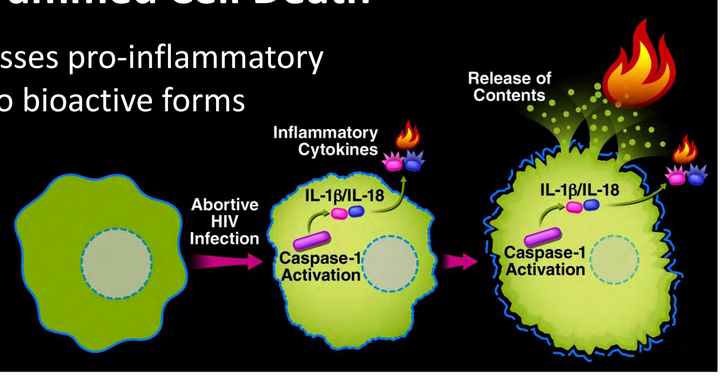
In Vivo Evidence for a Role of Pyroptosis in HIV Pathogenesis

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Pyroptosis: An Intensely Inflammatory Form of Programmed Cell Death

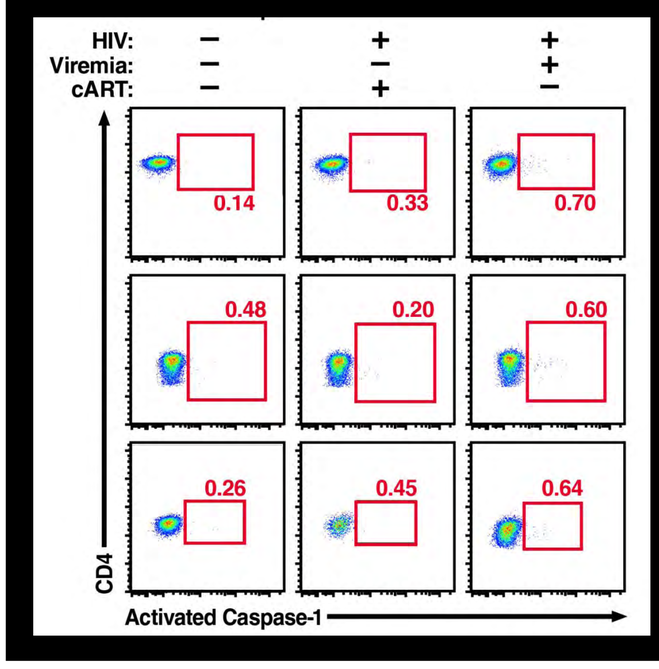
- Activated caspase-1 processes pro-inflammatory cytokines IL-1β and IL-18 to bioactive forms
- Intracellular content of dying cells is released through pores causing local inflammation



Previous Findings: Cell Death by Pyroptosis Drives Depletion of CD4 T Cells in Human Lymphoid Cultures

