POSTER 0213

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

Joseph M. Rocco[1], Zachary York[2], Janet McLaughlin[1], Luann Borowski[1], Jennifer Webster-Cyriaque[3], Caroline H. Shiboski[4], Judith Aberg[5], Charles R Rinaldo[1], **Bernard J Macatangay**[1]

[1]University of Pittsburgh, [2]University of Wisconsin-Madison, [3]University of North Carolina at Chapel Hill, [4]University of California San Francisco, [5]Icahn School of Medicine at Mt Sinai

BACKGROUND

- HIV-1 infection 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 soluble 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 $CD4^+$ T cell count.

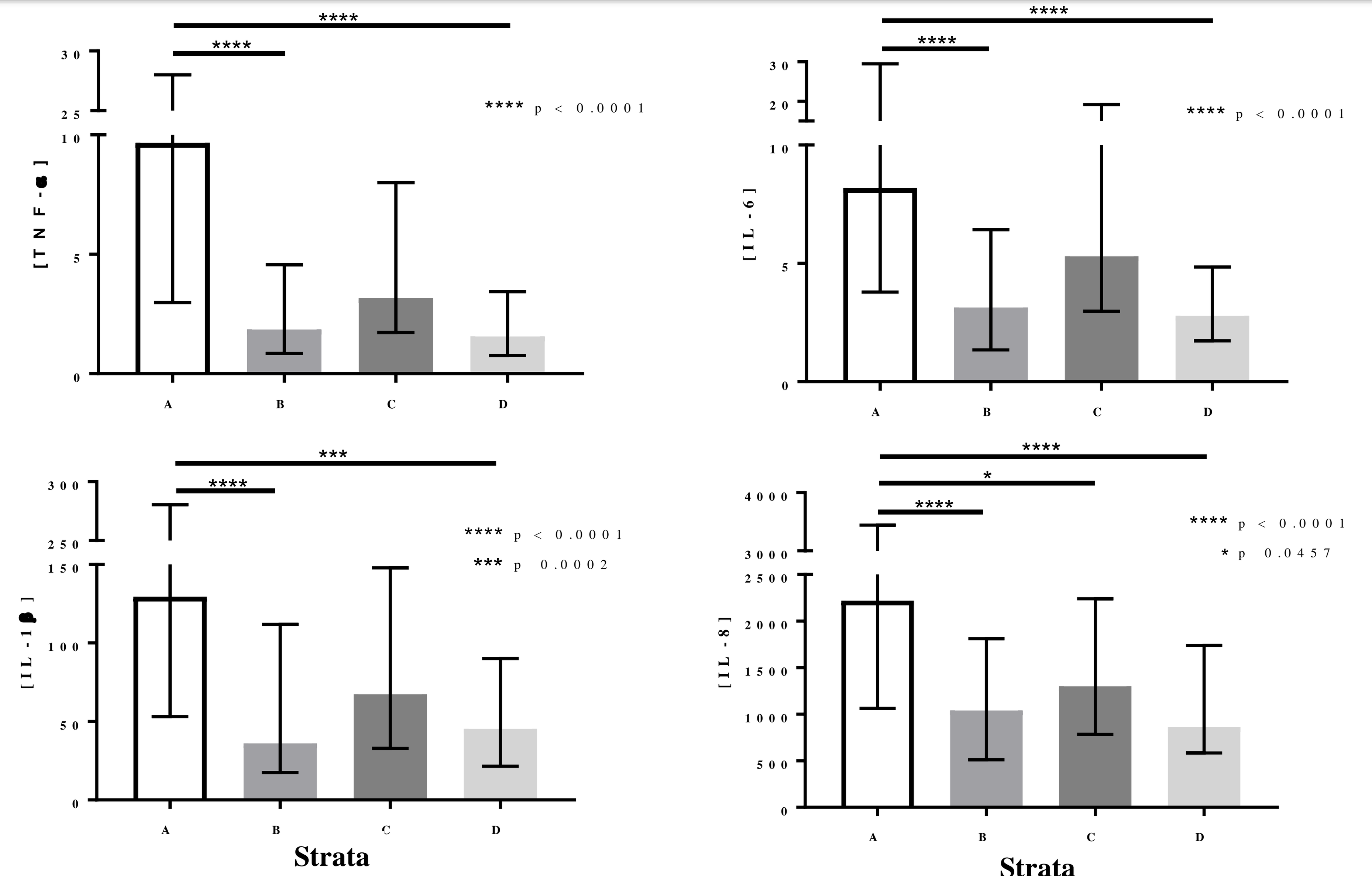
METHODS

- **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/mm³ (CD4) and HIV-1 cps/mL (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.
