HIV Gag-specific T cell responses in the female genital tract are detected in women with high magnitude blood responses during chronic HIV-1 infection

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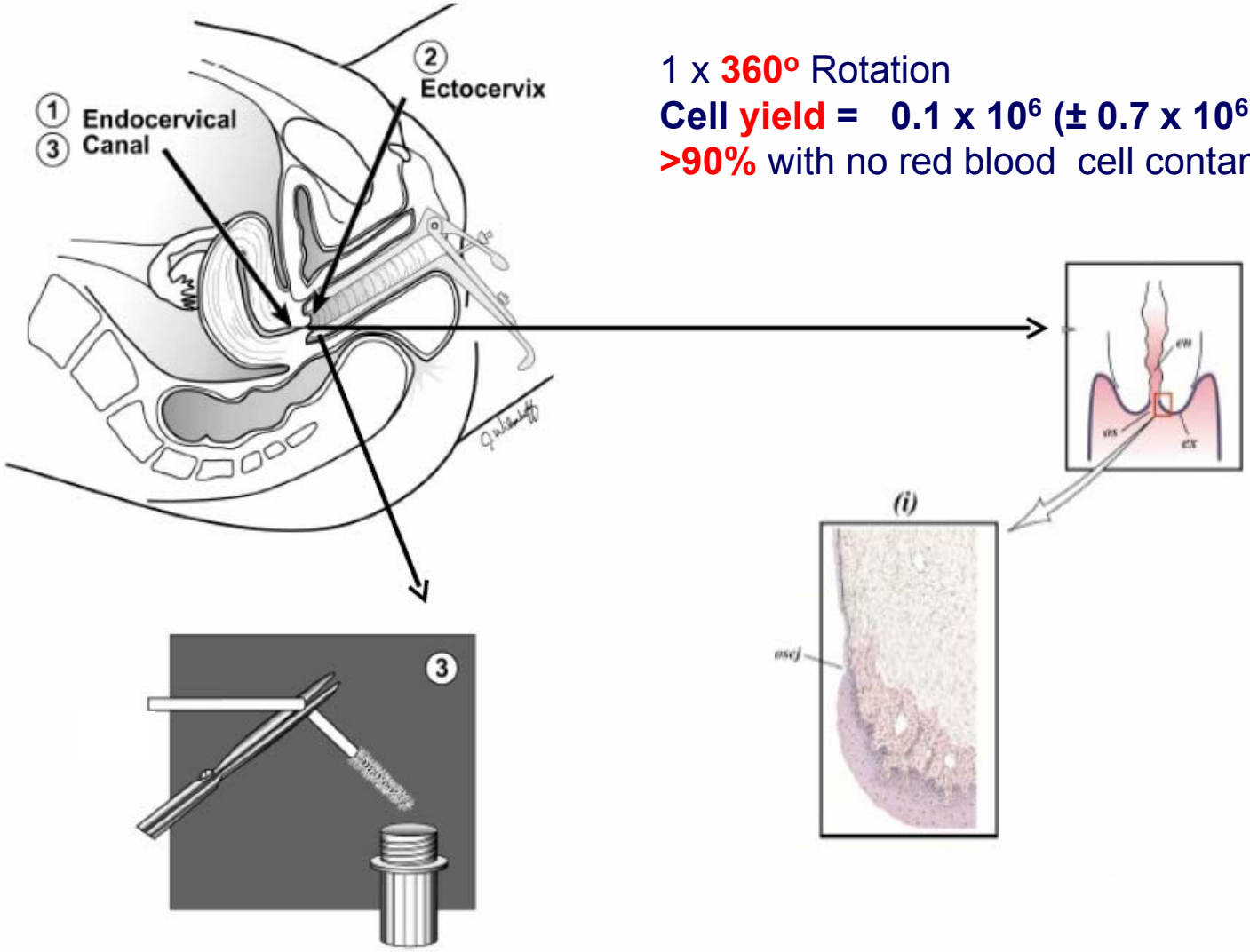
Introduction