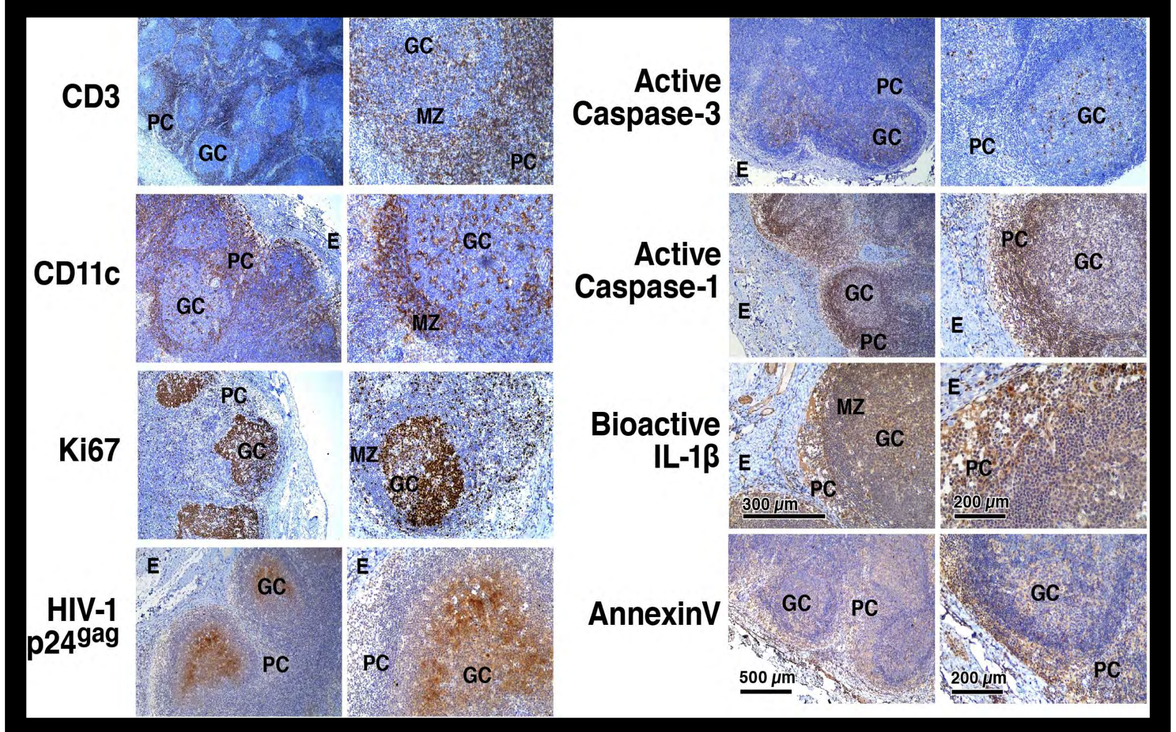
- Quiescent "bystander" lymphoid CD4 T cells, which are not permissive to productive infection by laboratory-adapted HIV-1 isolates, die in huge numbers as a result of **abortive viral infection** where incomplete viral DNA transcripts accumulate in the cytoplasm.
- DNA transcripts are sensed by **IFI-16**, which leads to **caspase-1 activation** in inflammasomes, triggering the processing and release of **bioactive IL-1β** and **induction of pyroptosis.**
- Pyroptosis provides a new nexus between CD4 T-cell death and inflammation—the two key drivers of HIV pathogenesis.
- HIV-1-mediated IL-1β secretion and CD4 T cell depletion by **pyroptosis can be completely blocked by caspase-1 specific inhibitors.**
- Interestingly, **blood CD4 T cells**, infected with laboratory-adapted HIV-1 isolates, **resist pyroptosis** partially due to a deeper resting state with **lower IFI16 level.**

Pyroptosis in blood cells from HIV-infected patients



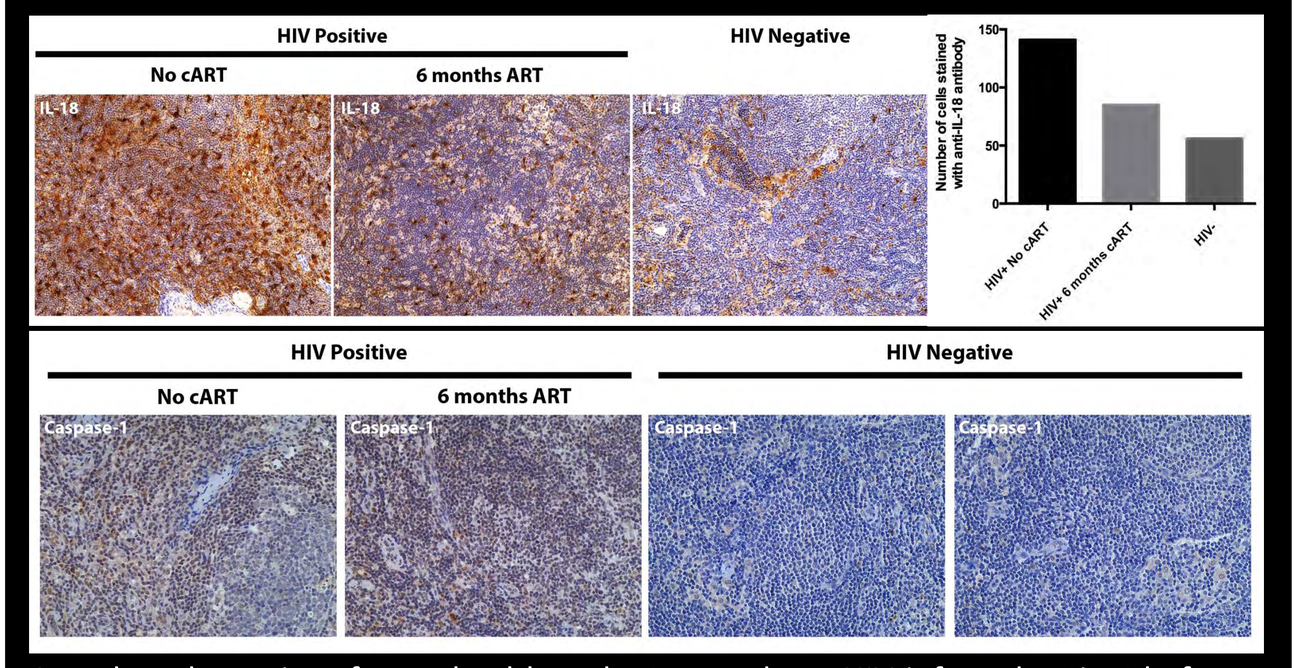
HIV-infected patient blood-derived CD4 T cells have undetectable levels of caspase-1 activation. CD4 T cells were purified from the blood of healthy volunteers or HIV-infected patients on or off cART and stained for activated caspase-1 with the CaspaLux system and analyzed by flow cytometry. We analyzed 11 healthy volunteers, 7 HIV patients on cART and 8 HIV patients off cART.

