Retrovirology

Poster presentation

P04-12. Characterization of leukopak PBMC phenotypes and biotypes for optimal performance in HIV-1 neutralization assays

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Background

PBMC assays are plagued with significant variability in neutralization titers due to leukopak differences. Here we assess PBMC surface phenotypes, genotypes and viral permissivity to identify leukopak characteristics that correlate with optimal performance in neutralization assays, as part of a standardization program.

Methods

Leukopaks (N = 51) were analyzed using multi-parametric flow cytometry (LSRII cytometer, Becton Dickinson) to quantitate immune cell populations and surface markers. Viral titrations were performed using infectious molecular clones (IMC) (293T cell-derived or PBMC-passaged) and IC50 values were calculated using the Spearman-Karber formula. Productive replication in leukopaks was analyzed by p24 or luciferase expression; phenotypic correlates of viral permissiveness were assessed by Spearman correlation analyses.

Results

Leukopaks were rank ordered using titrations of a panel of 6 IMC, and permissive versus resistant leukopaks were identified. Eight leukopaks from one donor showed a wide range in permissivity. As expected, permissive leukopaks showed higher CD4+ T cells (median = 50.7%), compared with more resistant leukopaks (median = 36.9%), and the converse was observed for CD8+T cells. Unexpectedly, permissive donors had fewer CCR5+CD4+ T cells (median = 5.8%), as compared with less permissive (median = 11.1%). Several surface markers (ie. CD38, CD69, HLA-DR and CXCR4) showed no difference between groups. For 2 reporter IMCs tested, titers correlated well for 293T-derived and PBMC passaged virus (NL LucR.T2A-SF162.ecto, p = 0.042; NL LucR.T2A BaL.ecto, p = 0.0006), suggesting utility of 293T stocks.

Conclusion

Use of CD8-depleted versus bulk PBMC for HIV neutralization may be warranted, as %CD4+ and %CD8+ T cells post-PHA stimulation was clearly associated with leukopak permissiveness. PBMC pooling may provide an option to overcome the within and between donor variation. No other phenotypic or genetic characteristics have as yet been associated with leukopak performance in viral assays; numerous analyses are ongoing. Regarding viral growth, use of 293T-derived IMCs may be warranted; studies comparing HIV neutralization titers using both sources will further inform this.