- Sexual transmission of HIV accounts for the majority of new infections worldwide, with the risk of becoming infected through heterosexual route being at least two times higher in women than men
- The mucosal surface of the female genital tract is ultimately the site of HIV transmission and shedding
- While there is clear evidence that HIV-specific cytotoxic T lymphocytes (CTLs) in blood play an important role in controlling HIV replication systemically, there is comparatively little known about CTL responses to HIV in the genital tract and factors that govern these
- Understanding the pathogenesis of genital infection and the role of protective immunity at the genital mucosa is of particular importance since it might provide insight into the development of an effective mucosal HIV vaccine

Introduction

- There is an urgent need for reliable and validated methods for investigating mucosal immune responses in the female genital tract. Such methods would be particularly valuable in HIV vaccine trial settings where intensive invasive sampling is not an option
- HIV-specific mucosal CTL literature has been dominated by biopsy approaches to isolating lymphocytes from rectal and gastrointestinal mucosal tissue
- Fewer approaches are available for sampling mucosal tissue from the female genital tract (cervical lavages and cervical cytobrushes)
- The usefulness of both these methods are, however, constrained by the low numbers of *ex vivo* lymphocytes they yield

How are these mucosal samples obtained ?



1 x **360°** Rotation

Cell yield = 0.1 x 10^6 (± 0.7 x 10^6) cells

>90% with no red blood cell contamination

Adapted from Coombs et al 2003

Direct ex vivo analysis of mucosal samples

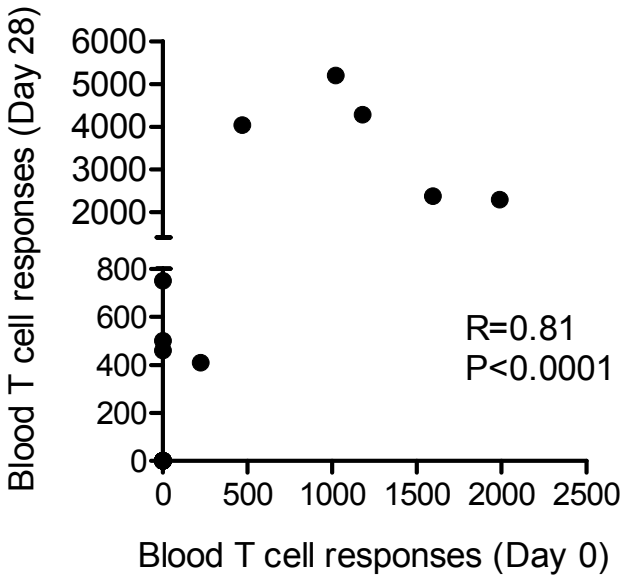
- Our laboratory (Gumbi et al., JVI, 2008) have shown that HIV Gag-specific T cell responses are detectable at the cervix immediately *ex vivo*
- Because of cervical yield constraints, this *ex vivo* study focused measuring a single function (IFN-γ production) of CTLs to a single antigen (Gag)
- Clearly, *ex vivo* lymphocyte yields from a single cytobrush are not sufficient for thorough mapping of HIV Gag genome
- Short term *in vitro* polyclonal stimulation was explored to expand cervical CD3+ T cells using anti-CD3 monoclonal antibodies and IL-2

Aim

The aim of this study was to investigate the feasibility and efficiency of polyclonal *in vitro* expansion of cervical cytobrush-derived T cells to study HIV-specific T cell responses in the female genital tract to overcome the impact of low cytobrush yield

Validation: Impact of expansion on HIV-specific T-cell responses in blood

- Initial experiments were conducted using PBMCs to investigated whether our polyclonal expansion protocol with anti-CD3 and IL-2 influenced the regions of Gag targeted by HIV specific T-cells
- PBMC from chronically HIV-infected individuals with well characterized *ex vivo* (day 0) Gag-specific ELISPOT responses were examined after 28 days of expansion for Gag-specific regions targeted
- In all individuals, dominant *ex vivo* Gag regions targeted were maintained following *in vitro* expansion ($p<0.0001$, $Rho=0.81$)



The Nyanga Day Hospital

Women were recruited from a cohort of chronically HIV-infected women enrolled in a longitudinal study of cervical diseases in relation to HIV infection attending the Nyanga Day Hospital

