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HCMV Pre-fusion like gB shows strong immunogenicity and virus neutralization

EVAXION

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Introduction

Human cytomegalovirus (HCMV) is a common virus infecting people of all ages, often causing mild or no symptoms in healthy individuals. However, infections can be serious for newborns and people with weakened immune systems, such as those undergoing organ transplantation. Glycoprotein B (gB) is a critical fusion protein, undergoing significant conformational changes during infection, transitioning from a metastable pre-fusion state to a stable post-fusion state to facilitate the fusion of the viral envelope with the host cell membrane. Because of this, gB has been intensively studied as a vaccine target, but traditional post-fusion gB vaccines elicit insufficient immunity due to its inherent conformational instability. This study lays the groundwork for developing pre-fusion stabilized HCMV vaccines with improved immunogenicity.

1. AI-guided Re-engineering of gB antigen

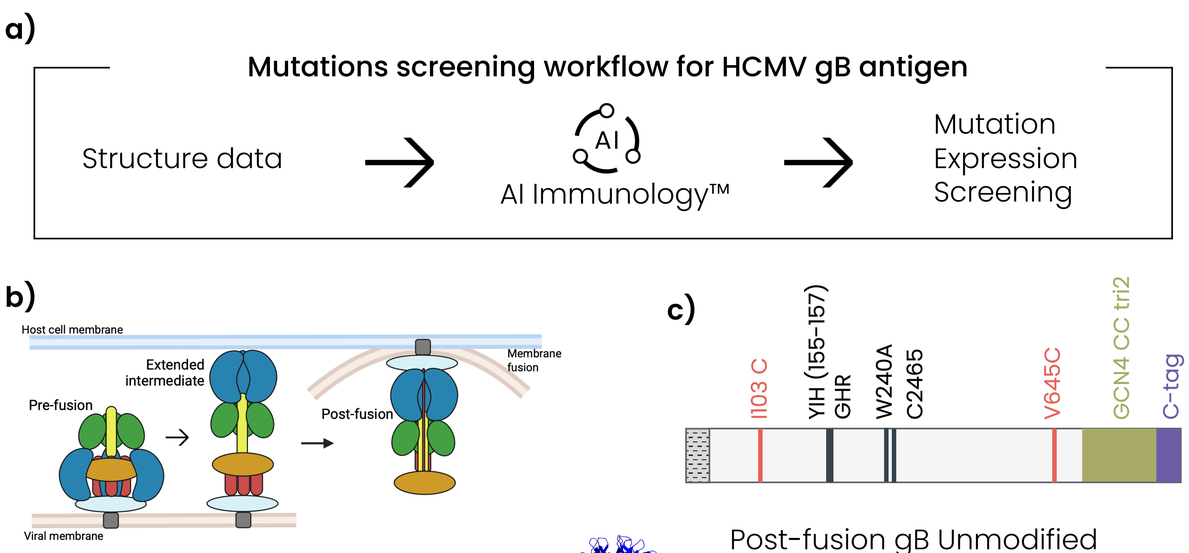


Figure 1: a) Workflow for Mutations screening in Post-Fusion gB Using AI-Driven Modelling **b)** Schematic illustration of gB conformational dynamics during membrane fusion. **c)** Schematic overview of mutations introduced to engineer gB structure. Transmission electron microscopy confirms that the mutated gB adopts a pre-fusion like structure.

