Mucosal-Associated Invariant T (MAIT) Cell Depletion and Exhaustion in HIV/HCV Co-infection.

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Presenter: Ali Fawaz

HIV and HCV Co-infection
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HIV/HCV Co-infection

• HCV targets the hepatocytes of the liver, and over time may cause cirrhosis.
• HIV/HCV co-infected patients progress more rapidly to end-stage liver disease, and compared to HCV mono-infection show:
  – Increased viral load.¹
  – Higher rate of viral persistence¹

• What is the immunological basis for this?

Mucosal Associated Invariant T (MAIT) Cells

• Anti-microbial, innate-like T Cells
  – React against vitamin metabolites produced by bacteria and yeast
  – Characterized by expression of invariant TCR Va7.2, along with CD161 and IL-18R.
• Found in mucosal tissues, the liver, and peripheral blood.
• Constitute up to 10% of peripheral blood T cells, 40% liver T cells²
• Secrete IFNγ (anti-fibrogenic), TNFα and IL-17 (pro-fibrogenic), IL-22 (hepatoprotective)

Question: Are MAIT Cells impaired in HIV/HCV?

- Due to their accumulation in the liver, and secretion of pro- and anti-fibrogenic cytokines, MAIT cells are of interest when examining liver disease progression.
- We wanted to know if:
  1. MAIT Cells are somehow impaired in HIV/HCV
  2. This impairment could explain why liver disease progresses more rapidly in co-infected individuals.
In HIV Mono-infection:

- MAIT cells are highly activated and exhausted (i.e. elevated Tim-3)
- Proportion of MAIT cells producing IFN\(\gamma\), IL-17, and TNF\(\alpha\) is lower.
- Reduced MAIT cell frequency in peripheral blood, static in rectal mucosa (potential recruitment?)
- Accumulation of less functional, CD161- V\(\alpha\)7.2+ MAIT cells as infection progressed.
Methods: Flow Cytometry

- Peripheral blood mononuclear cells (PBMCs) from uninfected, chronic HIV+, HCV+, and HCV+/HIV+ patients were stained with fluorescent antibodies.
- Analyzed using flow cytometry, which allowed for:
  1. Identification of MAIT Cells in a mixed PBMC population
### Flow Cytometry: Staining Panel

<table>
<thead>
<tr>
<th>Marker</th>
<th>Function</th>
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<tbody>
<tr>
<td>CD3</td>
<td>TCR Component</td>
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<tr>
<td>Vα7.2</td>
<td>Specific to MAIT TCR</td>
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<tr>
<td>CD161</td>
<td>IL-17 Producing Cell Marker. Identifies MAIT Cells</td>
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<tr>
<td>PD-1</td>
<td>Exhaustion Marker</td>
</tr>
<tr>
<td>Tim-3</td>
<td>Exhaustion Marker</td>
</tr>
<tr>
<td>IFNγ</td>
<td>Anti-fibrogenic cytokine, marker of functional capacity.</td>
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Gating Strategy

- Lymphocytes
- Live Cells
- Singlets
- CD3 +
- MAIT Cells

Flow cytometry plots showing gating strategy for lymphocytes, live cells, singlets, CD3+, and MAIT cells.
Depletion of MAIT Cells in Peripheral Blood of HIV, and HCV/HIV Patients

Two-tailed Mann-Whitney t test (95% confidence interval) was used for statistical analysis.
Depletion of CD161+/Vα7.2+ Subset in HIV/HCV

- **Healthy**: 11.2% CD161+ and 88.9% Vα7.2+
- **HCV+**: 5.29% CD161+ and 95.2% Vα7.2+
- **HIV+**: 5.11% CD161+ and 95.2% Vα7.2+
- **HIV+/HCV+**: 7.14% CD161+ and 93.1% Vα7.2+

\[ \text{p} = 0.0303 \]
MAIT Cells Show Exhaustion Phenotypes in HIV/HCV

**PD-1+ MAIT Cells:**

- Healthy N=5
- HIV+ N=4
- HCV+ N=4
- HIV+/HCV+ N=6

- p= 0.0303

**Tim-3+ MAIT Cells**

- Healthy N=5
- HIV+ N=4
- HCV+ N=4
- HIV+/HCV+ N=6

- p= 0.0190
MAIT Cell Production of IFNγ with PMA/Ionomycin Stimulation
What Do These Findings Tell Us?

Going back to the original questions:

Are MAIT Cells impaired in HIV/HCV?:
- They are depleted in the peripheral blood.
- There is a significant decline in the proportion of functional (Vα7.2+, CD161+) MAIT Cells in HIV/HCV.
- Greater proportion of MAITs expressing the exhaustion markers PD-1 and Tim-3 in HIV/HCV.

Can this impairment explain the more rapid progression of liver disease in HIV/HCV?
- Difficult to make any conclusions at this point.
- Will become more clear as their functional capacity is more comprehensively characterized.
Future Directions

• Assess MAIT cell phenotypes in the liver.
• Re-assess IFNγ production using a more physiological stimulus, such as *E. coli*.
• Expand our functional characterization to the remaining cytokines.
  – Are there compounding negative effects? (ex. Lower IFNγ production together with higher IL-17)
Acknowledgements

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