Inflammation in Acute HIV Infection Correlates with Blood and Gut CD4 T-cell Loss and HIV Viral Burden

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I. Introduction

- Biomarkers of inflammation, coagulation and fibrosis predict morbidity and mortality in patients with chronic HIV infection and predict disease progression in patients with primary HIV infection
- Evaluation of biomarkers in early stages of acute HIV infection (pre-seroconversion) could illuminate the timeline of viral reservoir establishment and immunologic damage
- Abundant CCR5$^+$ CD4 T cells in the gut-associated lymphoid tissue provide target cells resulting in intensive HIV replication with subsequent reservoir establishment and CD4 T cell depletion
- The sequence and timing of these events may determine the impact of early antiretroviral therapy and other interventions on viral reservoirs and therefore be critical for future eradication strategies

Objective: To study the levels of biomarkers in early acute HIV infection as defined by 4th generation staging and evaluate biomarker associations with the viral burden (plasma or colonic mucosa) and the early immunological insult (CD4 loss) in peripheral blood and colonic mucosa.

II. Methods

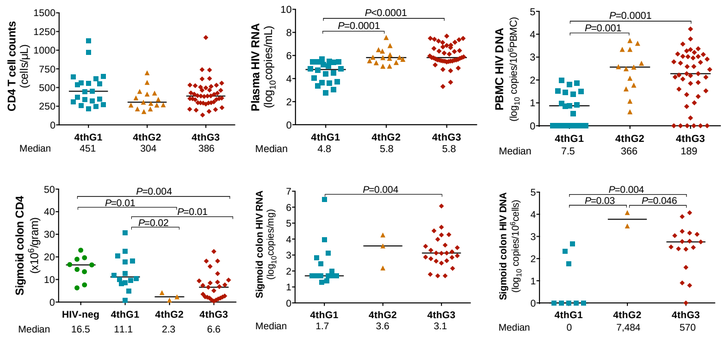
- **Population:**
  - Ongoing prospective, open-label study in Bangkok, Thailand
  - Subjects who seek voluntary HIV testing, many of whom are MSM or commercial sex workers
  - 78 subjects with acute HIV infection divided by 4th generation (4thG) staging
    - 4thG1 (EIA-, 3rdG EIA-)
    - 4thG2 (4thG +, 3rdG -)
    - 4thG3 (4thG +, 3rdG +, Western blot - or indeterminate).
  - 109 HIV-uninfected subjects
- **Assays:**
  - D-dimer by ELFA
  - Hyaluronic acid (HA), C-reactive protein (CRP), soluble CD14 (sCD14), IL-6, TNF, and intestinal fatty acid binding protein (I-FABP) by ELISA
  - Cell-associated HIV DNA and RNA in PBMCs and sigmoid colon by PCR and RT-PCR
- **Statistical analysis**
  - Comparisons between groups were performed using the Mann-Whitney U test
  - Correlations reflect the Spearman's coefficient
  - Robust regression and backward stepwise method were used to fit the multivariate model based on all variables for subjects with sigmoid colon CD4 T cell (n=42), except for sigmoid colon HIV DNA (n=27), TNF-alpha (n=29), IL-6 (n=29)

III. Results

Table 1. Baseline characteristics

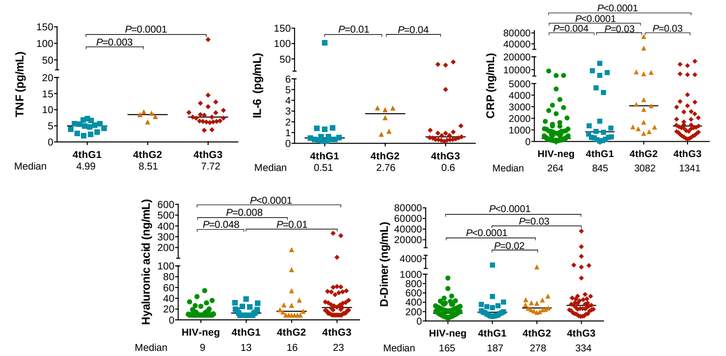
Baseline levels Median (IQR)	HIV-Uninfected (n=109)	Acute HIV-Infected (n=78)			
		All stage (n=78)	4thGstage 1 (n=20)	4thGstage 2 (n=15)	4thGstage 3 (n=43)
Age	27 (22 - 37)	28 (24 - 33)	28 (23 - 37)	30 (25 - 35)	27 (23 - 30)
Sex, male n(%)	84 (77.1)	72 (92.3)	18 (90.0)	14 (93.3)	40 (93.0)
Days since HIV exposure		16	12	16	18
CD4 T (cells/μL)	-	384 (293-525)	451 (316-592)	304 (265-426)	386 (298-506)
Plasma HIV RNA (log10copies/mL)	-	5.6 (5.1-6.3)	4.8 (3.7-5.4)	5.8**(5.4-6.4)	5.8** (5.5-6.7)

