Development of HIV-Neutralizing Antibody-Producing Glial Progenitor Cells

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Introduction

Human immunodeficiency virus (HIV) is an RNA lentivirus that infects through the recognition and binding of viral envelope glycoproteins to chemokine receptor 5 (CCR5) on $CD4^+$ T-cells. HIV will then integrate into the host genome for further replication and destruction of immune cells.

This virus affects around 1.2 million people in the US, with ethnicity, gender, and age all having an impact on prevalence rate. Although, due to the improvement of viral detection, $CD4^+$ cell count, and treatment, the mortality rate has dropped to only 2%. Yet, a person with HIV (PWH) is dependent on lifelong antiretroviral medications due to persistent viral reservoirs, primarily in T-cells, tissue macrophages, and glial cells of the brain, which can lead to re-infection in the host upon drug cessation. However, long-term drug treatment has been shown to cause adverse side effects often linked to changes in lipids in the gut, respiratory disease, liver and kidney toxicity and neurological complications. Unfortunately, damage to these organs over time is often irreparable, thus improvement on current treatments is necessary.

Project outline

This project aims to eradicate HIV from the brain by developing genetically engineered glial progenitor cells (GRPs) that produce a human broad neutralizing antibody (bNAb) targeting the CD4 binding site of the HIV gp120 protein. The modified cells will be delivered to the brains of humanized mice infected with HIV, to produce the antibody, which will neutralize free HIV particles and induce death of infected cells via antibody dependent cell cytotoxicity (ADCC), ultimately clearing the infection.

S = Signal peptide
HC = Heavy chain
LC = Light chain
Furin = cleavage site

GSG = linker
2A = peptide
tGFP = fluorescent protein

The constructs (CII and CIII) were designed by Mohammad Sajadi and Abdolrahim Abassi for the purpose of this project.

Aims and methods

Aim 1: To design and test HIV bNab construct expression *in vitro*.

2nd generation lentiviral packaging and transduction of the two constructs (CII and CIII) in mGRP cells confirmed through cell sorting.

Aim 2: To determine appropriate expression and folding of the bNAb's.

Antibody purification from the cell supernatants visualized by western blot and *in silico* prediction of antibody structures.

Aim 3: To show binding of secreted antibody to the HIV glycoprotein.

Flow cytometry of RAJI ENV-B1 cells expressing the HIV glycoprotein, after incubation with mGRP supernatants.

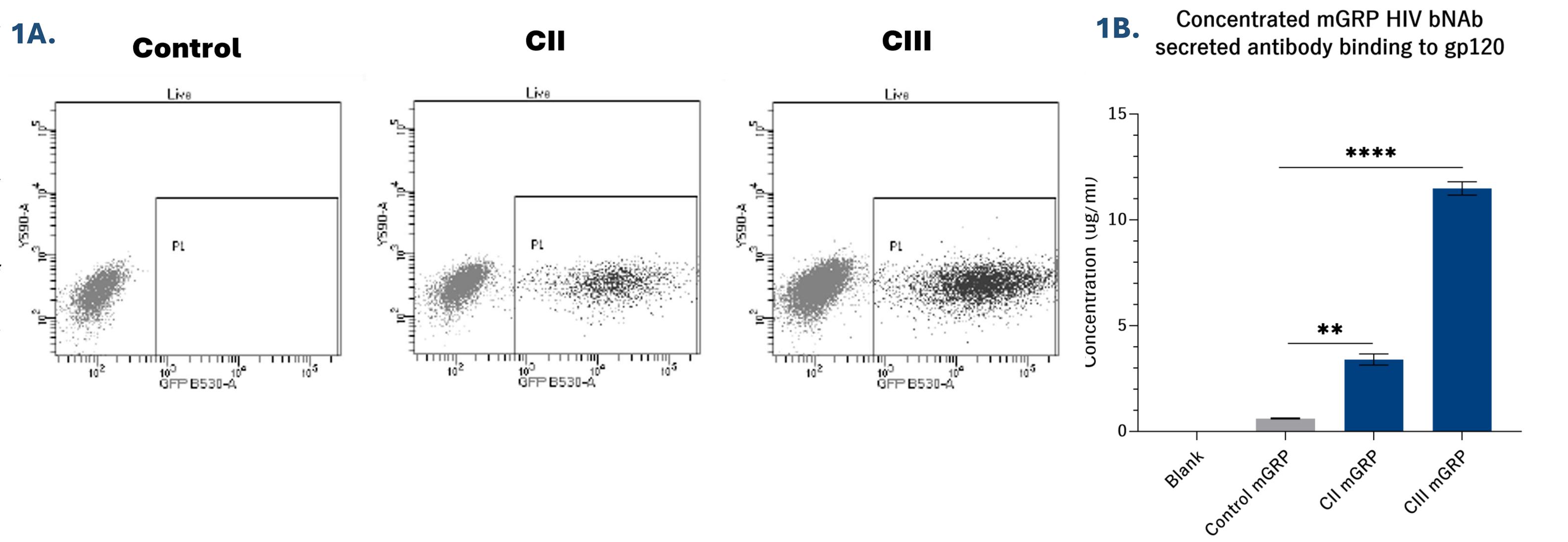
Aim 4: Injection of the mGRP cells *in vivo* for detection of antibody secretion.

