



# CAHR 2020 Virtual

May 1 & 2, 2020

Hope, Victories and Perseverance beyond 2020



**L'ACRV 2020 virtuel sur votre agenda**

**1 au 2 mai, 2020**

**Espoir, Victoires et Persévérance au-delà de 2020**

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

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### **Conflict of Interest Disclosure**

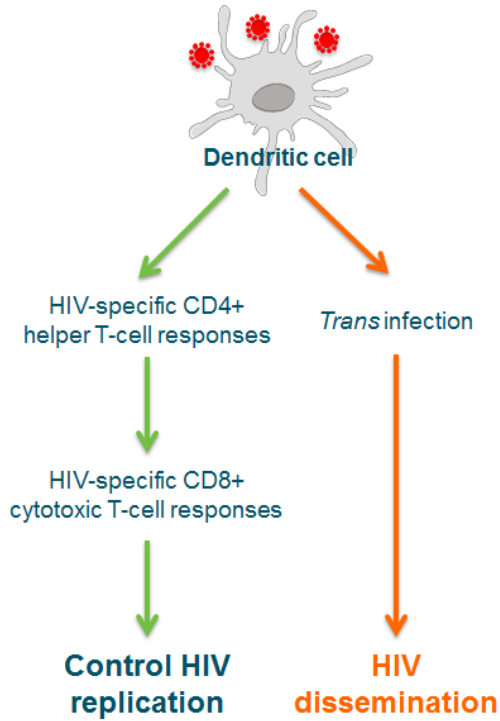
I have no conflicts of interest



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# BACKGROUND

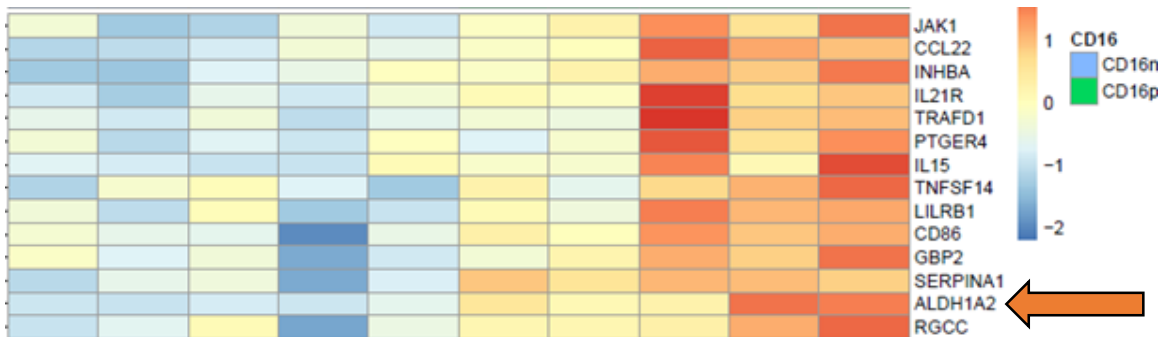
## Dual Role of Dendritic Cells During HIV-1 Infection



## Two main subsets of monocytes:

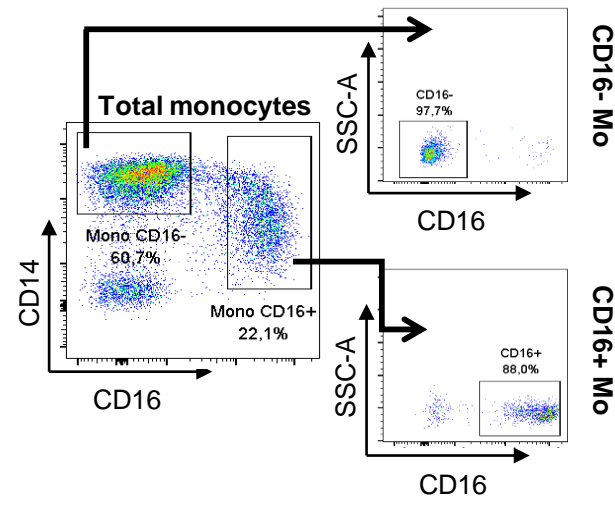
| Classical CD16-<br>Inflammatory                        | Non Classical CD16+<br>Resident  |
|--|--|
| CCR2 +++<br>→ Migrate to site of injury / inflammation | CX3CR1 +++<br>→ Patrolling   |
| TLR2 / TLR4<br>→ Recognition of LPS and lipopeptides   | TLR7 / TLR8<br>→ Sense ssRNA   |
|  | <b>Increased frequency in HIV infection</b><br>→ not normalized with ART |

