Activated Platelets Render Functionally Deficient Natural Killer Cells in People Living with HIV

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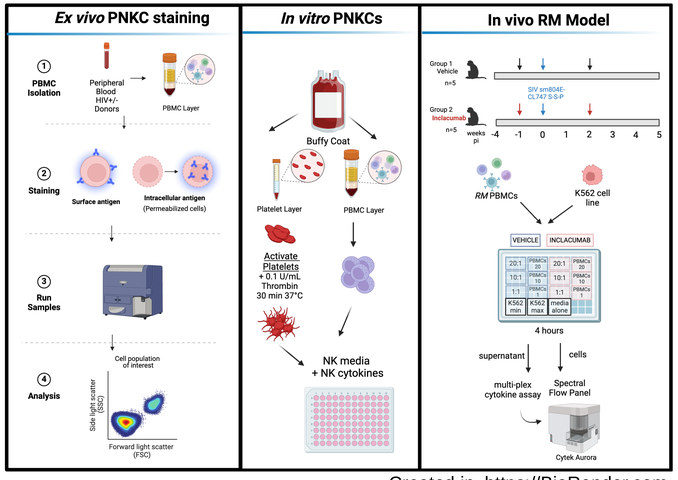
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BACKGROUND

Anti-retroviral therapy (ART) for people with HIV (PWH) is very effective in suppressing HIV replication and preventing disease progression. However, even while on ART, NK cells remain dysfunctional. Although the exact mechanism of this dysfunction is unknown, there is evidence of increased platelet-immune cell complex formation in PWH. These complexes alter immune cell functions, which could play a role in NK dysfunction. We propose another role of platelets during HIV infection through platelet-NK cell complexes (PNKCs). **We predict that PNKCs are a key factor in promoting NK dysfunction during HIV infection.**

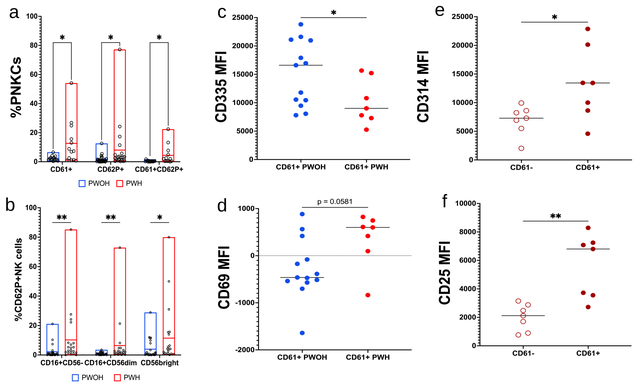
METHODS



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RESULTS

Figure 1. PNKCs are elevated in PWH and are phenotypically different from non-PNKCs and PNKCs from PWOH. (a) %PNKCs from PWOH vs PWH. (b) PNKCs by NK subset (c, d) CD335 and CD69 expression on PNKCs from PWOH and PWH (e, f) CD314 and CD25 expression on PNKCs and non-PNKCs from PWH.



Platelets form significantly more complexes with NK cells during HIV infection. Platelets enhance, and chronic PF-4 exposure reduces NK cytotoxicity *in vitro*. However, during an *in vivo* SIV infection model, platelets reduce NK effector functions at low E:T ratios.

Figure 2. Platelets form complexes with NK cells *in vitro* likely through PSGL-1 & CD62P interactions. (a) CLEM imaging of a platelet interacting with an NK cell (b) Types of PNKCs (c) quantification of PNKC types (d) CD40L and PSGL-1 expression on NK cells.

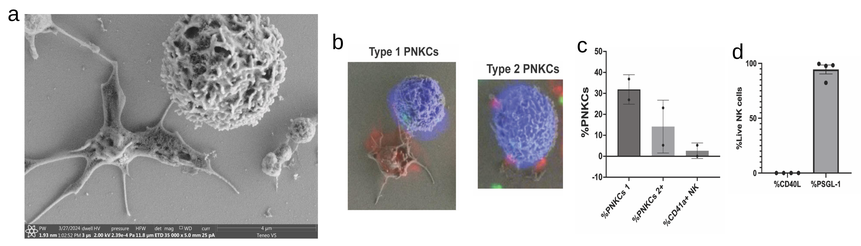


Figure 3. Platelet depletion prior to NK expansion alters the phenotype of NK cells. (a) NK sorting with and without platelet depletion (b, c, d) CD337, CD314, CD335 expression on days 0-14 of expansion for NK cells with platelets or with platelet depletion.

