Afucosylation of Broadly Neutralizing Antibodies to Eliminate HIV+ CD4+ T Cells and Macrophages

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BACKGROUND

The persistence of HIV reservoirs in T cells and myeloid cells precludes HIV cure. Some broadly neutralizing anti-HIV envelope (Env) antibodies (bNAbs) can bind HIV-infected cells and elicit natural killer (NK) cell antibody-dependent cellular cytotoxicity (ADCC). Removal of core fucose from the Fc region of antibodies can increase NK cell ADCC, but remains to be fully explored in HIV.

The aim of this study was to examine the utility of afucosylated bNAbs to enhance ADCC of HIV-infected CD4+ T cells and macrophages by NK cells.

METHODS

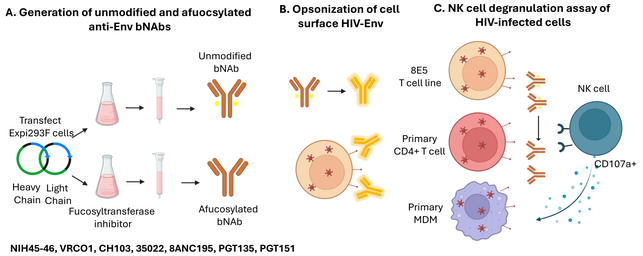


Figure 1. Schematic diagram of experimental methodology
A) A panel of seven bNAbs (NIH45-46, VRC01, CH103, 35O22, 8ANC195, PGT135, PGT151) were generated in the presence or absence of a fucosyltransferase inhibitor (2F-Peracetyl-Fucose) and purified using protein-G chromatography. B) Opsonization of cell-surface Env on primary CD4+ T cells and monocyte-derived macrophages (MDM) infected with HIV_{BaL} *in vitro* was evaluated by immunolabeling using flow cytometry. C) Natural killer (NK) cell ADCC responses to HIV Env-expressing targets in the presence of bNAbs was assessed by flow cytometry using a CD107a degranulation assay.

RESULTS

1. Generating unmodified and afucosylated bNAbs

A panel of bNAbs targeting multiple HIV Env epitope regions (Figure 2A) was generated. Afucosylated bNAbs demonstrated a significant reduction in core fucosylation compared to unmodified bNAbs (range 54.0% to 63.9%; Figure 2B).

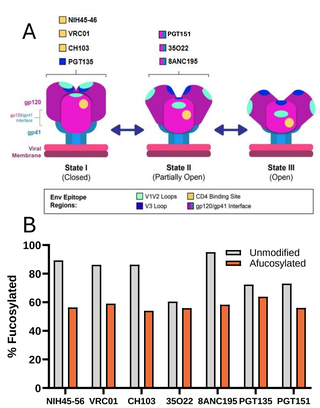


Figure 2. Generation of unmodified and afuocsylated bNAbs A) The epitope regions of different Env conformations targeting by bNAbs adapted from Kek et al. *Antiviral Research.* 2021;191:105085 B) Fucosylation levels of unmodified and afucosylated bNAbs measured using microcapillary electrophoresis.

Afucosylation modifications of anti-Env bNAbs can *enhance* NK cell-mediated ADCC against both HIV-infected primary CD4+ T cells and macrophages.

RESULTS CONT.

2. A panel of bNAbs opsonize HIV-infected T cells and macrophages with high specificity.

The bNAbs NIH45-46, VRC01, CH103, PGT151 and 35O22 opsonized both HIV-infected T cells and MDM with high specificity. Maximal opsonization was exhibited by 35022 for 8E5 cells (mean of 55.4% of p24+ cells opsonized, Figure 3A), PGT151 for CD4+ T cells (18.1%, Figure 3B) and NIH45-46 for MDM (39.6%, Figure 3C). PGT135 effectively opsonized p24+ T cells but demonstrated significant non-specific binding to uninfected MDM whilst 8ANC195 had minimal ability to bind HIV-infected cells.

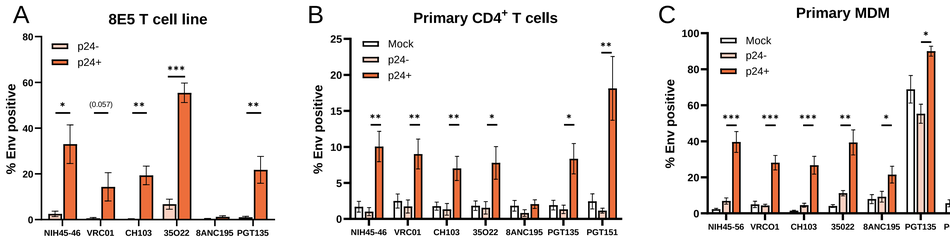


Figure 3. bNAb opsonization of HIV-infected cells. The proportion of mock, bystander (p24-) and HIV-infected (p24+) A) 8E5s, B) primary CD4+ T cells and C) MDM opsonized by each bNAb. Graphs show mean and standard error for 5 experimental replicates for 8E5 cells and 7-8 independent donors for primary cells. *p<0.05, **p<0.01 and ***p<0.001 by paired t test.