3. Pre-fusion gB immune sera inhibit membrane fusion and cellular entry

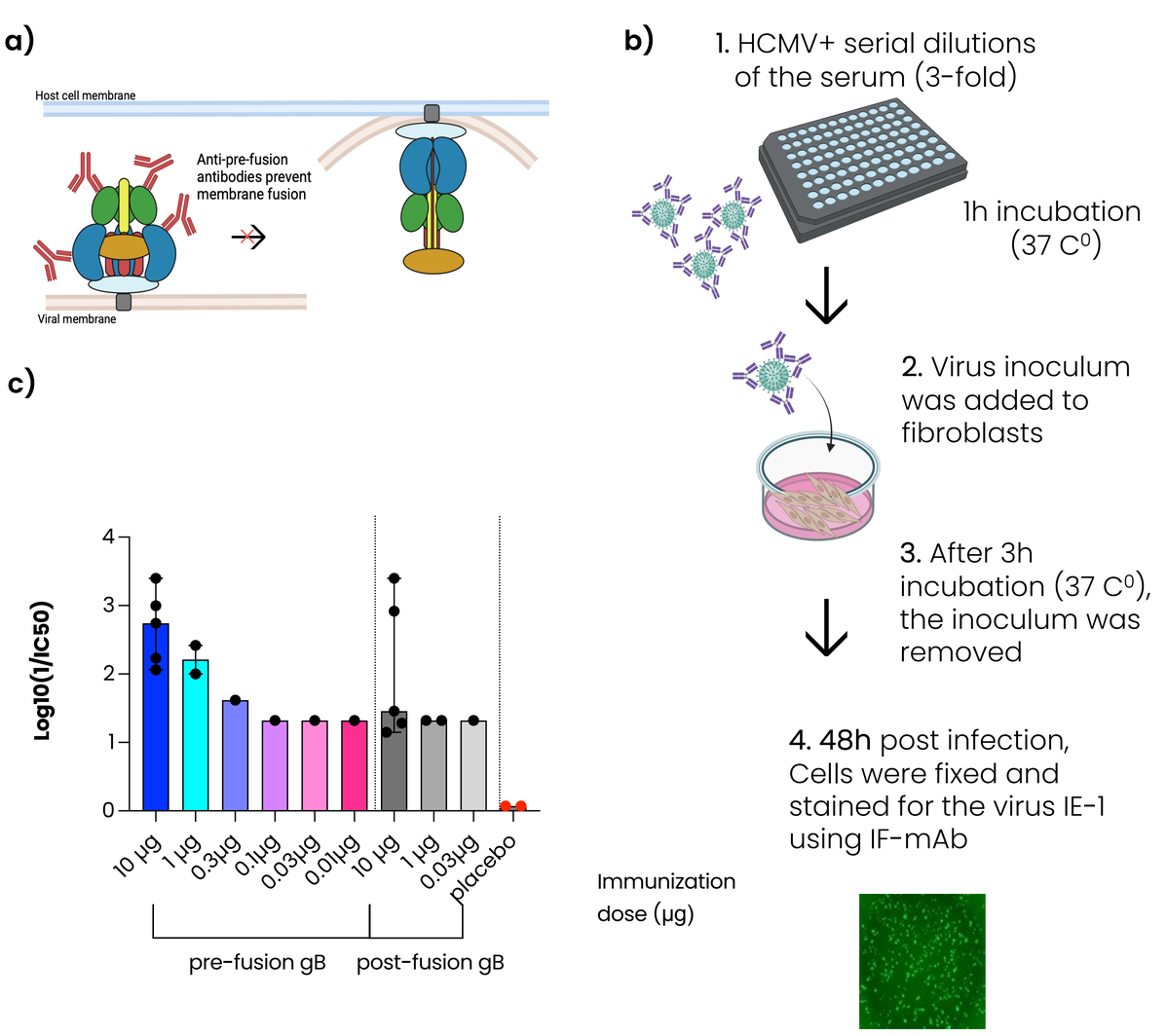


Figure3: a) Schematic representation illustrating how anti pre-fusion gB antibodies inhibit membrane fusion and viral entry. **b)** Design of the live virus microneutralization assay (rapid screening strategy), analyzing the percentage virus infected cells 48 hours post infection (first virus progeny). **c)** Comparison of neutralization efficacy between pre-fusion gB and post-fusion gB immune sera induced by different immunization doses. Mice were immunized with 0.01-10 µg gB protein (pre-fusion or post-fusion) formulated in Addavax®. The plot presents median with 95% CI.

Conclusions

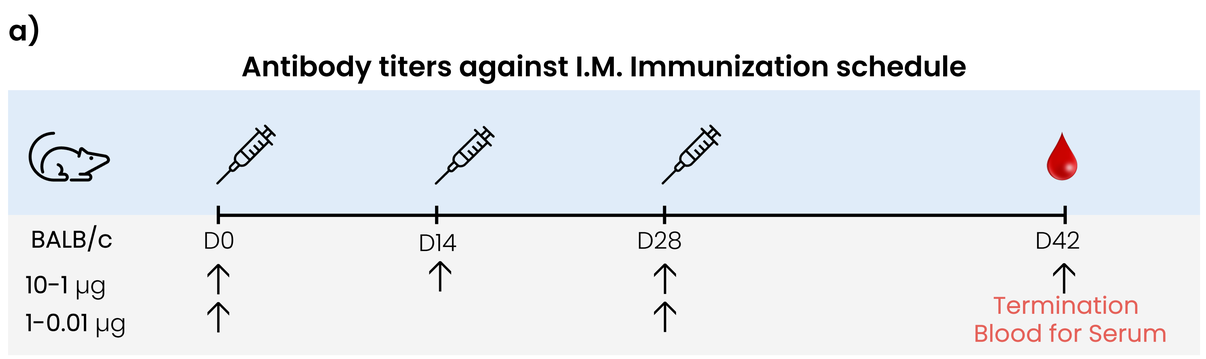
Here, we report:

- The stabilization of gB in a pre-fusion like conformation through cysteine mutagenesis, confirmed by electron microscopy.
- High-titer antibodies elicited by immunization with our pre-fusion stabilized gB in mice.
- Enhanced neutralization of HCMV in fibroblasts compared to post-fusion gB in both early and late stage of cell culture infection.