## ALDH1A2 is preferentially expressed at the RNA levels in CD16+ MDDC

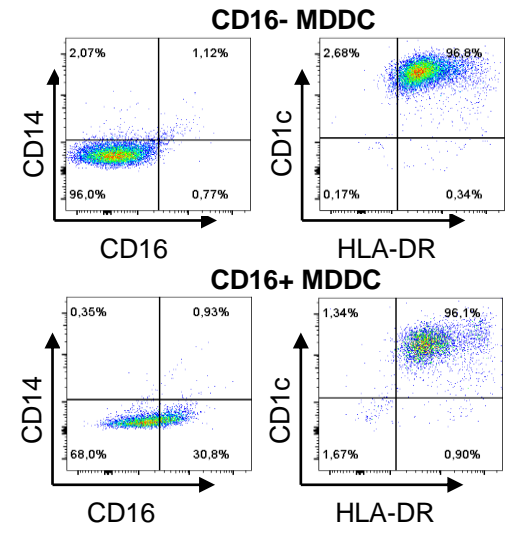


# EXPERIMENTAL APPROACH

## CD16+/CD16- Mo FACS sorting

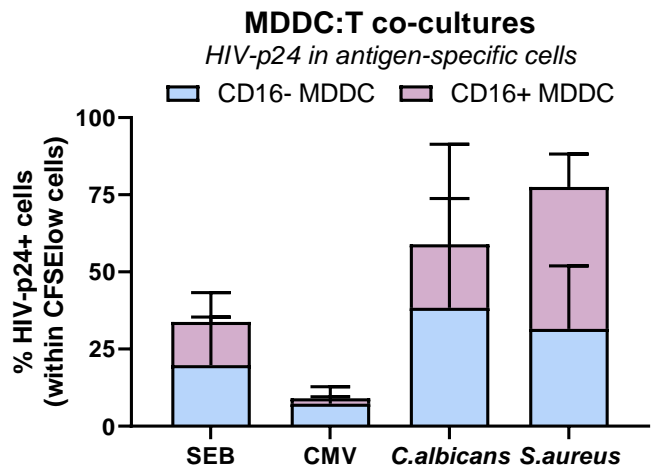
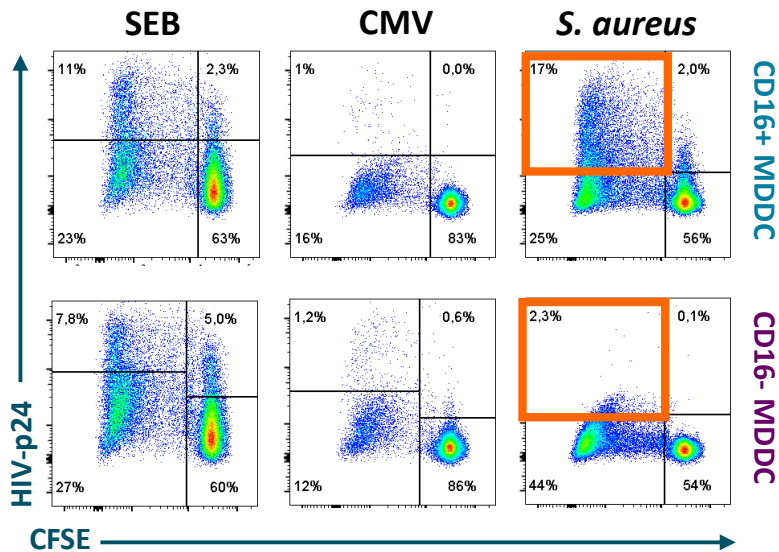