- Throat-wash sample analyses:
  → **HIV-1 viral load** and **HSV, CMV, EBV, KSHV** – real-time quantitative PCR (copies/ml)
  → **IL-1β, IL-2, IL-4, IL-6, IL-8, IL-10, IL-12, IL-13, IL-17, IFNγ, and TNFα**:
  Concentrations were measured using a multi-bead fluorescent platform (Meso Scale Discovery).
- Cytokine differences between strata were evaluated using Kruskal-Wallis with Dunn's post-test and adjusted for multiple comparison. Graphs show median with interquartile range.
- Linear regression was used to model the relationship between cytokine production and oral or plasma HIV VL while controlling for CD4 count, oral candidiasis, and human herpesviruses.

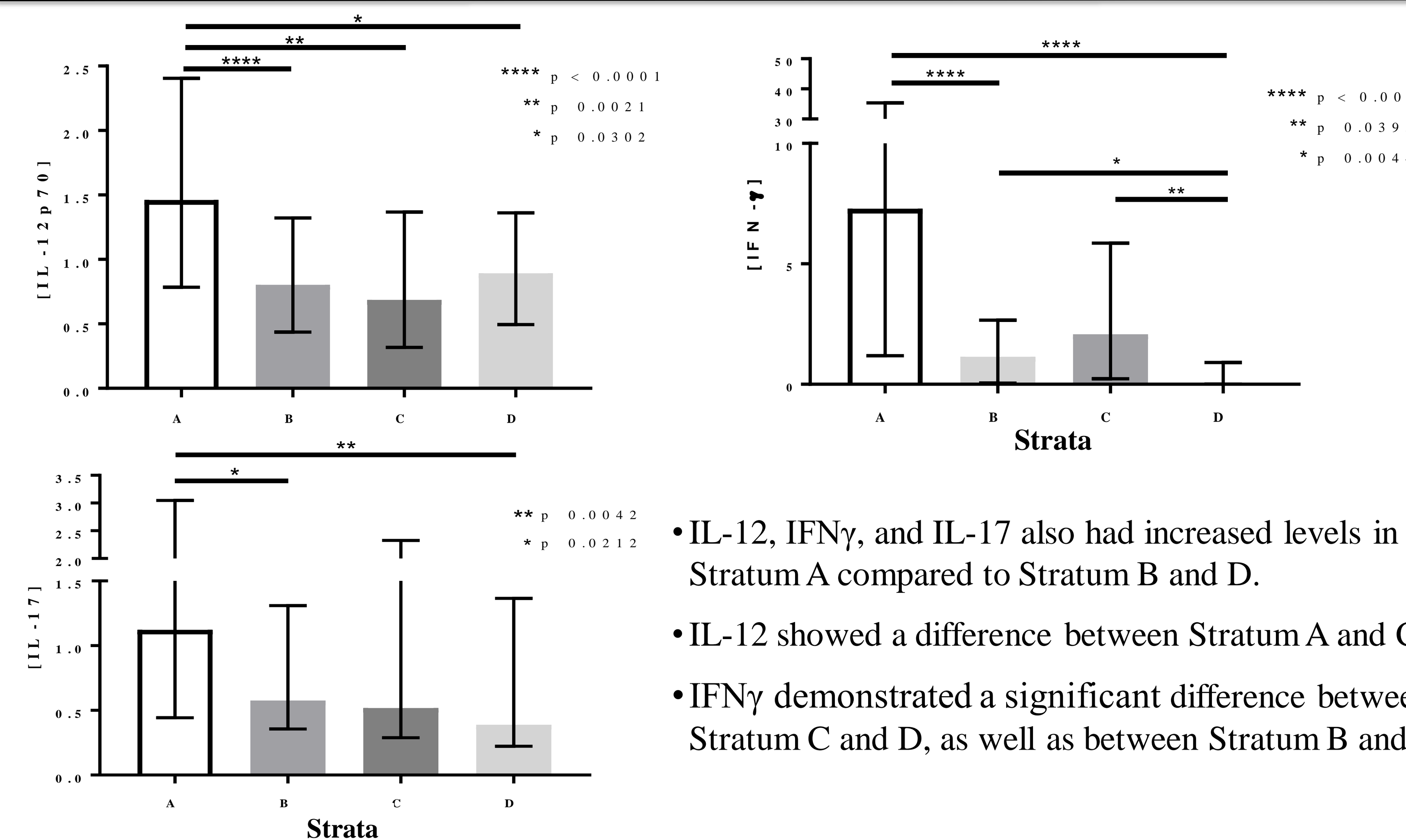
Table 1: Patient characteristics at study entry.

