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Productive HIV-1 infection of human cervical tissue ex vivo is associated with the secretory phase of the menstrual cycle

Saba E. et al.

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Introduction

HIV-1 deposited in mucosa of lower female reproductive tract (FRT)

Infects primary HIV-1 target cells and disseminates to the regional lymph nodes

Challenge: Lack of understanding of basic mechanisms of HIV-1 transmission + dissemination in FRT

Protection against sexually transmitted infections (STIs) versus enabling successful reproduction.

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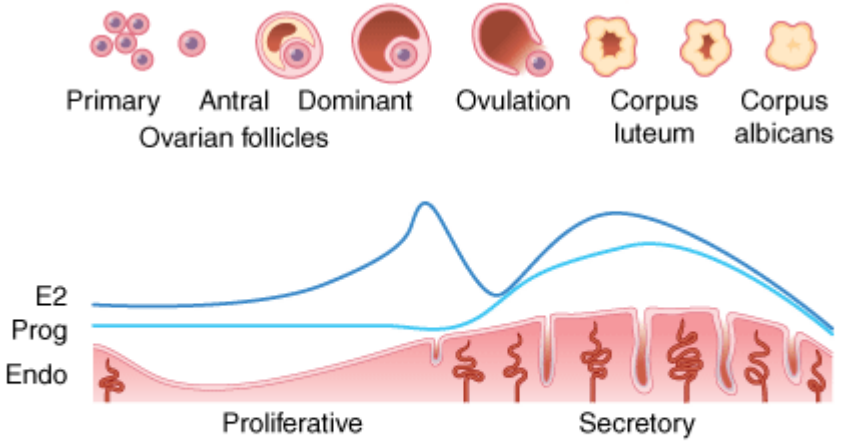
Window of Opportunity

Estradiol + progesterone modify functions of epithelial cells, fibroblasts and immune cells in the FRT

Innate and adaptive immune systems under hormonal control
-> immune protection in FRT varies with phase of menstrual cycle.

Immune protection dampened during secretory phase of cycle to optimize conditions for fertilization and pregnancy
-> "Window of Opportunity" for pathogens



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Mucosal Barrier

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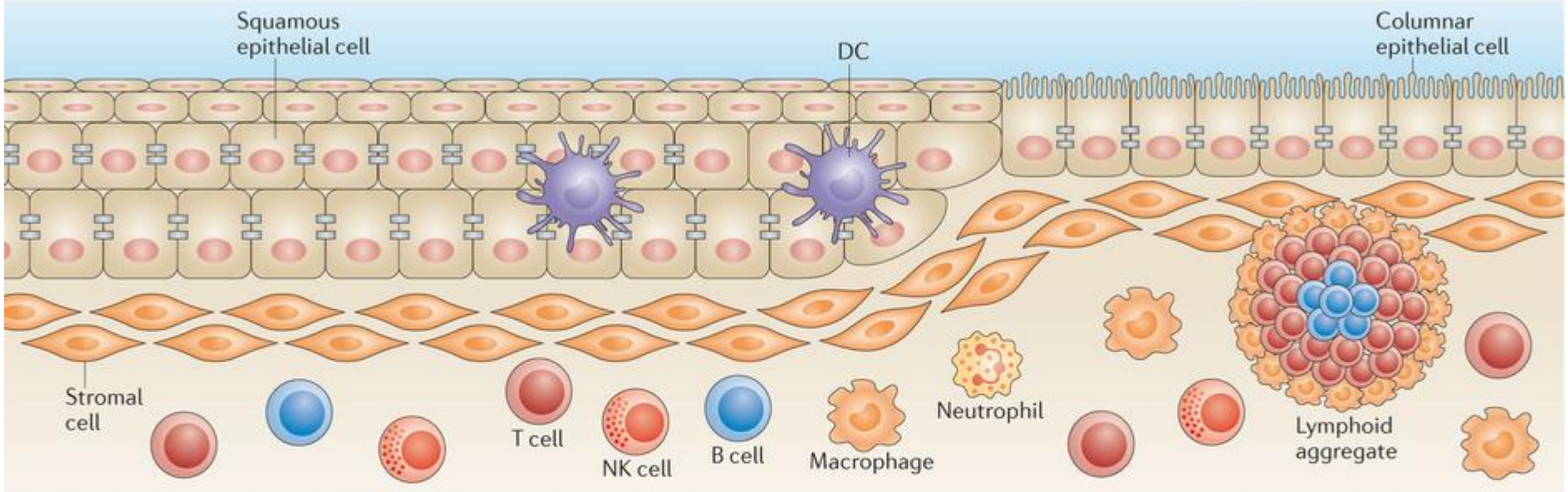
	Immune function	Effects of oestradiol and progesterone	
		Lower FRT	Upper FRT
Lumen	Pathogen killing and inactivation	↓ HBD2, elafin, IgA and IgG	↑ CCL20, HBD2, SLPI, elafin, IgA and IgG (pIgR and FcRn)
Epithelial cells	Immune cell recruitment, innate immune protection and tolerogenic nature	↓ CCL2, IL-8, HBD2 and elafin	↑ CX_3CL1, CCL2, IL-8, HBD2, CCL20, SLPI, elafin and TGFβ
Fibroblasts	Immune cell recruitment	↔ CXCL12	↑ CXCL12
	Regulation of epithelial cells	↔ HGF	↑ HGF
Immune cells	Pathogen killing	↔ $CD8^+$ CTL activity	↓ $CD8^+$ CTL activity
	Protection against viruses	↔ NK cell activity (IFNγ)	↓ NK cell activity (IFNγ)
	Protection against bacteria	$CD14^+$ macrophages	$CD163^+$ macrophages
	Infection by HIV	↓ $CD4^+$ T cell susceptibility	↓ $CD4^+$ T cell susceptibility



