HIV-2 IMPACT ON LYMPHOID ORGANS: EVIDENCE OF VIRAL REPLICATION AND TISSUE DISRUPTION

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BACKGROUND

The ability of HIV to replicate in secondary lymphoid organs (SLO) and disrupt lymph node (LN) architecture are considered main determinants of disease progression (1).

HIV-2+ individuals feature:

1) slow rate of CD4 T cell decline and AIDS progression with limited impact in patient survival (2),
2) maintain low to undetectable viremia in the presence of disseminated viral reservoirs (3),
3) progressive increase in immune activation markers in direct association with loss of circulating CD4 T cells (4,5),
4) progressive memory B cell depletion (6),
5) high titers of broadly neutralizing antibodies throughout the disease course (7,8).

Thus, this naturally-occurring attenuated infection provides a unique setting to investigate HIV-host interplay in SLO that has been poorly explored (9-11).

CONCLUSION

Our data support **continuous viral replication in SLO and an altered germinal center organization in HIV-2 infection** despite the relatively benign course of the disease and the low to undetectable viremia in the absence of ART.

Their contribution to the benign HIV-2 course deserves further investigation that may provide a better understanding of the generation of broadly neutralizing Abs and reveal new strategies to control HIV reservoirs.

RESULTS

Ex-vivo lymph node analysis

We performed a retrospective study of archived lymph node (LN) samples collected in several Portuguese hospitals for clinical reasons, mainly lymphadenopathy diagnosis.

PFA embedded LNs from 4 HIV-2-infected patients were included, irrespective of CD4 counts (744, 444, 255, 73 cells/µl without ART), age (55, 37, 58, and 46y, respectively), follow up time, and clinical stage excluding those with opportunistic infections or lymphoproliferative disease.

Samples from 12 HIV-1 infected individuals (age 36-66y; CD4 counts 810-50 cells/µl; 4 under ART), and from 8 seronegatives, with similar age and gender distribution, were included.

Immunohistochemistry staining was performed using Abs against CD3 (F7.2.38, Dako), CD4 (SP35, Cell Marque), CD8 (1A5, Leica), CD21 (1F8,Dako), KI67 (B56, BD), P24 (KAL-1,Dako) and P27 (ARP396/397, NIH), in addition to **haematoxylin-eosin (HE) and Gomori**., as previously described (12). Representative images are shown, with black scale bar representing 250 µm. Stained area was quantified in 100 bright-field images, automatically acquired using the Axiovert200M motorized inverted microscope and a Metamorph scan stage tool with a vertical zigzag grid of 10mmx10mm using previously optimized procedures (12). Non-germinal center area was identified by a trained pathologist, and measured using Fiji software.

The study was approved by the ethical boards of the involved institutions.

In-vitro infection of tonsil organ cultures (TOCs)

In parallel, we used a validated in-vitro system to investigate the impact of HIV infection in SLO, based on tonsillar tissue removed during routine pediatric tonsillectomy, and collected upon informed consent by the legal representatives.

Non-infected	*Primary isolates*			
Medium	HIV-2_{RS}	HIV-2_{X4}	HIV-1_{RS}	HIV-1_{X4}
	92US660	*92HT599*	*6041SK*	*20.04*



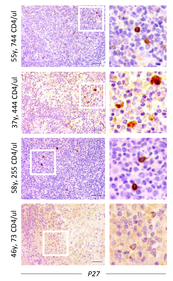
Tonsil Organ Cultures (TOCs, 7 days) → *Cell suspension* Phenotype, Total viral DNA, Viral mRNA

Tonsil organ cultures: *Fresh tonsil samples were cut in small pieces and placed on Millicel cell culture inserts (Millipore) in a 6-well plate containing 1250µL of tonsil organ culture (TOC) medium (complete medium with 15%FBS, 1mM sodium pyruvate, and 1% minimal essential medium nonessential amino acids [all from Gibco]).*
Viral infection and procedures*: A drop of 5µL containing 3ng RT of virus (table above) was applied on the top of each piece*[11]*. A third of the medium was replaced every 2 to 3 days. On day 7, TOCs were placed in 4% formaldehyde or mashed.*
Flow cytometry analysis of cell suspensions*:* Surface and intracellular staining was performed as previously described (13), using the following markers: CD3, CD4, CD8α, CD19, PD-1, CXCR5, FoxP3; CD25, CD45RA, CXCR4, CCR5, Ki67, and KC57 (Beckmann Coulter) to quantify HIV-1 and HIV-2 Gag protein. Cells were acquired using LSRFortessa cell analyzer (BD Biosciences) and analyzed with Flowlo Software (TreeStar).T-Distributes Stochastic Neighbor Embedding (tSNE) algorithm was used for unbiased representation of multi-parameter flow cytometry data. Death cell were always excluded using fixable viability dye (eBioscience).
Gag mRNA and proviral DNA were quantified by real-time PCR as previously described (13).
Statistical analysis*:* Comparison between infection conditions was performed using Repeated Measures ANOVA test , and Bonferroni post test. P values below 0.05 were considered significant and are shown.