## MDDC differentiation

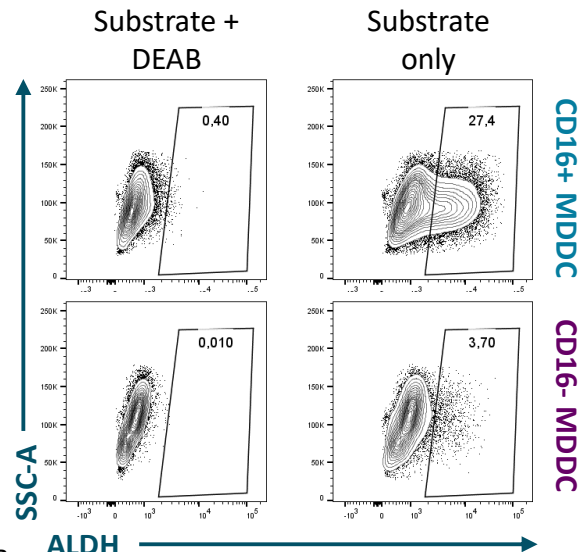


- Genome-wide transcriptional profiling were performed using the **Affymetrix technology** in matched CD16<sup>+</sup>/CD16<sup>-</sup> MDDC from 5 uninfected subjects (Wacleche *et al.*, Blood Adv. 2018)
- Trans*-infection ability was evaluated by co-culturing MDDC loaded with antigen (SEB, CMV, *S. aureus*) and infected with HIV (NL4.3BaL) with autologous CD4<sup>+</sup> 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<sup>+</sup>CD4<sup>+</sup> T-cells
  - Proliferation was measured by FACS
- RALDH activity was measured using the **ALDEFLUOR Assay** in matched CD16<sup>+</sup>/CD16<sup>-</sup> MDDC
- HIV reactivation was measured using a **MDDC-based Viral Outgrowth Assay**

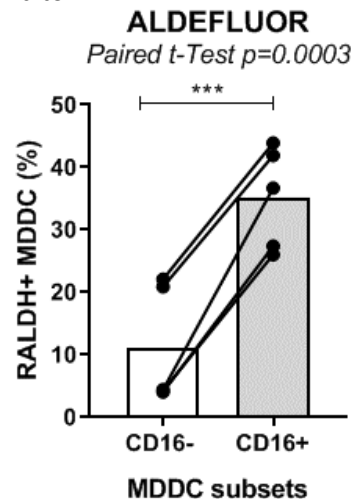
# RESULTS



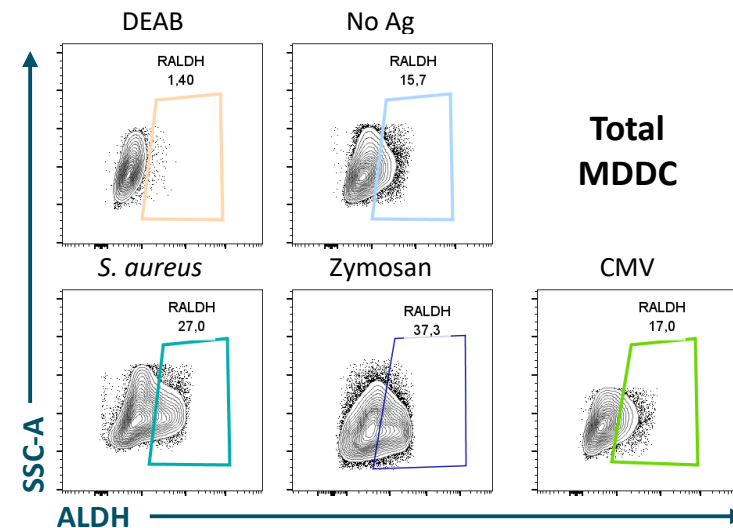
**MDDC Loaded with *S. aureus* or *C. Albicans* vs SEB or CMV Exhibit a Superior Ability to Transmit HIV to CD4+ T-cells**