The role of sex hormones in immune protection of the female reproductive tract; Charles R. Wira, Marta Rodriguez-Garcia & Mickey V. Patel
Nature Reviews Immunology 15, 217–230 (2015) doi:10.1038/nri3819

Cervico-vaginal tissue explants (CTE)

Retain the *in vivo* cyto-architecture and some tissue functions for several days

Previous CTE experiment: activated tissue-associated CD4+ T cells are major targets ->
likely source of CCR5-dependent (R5), but not CXCR4-dependent (X4), HIV-1

Contribution of resident macrophages needs more research

CTEs obtained from HIV-seronegative women undergoing hysterectomy for benign gynecological conditions.

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R5 HIV-1 replication

CTEs dissected into ~2-mm^3 blocks, cultured on collagen sponge gel rafts for up to 12 days

CTEs inoculated with R5 HIV-1_{BaL}

Virus replication evaluated by $p24_{Gag}$ release into culture supernatant and by accumulation of HIV DNA in tissue-associated cells.

Control donor-matched tissue blocks incubated with R5 HIV-1 in presence of the RT inhibitor 3TC

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R5 HIV-1 replication

Virus replication evident on day 9 post-HIV-1 inoculation

Increased up to day 12 as evaluated by $p24_{Gag}$ release in CTE culture supernatants

No increase of virus release in 3TC-treated HIV-1-exposed cultures

"**Productive**": CTEs with progressive accumulation of $p24_{Gag}$ into culture supernatants (n=8)

"**Nonproductive**": CTEs showing no difference in $p24_{Gag}$ release in the presence or absence of 3TC (n=14)

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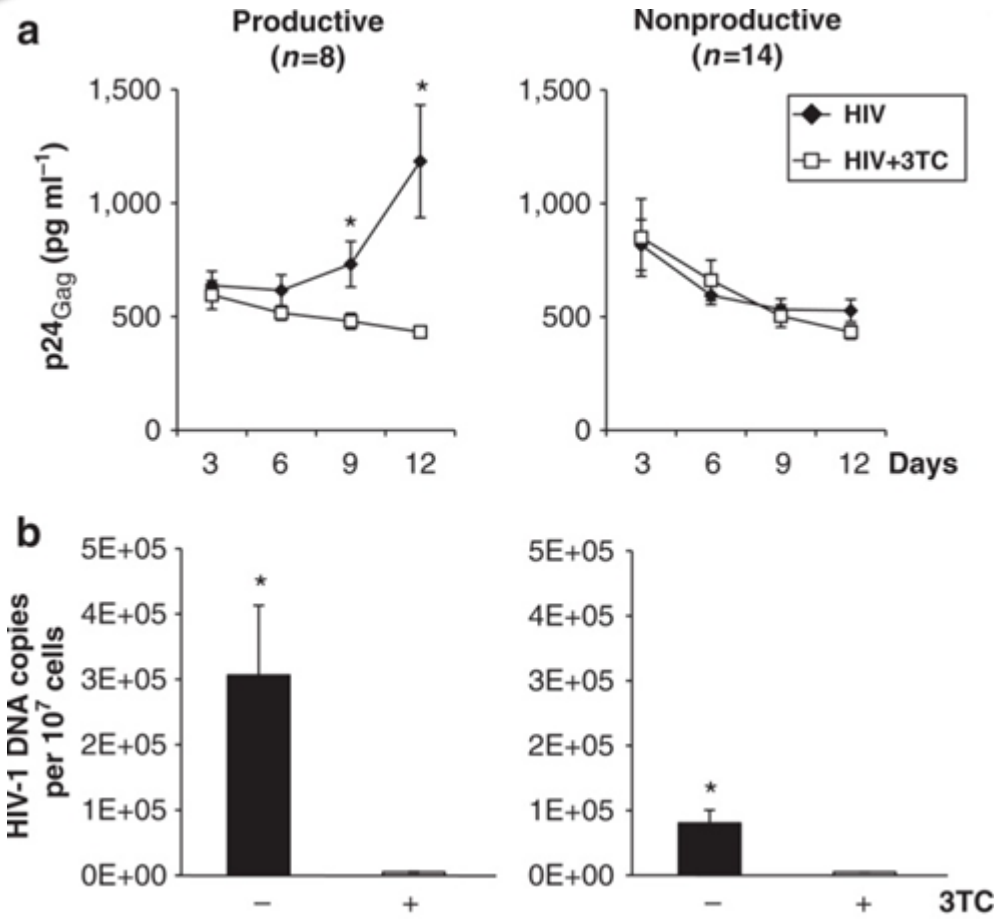
R5 HIV-1 replication