Stereotactic injection of the mGRP and RAJI B1-ENV cells into the brains of mice to identify antibody binding.

Results

Aim 1: The CII and CIII constructs were transduced into mouse glial progenitor cells (mGRP) and FACS sorted to obtain GFP positive CII (18% of live cells) and CIII (19% of live cells) stable cell lines, each with starting populations of ~50,000 (Fig. 1A). The control cells were transduced with the packaging plasmids only and therefore do not express GFP.

*Figure 1: **A.** FACS cells sorting of the transduced mGRP cells produced stable construct expressing cell lines. The cells were sorted via GFP expression in the live population to identify successful transduction in 18% of CII and 19% of CIII cell populations. **B.** ELISA of concentrated supernatant from these cells shows antibody detection in both cell lines, with CIII producing a greater amount of antibody (11.5ug/ml) than CII (3.4ug/ml), n=3 **p<0.001, ****p<0.00001.*



We performed ELISA's (Fig. 1B) of cell supernatants to detect secreted antibody and calculate the concentration of CII (3.4ug/ml) and CIII (11.5ug/ml). Additionally, flow cytometry identified a correlation between the expression of bNAb (APC) and construct (GFP) via population data percentage of double fluorescent (APC and GFP) cells in the mGRP CII (91.6%) and CIII (89.9%) cultures (Fig. 2) .