~75% women have CD4 counts >300 cells/μl have been enrolled in our study since July 2004

Clinical description of HIV-infected women

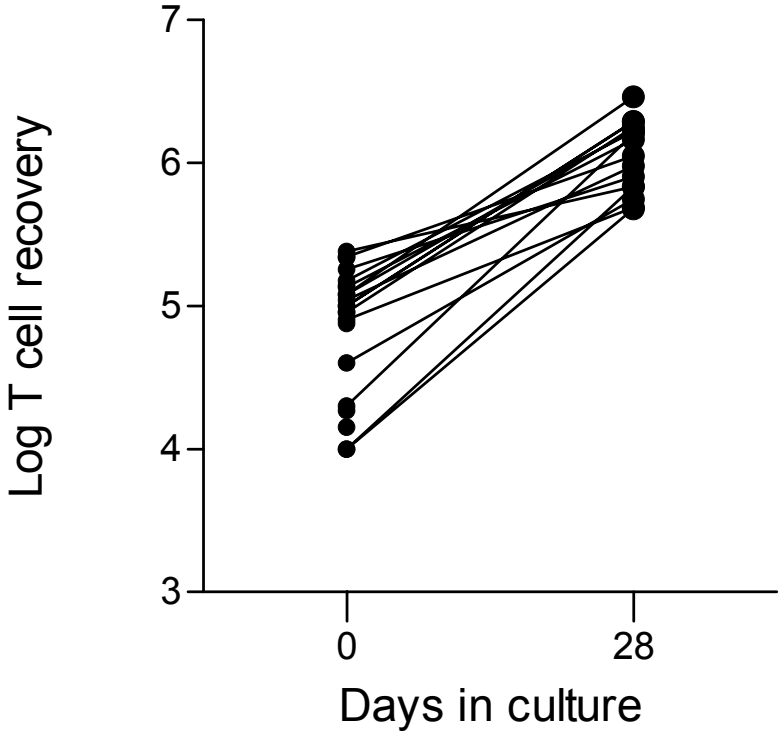
N= 22

ARV Naïve

	Mean (± SD)	Range
Age	32 (± 5.7)	22-39
Absolute CD4 Count (cell/μl)	501 (± 223)	300 -1300
HIV Viral Load (RNA copies/ml)	90308 (± 294363)	970 -1300000