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susceptibility to HIV-1 infection of CD4+ T cells and macrophages

CTEs digested with collagenase IV

Cell suspensions stained for different surface antigens

Flow cytometric analysis -> % of CD4+ T cells and resident macrophages not significantly different between productive and nonproductive tissues

Most CD3+CD4+ T cells were CCR5+ and showed an “effector memory” phenotype, as defined by lack of expression of CCR7 and CD45RA

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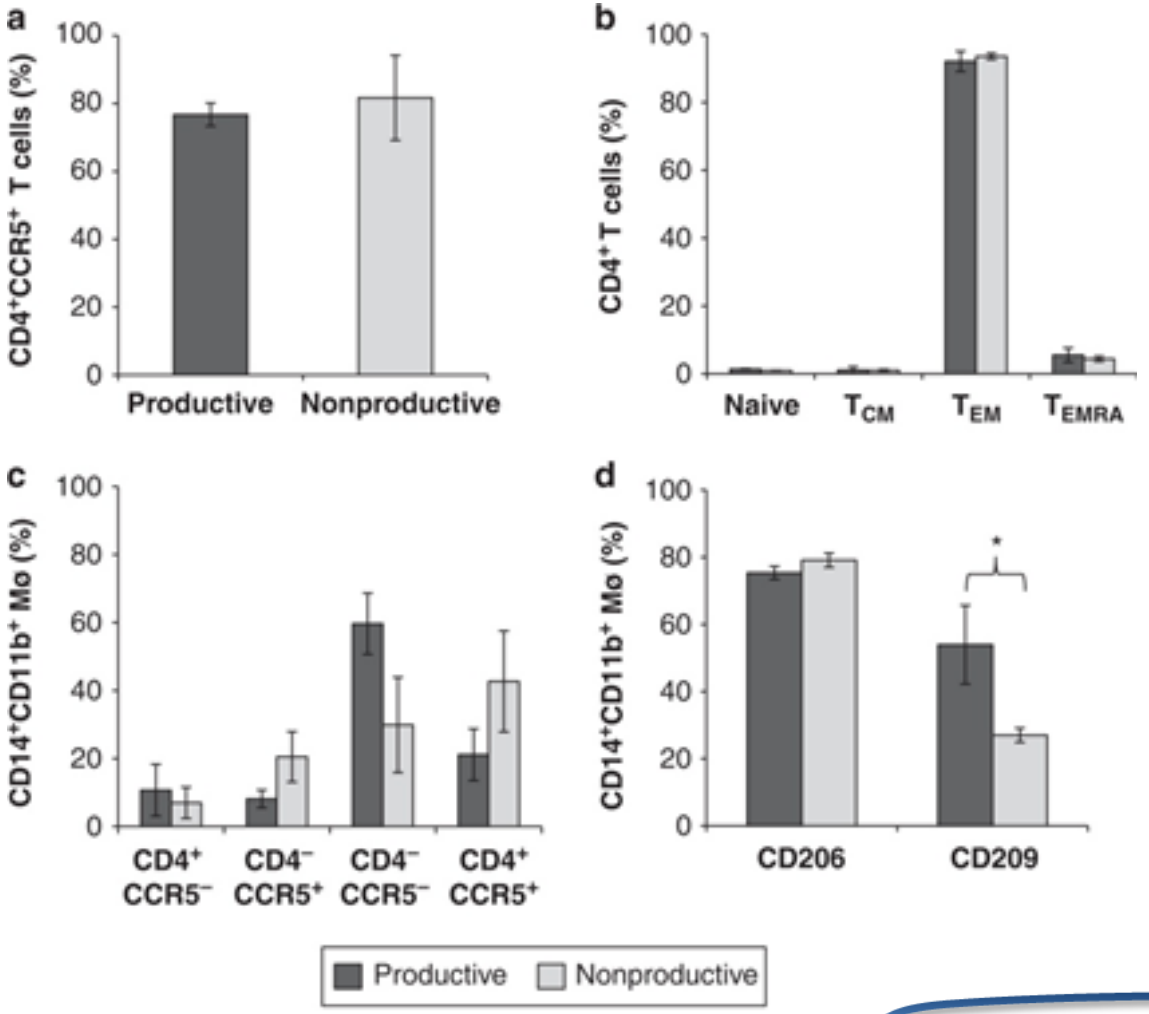
susceptibility to HIV-1 infection of CD4+ T cells and macrophages