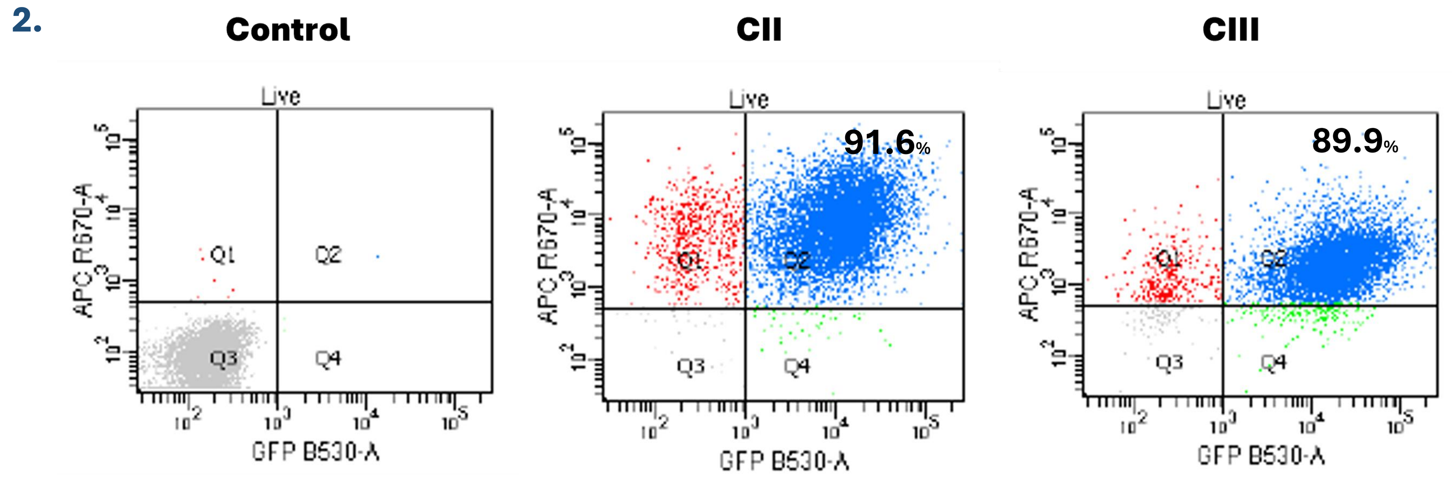


*Figure 2: Flow cytometry data of APC anti-human secondary antibody stained GFP-positive mGRP cells found 91.6% and 89.9% of the CII and CIII populations were double positive, respectively, both significantly higher than the control populations (0%). The CII and CIII populations also had an increased percentage of APC and GFP single stained cells when compared to the controls, possibly explained by a lack of antibody production (GFP only) or low GFP protein expression (APC only). Due to uncertainty, we do not consider these single stained cells to be expressing bNab's. n=3, **p<0.001, ***p<0.0001, scale bar = 100μm.*

Aim 2: The antibodies were purified on protein A/G beads and identified by non-denaturing western blot using a primary antibody raised against the CII and CIII bNAb's (Fig. 3A). Additionally, we've run the amino acid ORF sequence for each antibody through a prediction software, AlphaFold, to determine the protein domains (Fig. 3B).

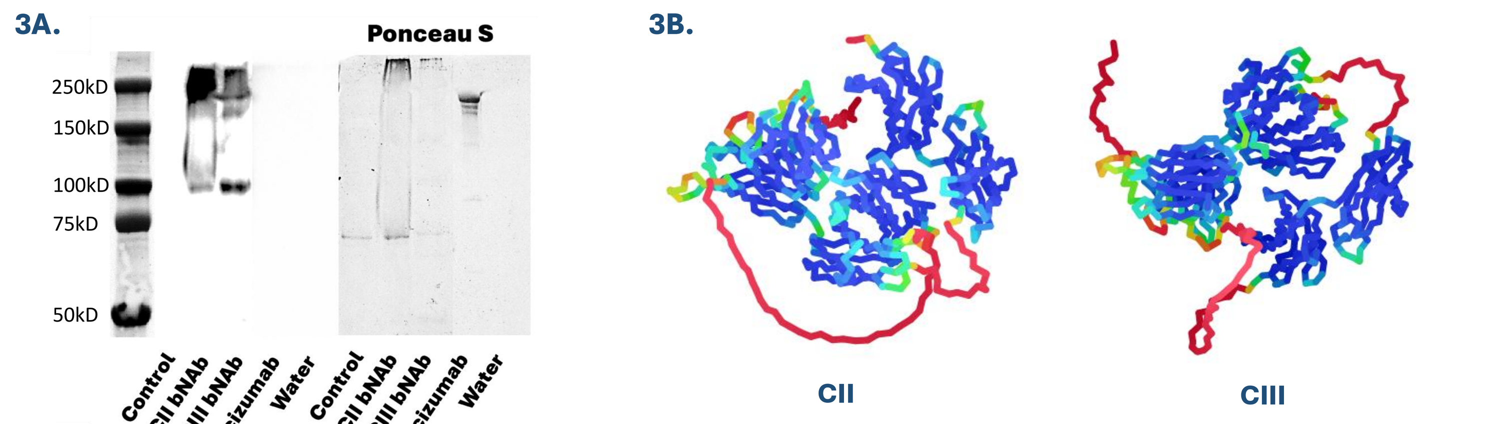


*Figure 3: **A.** Western blotting of the purified antibody product produced the most prominent bands at ~75kD as expected for the CII and CIII bNAb's, while the larger bands are possibly due to precipitation of the antibody. n=2 **B.** AlphaFold has been used to predict the most likely folding outcome for our CII and CIII antibodies allowing us to visualize the protein.*

