

High level persistence of HIV-1 antigen-specific CD4+ T cells in untreated chronic infection, as detected by a novel flow cytometric assay

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Introduction

- HIV antigen-specific CD4+ T cells are believed to be preferentially targeted and deleted by cytopathic infection.
- In most cases, HIV-1 antigen-specific CD4+ T cells are barely detectable using either proliferation assays or MHC Class II tetramers, and only found at low levels by interferon- γ production
- We have developed a novel flow cytometric assay of antigen-specific CD4+ T cells which measures co-expression of CD25 and CD134 and does not rely on either proliferation or cytokine production

Aim

- We have used our novel CD25+CD134+ assay to reassess the level of HIV-specific CD4+ T cells in untreated chronic infection.

Methods

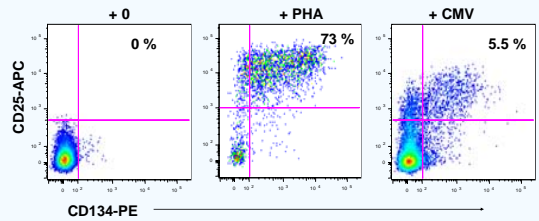
- We obtained Na Heparin-anticoagulated fresh whole blood samples from 17 healthy adult controls and 13 consecutive untreated subjects with established HIV-1 infection (median CD4 cell count: 158 cells/ μ l; median plasma RNA viral load: 4.7 log₁₀ copies/ml).
- 0.25 ml of NaHep fresh whole blood + 0.25 ml of Iscove's Modified Dulbecco's medium was incubated for 40-44 hr with: culture medium alone (negative control); PHA (positive control); CMV lysate; *M avium* lysate; UV-inactivated HSV-1; and a pool of overlapping 15-mer peptides covering HIV-1 Gag.

Methods (cont'd)

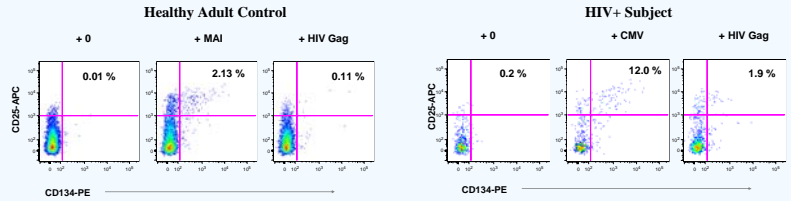
- At the end of the incubation, 100 μ l of whole blood cultures were stained with CD3-PerCP-Cy5.5, CD4-AlexaFluor 700, CD25-APC and CD134-PE monoclonal antibodies (Becton Dickinson), using a standard whole blood staining protocol and analysed on an LSR II flow cytometer (Becton Dickinson).
- Antigen-specific CD4+ T cells were identified as CD25+CD134+ and measured as a percentage of CD4+ T cells.

Results

- Figure 1. Representative results from a healthy adult control showing background co-expression of CD25 and CD134 when whole blood was incubated in the absence of any added antigen (left) and when incubated in the presence of PHA (middle) or CMV lysate (right).
- CD25+CD134+ results in healthy adult controls correlated with PBMC proliferative responses.

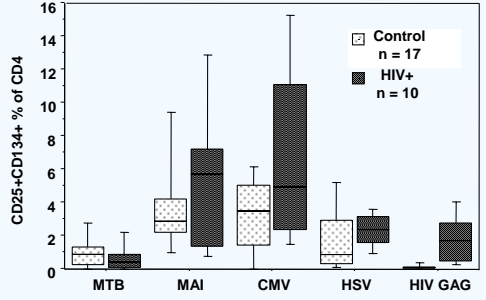


- Figure 2. Representative results from a healthy adult control (left) and from an HIV+ subject (right). The HIV+ subject had a CD4 cell count of 120 cells/ μ l, and a plasma viral load of 504,900 copies/ml



Results (cont'd)

- Figure 3. Summary of results for all healthy adult controls and HIV+ subjects, respectively. The box plots represent 10th, 25th, 50th, 75th and 90th percentiles for each patient group. The HIV Gag responses for HIV+ subjects was significantly higher than for healthy adult controls by Mann-Whitney U test (p<0.0001).



Conclusions

- The novel CD25+CD134+ assay has revealed that there are still relatively large populations of antigen-specific CD4+ T cells that respond to recall antigens, even in relatively advanced chronic HIV-1 infection.
- In particular, our results suggest that there are 5-10 times more HIV Gag-specific CD4+ T cells present in peripheral blood than previously estimated by proliferation, tetramers or IFN- γ production

Acknowledgments

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