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Infection of HIV-1 target cells

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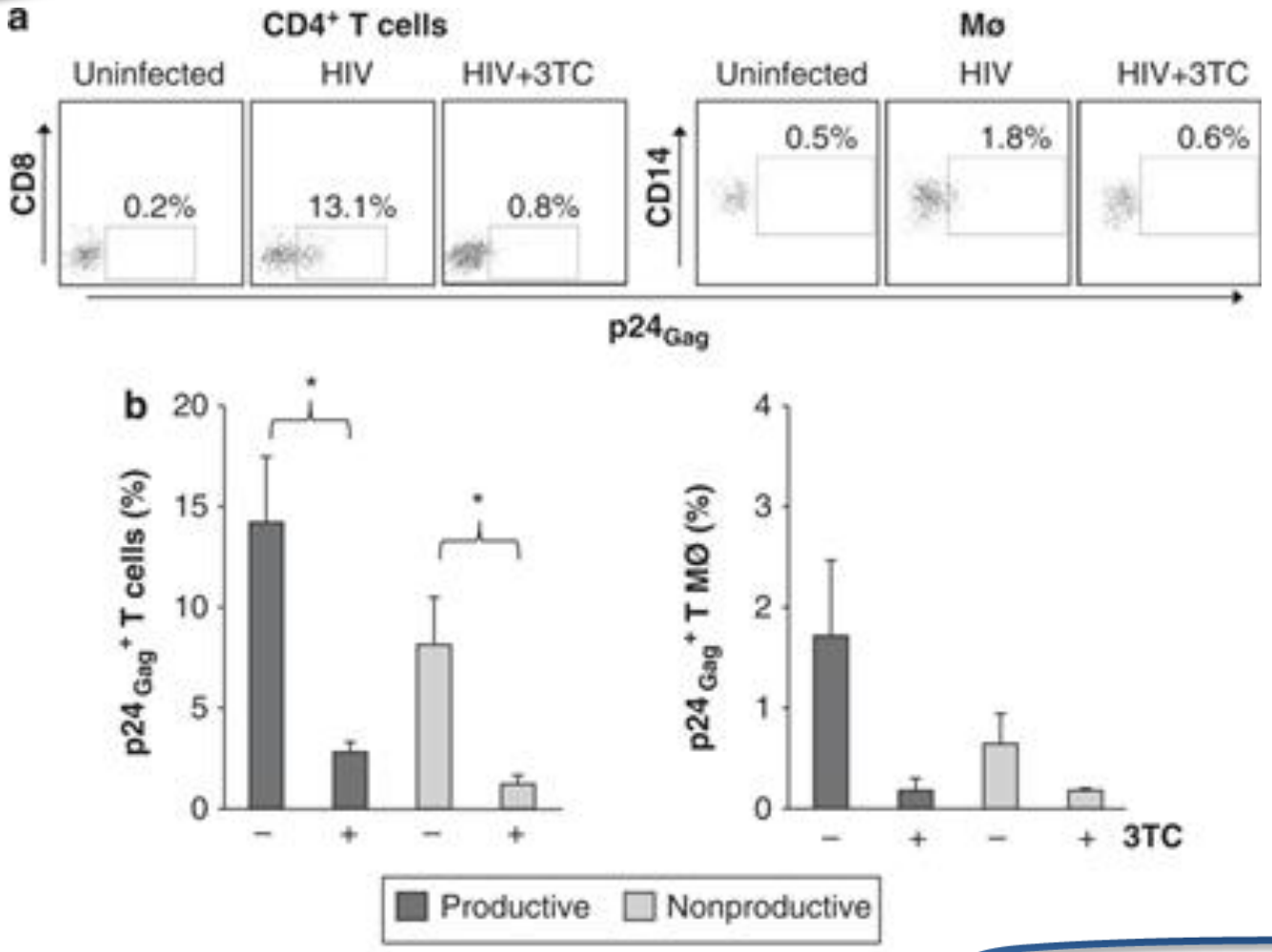

