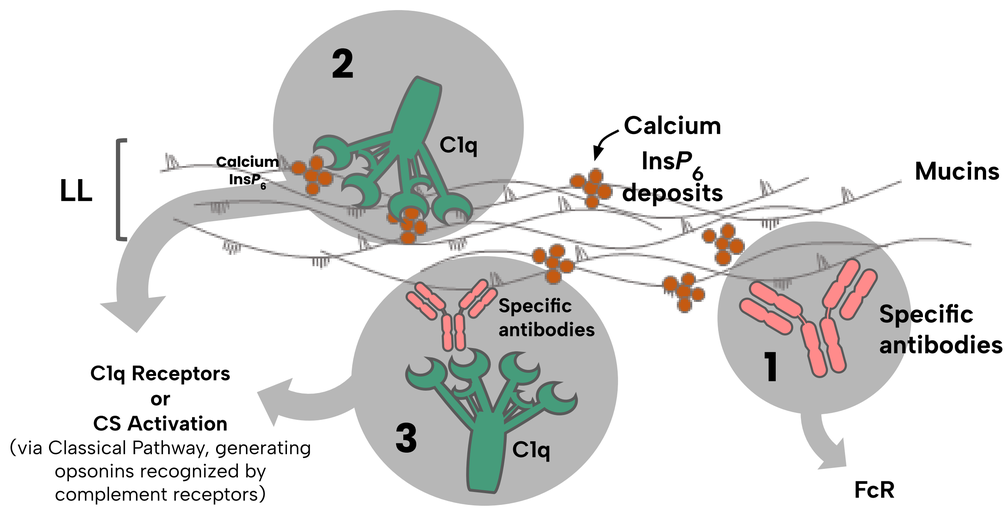
BACKGROUND

- ***Echinococcus granulosus*** is the cestode parasite responsible for cystic echinococcosis.
- Its larval stage (hydatid) develops within the host's organ parenchyma shielded by a thick acellular layer called the **laminated layer (LL),** mainly composed of **highly O-glycosylated mucins** and **calcium myo-inositol hexakisphosphate (InsP_6) deposits**.
- LL material circulates systemically during infection and accumulates mainly in liver Kupffer cells via the lectin receptor Clec4F.
- Humans lack a functional Clec4F, making **Clec4F-independent uptake pathways** especially relevant to human disease.

Candidate Clec4F-independent routes for uptake of LL materials by host phagocytes



1 Opsonization by LL-specific antibodies → **Fc Receptors**

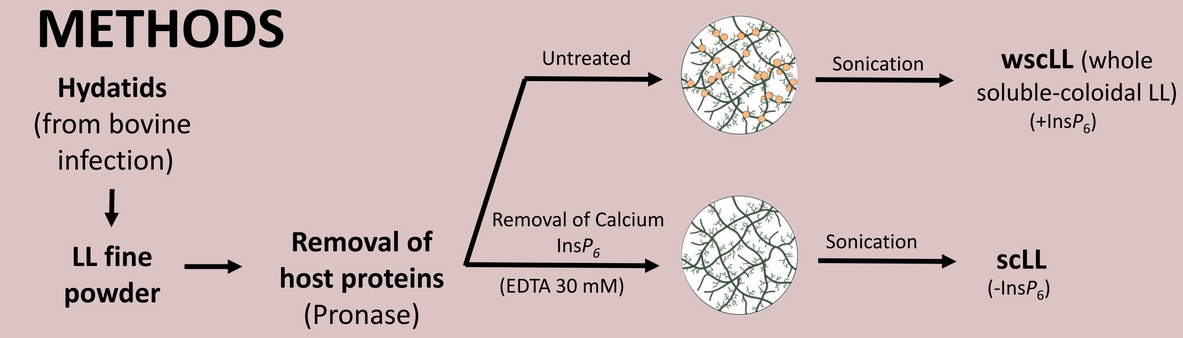
2 Direct binding of C1q to Calcium InsP_6

3 Binding of C1q to antibodies specifically bound to the LL

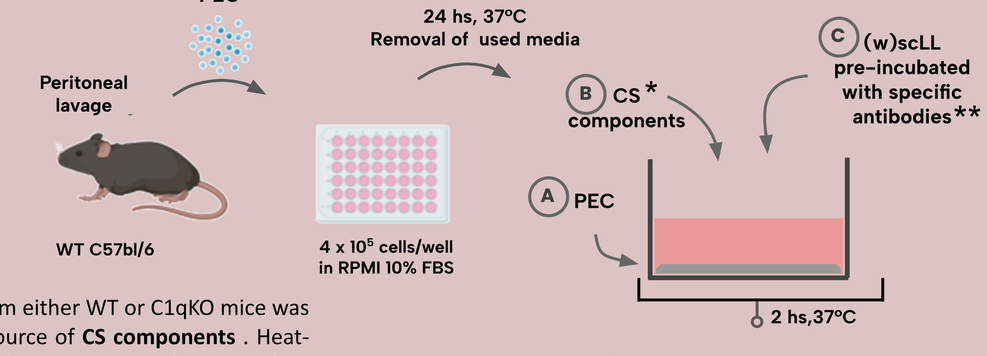
→ **C1q Receptors**

→ **C3b/C4b/iC3b/iC4b Receptors:** Complement System (CS) activation via the Classical Pathway (CCP) and engagement of C3b/C4b/iC3b/iC4b receptors

METHODS



Cell model: Mouse resident peritoneal macrophages (large peritoneal macrophages, **LPM**) were selected as a Clec4F-independent phagocytic model.