Figure 1. CD4 and HIV viral burden



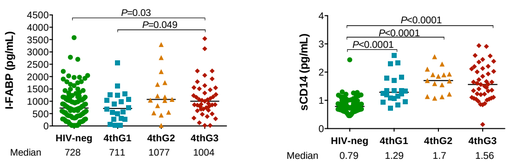
- Sigmoid colon CD4 T cell counts were significantly lower in 4thG2 and 4thG3 than in 4thG1 and HIV-uninfected subjects.
- Plasma HIV RNA and sigmoid colon HIV DNA levels were significantly higher in 4thG2 and 4thG3 than in 4thG1 subjects.

Figure 2. Biomarkers of inflammation and fibrosis



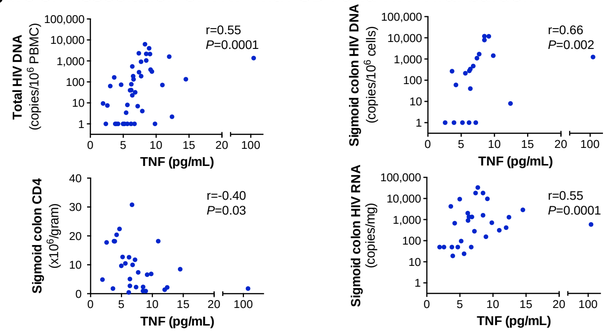
- Subjects with 4thG1 stage acute HIV infection had less inflammation than subjects with later stage acute HIV infection, as reflected by lower TNF, IL-6, CRP, hyaluronic acid, and D-dimer levels.
- Subjects with 4thG2 stage acute HIV infection had higher IL-6 and CRP levels than subjects with earlier or later stage acute HIV infection.

Figure 3. Biomarkers of intestinal damage and microbial translocation



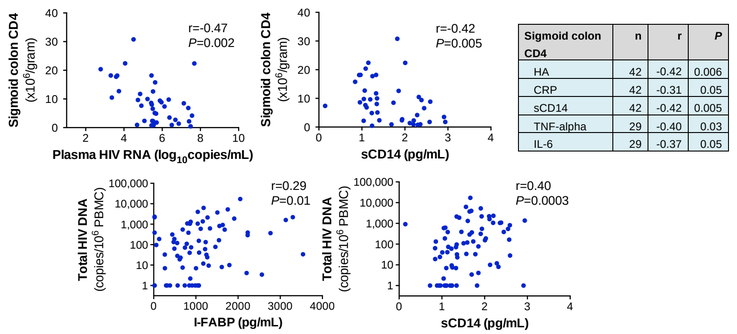
- Intestinal fatty acid binding protein (I-FABP) levels were significantly higher in 4thG3 stage HIV infection than subjects with 4thG1 stage infection and HIV-negative subjects.
- Soluble CD14 (sCD14) levels were significantly increased at all stages of acute HIV infection.

Figure 5. Association of TNF with CD4 and HIV viral burden



High circulating TNF levels are associated with high plasma HIV RNA (r=0.62, P<0.001), high HIV DNA in PBMCs and the sigmoid colon, high HIV RNA in the sigmoid colon, and low CD4 T cells in the sigmoid colon.

Figure 6. Intestinal CD4 depletion, HIV, inflammation, and MT



Sigmoid colon CD4	n	r	P
HA	42	-0.42	0.006
CRP	42	-0.31	0.05
sCD14	42	-0.42	0.005
TNF-alpha	29	-0.40	0.03
IL-6	29	-0.37	0.05

- Sigmoid CD4 T cell depletion is associated with greater viremia, microbial translocation (MT), and inflammation.
- Higher viremia and sCD14 levels predict greater sigmoid CD4 T cell depletion, even after adjusting for peripheral blood CD4 T cell counts.
- High HIV DNA in PBMCs is associated with high intestinal fatty acid binding protein (I-FABP), reflecting enterocyte loss, and high sCD14 levels, reflecting LPS-induced monocyte activation.

IV. Conclusions

- The earliest (4thG1) stage of acute HIV infection is associated with lower cellular HIV burden in both peripheral blood and colonic mucosa and less immunologic damage (CD4 loss) in colonic mucosa.
- Biomarkers of inflammation and microbial translocation increase early in acute HIV infection and are lowest in the 4thG1 and often highest at the 4thG2 stage of acute HIV infection.
- Plasma HIV RNA and sCD14 levels are independently associated with sigmoid CD4 T cell depletion, suggesting ongoing viral replication accelerates CD4 depletion and LPS-induced monocyte activation.
- Together, these data suggest that days matter when halting viral replication to reduce the viral reservoir, intestinal CD4 depletion, and inflammation.

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