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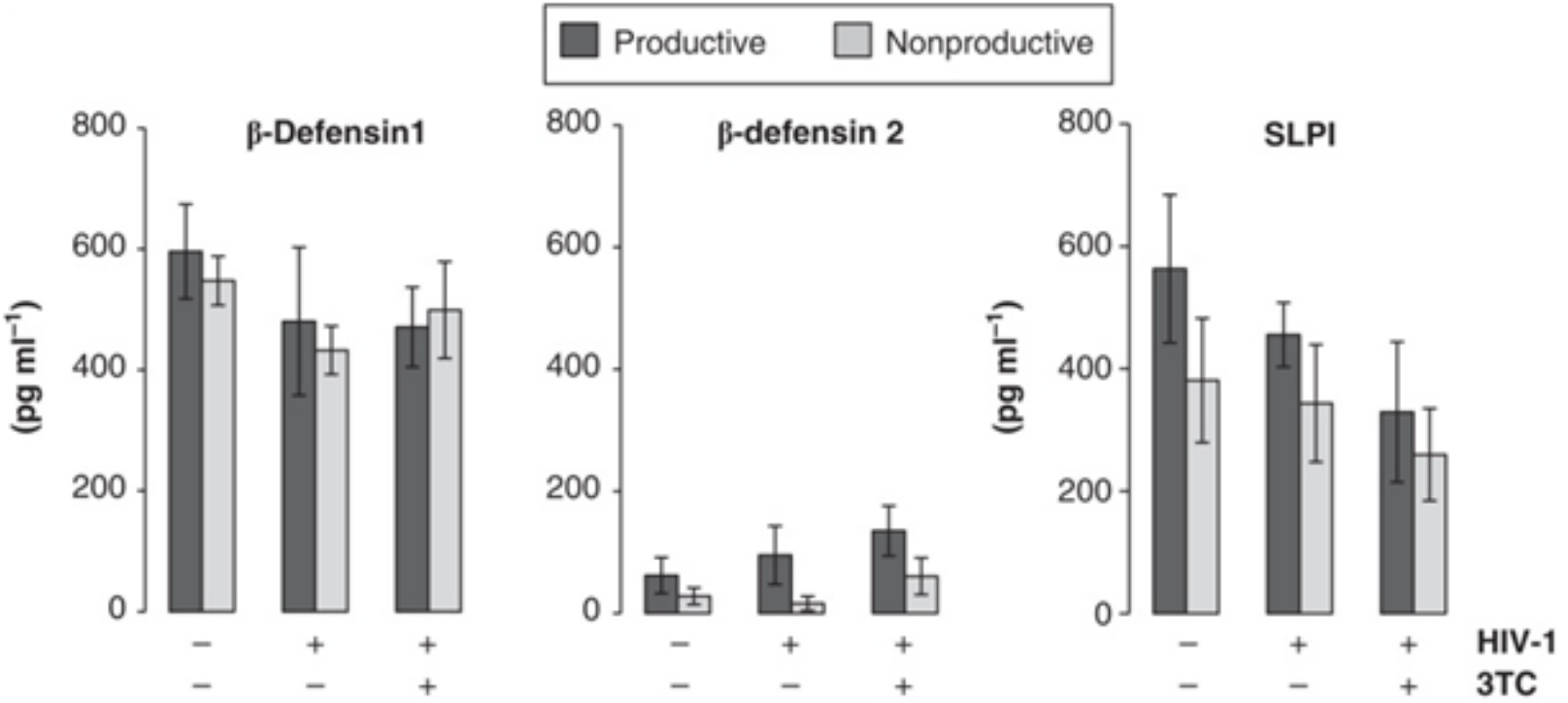
Anti-viral peptides



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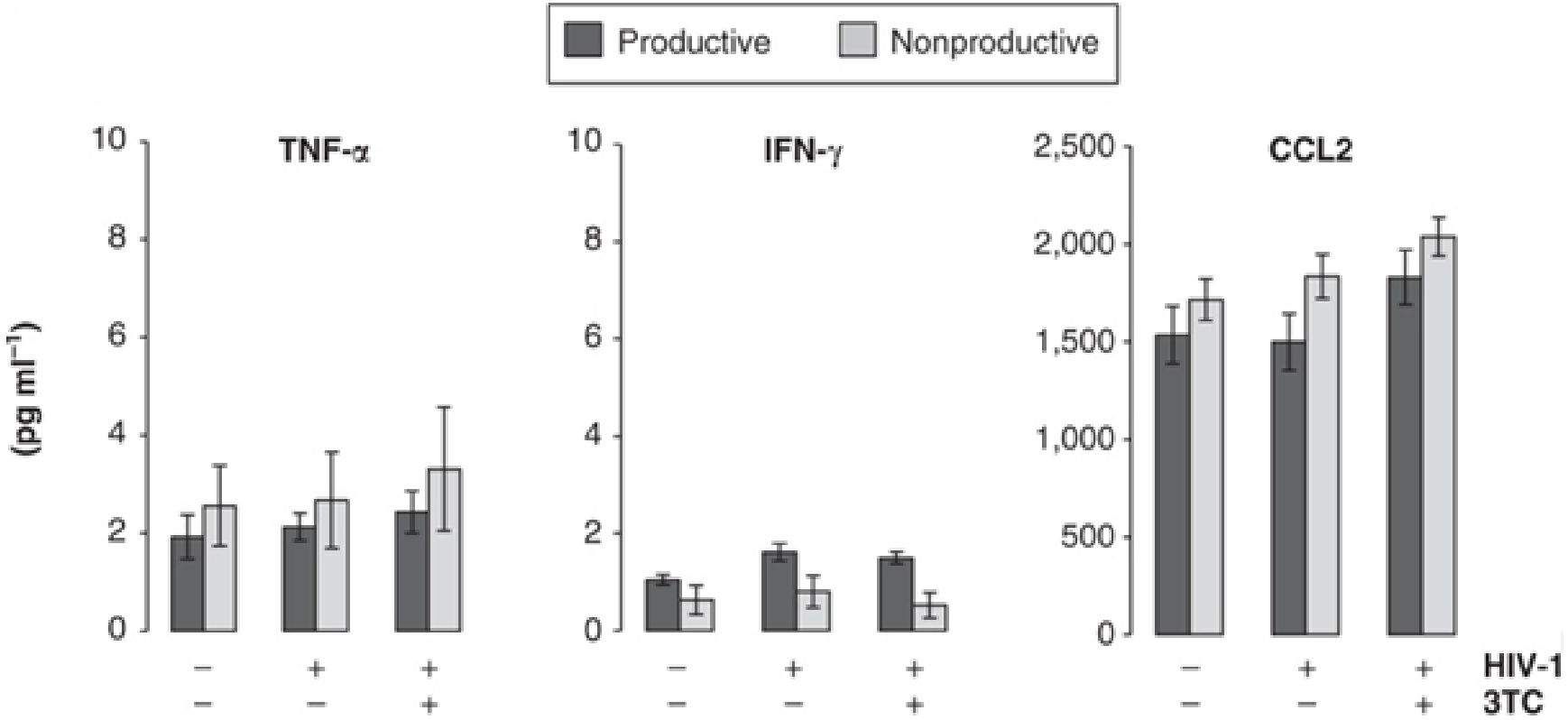
Pro-inflammatory cytokines



