Characterizing the Role of PSGL-1/CD162 in the HIV-1 Envelope

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Introduction: P-selectin glycoprotein ligand-1 (CD162/PSGL-1)

- Transmembrane protein present on all leukocytes
- Large homodimer with a 50 nm extracellular domain
- Facilitates binding to P-, E-, and L-selectins to mediate rolling on endothelium and extravasation into inflamed tissues

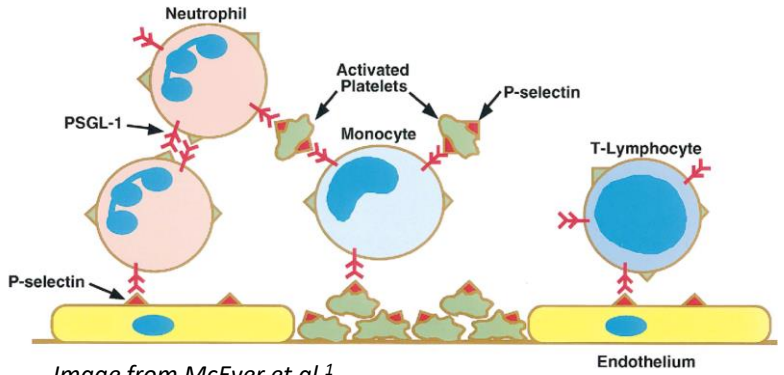
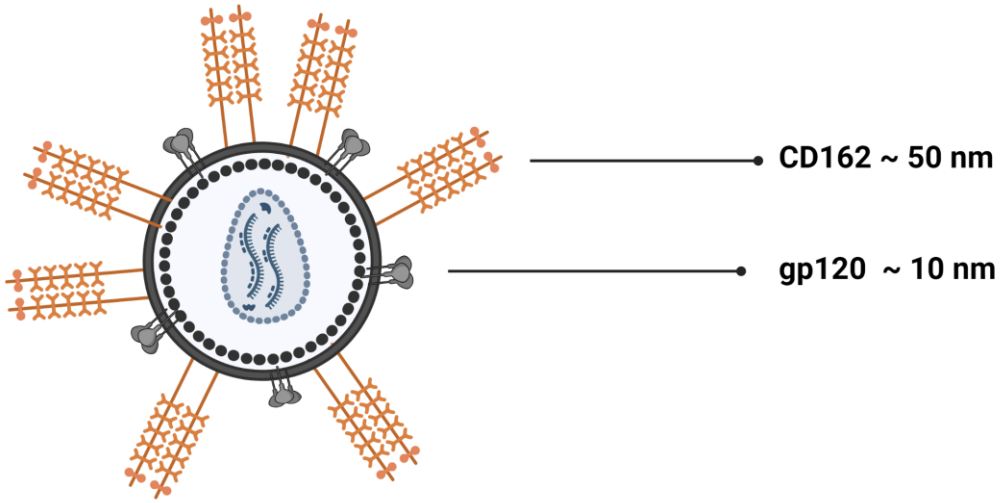


Image from McEver et al.[1]

Introduction: CD162 as a novel HIV host restriction factor

- Restriction factor identified in CD4 $^{+}$ T cells in 2019[2]
- Induced by IFN-γ and antagonized by HIV Vpu & Nef
- Antiviral activities
  - Inhibits HIV reverse transcription
  - Inhibits incorporation of Env into virions
  - Incorporates in virions to reduce infectivity [2-4]
- Largely characterized in transfected viruses