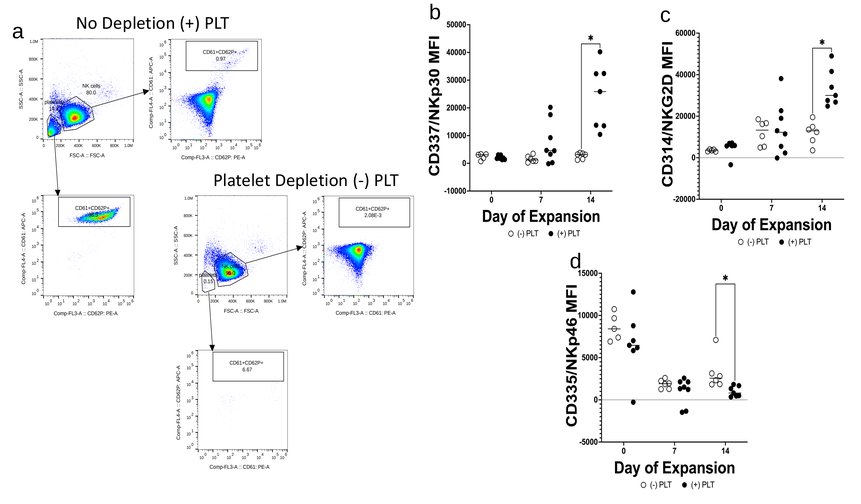


Figure 4. Platelets enhance and PF-4 reduces NK cytotoxicity *in vitro*. Expanded Day 14 NK %specific lysis of (a) U937 (b) U1 (c) Jurkat and (d) JLAT 10.6 cell lines using an EuTDA based assay.

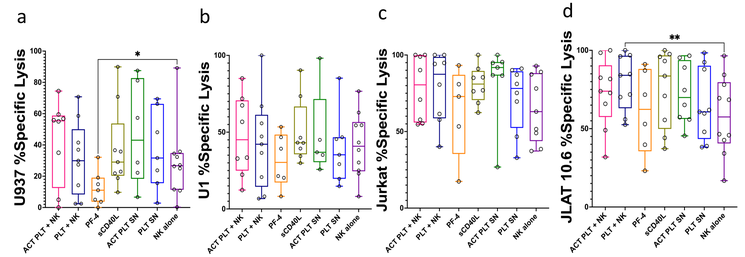
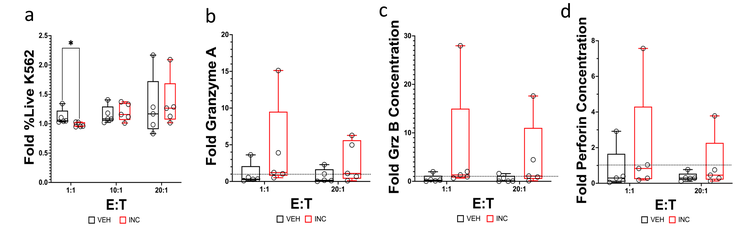


Figure 5. In an SIV RM model, platelet inhibition saves NK cells from dysfunction at low E:T ratios. (a) Fold %Live K562 (Nx NK compared to -28 NK)after 4h co-culture (b, c, d) Fold granzyme A concentration (Nx NK compared to -28 NK) in the supernatant after 4 h co-culture.



CONCLUSIONS

- PWH have increased levels of PNKCs compared to PWOH.
- Platelet-NK complexes and PNKCs may alter NK phenotypes during expansion (*in vitro* & *ex vivo*)
  - ↑CD337/NKp30, CD314
  - ↓CD335/NKp46
- PNKCs can be formed *in vitro* and likely occur through PSGL-1 & CD62P.
- *In vitro,* platelets enhance NK cytotoxicity; however, chronic PF-4 exposure reduces NK cytotoxicity.
- In an *in vivo* RM study, platelet inhibitor (inclacumab) treatment prevents NK dysfunction at low E:T ratios.

Future studies may investigate how specifically HIV infection itself may alter PNKCs and influence NK dysfunction.

ACKNOWLEDGEMENTS

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