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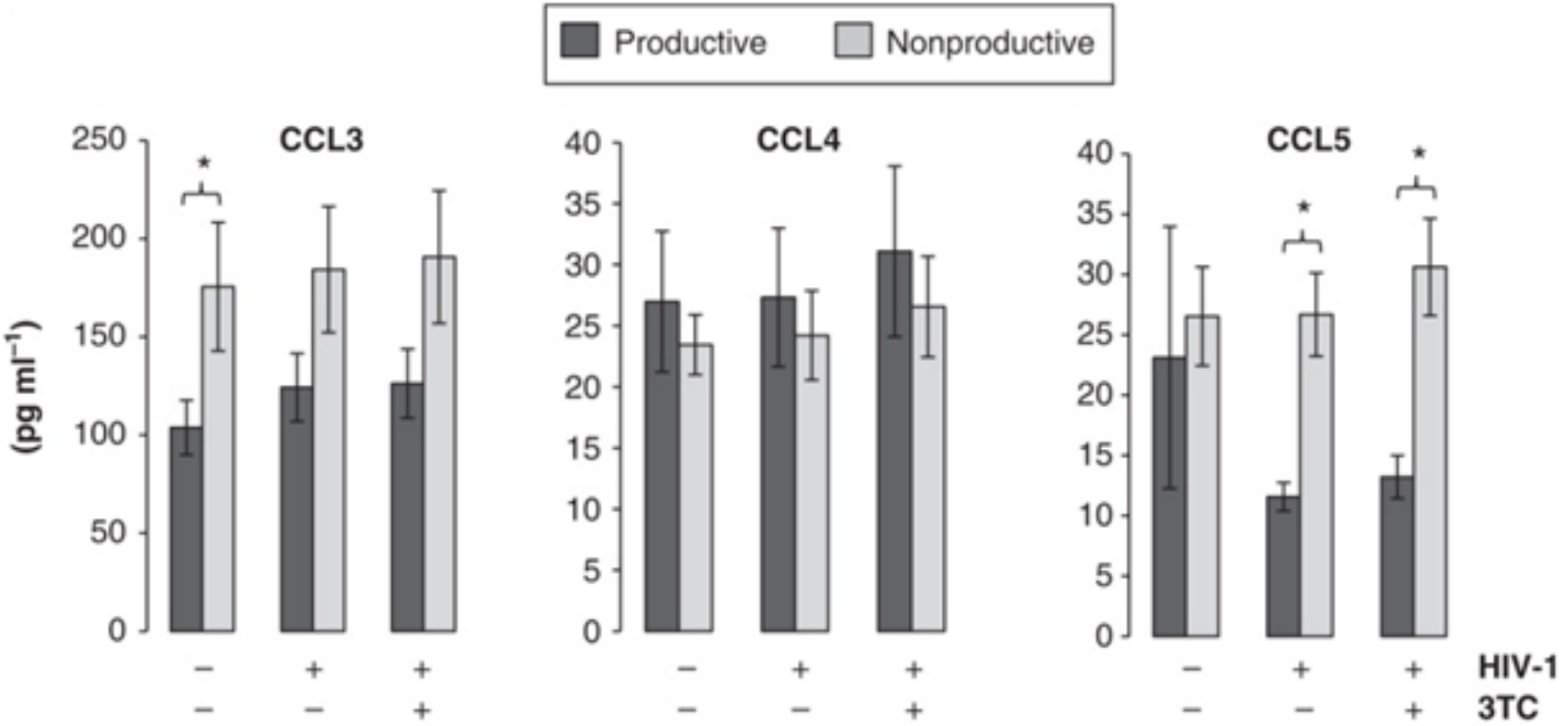
CCR5 ligands