	N	A (*n*=148)	B (*n*=82)	C (*n*=29)	D (*n*=29)
Age (years); median	44	41	47	38	45
Sex; n (%)					
male	67	56	85	69	74
female	33	44	15	31	26
Race; n (%)					
Black, non-Latino	72	89	59	62	41
White, non-Latino	17	5	7	24	17
Latino	9	4	32	14	38
other	2	2	0	7	3
Intravenous Drug Use; n (%)					
never used	85	90	77	83	79
current/past use	15	9	21	17	20
Currently on ART; n (%)	67	52	98	21	100

DIFFERENCES IN ORAL PRO-INFLAMMATORY CYTOKINE PRODUCTION

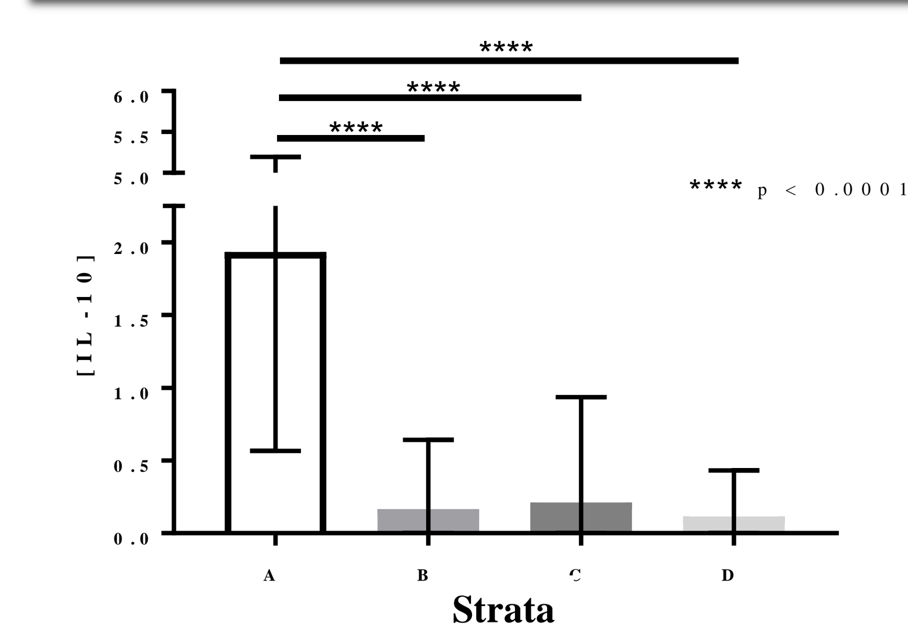


- Pro-inflammatory (TNFα, IL-1β, IL-6, IL-8) oral cytokine production showed significant differences in Stratum A compared to Stratum B and D. Only IL-8 demonstrated a notable difference between Stratum A and C (p=0.046).
- HIV-1 VL appears to primarily drive oral production of TNFα, IL-1β, IL-6, and IL-8. Suppression of VL without CD4 reconstitution (Stratum B) was not different than Stratum D (CD4>200, VL<1000)



- IL-12, IFNγ, and IL-17 also had increased levels in Stratum A compared to Stratum B and D.
- IL-12 showed a difference between Stratum A and C.
- IFNγ demonstrated a significant difference between Stratum C and D, as well as between Stratum B and D.