Aim 3: To further determine antibody specificity, we incubated RAJI B1-ENV cells, which express HIV glycoprotein on the cell surface, with supernatants from the mGRP CII and CIII cells. The RAJI cells were then stained with the anti-human (APC) antibody for flow cytometry (Fig. 4A). We found that both CII and CIII antibodies successfully bound to the RAJI cells, with CIII (91%) consistently showing higher binding than CII (83%) (Fig. 4B).

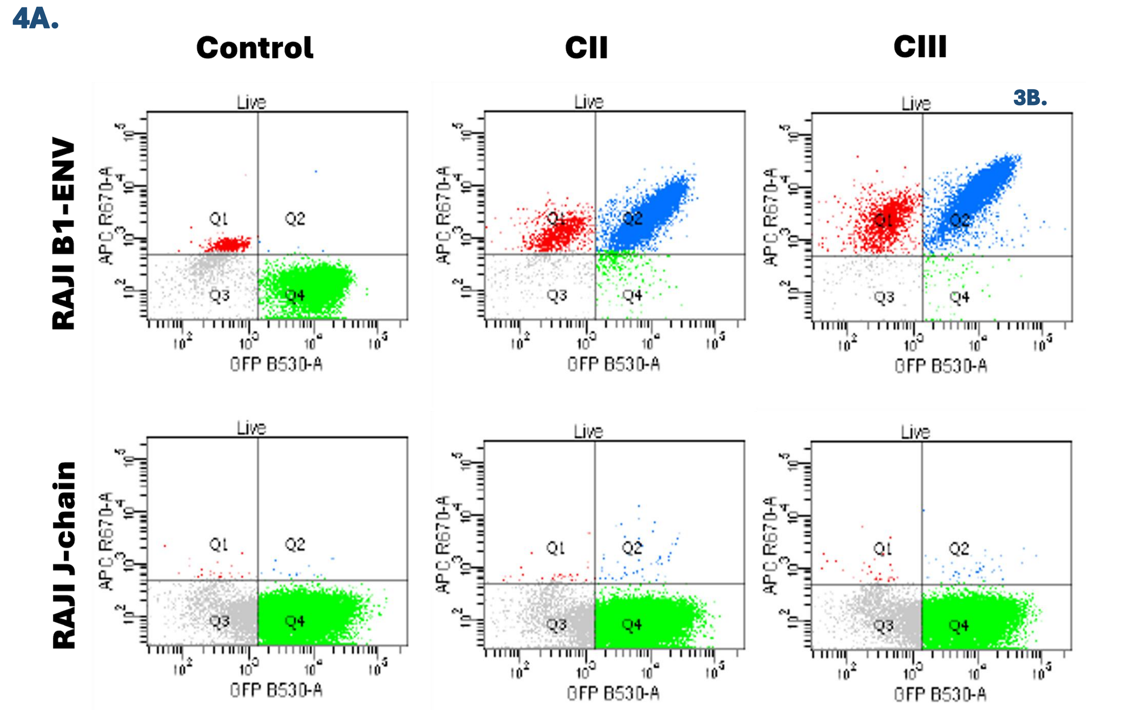
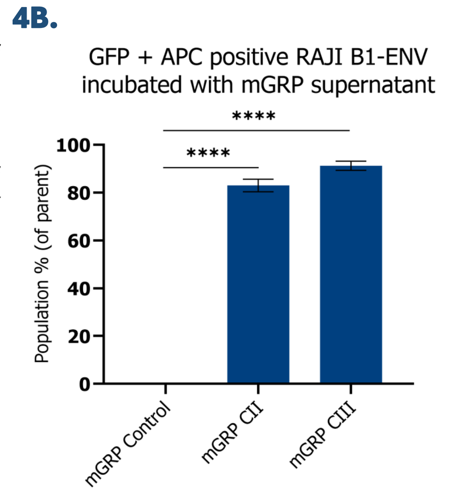
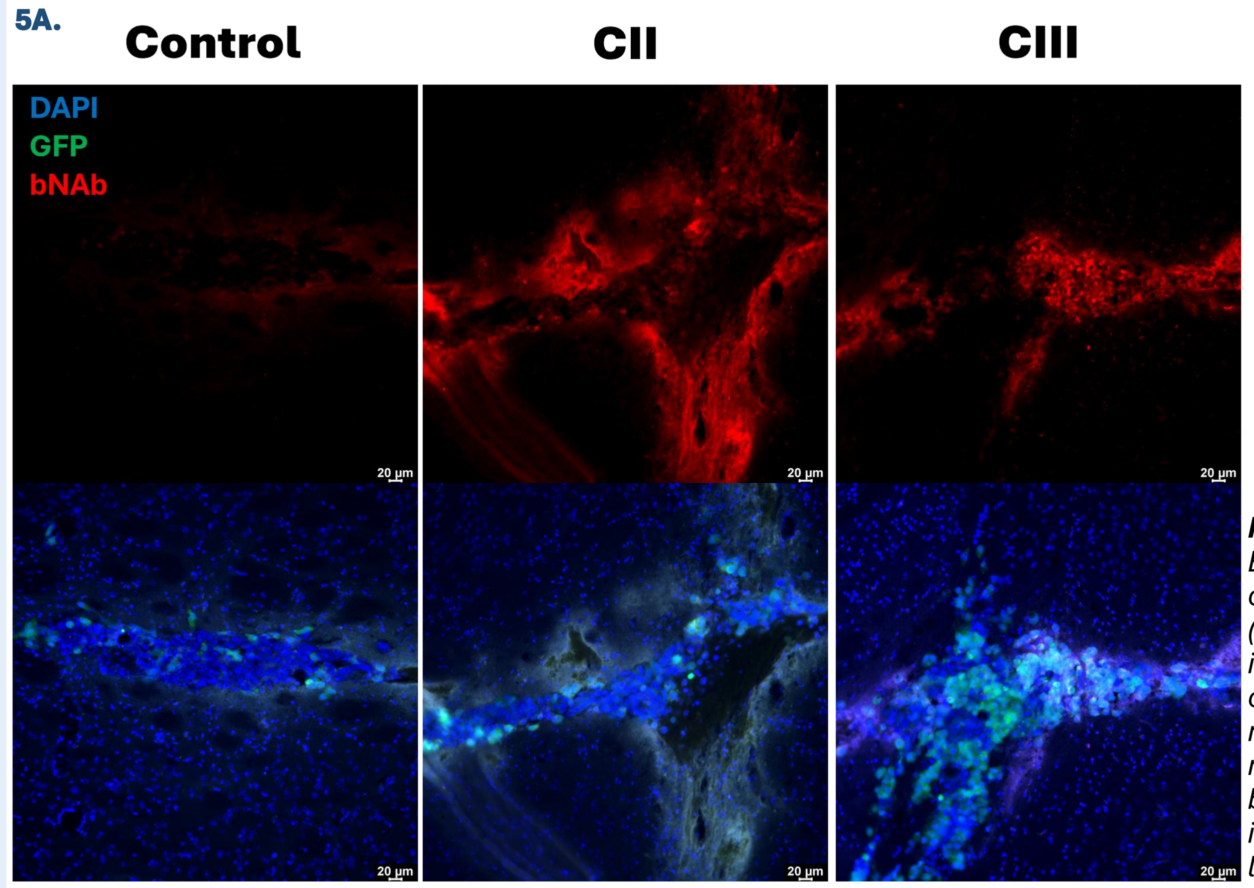


