Comprehensive analysis of viral persistence and immune activation in lymph nodes of HIV-1 infected individuals during HAART

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Some basics about lymph node structure
Viral replication & immunopathology in lymph nodes
Effects of HAART in lymph nodes
Persisting immune activation in aviremic patients
Aberrant immune responses in lymph nodes
Questions

- Where does the virus replicate within lymphoid tissues?
- What is the effect of HAART on viral replication and immunopathology in LN?
- How much immune activation persists?
- What are the consequences?
Lymph Node Pathology in HIV

HIV -

- Mantle zone
- CD4⁺ T cells
- CD8⁺ T cells
- FD cells
- GC

HIV +

- IgD⁺, IgM⁺
- Invagination of MZ
- B-cell sinus reaction
- Granulocytic infiltrates
- Proliferation of blood vessels
- Giant cell
- CD4⁻CD8⁺

Stellbrink & van Lunzen, Curr Opin Infect Dis 2001
Immunopathology in LN


- CD4+Ki-67+
- CD8+
Follicular hyperplasia, FDC-network intact

Follicular involution, FDC-network destroyed

Productive HIV Infection of FDC?

HIV-1 RNA in LN during HAART


van Lunzen J, Ruiz L et al., AIDS 1998
Persistence of HIV-1 structural proteins in the GC of patients under HAART

Popovic, M et al. (2005) Proc. Natl. Acad. Sci. USA 102, 14807-14812
Residual replication in non-proliferating and resting cells

a: HIV RNA$^+$ Ki-67$^-$ cell (non-proliferating)

b: HIV RNA$^+$ p27$^+$ cell (resting)
T cell proliferation in LN during HAART

van Lunzen J, et al., CROI 1999
CTL in Lymph Nodes and Blood

- Pre-Rx
- On Rx
- After TI

Graph showing SFC/Mill cells with bars for each condition:
- Pre-Rx: 29
- On Rx: 33
- After TI: 38

Results:
- p < 0.05
- p = 0.001

Altfeld M, van Lunzen J et al., J Clin Invest 2003
CTL frequency decreases during HAART in PB but not in LN.

Altfeld M, van Lunzen J et al., J Clin Invest 2003
Summary I

- HIV is a highly replicating virus even during clinical latency
- HIV mainly resides in lymphoid tissues and causes severe pathology in particular in GC`s
- HIV replication leads to hyperactivation of the immune system
- As a consequence T cell turnover is massively increased
- Regenerative capacity of the immune system gets exhausted
- Disbalance of consumption and renewal of T cells
- HAART partially restores pathology but fails to improve HIV specific immune responses
- Some degree of immune activation persists, antigen present for prolonged time on FDC
Tregs in HIV infection

Loss of CD4+ T cells

WHY?

- direct HIV-mediated killing
- chronic immune activation with high turnover of T cells
- beneficial?
Relative frequency of $T_{\text{regs}}$ increases with disease progression

Schulze zur Wiesch et al., J Virol, 2011
Longitudinal analysis of Tregs before and after HAART

Viral load (copies/ml)  CD4 count (copies/ml)  Frequency of Tregs

Schulze zur Wiesch et al., J Virol, 2011
Increased proliferation of HIV-specific helper cells after depletion of T\(_{\text{regs}}\)
Higher relative $T_{\text{reg}}$ frequencies in Lymph Nodes compared to PBMC
Expression of CD39 and CD73 on peripheral and LN T cells
Down-regulation of the 5’-Ectonucleotidase CD73 of CD8+ T cells correlates with immune activation and T cell exhaustion.
CD4 $T_{FH}$ cells are critical for effective antibody responses

- Within the follicle
- Express CXCR5, PD1, ICOS
- Bcl6$^+$
- IL-21 (cardinal cytokine)

- B cell proliferation
- Antibody maturation (SHM)
- Class switching (CSR)
- Memory development
Infection rate of CD57+ and CD57- CD4+ T cells in PB and LN

Ratio of the viral loads: CD57+CD4+ T cells [%]/CD57− CD4+ T cells [%]

LN:
10.5  3.3  8.0  9.5  3.0

PBL:
0.3  2.2 <0.1  2.5  2.5

High frequency of $T_{FH}$ (CXCR5+PD-1$^{\text{high}}$) cell subset in the lymph node

Lindqvist M. et al. accepted J Clin Invest 2012
IL-21 preferentially produced by $T_{FH}$ cells

Lindqvist M. et al. accepted J Clin Invest 2012
Expansion of $T_{FH}$ cells correlates with skewing of B cell subsets

Lindqvist M. et al. accepted J Clin Invest 2012
Hypersecretion of IgG is associated with BCL6 expression in $T_{FH}$ cells

Lindqvist M. et al. accepted J Clin Invest 2012
• Accumulation of functionally impaired $T_{\text{regs}}$ occurs in LN and may impair appropriate CTL responses

• GC derived T helper cells are the major cellular source of viral infection, infection rates LN$>>$PB

• HIV-specific $T_{\text{FH}}$ cells are expanded in chronic infection

• Expansion of $T_{\text{FH}}$ cells correlates with skewing of B cell subsets in chronic HIV infection

• Hypersecretion of IgG1 is associated with BCL6 expression in $T_{\text{FH}}$ cells
Hypothesis Model

- Lymph node B cell follicle
- Germinal center
- TFH
- Plasma cell
- Memory B cell
- Germinal center B cell
- HIV viremia
- IL-21

B cell follicle
Lymph node
Loss of neutralizing Ab

Neutralizing V3-loop specific Ab

C1-01, C2-07, C2-13

Cutoff

1986, 1992

Cutoff

1988, 1993

Conclusions I

• HIV pathology is driven by viral replication and hyperactivation of the immune system in lymphoid tissue (GC`s primarily involved)

• Viral replication is rapidly controlled by HAART in LN but some replication is persisting in resting and non-proliferating CD4 T cells in GC.

• The persistence of HIV structural proteins within GC may lead to chronic persisting immune activation even in the absence of viral replication
Conclusions II

- Abnormalities in $T_{reg}$ frequencies and function is primarily found in LN.

- Malfunction of $T_{regs}$ is associated with altered expression of ectonucleotidases (CD39, CD73).

- HIV-specific $T_{FH}$ cells are expanded in chronic infection and are associated with skewing of B cell subsets.

- GC of LN are major sources for viral persistence and immune activation during successful HAART.
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Expression of CD73 on T cells is higher in the lymph nodes than in the peripheral blood.