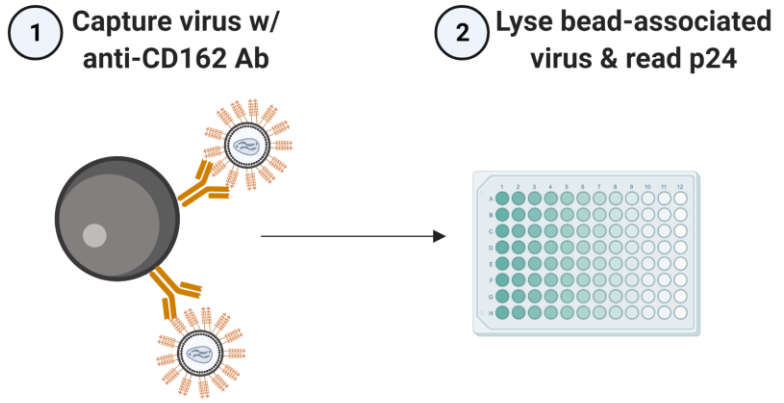


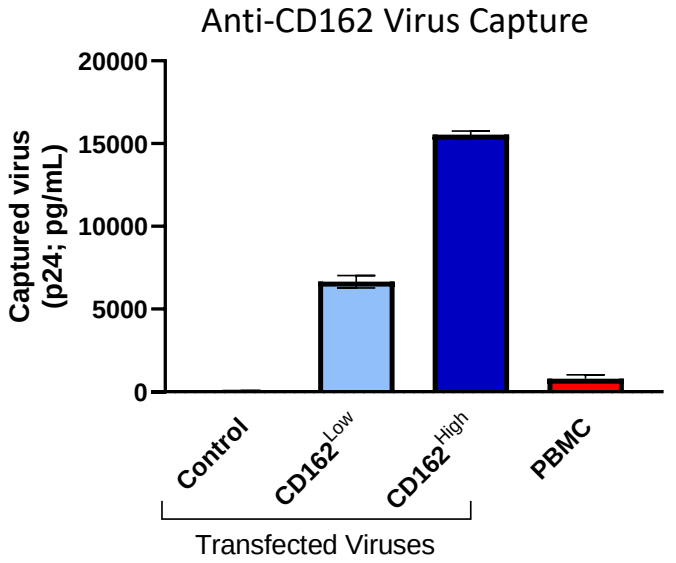
Research questions

1. Are transfected viruses a representative model of viruses produced by infection in primary cells (PBMC) for studying virion-incorporated CD162?
2. Which viral determinants are involved in CD162 incorporation into virions?

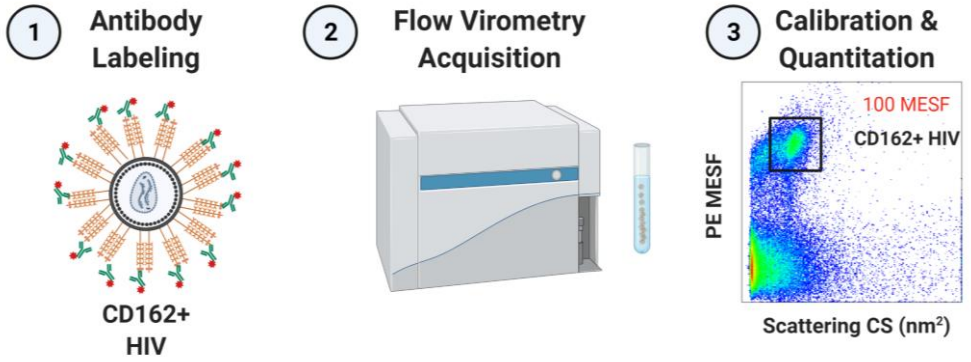
Results: CD162 is more abundant on transfected viruses than PBMC viruses

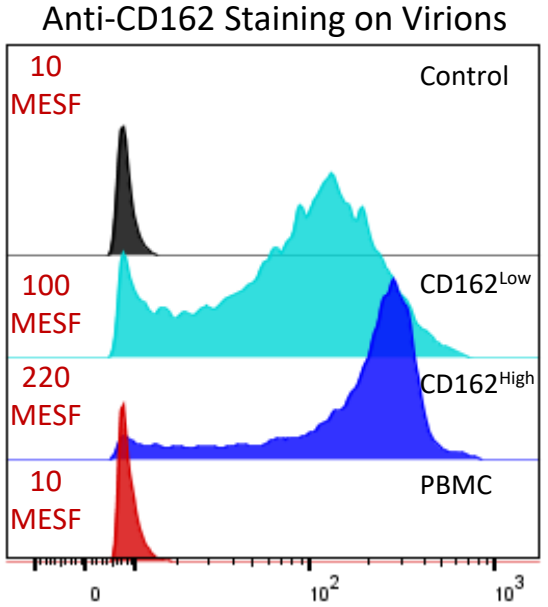
- Transfected full-length viruses produced with no ('Control'), low or high levels of CD162 plasmid DNA were compared to viruses produced through PBMC infection to assess levels of virion-incorporated CD162





- CD162 on PBMC viruses is present at much lower levels than on transfected viruses as detected by CD162 antibody capture and flow virometry staining[5]