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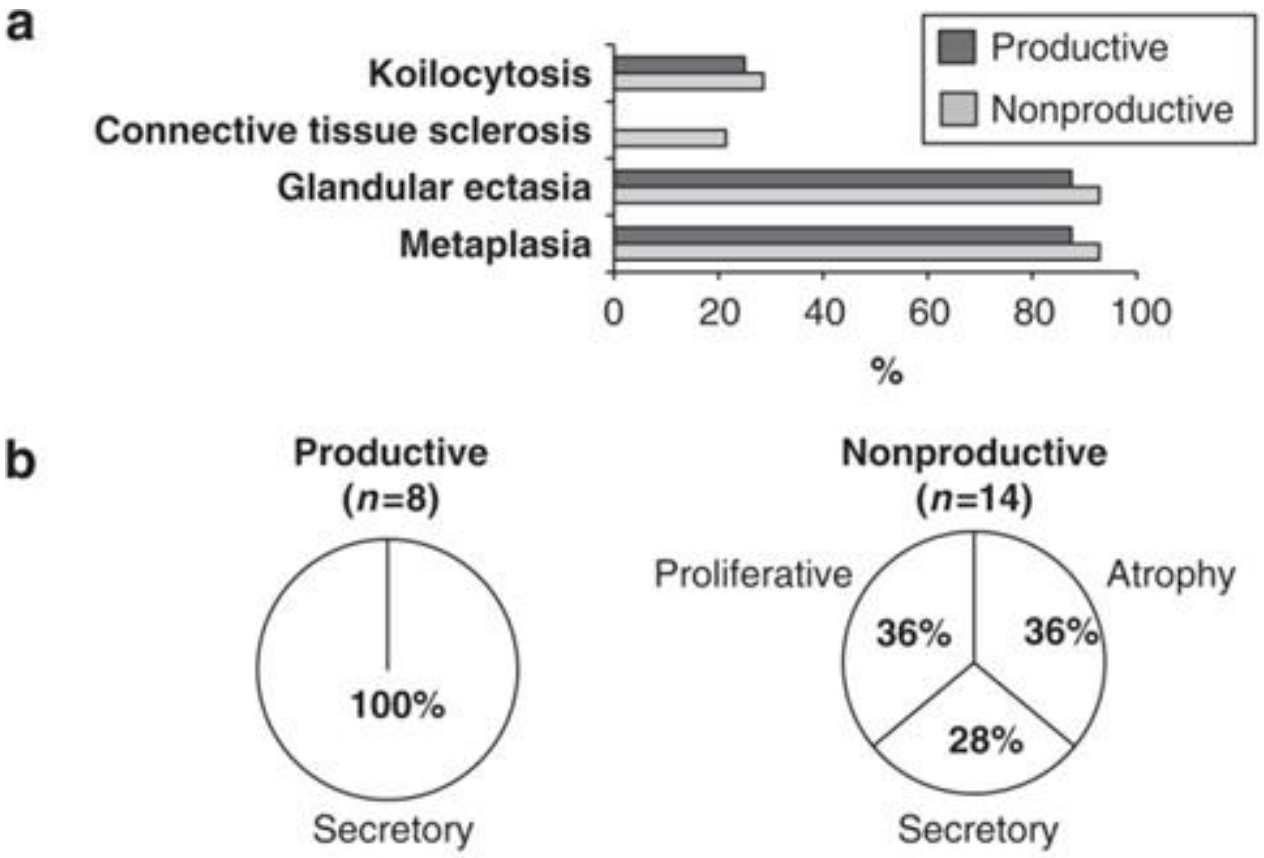
Productive and nonproductive CTE tissue architecture