Using this combined approach we assessed:

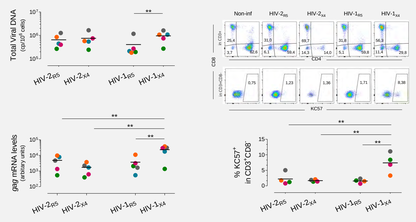
1) HIV-2 replication in SLO
2) HIV-2 ability to deplete CD4 T cells
3) Impact of HIV-2 on GC

LN from HIV-2+ individuals



HIV-2 gag was found in all LN, irrespective of the disease stage, as assessed by immunochemistry staining to P27.

Infected TOCs

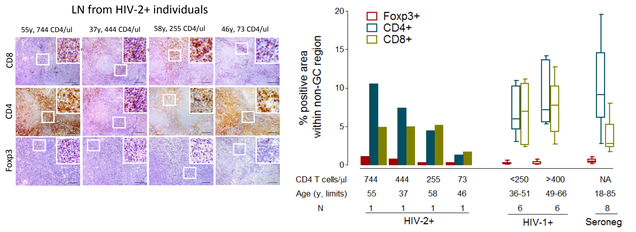


Comparable levels of HIV-2 and HIV-1 replication in terms of proviral DNA and gag mRNA levels, although the amount of cells producing virus (KC57+) was significantly lower in HIV-2_{X4} than in HIV-1_{X4} isolates.

Evidence of:

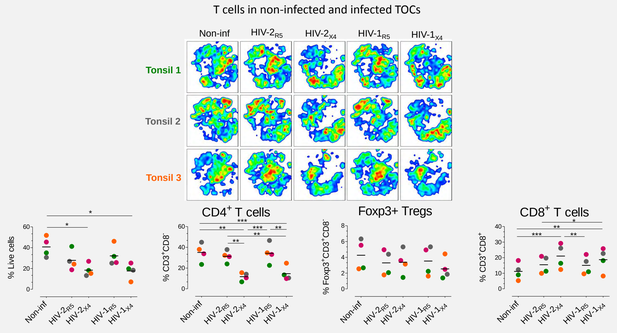
1) HIV-2 replication in LN from HIV-2+ subjects
2) Productive in-vitro HIV-2 infection of tonsils

LN from HIV-2+ individuals



LN from HIV-2+ patients featured a progressive CD4 T cell depletion in non-germinal center (GC) areas, in agreement with blood CD4 T cell decline.

T cells in non-infected and infected TOCs

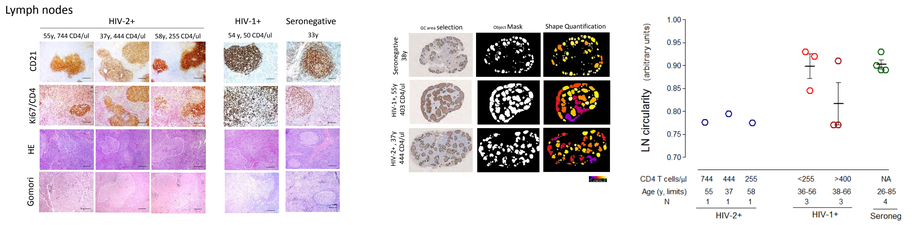


The pattern of impact of the HIV strains on T cells was remarkably reproducible between TOCs, with preferential depletion of CD4 T cells by virus using CXCR4.

HIV-2 infection was associated with:

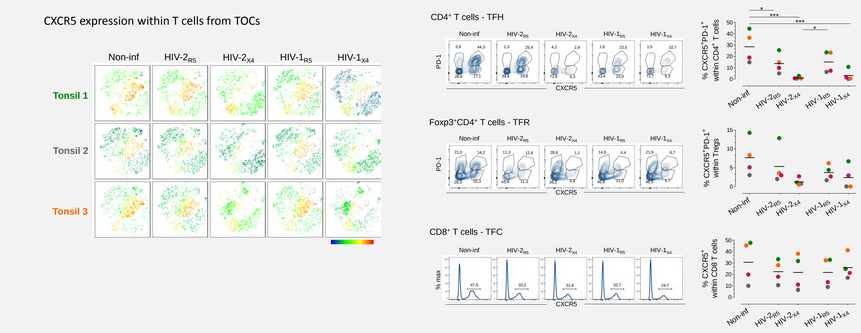
1) Progressive CD4 depletion in the T cell area of the LN from HIV-2+ subjects in agreement with the degree of peripheral blood CD4 depletion
2) Major co-receptor dependent depletion of CD4 T cells upon in-vitro infection

Lymph nodes



Regarding LN architecture, we found an apparent effacement of germinal centers (GC) with significant disruption of the reticulin network (Gomori staining) with reduced circularity (assessed using Fiji Shape Descriptor Maps plugin), even in early stage HIV-2 infection.

CXCR5 expression within T cells from TOCs



In infected TOCs there was a preferential depletion of follicular CD4 T cells (TFH), particularly with HIV-2_{X4}. Interestingly follicular regulatory CD4 T cells (TFR) were less depleted and CXCR5+CD8+ T cells were maintained.

Overall, HIV-2 infection leads to:

1) an effacement of the GC and loss of circularity in the LN irrespective of circulating CD4 T cell counts;
2) preferential target of follicular helper T cells in infected TOCs, particularly in CXCR4-using viruses, at levels comparable to HIV-1 primary isolates.

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