

Role of TLR in HIV-1 Pathogenesis

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Abstract # D-219

Background: Interactions of HIV-1 with other microbes (co-pathogens) is an important factor in HIV disease progression. Innate response is the first line of the host defense to an incoming infection. Toll-like receptors (TLRs) are one of the key elements in this response. Their engagement by microbial ligands leads to the induction of a variety of cellular factors that interfere with the life cycle of the pathogen. Any other, present or incoming, pathogen may be also affected by these factors.

Methods: To investigate how the co-pathogens-induced engagement of TLRs affects HIV-1 replication, we studied the effect of ligands specific for TLR 2, 3, 4, 5, 7, 8, and 9 on HIV-1 replication in human lymphoid tissue *ex vivo*. Replication of CCR5-tropic (SF162) and CXCR4-tropic (LAL04) HIV-1 viral strains was measured by p24 ELISA. Cell activation (CD69 and CD38) and cell death (annexin V, Live/Dead fixable dead cell stain (Invitrogen)) was monitored in various cell subpopulations (CD3, CD4, CD8) using flow cytometry. Changes in the production of nineteen cytokines/chemokines were determined in multiplex bead-based assay. Student's t test was used to determine statistical significance of the differences in cumulative p24 values between untreated and TLR ligand treated tissues.

Results: We demonstrate differential effect of various TLR ligands on both HIV-1 replication and cell activation/death. Effect on HIV-1 replication depends on: 1) TLR receptor engaged; 2) HIV-1 viral strain and 3) the duration of the presence of a TLR ligand during the infection. Our results indicate that the altered cytokine profiles (namely MIP1 α , MIP1 β , and SDF-1) have the major direct effect on HIV-1 replication *in vivo* lymphoid tissue while the effect of cell activation/death is mainly indirect.

Conclusion: HIV-1 disease progression may be affected by the engagement of TLR by microbial components derived from HIV-1 co-pathogens.

Methods

TLR ligands

- TLR2 – LPS from *Porphyromonas gingivalis* (3 μ g/ml)
- TLR3 – poly(I:C) (25 μ g/ml)
- TLR4 – LPS from *E. coli* K12 (Sf162) (5 μ g/ml)
- TLR5 – Flagellin (*S. typhimurium*) (5 μ g/ml)
- TLR7 – Imiquimod (R837) (20 μ g/ml)
- Loxoribine (10nM)

- TLR8 – ssRNA (ssRNA4A/LyoVec) (10 μ g/ml)
- TLR9 – CpG oligonucleotide type C (ODN M362) (5 μ M)

Viruses

- HIV-1 SF162 (CCR5-tropic)
- HIV-1 LAL04 (CXCR4-tropic)

Lymphokines tested

- RANTES, MIP-1 α , MIP-1 β , SDF-1, IL-1 α , IL-1 β , IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-15, IL-16, IP-10, MIG, TNF- α , IFN γ , and GM-CSF

Luminex technology (Bio-Plex system – Bio-Rad)

HIV-1 p24 assay

ELISA (Pierce/Amersham)

Luminex technology (Bio-Plex system – Bio-Rad)

Student's t test was used to determine statistical significance of the differences in cumulative p24 values

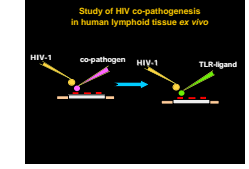
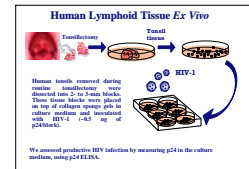
Cellular markers tested

Activation: CD38, CD69

Apoptosis/cell death: annexin V, Live/Dead fixable dead cell stain (Invitrogen)