Cervical expansion kinetics and efficiency

T cell Recovery

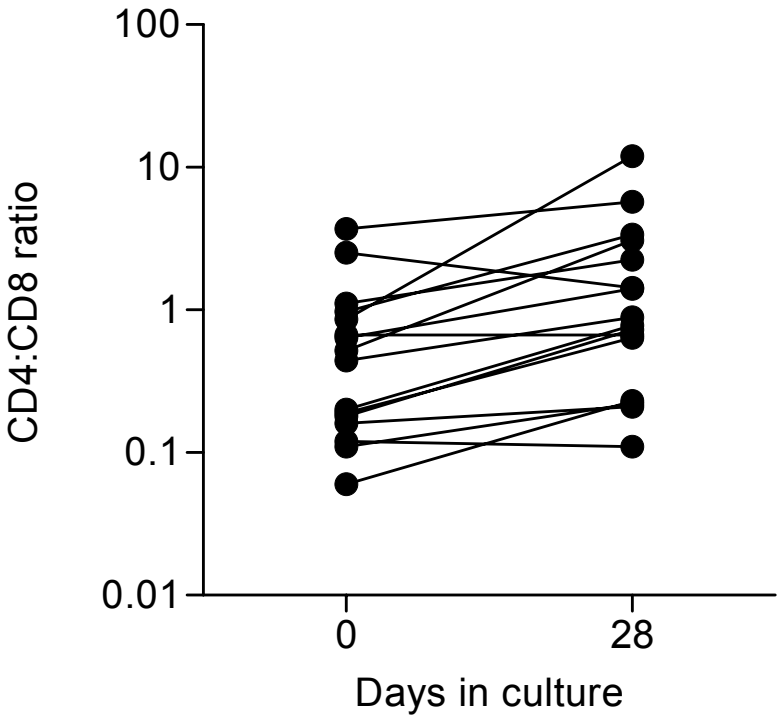


Mean ± SD

Day 0 = 0.11 x 10^6 (± 0.69 x 10^6) cells

Day 28 = 1.38 x 10^6 (± 0.63 x 10^6) cells 22.9-fold

CD4:CD8



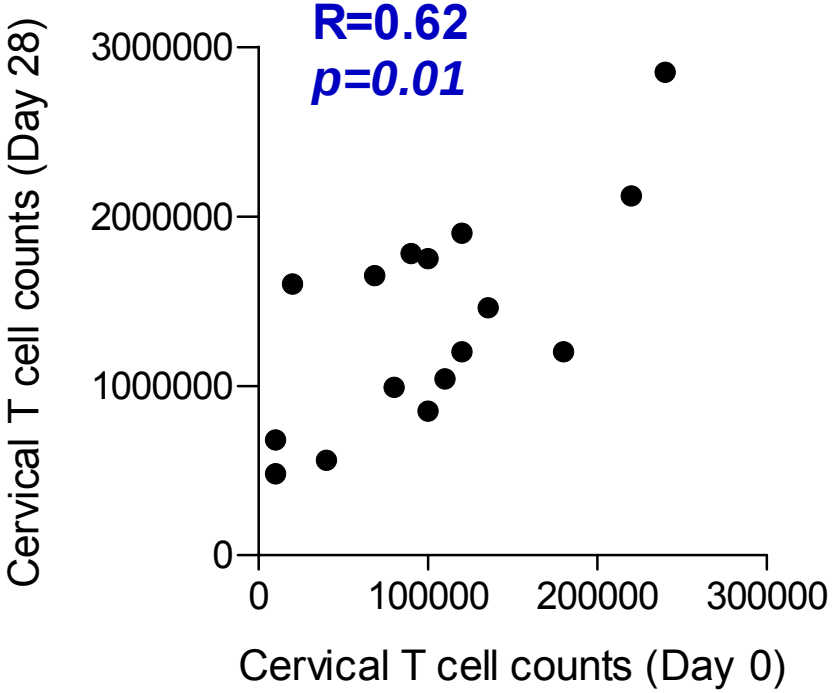
Median CD4:CD8

Day 0 = 0.48

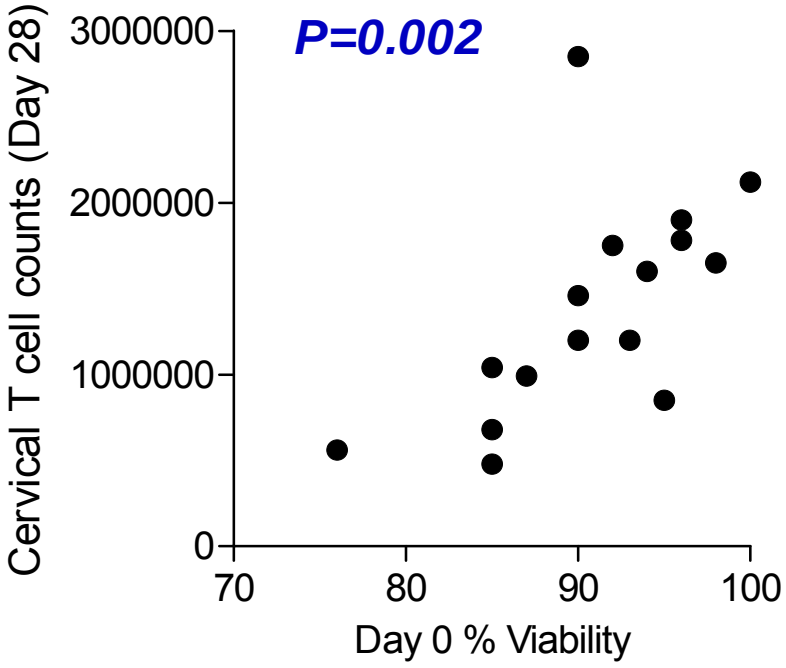
Day 28 = 0.83

Impact of *ex vivo yield and viability on expansion of cervical* cytobrush-derived CD3+ T cells