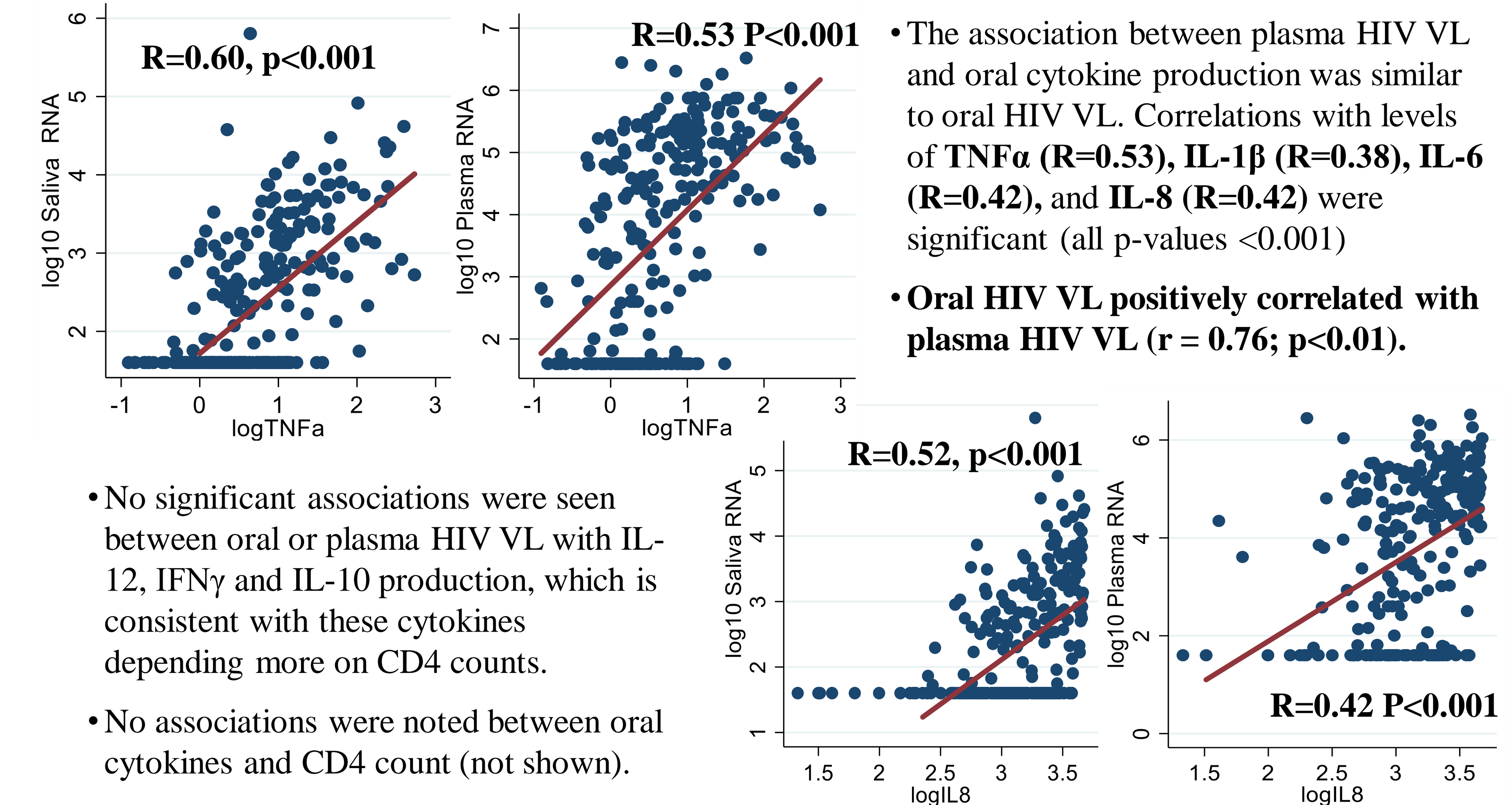
DIFFERENCES IN ORAL ANTI-INFLAMMATORY CYTOKINE PRODUCTION



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

- Linear regression modeling demonstrated the strongest association between oral HIV VL and the production of **TNFα (R=0.60), IL-1β (R=0.45), IL-6 (R=0.46),** and **IL-8 (R=0.52)** while controlling for CD4 count and OIs (all p-values <0.001).



- 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=0.38), IL-6 (R=0.42),** and **IL-8 (R=0.42)** were significant (all p-values <0.001)
- **Oral HIV VL positively correlated with plasma HIV VL (r = 0.76; p<0.01).**
- No significant associations were seen between oral or plasma HIV VL with IL-12, IFNγ and IL-10 production, which is consistent with these cytokines depending more on CD4 counts.
- No associations were noted between oral cytokines and CD4 count (not shown).

CONCLUSIONS

- **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.**
- **Control of HIV viremia (VL<1000) without immune reconstitution (CD4>200) was associated with a significant decrease in cytokine production, suggesting that dysregulation is primarily driven by viral antigenic stimulation.**
- **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.**

<u>IMPLICATIONS</u>: 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.