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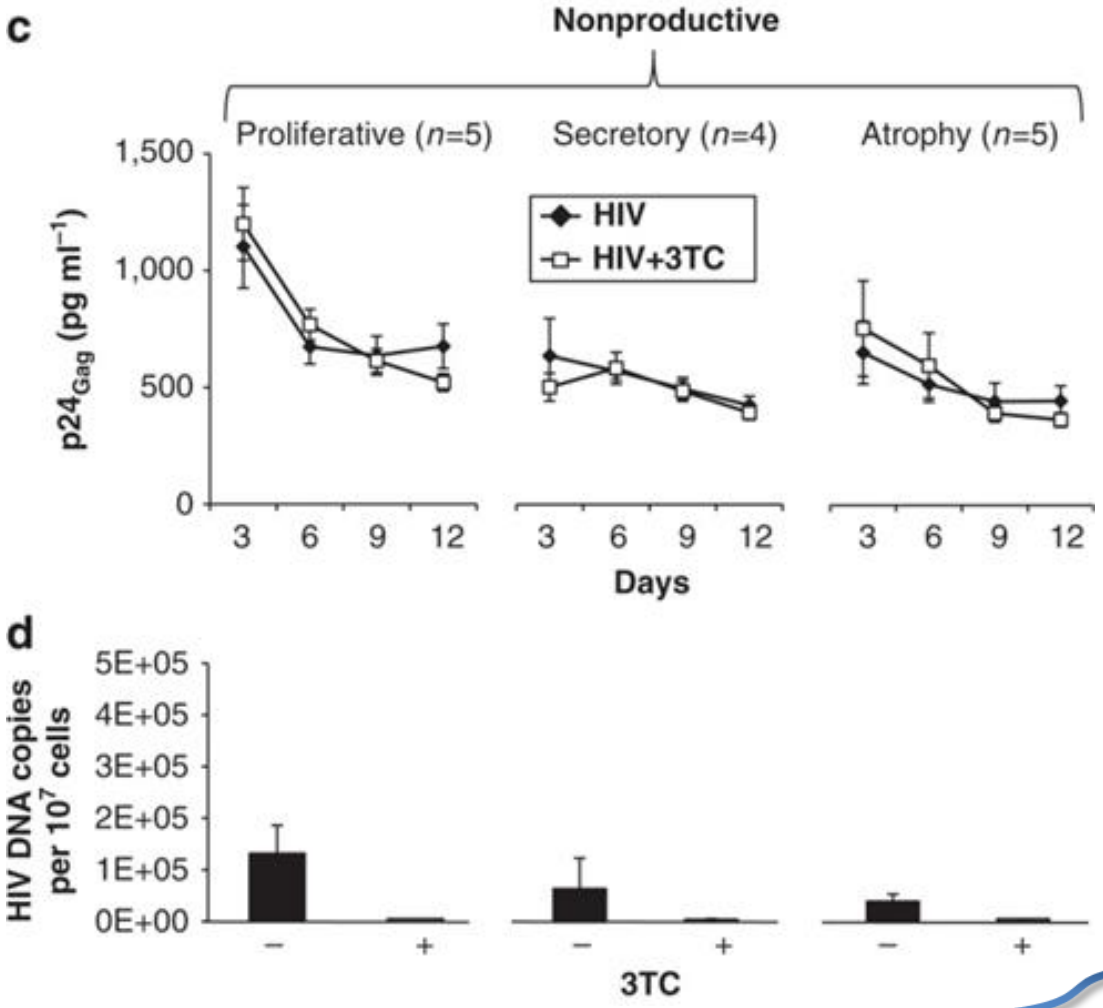
Productive and nonproductive CTE tissue architecture



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Summary

Investigated susceptibility to productive R5 HIV-1 infection of CTEs *ex vivo* from 22 HIV-1 seronegative women

8 out of 22 CTEs (36%) showed virus replication
14 out of 22 CTEs (64%) in presence or absence of 3TC did not

-> “productive” and “nonproductive” infections.

Both CD4+ T cells and resident macrophages supported HIV-1 replication, although macrophages to lesser extent than T lymphocytes

Outcome infection (productive vs. nonproductive) did not correlate with number of HIV-1 target cells

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Summary

Functional distributions of CD4+ T cells similar in productive and nonproductive CTEs, with CD4+ T cells displaying an “effector memory” phenotype representing the dominant subset

Resident macrophages: % expressing CD206 (mannose receptor) comparable in both productive and nonproductive CTEs
but significantly higher % of cells expressing CD209 (DC-SIGN) on surface in productive CTEs than in nonproductive ones

No significant differences in secreted levels of innate anti-viral peptides (β-defensins, SLPI), pro-inflammatory (TNF-α, IFN-γ, CCL2), or anti-inflammatory (IL-4, IL-10) cytokines between productive and non-productive

Some CCR5-binding chemokines secreted at higher levels by nonproductive vs. productive CTEs

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Summary

Post-hoc analysis of *ex vivo* outcomes of HIV-1 exposure of CTEs (productive vs. nonproductive) did not reveal a correlation with histological alterations of mucosa and submucosa.

Strong correlation between productive HIV-1 infection of CTEs and menstrual cycle phase of the donor at the time of surgery

-> infected CTEs all from women in secretory phase, nonproductive tissues from women in different phases of menstrual cycle

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Ideas and Suggestions

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Menstrual cycle of women and/or their consumption of hormonal-based contraceptives should be taken into consideration when new preventative strategies against HIV transmission are developed

CTEs can represent an adequate experimental system to study the mechanisms and replication of HIV-1 infection

CTEs could be adopted as low-cost platform for testing novel antivirals and microbicides before moving towards more expensive NHP models of HIV-1 transmission and clinical trials in women.

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Thank you for your attention!

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