ORAL CYTOKINE EXPRESSION IS LINKED TO ORAL HIV-1 LEVELS IN ACTG A5254

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BACKGROUND

• HIV-1 infections disrupts oral mucosal immunity, but the pathogenesis of this immune dysregulation remains unclear.

• Here we characterized the mucosal immune response at variable stages of HIV infection by measuring 11 soluable immune mediators in oral washings of people with HIV (PWH).

• We investigated changes in this oral cytokine expression in the setting of oral opportunistic infections (OIs) and anti-retroviral treatment (ART), including the role of HIV-1 viral load and CD+ T cell count.

METHODS

• ACTG PROTOCOL A5254 - Multicenter, cross-sectional, single–visit study of PWH.

• Plasma and throat-wash samples were collected and oral examination for OIs performed at visit.

• Participants were divided into strata by plasma CD4+ T cells/µl (CD4) and HIV-1 viral load (VL).

• Stratum A (CD4 <200, VL >1000), Stratum B (CD4 >200, VL <1000), Stratum C (CD4 >200, VL >1000), Stratum D (CD4 >200, VL <1000).

• Throat-wash samples were obtained through a standardized 1-minute rinse with 10ml of sterile saline. Samples were collected, frozen, and stored at −80°C.

• Cytokine differences between strata were evaluated using Kruskal–Wallis test.

• Correlation analyses were performed using Pearson’s correlation test.

RESULTS

1. Levels of IL-10, an anti-inflammatory cytokine, were significantly different between Stratum A and the other three strata.

2. IL-12, IFNγ, and IL-10 showed differences between Strata A and C or Strata B and D, suggesting CD4 count impacted the production of these cytokines greater than TNFα, IL-1β, and IL-6 (not shown).

3. No differences were noted between the strata in IL-2, IL-4, or IL-13 levels (not shown).

4. Multicenter, cross-sectional, single-visit study of PWH.

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6. No differences were noted between the strata in IL-2, IL-4, or IL-13 levels (not shown).

Table 1: Patient characteristics at study entry

<table>
<thead>
<tr>
<th>Age (years); median</th>
<th>A (n=48)</th>
<th>B (n=52)</th>
<th>C (n=29)</th>
<th>D (n=29)</th>
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<tbody>
<tr>
<td>N</td>
<td>44</td>
<td>41</td>
<td>47</td>
<td>45</td>
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<tr>
<td>Sex; n (%)</td>
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<td>85</td>
<td>69</td>
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<tr>
<td>female</td>
<td>33</td>
<td>44</td>
<td>15</td>
<td>31</td>
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<td>Race; n (%)</td>
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<tr>
<td>Black, non-Latino</td>
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<td>89</td>
<td>59</td>
<td>62</td>
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<td>5</td>
<td>7</td>
<td>24</td>
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<td>Latino</td>
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<td>4</td>
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<td>14</td>
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<tr>
<td>other</td>
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<td>2</td>
<td>0</td>
<td>7</td>
</tr>
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<td>Interventional Drug Use; n (%)</td>
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<tr>
<td>never used</td>
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<td>90</td>
<td>77</td>
<td>85</td>
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<tr>
<td>current/past use</td>
<td>15</td>
<td>9</td>
<td>21</td>
<td>17</td>
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<td>Currently on ART; n (%)</td>
<td>61</td>
<td>52</td>
<td>98</td>
<td>21</td>
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</tbody>
</table>

DIFFERENCES IN ORAL PRO-INFLAMMATORY CYTOKINE PRODUCTION

1. IL-12, IFNγ, and IL-17 also had increased levels in Stratum A compared to Stratum B and D.

2. IL-12 showed a difference between Stratum A and C.

3. IFNγ demonstrated a significant difference between Stratum C and D, as well as between Stratum A and D.

DIFFERENCES IN ORAL ANTI-INFLAMMATORY CYTOKINE PRODUCTION

1. Levels of IL-10, an anti-inflammatory cytokine, were significantly different between Stratum A and the other three strata.

2. IL-12, IFNγ, and IL-10 showed differences between Strata A and C or Strata B and D, suggesting CD4 count impacted the production of these cytokines greater than TNFα, IL-1β, and IL-6 (not shown).

3. No differences were noted between the strata in IL-2, IL-4, or IL-13 levels (not shown).

RELATIONSHIP BETWEEN HIV-1 VL AND CYTOKINE PRODUCTION

1. Linear regression modeling demonstrated the strongest association between oral HIV VL and the production of TNFα (R=0.45, IL-1β (R=0.48), IL-6 (R=0.46), and IL-8 (R=0.52) while controlling for CD4 count and OIs (all p-values <0.0001).

2. The association between plasma HIV VL and oral cytokine production was similar to oral HIV VL. Correlations with levels of TNFα (R=0.53), IL-1β (R=0.39), IL-6 (R=0.42), and IL-8 (R=0.42) were significant (all p-values <0.001).

3. Oral HIV VL positively correlated with plasma HIV VL (r=0.76; p<0.01).

CONCLUSIONS

1. Severe HIV infection (CD4<200, VL>1000) is associated with mucosal immune dysregulation characterized by elevations in both pro-inflammatory (Th1, Th17) and anti-inflammatory (Th2) cytokines.

2. Control of HIV viremia (VL<100) without immune reconstitution (CD4<200) was associated with a significant decrease in cytokine production, suggesting that dysregulation is primarily driven by viral antigenic stimulation.

3. The strongest association between oral and plasma HIV VL was with TNFα, IL-1β, IL-6, and IL-8 production; these cytokines are released early in the innate immune response by both immune and non-immune cells and are potentially less affected by a decrease in CD4 T-cells.

4. Implications: Control of HIV viremia appears to resolve most of the oral immune dysregulation. PWH controlled on ART are likely at significantly lower risk of oral OIs even before CD4 counts increase.