Staphylococcus aureus and Candida albicans Facilitate HIV Reservoir Establishment in CD4+ T-Cells by Promoting RALDH Activity in Dendritic Cells

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**BACKGROUND**

**Dual Role of Dendritic Cells During HIV-1 Infection**

Two main subsets of monocytes:

<table>
<thead>
<tr>
<th>Classical CD16-</th>
<th>Non Classical CD16+</th>
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<td><em>Inflammatory</em></td>
<td><em>Resident</em></td>
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<tr>
<td>CCR2 +++</td>
<td>CX3CR1 +++</td>
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<tr>
<td>→ Migrate to site of injury / inflammation</td>
<td>→ Patrolling</td>
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<td>TLR2 / TLR4</td>
<td>TLR7 / TLR8</td>
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<td>→ Recognition of LPS and lipopeptides</td>
<td>→ Sense ssRNA</td>
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**ALDH1A2 is preferentially expressed at the RNA levels in CD16+ MDDC**

- Genome-wide transcriptional profiling were performed using the **Affymetrix technology** in matched CD16+/CD16- MDDC from 5 uninfected subjects (Wacleche et al., Blood Adv. 2018)

- Trans-infection ability was evaluated by co-culturing MDDC loaded with antigen (SEB, CMV, *S. aureus*) and infected with HIV (NL4.3BaL) with autologous CD4+ T-cells
  → Levels of p24 were measured by ELISA and FACS

- Immunogenic potential was evaluated by co-culturing MDDC loaded with antigen (SEB, CMV, *S. aureus*) with autologous CFSE+CD4+ T-cells
  → Proliferation was measured by FACS

- RALDH activity was measured using the **ALDEFLUOR Assay** in matched CD16+/CD16- MDDC

- HIV reactivation was measured using a **MDDC-based Viral Outgrowth Assay**

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**EXPERIMENTAL APPROACH**

**CD16+/CD16- Mo FACS sorting**

**MDDC differentiation**

- [Flow cytometry plots for CD16- and CD16+ MDDC differentiation]

- [Control HIV replication]

- [HIV dissemination]
**RESULTS**

**MDDC Loaded with S. aureus or C. Albicans vs SEB or CMV Exhibit a Superior Ability to Transmit HIV to CD4+ T-cells**

**CD16+ vs. CD16- MDDC exhibit superior RALDH2 Activity**

**S. aureus but not CMV Promotes RALDH2 Activity in MDDC of PLWH**
MDDC Ability to trans Infect S. aureus-Specific CD4+ T-cells is Dependent on RALDH2 Activity and retinoic acid Signalling

Viral Outgrowth Assay in a MDDC: T Cell Co-Culture System

Zymosan = TLR2 agonist

Zymosan Promotes HIV Reactivation and the Inhibition of the retinoic acid pathway reduces zymosan-induced reservoir reactivation
• **CD16+ versus CD16- MDDC** exhibit higher RALDH activity and superior capacity to transmit HIV to *S. aureus*-specific CD4+ T-cells

• RALDH2 activity in MDDC is upregulated by *S. aureus* and zymosan likely due to TLR2 triggering

• Zymosan promotes viral reservoir reactivation in a MDDC-based VOA performed with cells of ART-treated PLWH

• Blocking the retinoic acid production (DEAB) and signaling (LE540) reduced zymozan-induced reservoir reactivation

**CONCLUSION**

By hijacking the RALDH/RA pathway, HIV reservoir reactivation may occur in memory CD4+ T-cells of ART-treated PLWH upon interaction with MDDC loaded with fungi/bacteria at mucosal barrier surfaces (e.g., GALT)