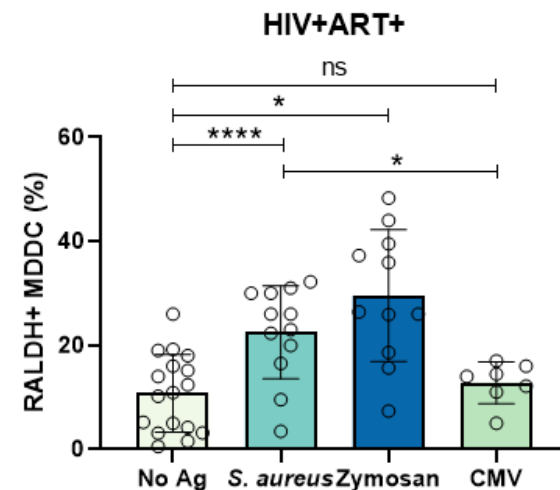
DEAB = RALDH inhibitor



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



Zymosan = TLR2 agonist

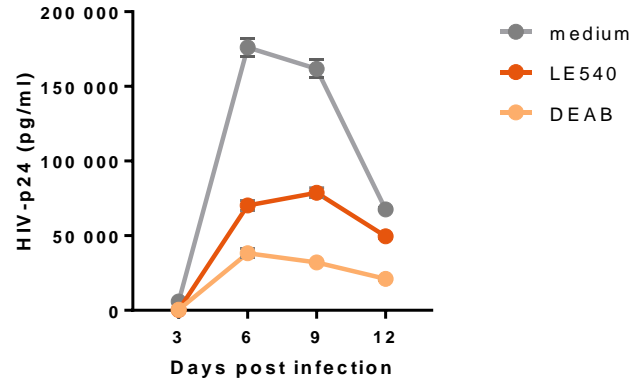


***S. aureus* but not CMV Promotes RALDH2 Activity in MDDC of PLWH**

# RESULTS

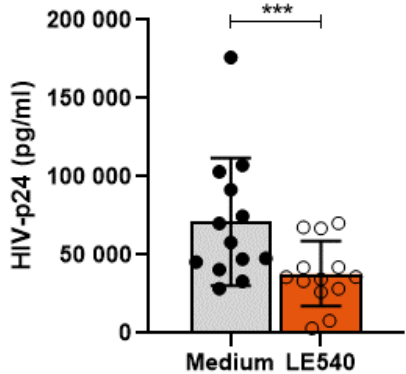
**LE540** Retinoic acid receptor antagonist  
**DEAB** RALDH inhibitor

MDDC to CD4+ T-cells  
*S. aureus*



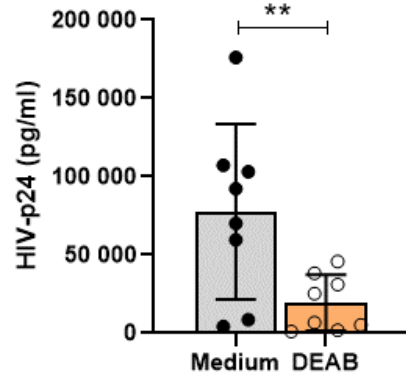
Day 6

Wilcoxon test  
 $p=0.0002$



Day 6

Wilcoxon test  
 $p=0.0078$



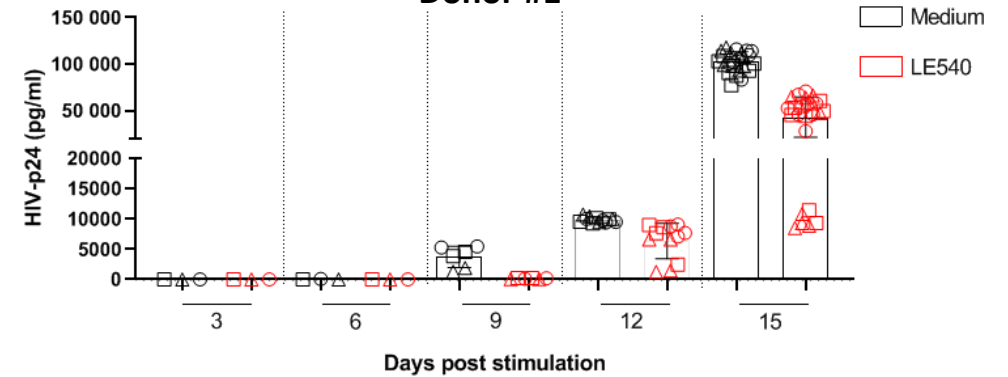
MDDC Ability to *trans* Infect *S. aureus*-Specific CD4+ T-cells is Dependent on RALDH2 Activity and retinoic acid Signalling

