HIV-1 Env Exhibits Distinct Conformations and Neutralization Profiles in CSF and Blood

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BACKGROUND

- HIV-1 replicates in different body compartments characterized by distinct solute compositions.
- Our previous studies showed that the microenvironment of HIV-1 replication impacts the interaction between HIV-1 Env and inhibitory ligands.
- For example, Env recognition by agents that target high-mannose glycans is reduced by fructose in human semen (Johnson *et al*., JVI 2020). Similarly, cholesterol content of the lipid membrane, which varies between different cell types, determines the conformation of Env (Salimi *et al*., JBC 2020).
- We sought to establish if the neutralization profile of HIV-1 differs between two microenvironments that harbor HIV-1 replication; blood serum and cerebrospinal fluid (CSF).
- These fluids differ most notably in the content of albumin; 35-50 mg/mL in blood versus 0.2 mg/mL in CSF and interstitial fluid (ISF) of brain. Given such differences, and the promiscuous interaction of albumin with diverse solutes, we also tested the isolated effects of this protein on Env recognition and neutralization by antibodies.

METHODS

- **Biological fluids:** De-identified CSF and blood serum from HIV-negative donors, and plasma from PLWH donors were obtained by informed consent at the Univ. of Iowa clinics.
- **Pseudoviruses** that express luciferase and contain the Envs of different HIV-1 strains were produced in 293T cells cultured in: **(i)** Pooled whole CSF, **(ii)** Whole serum, **(iii)** DMEM containing 5% fetal calf serum (D/FCS), or **(iv)** D/FCS with blood-like levels of human serum albumin (50 mg/mL).
- **Neutralization assays** were conducted using TZM-bl target cells in the same fluids used for virus production, with plasma from PLWH or monoclonal antibodies (mAbs).
- **Env binding by mAbs** was tested using a cell-based ELISA system, whereby AD8 strain Envs were expressed on human osteosarcoma (HOS) cells. Binding was quantified as previously described (Rojas Chavez *et al*., JVI 2022)
- **Env Stability** was tested by incubating virus samples in the different fluids on ice for 0 - 48 hours, followed by inoculation of the TZM-bl cells (Johnson *et al*., JVI 2017).

RESULTS

1. HIV-1(AD8) exhibits distinct neutralization profiles in hSerum and hCSF

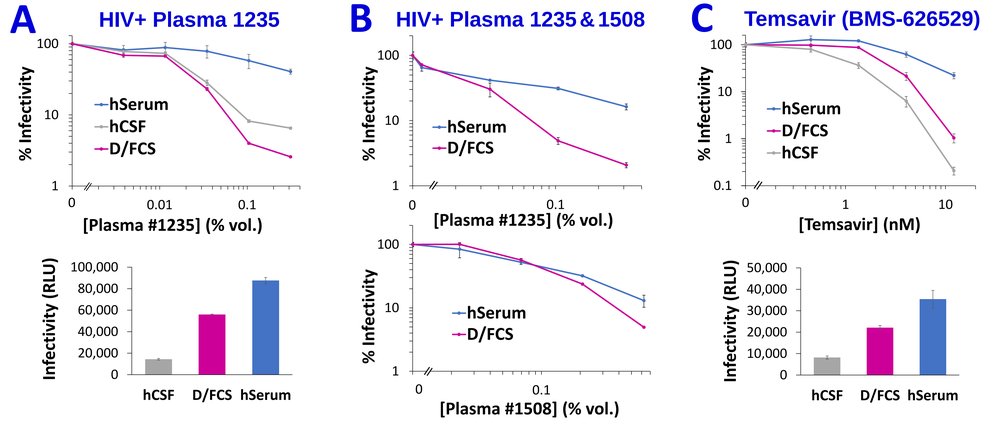


Figure 1. Neutralization of luciferase-expressing HIV-1 that contains the Envs of AD8 strain by plasma from HIV+ donors and by Temsavir. Viruses were produced and tested in the indicated fluids. Infectivity is expressed as a percent of that measured in the absence of the inhibitors. RLU, relative light units.

