Th2 Cytokines are Associated with Higher Levels of Intact Proviruses on ART

Joshua Cyktor [1], Hanna Mar [2], Gregory M. Laird [3], Ronald Bosch [2], Albine Martin [3], Joseph Eron [4], Bernard J.C. Macatangay [1], Deborah McMahon [1], Rajesh T. Gandhi [5], John Mellors [1] for the A5321 Team

[1] Univ of Pittsburgh, Pittsburgh, PA, USA, [2] Harvard TH Chan Sch Public Health, Boston, MA, USA; [3] AccelevirDx, Baltimore, MD, USA; [4] Univ of North Carolina, Chapel Hill, NC, USA; [5] Mass General Hospital, Boston, MA, USA

Introduction

Th2 cytokines, such as interleukin (IL)-4 and IL-13, regulate humoral immunity, promote production of neutralizing antibodies, and can suppress Th1 and Th17 responses by upregulating repressors of interferon (IFN)-γ and IL-17 transcription.

Th2 cytokines could affect the persistence of HIV-1 on ART, but their effects on the HIV-1 reservoir have not been defined.

Methods

Participants who initiated ART during chronic infection with sustained virologic suppression had virologic measurements of **plasma HIV RNA by single copy assay (SCA), PBMC-associated HIV DNA and RNA (CA-DNA, CA-RNA), and intact proviral DNA (IPDA).**

Immunologic measurements included **soluble markers IL-6, IP-10, neopterin, sCD163, sCD14, and TNF;** and cellular markers **CD38, HLA-DR, Ki-67, and PD-1.**

As well as plasma levels of **IL-1RA, IL-4, IL-10, IL-11, IL-13, CCL-22, and TGFβ** as measured by ELISA or Luminex.

All measurements of HIV persistence, inflammation, and T cell activation were performed on blood samples while on ART. A5321 study entry occurred at a median of 7 years after initiation of ART (**Table 1**). Participant characteristics were assessed at the timepoint that virologic and inflammatory/activation markers were measured.

Table 1 Participant Characteristics

		Total (N=98)
Age at A5321 entry (years)	Median	46
	Q1, Q3	37, 53
	Min, Max	23, 74
Sex	Male	77 (79%)
	Female	21 (21%)
Race/ethnicity	White Non-Hispanic	50 (51%)
	Black Non-Hispanic	18 (18%)
	Hispanic (Regardless of Race)	28 (29%)
	Asian, Pacific Islander	
	American Indian, Alaskan Native	2 (2%)
	More than one race	
Years on ART at A5321 entry	Median	6.7
	Q1, Q3	4.4, 7.5
	Min, Max	4.2, 14.8
Height (cm)	Median	174
	Q1, Q3	167.6, 180.3
	Min, Max	152.0, 200.2
Weight (kg)	Median	81.6
	Q1, Q3	70.8, 94.1
	Min, Max	46.3, 149.0
Pre-ART CD4⁺ T cell count (cells/mm³)	Median	289
	Q1, Q3	162, 379
	Min, Max	8, 981
A5321 entry CD4⁺ T cell count (cells/mm³)	Median	682
	Q1, Q3	518, 840
	Min, Max	149, 1,413
Pre-ART CD8⁺ T cell count (cells/mm³)	Median	796
	Q1, Q3	637, 1,200
	Min, Max	196, 2,566
A5321 entry CD8⁺ T cell count (cells/mm³)	Median	705
	Q1, Q3	531, 926
	Min, Max	268, 2,065

Table 1 *contains participant characteristics at pre-ART and A5321 study entry for participants with at least one HIV persistence measure.*

Table 2 Associations between measures of HIV-1 persistence and Th2 or anti-inflammatory cytokines

	Total HIV-1 DNA n = 95		CA-RNA n = 90		Plasma SCA n = 95		IPDA n = 48	
	r	p	r	p	r	p	r	p
IL-4	0.14	0.18	0.16	0.14	**0.2**	**0.06**	**0.37**	**0.009**
IL-10	0.04	0.69	0.07	0.49	-0.01	0.93	-0.02	0.91
IL-11	0.14	0.17	0.15	0.16	0.04	0.73	0.04	0.8
IL-13	0.05	0.65	0.14	0.18	0.16	0.13	**0.26**	**0.07**
IL-1RA	0.09	0.39	0.05	0.62	0.07	0.52	0.09	0.55
CCL-22	0.04	0.7	0.02	0.89	-0.13	0.19	0.04	0.78
TGF-β	0.14	0.18	0.11	0.29	-0.08	0.41	-0.06	0.67

Table 2 shows Spearman associations (r) of total HIV-1 DNA, cell-associated HIV-1 RNA, plasma HIV-1 RNA by single copy assay, and intact proviral DNA with plasma cytokine levels (pg/mL).

Results

98 participants (21 females) evaluated after a median of 7 years on ART (**Table 1**).

1. **Plasma levels of IL-4 were associated with the levels of intact proviral DNA (r=0.37, p=0.009),** and the other main Th2 cytokine, IL-13, showed a trend towards a positive association with intact proviral DNA (r=0.26, p=0.07)

2. There was also a trend towards an association of IL-4 levels with SCA HIV-1 RNA (r=0.2, p=0.06) but not total HIV-1 DNA (r=0.14, p=0.18) or CA-RNA (r=0.16, p=0.14).

3. **IL-1RA, IL-10, IL-11, IL-13, CCL-22, and TGFβ were not significantly associated with plasma SCA (N=95), total HIV-1 DNA (N=95), CA-RNA (N=90), or IPDA**

Conclusions

1. Th2 cytokines **IL-4 and IL-13** are associated with a higher frequency of intact proviral HIV-1 DNA but not total HIV-1 DNA,

2. Anti-Inflammatory cytokines including **IL-10** were not associated with intact or total HIV-1 DNA.

3. There was a weaker association of **IL-4 with residual viremia**, which likely arises from cells with intact proviruses.

4. This demonstrates the value of measuring intact proviral HIV-1 DNA when evaluating the relationship between immune responses and the HIV-1 reservoir.

5. **While the mechanistic link between IL-4 and IL-13 levels and cells carrying intact proviruses is undefined, these findings suggest that the dampening effect of Th2 cytokines on Th1 and Th17 responses could promote persistence of the HIV-1 reservoir.**

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