Pyroptosis is observed *in vivo* in lymph nodes from HIV-infected patients

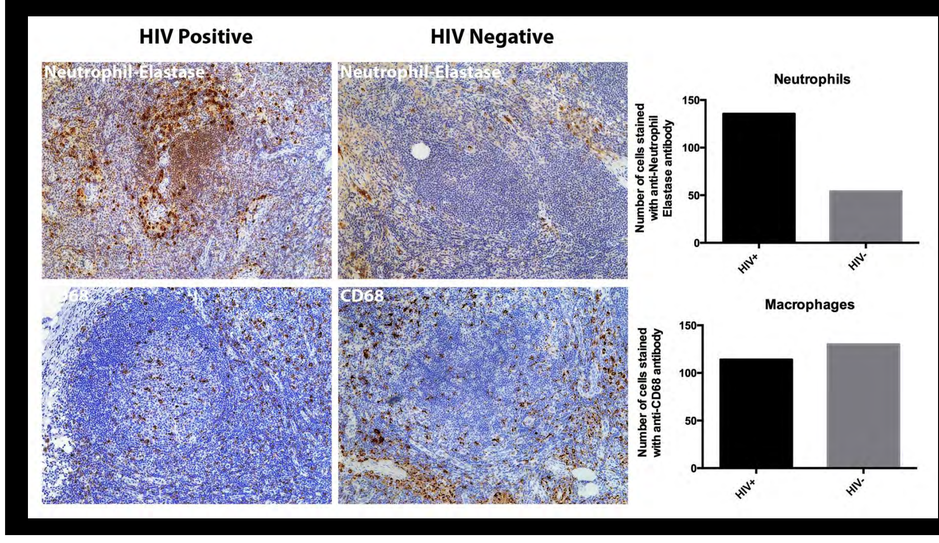


Immunohistochemistry analysis reveals regions of caspase-1 and IL1-β activity in lymph node of a patient chronically infected with R5-tropic HIV. Inguinal lymph node was collected from a 50-year-old immunosuppressed HIV-1 infected subject during the chronic phase of disease. The patient was first identified with HIV in 1985, has not been on antiretroviral therapy and displayed CD4 count of 156 cells/□l and viral load of 85,756 copies/ml at the time of lymph node resection. Staining of uninfected tonsil or spleen tissues revealed no such positive signals (not shown). GC, germinal center; MZ, mantle zone; PC, paracortical zone; E, epithelium.