Input cell yield

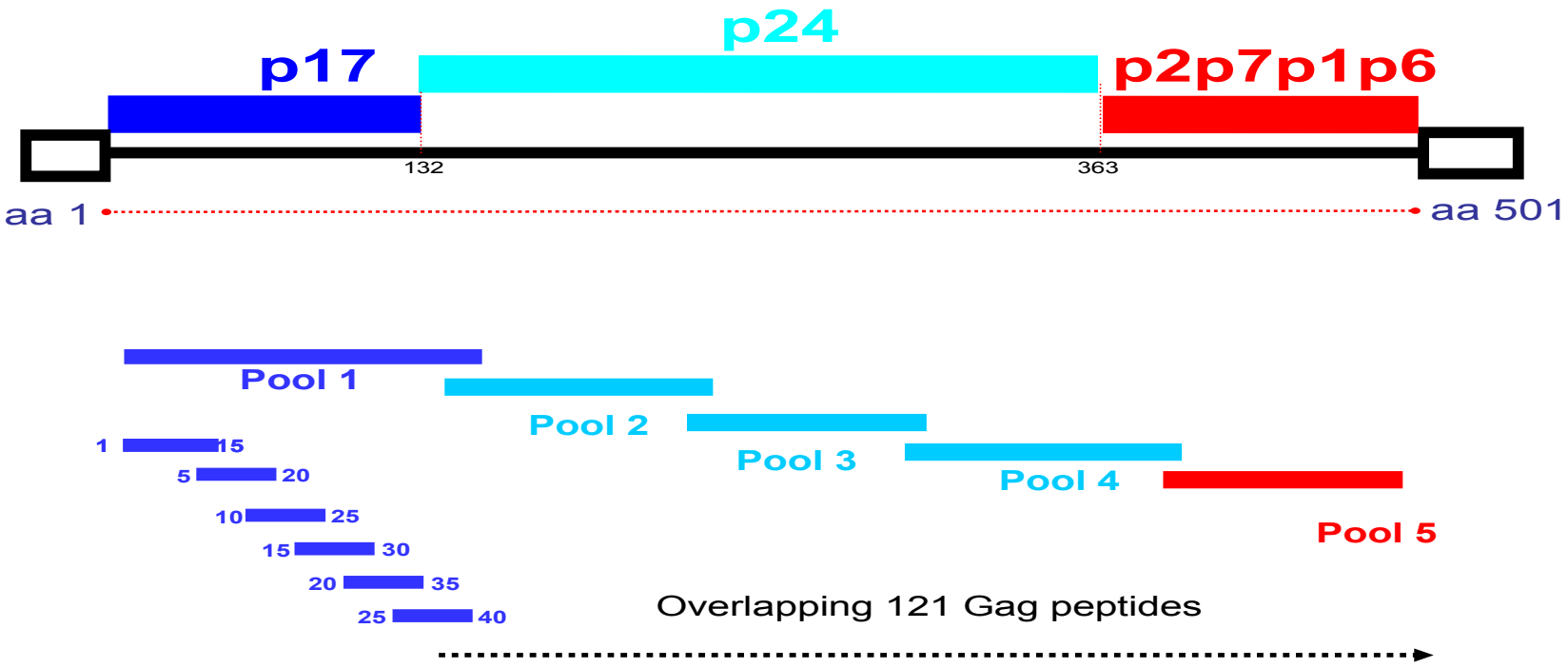


Viability



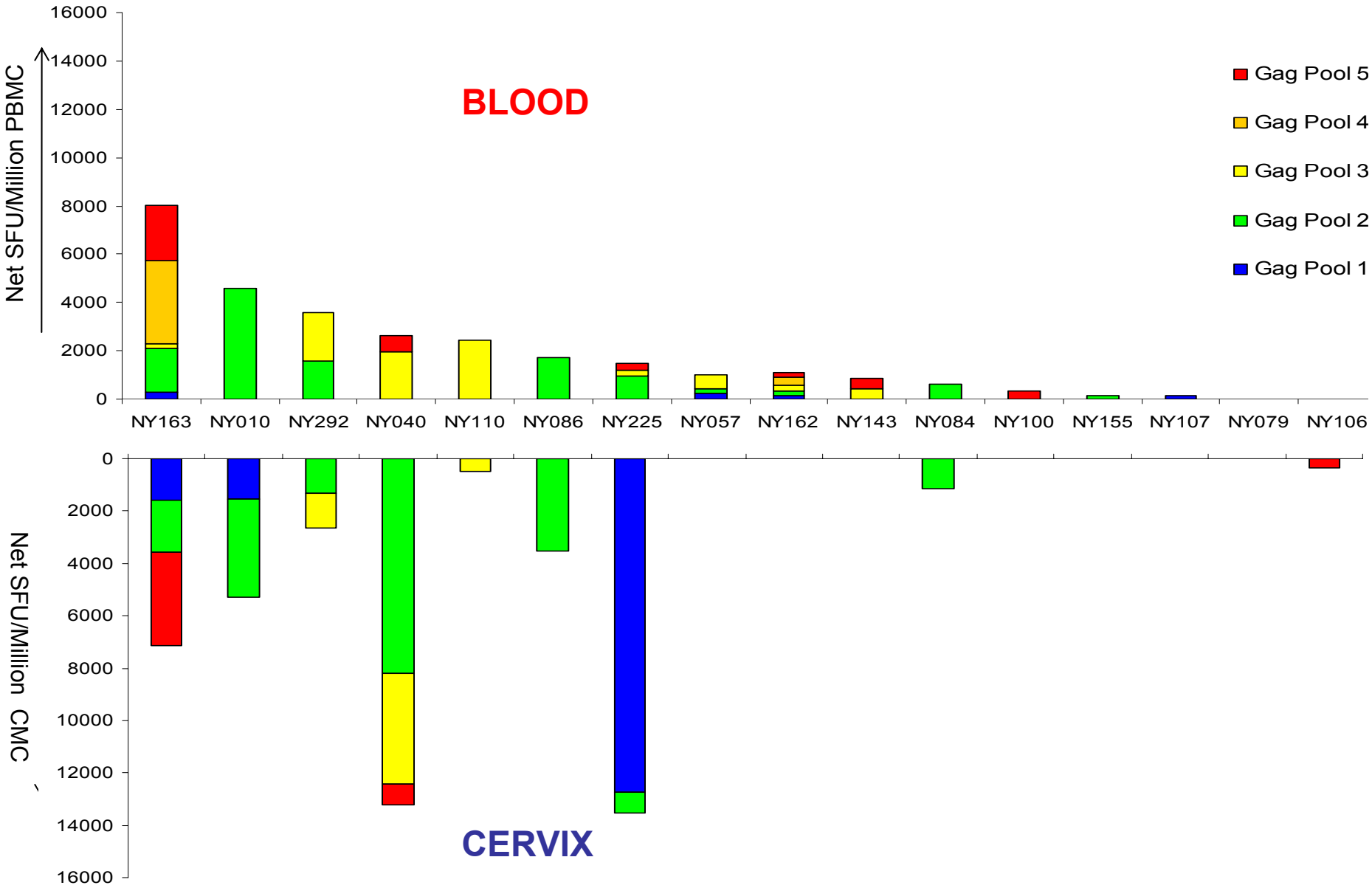
Ex vivo cervical samples will (1) yields of **≤100,000** cells and/or (2) viability of **≤85%** are unlikely to expand in culture

Comparison of cervical and blood HIV Gag-specific IFN-γ responses in women with chronic HIV-1-infection.



HIV-1 subtype C Du422 Gag overlapping peptides spanning the entire Gag sequence

Comparison of cervical and blood HIV Gag-specific IFN-γ responses in 16 women with chronic HIV-1-infection.