Cell subpopulations: CD3, CD4, CD8, CCR5, CXCR4

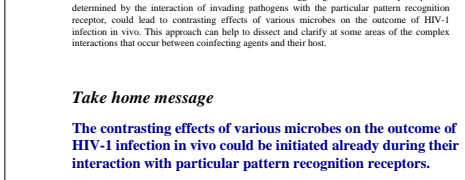
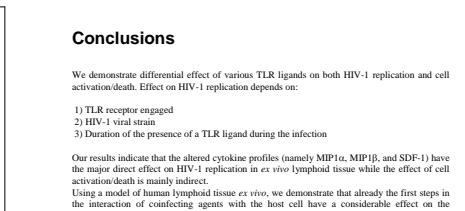
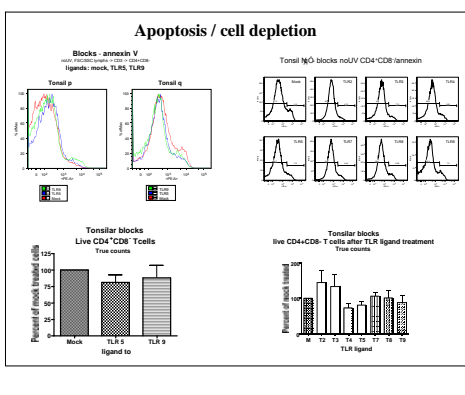
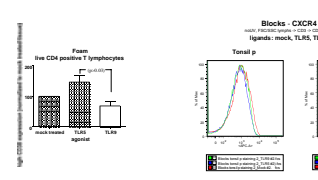
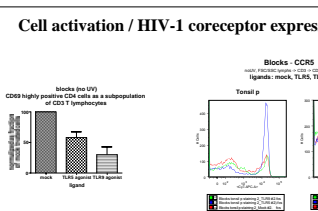
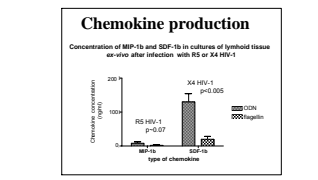
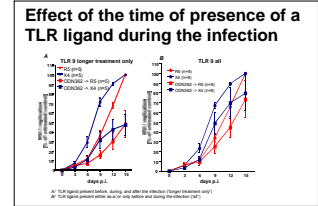
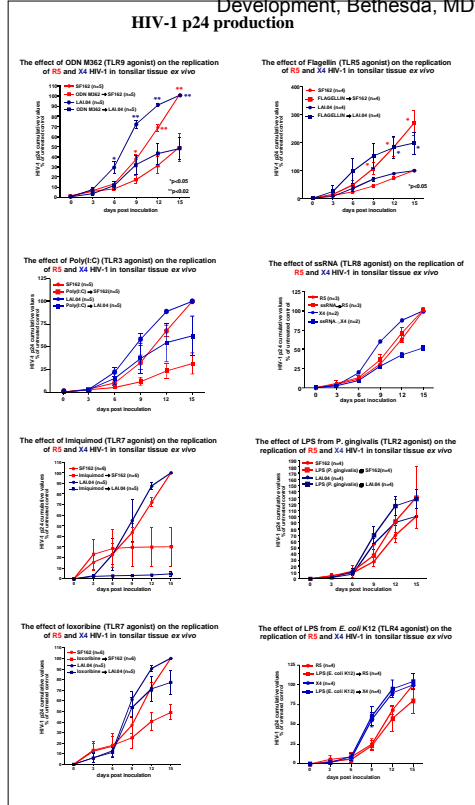
Flow cytometry (BD LSR II \rightarrow Diva \rightarrow FlowJo 6.5.3)



Introduction

HIV-1 infection as well as progression of HIV-1 disease is usually occurring in the presence of other microbes. These infection agents typically facilitate the transmission of the virus and aggravate the clinical course of HIV-1 disease. However, it has been suggested recently that some infection agents may have a repressive effect and could in fact alleviate the course of HIV-1 infection as GBV, O. tsutsugamushi, dengue, measles, or HTLV. One of the first responses to the invading microorganism is mediated by the activation of cellular pathways triggered by their engagement of cellular Pattern Recognition Receptors. Among the most potent mediators of this response are Toll Like Receptors (TLRs). A set of receptors that activate host defenses responsible for local inflammation, the recruitment of effector cells, and the secretion of cytokines that modulate both the innate and adaptive immune responses.

To investigate the possible contribution of microbial interaction with host TLRs on the outcome of HIV-1 infection, we studied the effect of TLR ligands on HIV-1 replication in human lymphoid tissue *ex vivo*.



Conclusions

We demonstrate differential effect of various TLR ligands on both HIV-1 replication and cell activation/death. Effect on HIV-1 replication depends on:

- 1) TLR receptor engaged
- 2) HIV-1 viral strain
- 3) Duration of the presence of a TLR ligand during the infection

Our results indicate that the altered cytokine profiles (namely MIP1 α , MIP1 β , and SDF-1) have the major direct effect on HIV-1 replication *in vivo* lymphoid tissue while the effect of cell activation/death is mainly indirect. Using a model of human lymphoid tissue *ex vivo*, we demonstrate that already the first steps in the interaction of confining agents with the host cell have a considerable effect on the outcome of HIV-1 infection. The differences in triggering innate immunity responses, determined by the interaction of invading pathogens with the particular pattern recognition receptor, could lead to contrasting effects of various microbes on the outcome of HIV-1 infection *in vivo*. This approach can help to dissect and clarify at some areas of the complex interactions that occur between confining agents and their host.

Take home message

The contrasting effects of various microbes on the outcome of HIV-1 infection *in vivo* could be initiated already during their interaction with particular pattern recognition receptors.