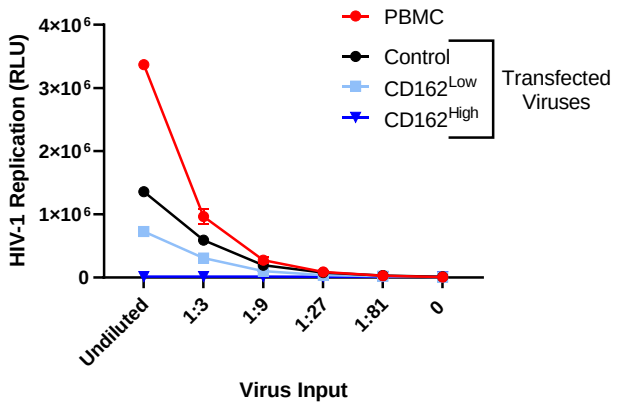




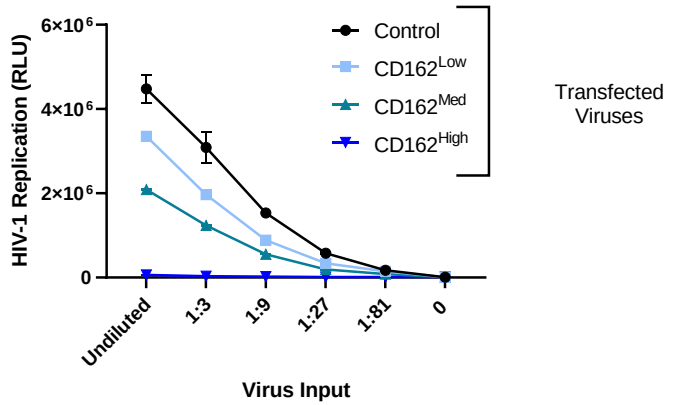
Results: CD162 levels correlates with reduced virus infectivity

- The infectivity of transfected full-length and pseudoviruses produced with no ('Control'), low, medium or high levels of CD162 pDNA were compared to viruses produced through PBMC infection using TZM-bl titration
- Increasing amounts of CD162 in transfected viruses correlate with decreased virus infectivity in both full-length and pseudoviruses
- However, PBMC virus remains highly infectious despite CD162 being present at low levels in the envelope

Full-Length Virus Infectivity



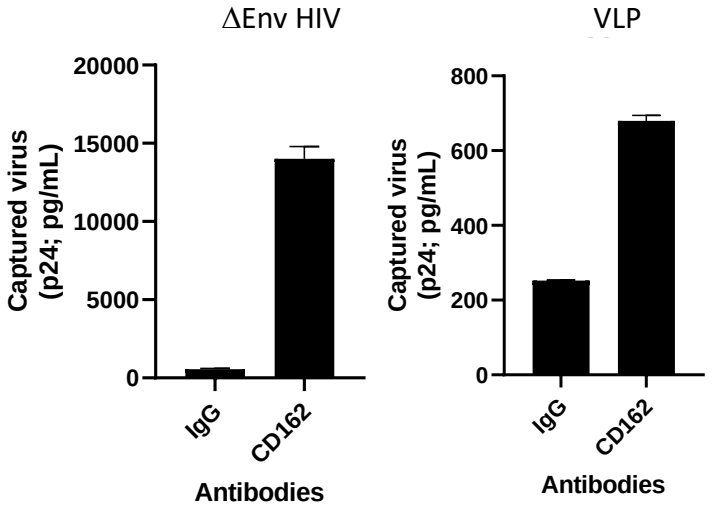
Pseudovirus Infectivity



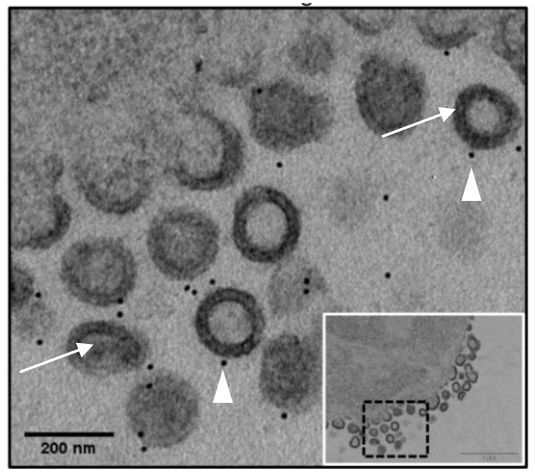
Results: CD162 incorporation is Env independent

- HIV pseudoviruses deficient in gp120/gp41 ('ΔEnv') and virus-like particles (VLP) were produced through co-transfection in HEK293 cells with a CD162 plasmid
- $Pr55^{Gag}$ alone is sufficient for the incorporation of CD162 into VLPs and Env is dispensable for this process
- Other viral proteins besides Gag likely facilitate increased levels of CD162 incorporation

Antibody Virus Capture



Immunogold Labelling



Arrow: $CD162^{+}$ Pr55 VLP
Arrow head: CD162 (10 nm gold)

Conclusions

- CD162 is a highly potent host restriction factor present in the HIV envelope but its effects are more notable in transfected viruses
- Differential amounts of CD162 are incorporated in transfected and PBMC viruses suggesting that other models may be more relevant for characterizing this host restriction factor
- While CD162 incorporation into virions doesn't require Env, the incorporation may be enhanced by other viral proteins besides Gag

Significance

- While CD162 has been well characterized as an antiviral factor using transfected viruses, PBMC viruses contain lower levels of the restriction factor which may explain the reduced potency of CD162 as an antiviral factor in PBMC viruses
- The choice of model used to characterize host restriction factors is important to ensure the generated results are physiologically relevant

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NSERC CRSNG

Figures

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