3. Afucosylated bNAbs elicit enhanced NK cell degranulation against HIV-infected cells

Afucosylated bNAbs significantly increased ADCC responses from NK-cells against HIV-infected 8E5 cells (p<0.05 for all except NIH45-46; Figure 4A), CD4+ T cells (p<0.05 for all; Figure 4B), and MDM (p<0.05 for all except VRC01; Figure 4C) when compared to unmodified bNAbs. A combination of afucosylated antibodies targeting multiple Env epitope regions (NIH45-46, CH103 and 35O22) elicited enhanced NK cell degranulation against HIV-infected cells as compared to an unmodified antibody combination, but did not elicit enhanced degranulation responses when compared to individual afucosylated bNAbs alone.

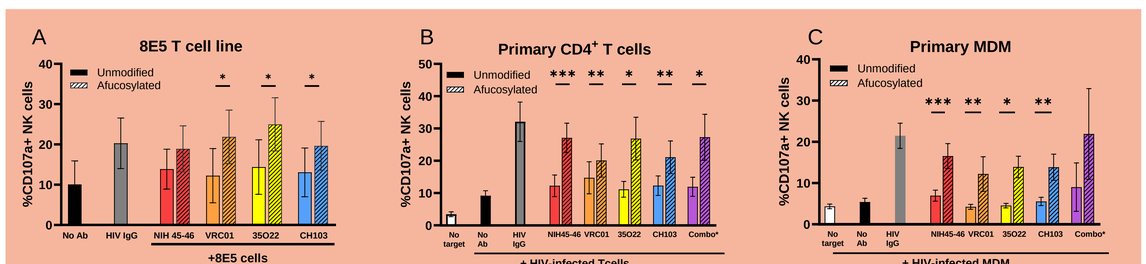


Figure 4. NK cell degranulation in response to HIV-infected cells. The proportion of degranulating CD107a+ NK cells in the presence of 5 ug/mL unmodified and afuocsylated bNAbs are shown for A) 8E5s, B) primary CD4+ T cells and C) MDM. No target (cells) and no antibody controls were included. Pooled HIV IgG was included as a positive control. Data shows the mean and standard error for 6 experimental replicates for 8E5 cells and 6-9 independent donors for primary cells *only n=2 included for MDM combination bNAbs. *p<0.05, **p<0.01 and ***p<0.001 by paired t test.

RESULTS CONT.

4. A combination of afucosylated bNAbs enhances death of HIV-infected T cells.

Assessment of NK cell mediated death of HIV-expressing cells (Figure 5A) demonstrated a combination of afucosylated NIH45-46, CH103 and 35O22 induced a significant increase in the proportion of non-viable p24+ 8E5 target cells following incubation with purified NK cells (171% increase, p=0.02) when compared to a no antibody control (Figure 5B). No significant difference in viability of p24- 8E5 targets was observed.

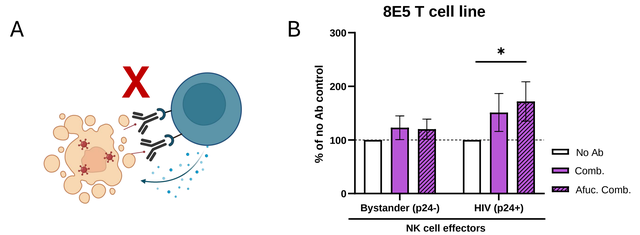


Figure 5. Killing of HIV-infected T cells by afucosylated bNAbs. A) 8E5 T cells were co-cultured with purified NK cells in the presence of 5 ug/mL total unmodified or afucosylated combination of bNAbs (NIH45-46, CH103 and 35022) and cell death (determined by Near-IR+ expression) assessed by flow cytometry. B) The proportion of non-viable bystander (p24-) and HIV-expressing (p24+) 8E5 cells normalized to the no antibody control when incubated with purified NK cell effectors. Graphs show mean and standard error for 6-7 experimental replicates *p<0.05, **p<0.01 by paired t test.

CONCLUSION

We have identified a panel of candidate anti-Env bNAbs that bind HIV-Env on both HIV-infected CD4+ T cells and macrophages. Opsonisation of HIV-infected cells with afucosylated bNAbs can increase NK cell ADCC, and combination afucosylated bNAbs can enhance death of HIV-infected cells. These findings support further examination of anti-Env bNAbs as a promising immunotherapy tool for ART-free HIV control.

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