CD4+ T Cell Death Mediated by Pyroptosis in Early SIV Infected Lymphatic Tissues

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Abstract

Lymphoid tissues (LTs) are principal sites for human immunodeficiency virus type 1 (HIV-1) replication, host-virus interaction and CD4+ T cell loss. However, the current understanding of the molecular virus-host interaction in lymphatic tissues, especially in early infection, is limited. We thus investigated virus-host interaction in the lymph nodes (LNs) of rhesus macaques in early simian immunodeficiency virus (SIV) infection. Transcriptome analysis showed that the hosts had clear responses at 3 dpi, with 103 differentially expressed genes (DEGs). Along with the increase of viral replication, host mobilized stronger responses, with 366 and 1350 DEGs at 6 dpi and 10 dpi, respectively. Pathway analysis of DEGs at 6 dpi and 10 dpi points to the activation of pyroptosis pathway in addition to innate immune response and inflammation pathways. The multiple genes of pyroptosis pathway (IFI16, DAI, RIG-I, MDA5, capsase-1 and IL-1β) were significantly up-regulated in expression. To further validate the activation of pyroptosis, the active form of capase-1, the hallmark of pyroptosis activation, was quantified in CD4+ T cells in rectal draining LNs using flow cytometry. The results showed that, concurrent with loss of CD4+ T cells in draining LNs in early infection, the level of caspase-1+ CD4+ T cells were significantly increased, indicating that the pyroptosis mediated CD4+ T cell death occurs in vivo in early SIV infected lymphatic tissues. Since CD4+ T cell loss and immune activation is the hall mark of HIV pathogenesis, the CD4+ T cell loss mediated by pyroptosis and associated immune activation in vivo in early SIV infection identified in this study opens a new avenue to design interceptive strategy to prevent CD4+ T cell death.

Experimental Design

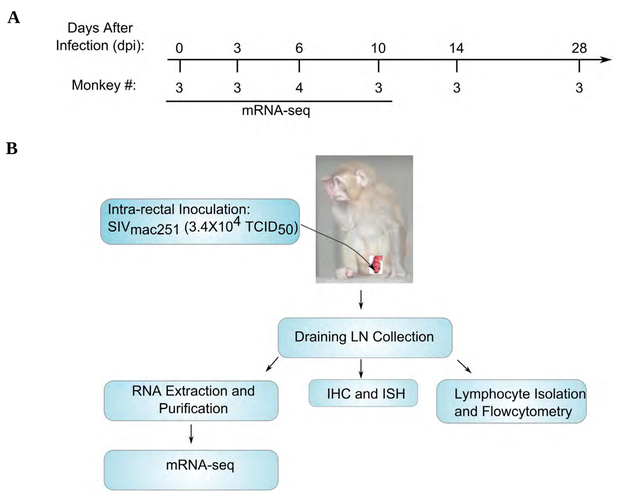


Figure 1. Schematic representative of experimental design. (A). Indian rhesus macaques were sacrificed at 3, 6, 10, 14 and 28 dpi, and macaques without inoculation were used as controls (0 dpi.). (B) Atraumatic rectal inoculation of macaques with SIV*mac*251. After collection, draining LNs were immediately frozen in liquid nitrogen for mRNA-seq, or fixed for tissue staining (IHC and ISH), or used for lymphocyte isolation. Thirteen samples were sequenced with 30 million reads per sample with 75 nt in length for each read.

Results

Inoculated SIV Established Productive Infection

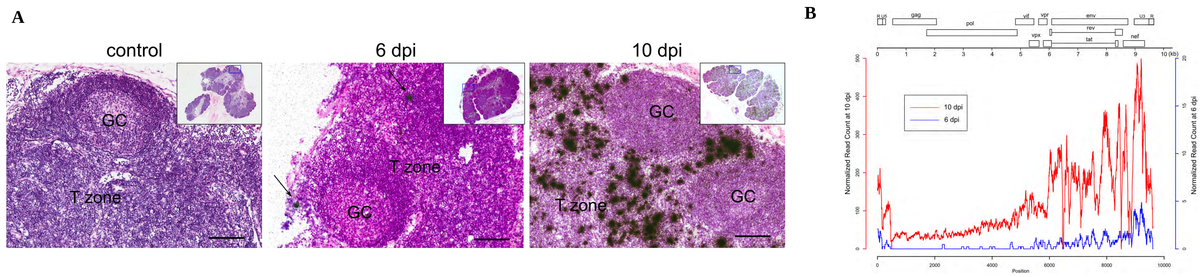
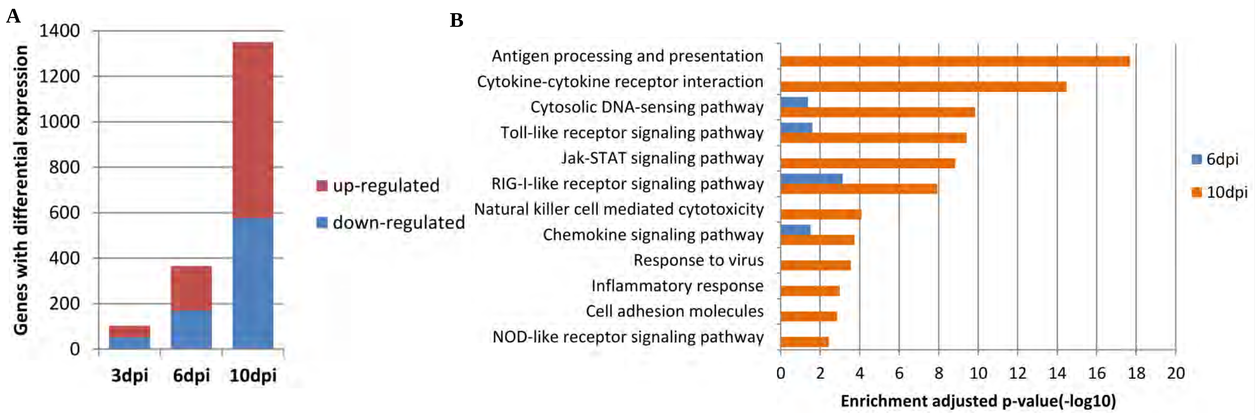