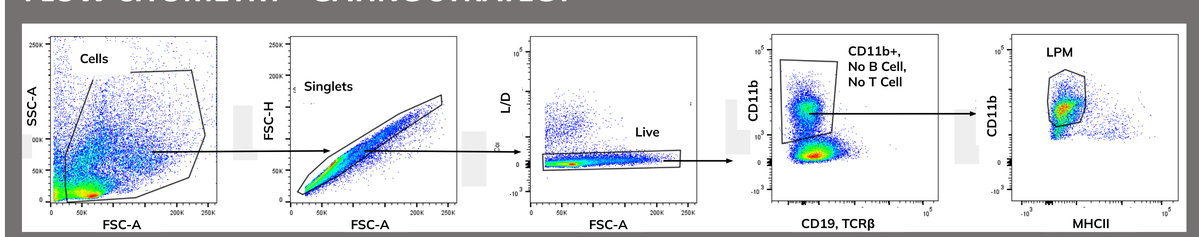


***** Serum from either WT or C1qKO mice was used as a source of **CS components** . Heat-inactivated aliquots were used as control.

****** Serum of *E. granulosus*-infected mice was used as a source of **LL-specific antibodies.**

1) Wash with PBS 0.1 mM $CaCl_2$
2) Macrophage detachment with Accutase
3) Flow cytometry analysis: LL was detected by intracellular staining with the E492 monoclonal antibody, which recognizes LL-specific carbohydrate motifs.

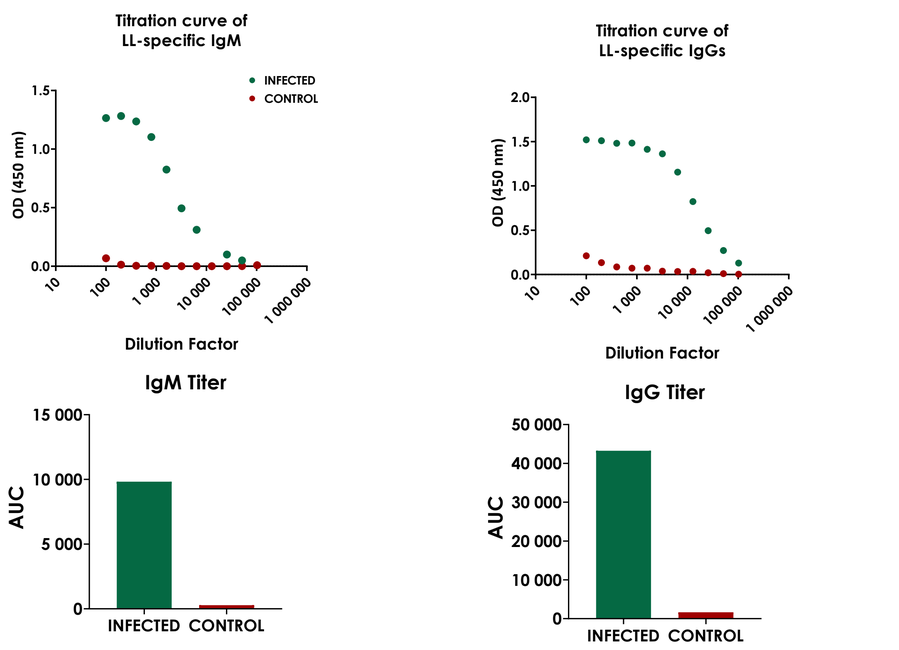
FLOW CITOMETRY - GATING STRATEGY



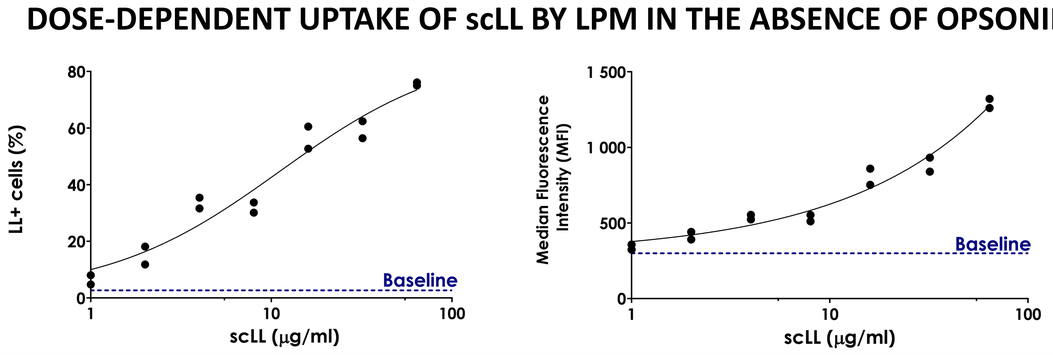
RESULTS

CHARACTERIZATION OF SERUM AS A SOURCE OF LL-SPECIFIC ANTIBODIES

Obtained from E. granulosus-infected mice (or control group)