❖ **Future work includes:**

- Evaluation of antibody-mediated inhibition of transmission from infected to uninfected fibroblast and epithelial cells in cell-to-cell neutralization assay.
- Epitope mapping to identify unique antibody binding patterns targeting pre-fusion specific domains to better understand the vaccine antigen potential.

2. Animal design and induction of antibodies of the pre-fusion gB versus post-fusion gB



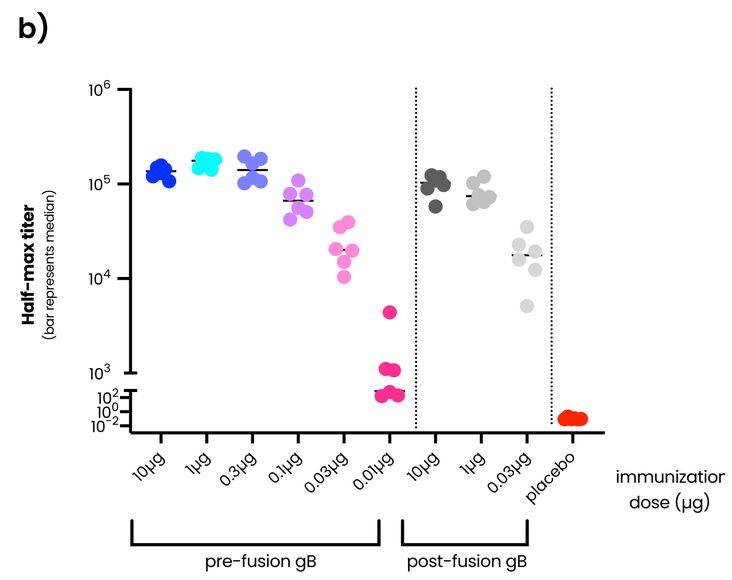


Figure 2: a) Immunization strategy schedule. For the study with 10-1 µg/ immunization dose, the mice received three times doses and for study with 1-0.01 µg/ immunization dose, the mice received two times doses.
b) ELISA results indicating immunogenicity and antibody induction against pre-fusion gB and post-fusion gB protein constructs.

4. Pre-fusion gB anti-sera effect in $TCID_{50}$ assay

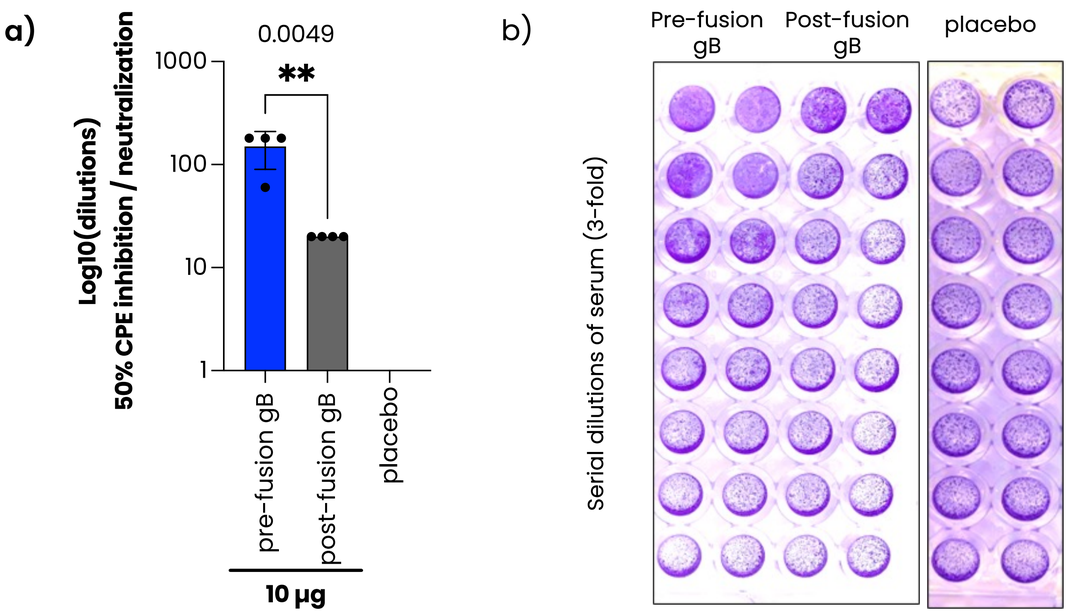


Figure 4: a) Comparison of 50% CPE inhibition between pre-fusion gB and post-fusion gB anti-sera in a $TCID_{50}$ assay. The graph displays mean values with standard deviations.
b) Illustration of $TCID_{50}$ plate results. Cytopathic effect (CPE) was assessed 6 days post-infection in cell cultures treated with anti-sera under 3-fold serial dilutions.

Acknowledgment and Contact

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