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

Zymosan = TLR2 agonist

Zymosan  
MDDC : mCD4+ T-cells

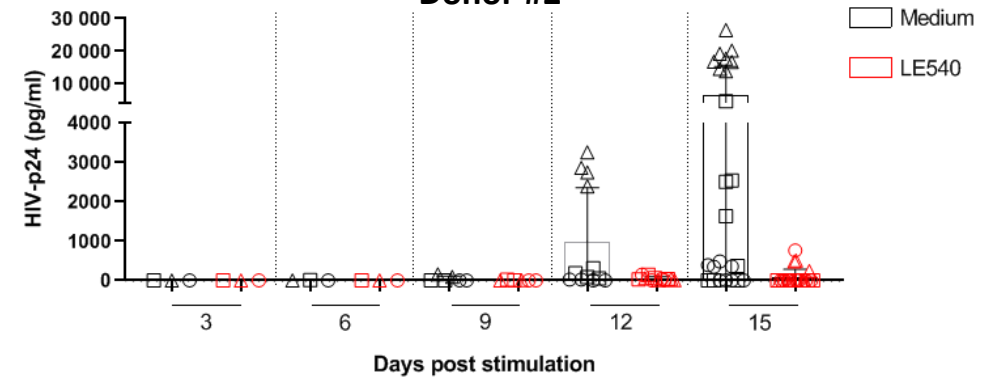
Donor #1



Zymosan

MDDC : mCD4+ T-cells

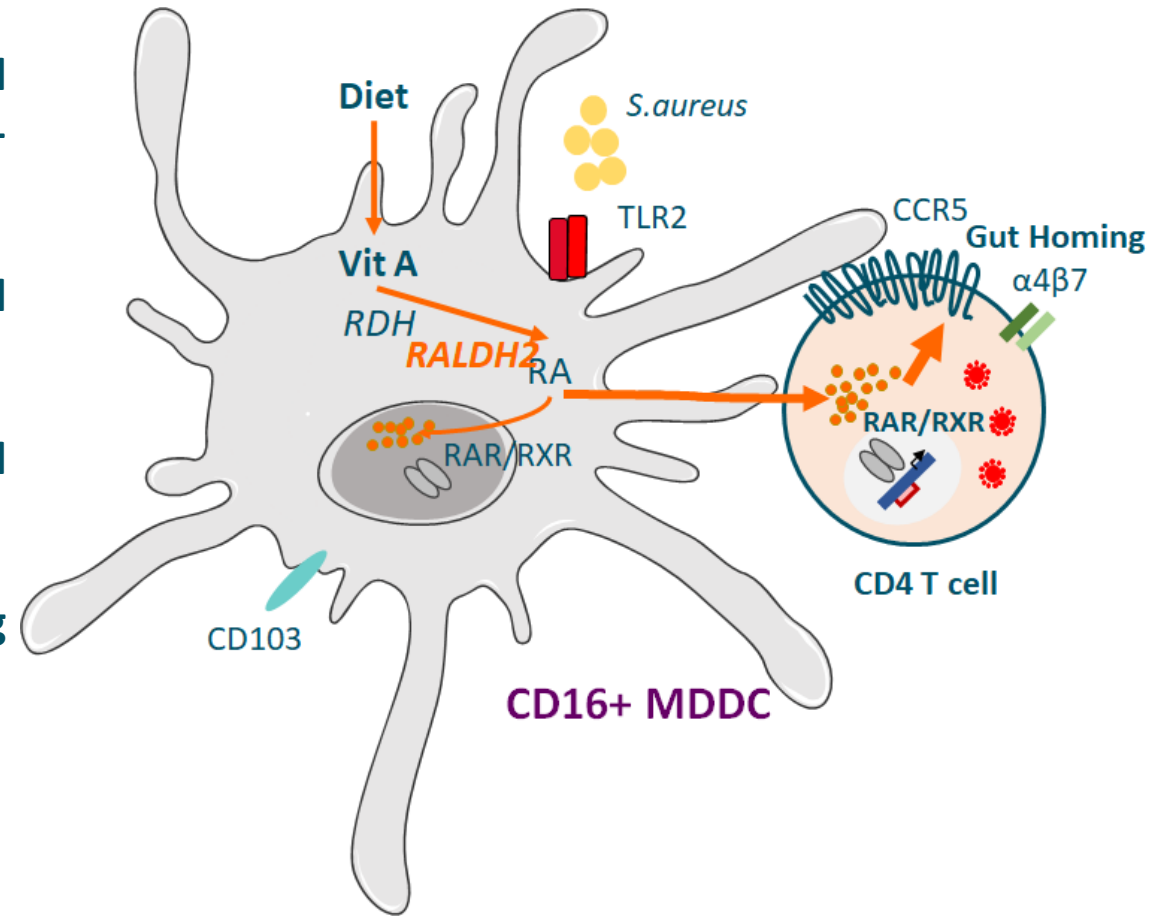
Donor #2



Zymosan Promotes HIV Reactivation and the Inhibition of the retinoic acid pathway reduces zymosan-induced reservoir reactivation

# CONCLUSION

- CD16+ versus CD16- MDDC exhibit higher RALDH activity and superior capacity to transmit HIV to *S. aureus*-specific CD4<sup>+</sup> 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 zymosan-induced reservoir reactivation



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