Pyroptosis is observed *in vivo* in lymph nodes from HIV-infected patients

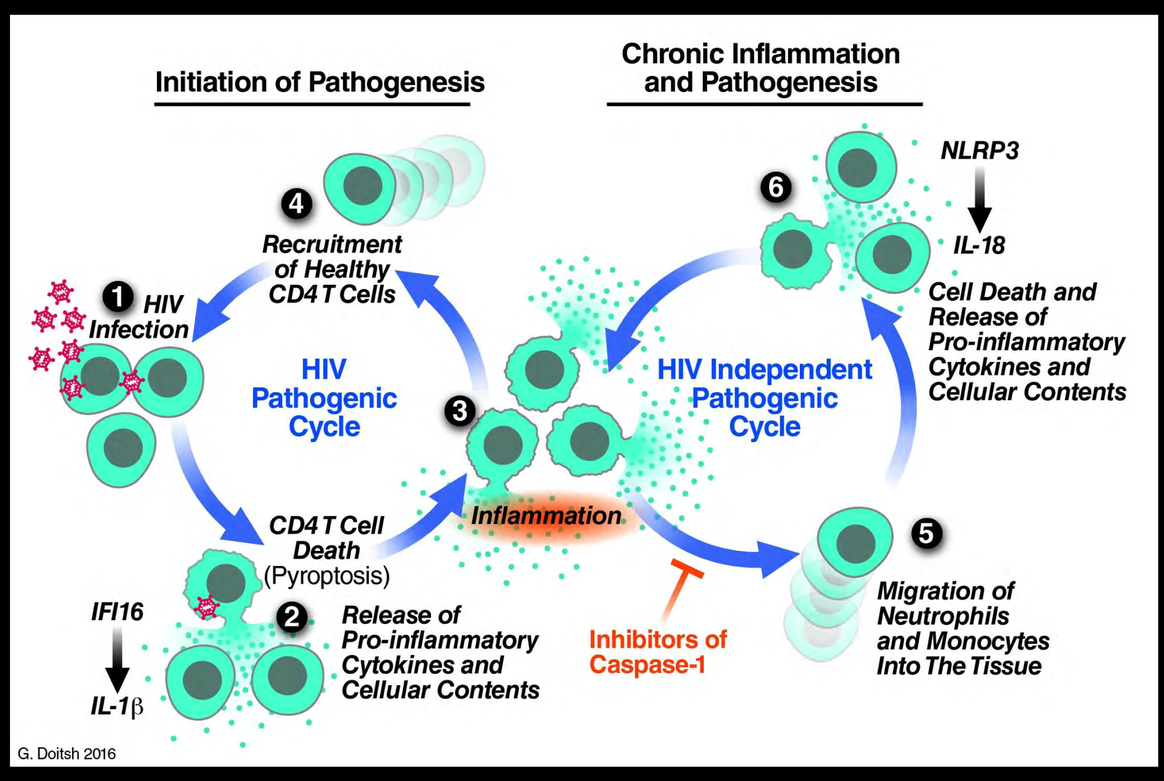


Lymph node sections from a healthy volunteer and one HIV-infected patient before cART and 6 months after cART initiation stained for bioactive IL-18 and caspase-1. Right upper panel shows the quantification of lymphoid cells expressing IL-18.



Lymph node sections from a healthy volunteer and one HIV-infected patient off cART were stained for neutrophil-elastase and CD68 (macrophages). Graphs show quantification of lymphoid cells expressing neutrophil-elastase and CD68.

Potential Role of Pyroptosis in HIV Pathogenesis



Step 1 and 2: Abortive HIV infection of CD4 T cells in lymphoid tissues results in sensing of the viral cytosolic DNA products by IFI16 leading to caspase-1 activation in inflammasomes and pyroptosis. Dying cells release large amounts of pro-inflammatory cytokines and cellular contents into the extracellular milieu.

Step 3 and 4: These events promote local inflammation, which attracts new circulating CD4 T cells into the lymph node and establish a vicious cycle of HIV spread, CD4 T-cell death and inflammation.

Step 5: Inflammatory signals induce transendothelial migration of neutrophils and monocytes; the latter cells differentiate into tissue-resident macrophages or DCs.

Step 6: These tissue-resident cells are primed to mount inflammatory responses, and constitutively express high levels of cytoplasmic pro-IL-18, and NLRP3 inflammasome. The release of proinflammatory cellular contents and ATP by nearby pyroptotic cells may activate the NLRP3 inflammasome in nearby primed cells, leading to new rounds of pyroptosis. Such an "**auto-inflammation**" scenario could generate persistent rounds of pyroptosis, chronic inflammation, and loss of CD4 T cells even when viral replication is suppressed by antiretroviral therapy.

Interestingly, a potential new therapy could curb this auto-inflammation process: **VX -765 - A Highly Selective, Orally Bioavailable Caspase-1 Inhibitor.** It is 100-10,000 fold more active against caspase-1 than caspase-3, -6, or -9 **and** safe and well tolerated in Phase 2a human trial of drug resistant epilepsy.

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