2. Neutralization and stability profiles of Env are altered by serum-like levels of hAlbumin

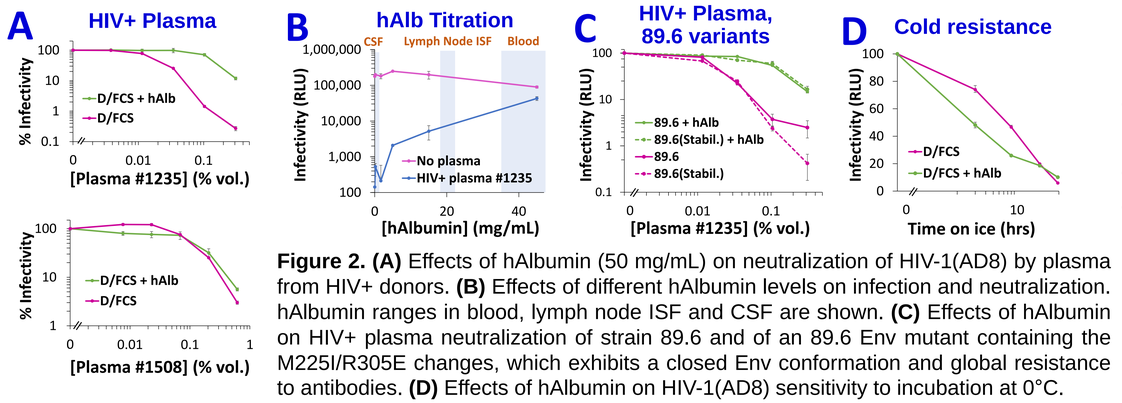


Figure 2. (A) Effects of hAlbumin (50 mg/mL) on neutralization of HIV-1(AD8) by plasma from HIV+ donors. **(B)** Effects of different hAlbumin levels on infection and neutralization. hAlbumin ranges in blood, lymph node ISF and CSF are shown. **(C)** Effects of hAlbumin on HIV+ plasma neutralization of strain 89.6 and of an 89.6 Env mutant containing the M225I/R305E changes, which exhibits a closed Env conformation and global resistance to antibodies. **(D)** Effects of hAlbumin on HIV-1(AD8) sensitivity to incubation at 0°C.

3. hAlbumin reduces binding of mAbs that target the base of the Env trimer

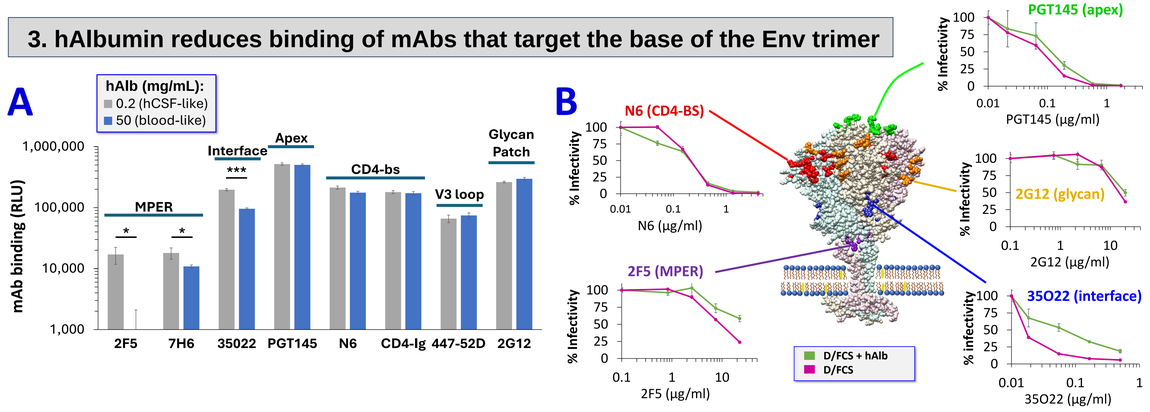


Figure 3. (A) mAb binding to Env AD8 expressed on the surface of HOS cells in buffer containing the indicated concentrations of hAlbumin. P-values in a two-tailed T-test: *, <0.05; ***, <0.0005. **(B)** Neutralization of HIV-1(AD8) by mAbs. Infections were performed in D/FCS or D/FCS supplemented with 50 mg/mL hAlbumin.

4. Supplementation of CSF by hAlbumin only partially recapitulates the neutralization profile of hSerum

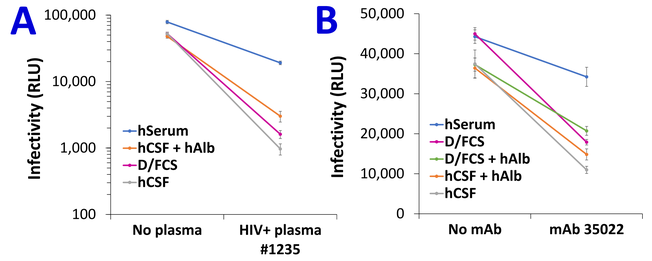


Figure 4. HIV-1(AD8) was suspended in the indicated fluids. Some samples were then supplemented with HIV+ plasma from donor 1235 (0.1% volume) or with mAb 35O22 (0.15 µg/ml). Samples were used to inoculate TZM-bl cells and infectivity was measured 2 days later.

CONCLUSIONS

- Antibodies in plasma from some PLWH inhibit HIV-1 infection more potently in hCSF relative to hSerum, while the potency of other plasma is unaffected by the medium.
- The epitopes impacted appear to be in the trimer base, including the gp120-gp41 interface and MPER, which bind antibodies more efficiently in low-hAlbumin (CSF-like) fluids relative to high-hAlbumin (blood-like) fluids.
- The distinct neutralization profiles in hCSF and hSerum can be attributed only in part to their content of hAlbumin.
- The small-molecule Temsavir is also more potent in CSF.
- Effects of the microenvironment in compartments that harbor HIV-1 replication should be considered in design and dosing of Env-targeting therapeutics.

ADDITIONAL INFORMATION

References

- Johnson *et al*., J Virol 2020, PMID 32102878.
- Salimi *et al*., J Biol Chem 2020, PMID 31757809.
- Rojas Chávez *et al*., J Virol 2022, PMID 35658529.
- Johnson *et al*., J Virol 2017, PMID 28490588.

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