*Figure 4: **A.** Flow cytometry of the GFP+ RAJI B1-ENV cells after incubation with the mGRP (control, CII and CII) cell supernatants shows binding of the bNAb (APC+) to the HIV glycoprotein on the cell surface. This was determined by the small percentage of APC+ RAJI J-chain cells, which lack the glycoprotein. The APC+ cells in these populations are mGRP cells that carried over in the supernatants, identified by the single stained FACS controls.*



***B.** Normalization of the RAJI B1-ENV cells to the FACS control (APC+, media -) shows no APC+ GFP+ double fluorescent cells when incubated with control supernatant, while CII antibody bound to 83% of the GFP+ RAJI population and CIII antibody bound to 91%. n=3, ****p<0.00001*

Aim 4: We transplanted the modified mGRP cells parallel with RAJI B1-ENV cells into the brains of IL34+ NOG mice to determine antibody binding after 24 hours *in vivo*. The brains were harvested, sliced and stained with anti-human IgG (APC) to detect antibody on the RAJI cells (Fig 5A and B). This was also performed on the non-glycoprotein expressing RAJI J-chain cells where no antibody was detected.



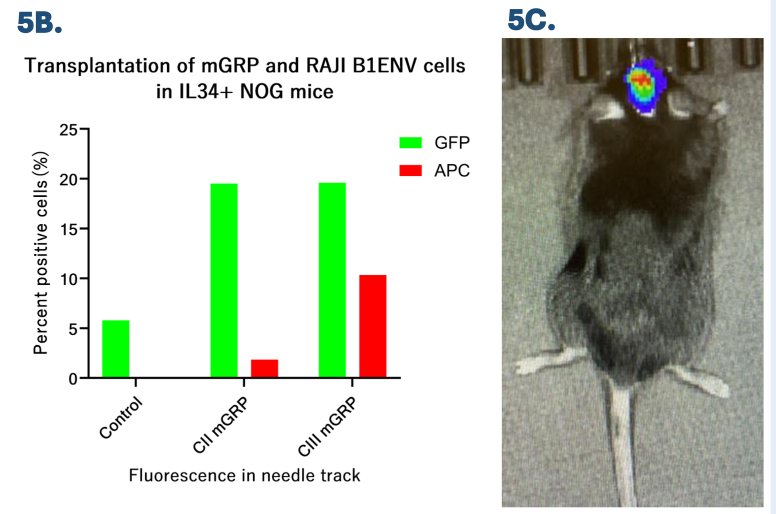


*Figure 4: **A.** Stereotactic injection of mGRP and RAJI B1-ENV cells into the brains of IL34+ NOG mice has shown construct (GFP) expression and the presence of bNAb's (APC). Scale bar = 20μm. **B.** Analysis of the immunofluorescent images identified 19% GFP positive cells in both CII and CIII, whereas 2% of cells in the needle track of CII mice were APC positive and 10% CIII mice. **C.** Luciferase positive mGRP cells injected into the brains of C57BL/6 mice underwent bioluminescent imaging 72-hours post injection. The cells showed high luminescence intensity indicating active metabolism and therefore cell survival.*

Conclusions

The packaging and transduction of CII and CIII constructs into mouse glial progenitor (mGRP) cells has generated a cell line that produces a broad neutralizing human antibody (bNAb) against HIV glycoprotein. This was verified through ELISA by calculating secreted bNAb concentration, as well as antibody staining to identify expression via flow cytometry. Additionally, RAJI B1-ENV cells were incubated with the mGRP cell supernatants prior to flow cytometry to demonstrate bNAb binding to the HIV glycoprotein expressed on the RAJI cell surface. To identify cell viability *in vivo* the mGRPs were injected into the brains of mice alongside RAJI B1-ENV's for fluorescent imaging of the needle track in the brain. BLI imaging of BL/6 mice which underwent stereotactic injection of mGRP's also provided evidence of cell survival after 72-hours.