Figure 2. SIV replication in draining LNs after inoculation. (A) In situ hybridization of SIV vRNA+ cells in draining LNs after 6 and 10 dpi. The LNs from un-infected monkeys were used as control. (B) Level of SIV mRNA in draining LNs at 6 and 10 dpi using mRNA-seq. Bar= 100 μm

mRNA-seq Revealed Robust Host Responses in Early SIV Infection



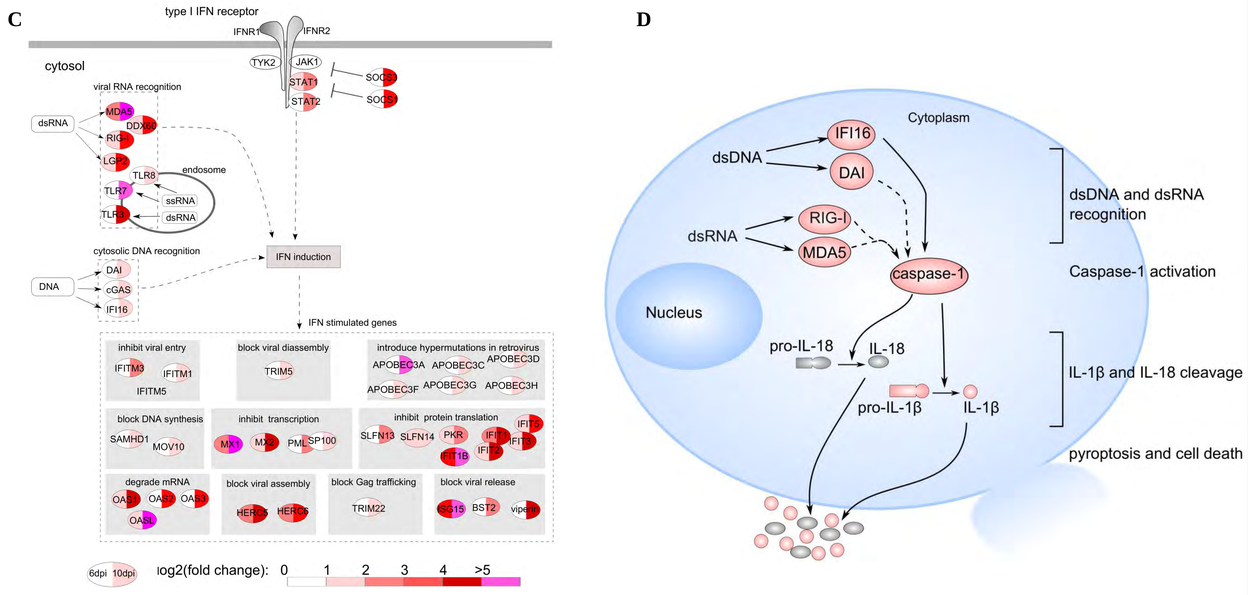


Figure 3. SIV infection induced robust responses in host LNs, characterized by increased inflammation and antiviral responses . (A) Number of differentially expressed genes at 3, 6 and 10 dpi in LNs. (B) Enriched gene categories at 6 dpi and 10 dpi. (C) Differentially expresses genes mapped to interferon signaling pathway. The log2 value of the fold change are color coded, as shown in the bar. (D) Schematic representation of pyroptosis pathway, genes with up-regulation were colored in red.

Pyroptosis Was Activated During Early SIV infection

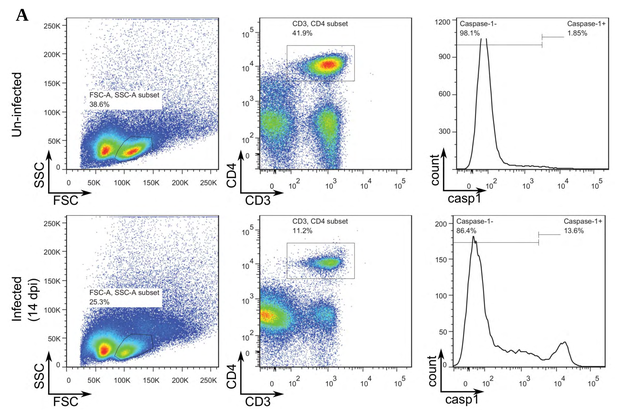


Figure 4. Increase of activated-caspase-1 protein level in LN CD4+ T cells during acute SIV infection. (A) Representative flow results of activated-caspase-1+ CD4+ T cells from un-infected and SIV infected monkeys. FAM-YVAD-FMK was used to stain activated-caspase-1. (B) Levels of caspase-1 activation and CD4+ T cells during SIV infection. *P<0.05 (Mann-Whitney U test)

Conclusions

- There are robust inflammatory and anti-viral responses in macaque's LNs during very early SIV infection.
- Pyroptosis pathway was activated in LNs, more specifically in CD4+ T cells, which correlated with the decline of CD4+ T cells.
- Pyroptosis contributes to CD4+ T cell death in vivo in early SIV infection.

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