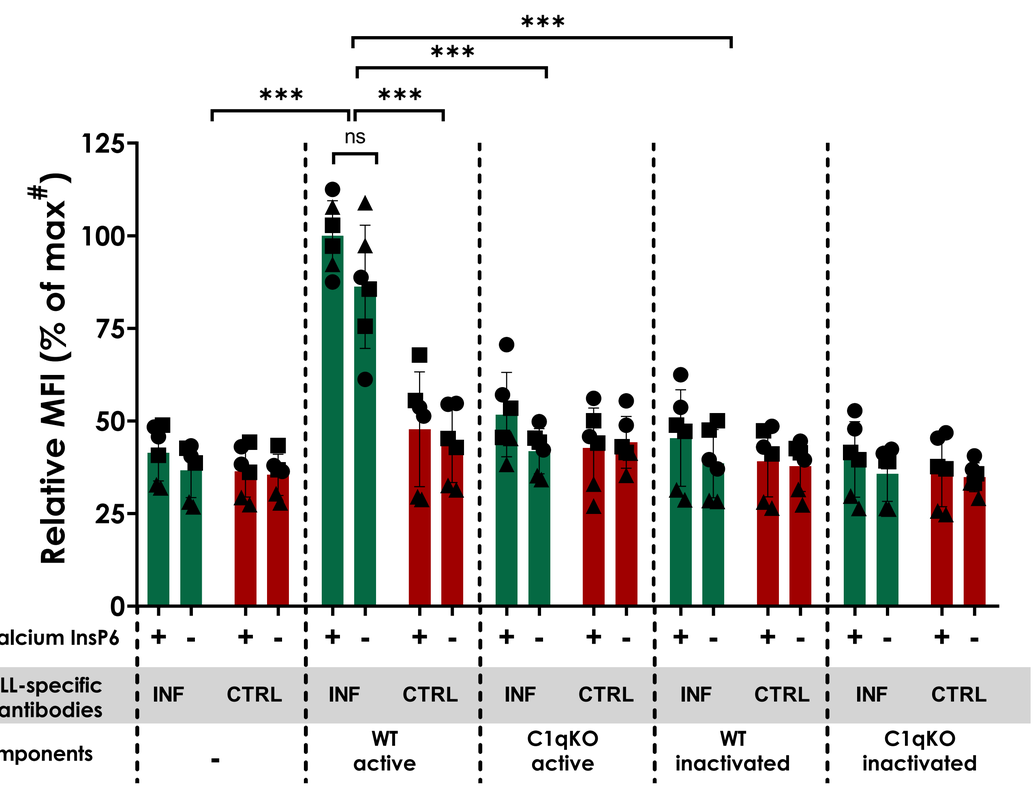


DOSE-DEPENDENT UPTAKE OF scLL BY LPM IN THE ABSENCE OF OPSONINS



UPTAKE OF (w)scLL REQUIRES CCP ACTIVATION BY ANTIBODIES BOUND TO LL MUCINS

Significant uptake was only evidenced in the combined presence of LL-specific antibodies, C1q and active CS components



MFI relative to the maximum uptake condition (wscLL + WT active serum + LL-specific antibodies) was calculated for each experimental replica.

Statystical análisis: Two-way parametric statistics was applied to raw data. Specifically, a robust linear mixed-effects model was used, followed by post-hoc tests, and adjustment for multiple comparisons using the Benjamini–Hochberg method. ***: $p < 0.001$; ns: non-significant ($p > 0.05$).

DISCUSSION AND CONCLUSIONS

- At high concentrations of (w)scLL there is a strong opsonin-independent uptake (possibly via macropinocytosis), overshadowing the contribution of opsonin-dependent pathways. Therefore, the range of 1-5 µg/ml was selected for further assays.
- Neither the interaction of C1q with calcium InsP_6 nor the opsonization by specific antibodies by themselves promote LL uptake. However, the **combined presence of both C1q and specific antibodies, in the presence of active CS components**, significantly **increased LL uptake by LPM**.
- Our results suggest that opsonization dependent on antibody-triggered CCP activation may be a major mechanism for macrophage uptake of LL materials in human cystic echinococcosis.

ONGOING EXPERIMENTS

ALTERNATIVE PATHWAY ACTIVATION

Preliminary assays have shown that alternative complement pathway may promote macrophage uptake when using higher concentrations (1:3 dilutions) of active WT serum as a source of CS components.

IN VIVO LL BIODISTRIBUTION ASSAYS

Ongoing experiments are assessing whether antibody opsonization affects cellular uptake of LL material *in vivo*, in WT, C1qKO and Clec4F mice.

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