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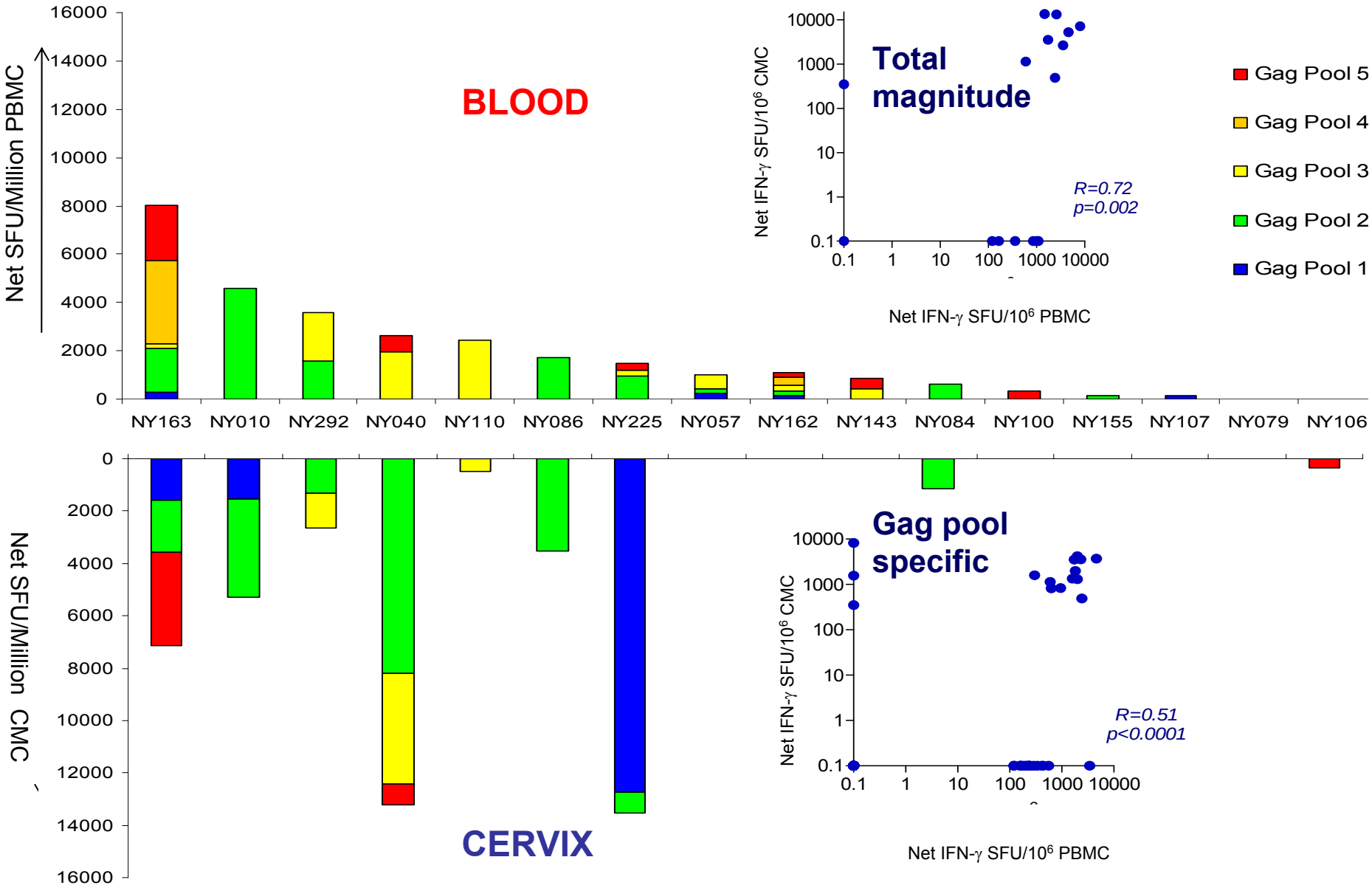

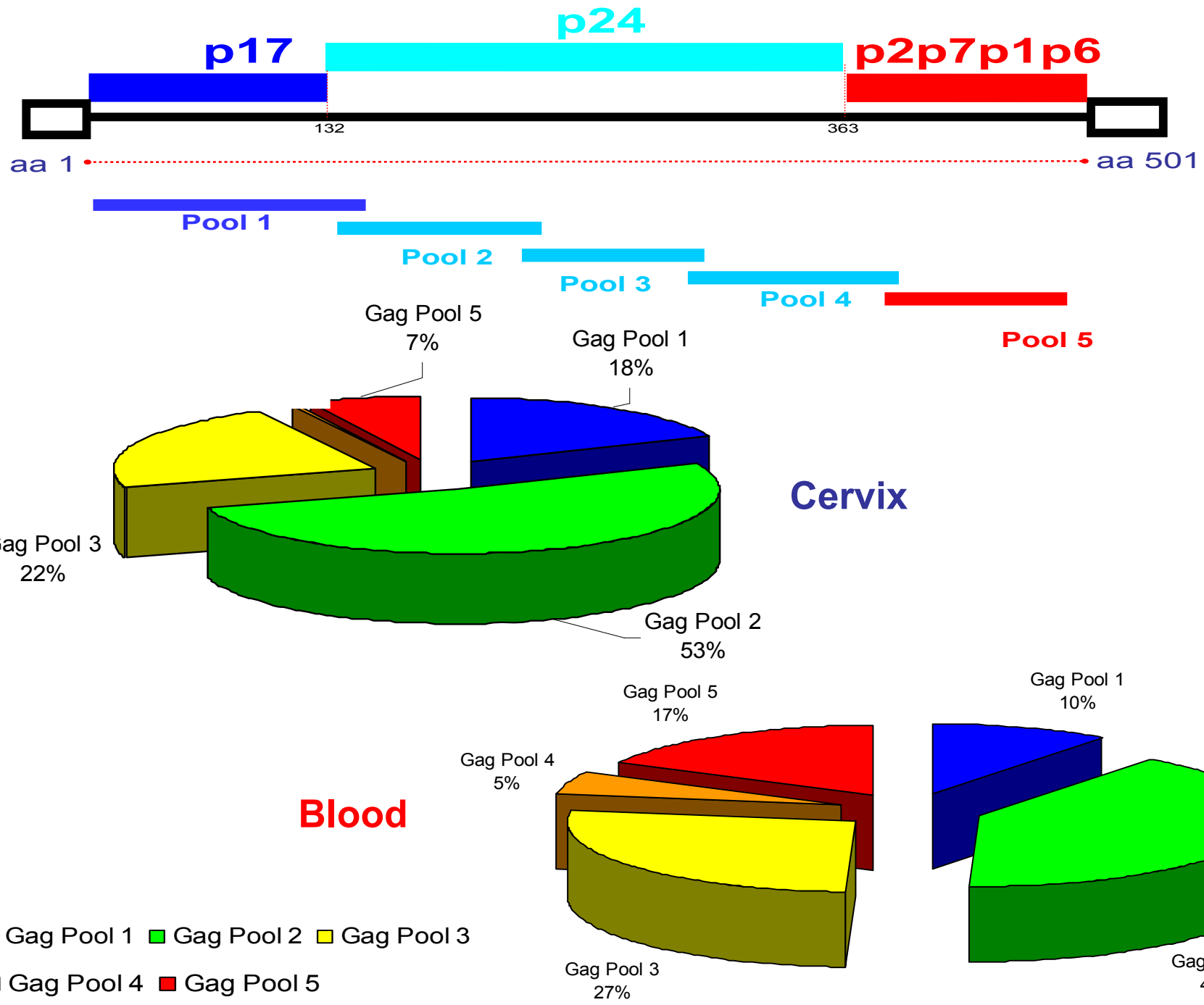
p24
p17
p2p7p1p6
132
363
aa 1
aa 501
Pool 1
Pool 2
Pool 3
Pool 4
Pool 5
Gag Pool 5
7%
Gag Pool 1
18%
Gag Pool 3
22%
Gag Pool 2
53%
Cervix
Gag Pool 5
17%
Gag Pool 1
10%
Gag Pool 4
5%
Blood
Gag Pool 3
27%
Gag Pool 2
41%
Gag Pool 1
Gag Pool 2
Gag Pool 3
Gag Pool 4
Gag Pool 5

Conclusions

- We found that *ex vivo* cervical cell numbers and viability are important determinants in the efficiency of cervical T cell expansion
- The magnitude and specificity of cervical T cell responses to HIV Gag correlate significantly with those detected in blood
- Women were more likely to have detectable cervical responses to Gag if they had matching but relatively high magnitude blood responses

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