Colon Polyps in HIV Patients Associated with Systemic Immune Activation

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Abstract

Background:
As human immunodeficiency virus (HIV) patients now commonly survive for decades, the sequelae of prolonged inflammation, including gastrointestinal tract cancers, has emerged. Data support an increased prevalence of colon neoplasia in HIV infected patients with these tumors occurring at younger ages than expected. Damage to the gut, with increased microbial translocation and inflammation are central to HIV pathogenesis. Our study aimed to identify the role of persistent immune activation, microbial translocation, and inflammation associated with adenomatous colon polyps among patients with HIV infection.

Study Design:
This study was performed as a parallel group design with three arms. Patients having completed colonoscopy at Jackson Memorial Hospital (Miami, FL) were prospectively enrolled into one of three groups: HIV positive with adenomatous polyps, HIV positive without adenomatous polyps, and HIV negative with adenomatous polyps. Plasma was collected after review of colonoscopy results and analyzed with a pro-inflammatory cytokine multiplex panel. Peripheral blood mononuclear cells were processed for flow cytometry. Differences among groups were analyzed by ANOVA or Fisher's exact test according to data distribution. Plasma microbiome analysis was performed following nucleic acid isolation, amplification, library preparation, and sequencing using SecondGenomeR.

Results:
36 patients were enrolled (n=3 excluded with inflammatory polyps). All patients with HIV were virally suppressed. No difference in absolute CD4 and CD8 counts was noted between HIV patients with and without polyps. Compared to HIV negative patients with polyps (n= 10), HIV patients with polyps (n= 10) had higher frequencies of activated T cells (CD38+ DR+) involving CD4 T cells (1.34 vs 4.00 p=0.017, resp), as well as CD8 T cells (4.67 vs 11.19, p=0.022, resp). Levels of several cytokine markers of Immune activation differed among groups, including IL17, IL1A, and IP10. sCD14, in particular, was elevated in HIV patients with adenomas (2252ng/mL) as compared to HIV patients without polyps (n=13); 1717ng/mL, p=0.008) and non-HIV patients (1691ng/mL, p=0.008). Microbiome analyses are ongoing.

Conclusions:
Virally suppressed HIV patients with colon adenomas have evidence for persistent systemic immune activation. Further work identifying a link between these findings, including the potential for microbial translocation, is ongoing.

Background

- **As human immunodeficiency virus (HIV) patients now commonly survive for decades with this chronic infection, the sequelae of prolonged inflammation, including GI tract cancers, has emerged.[1-2]**
- **Data support an increased prevalence of colon neoplasia in HIV infected patients with these tumors occurring at younger ages than expected.[3]**

Methods

- **Prospective, parallel group design**
- **3 groups:**
  - **HIV negative (HIV -) with adenoma**
  - **HIV positive (HIV +) without adenoma**
  - **HIV positive (HIV +) with adenoma**
- **Inclusion criteria**
  - **Informed consent**
  - **Completed high quality colonoscopy at Jackson Memorial Hospital or University of Miami/Sylvester Comp Cancer Center**
  - **HIV status confirmed**

Blood Draw

- **Plasma and peripheral blood mononuclear cells**
- **Pro-inflammatory cytokine multiplex panel for activation markers (Milliplex, EMD Millipore) acquired MAGPIX instrument (Luminex)**
  - **CD4 and CD8 T cells**
  - **Immune activation markers HLADR and CD38**

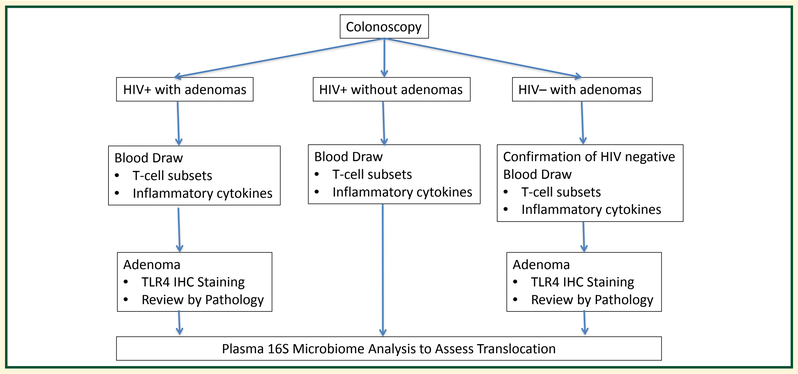
Adenomas

- **Immunohistochemical (IHC) staining for toll-like receptor 4 (TLR4) on adenomas, with recording intensity/percent staining**
- **Review by GI pathologist**

Plasma 16S Community Analysis

- **Next-generation sequencing of genomic DNA**
- **Analyzed with SecondGenomeR (San Francisco, CA)**

Recruitment/Patient Flow

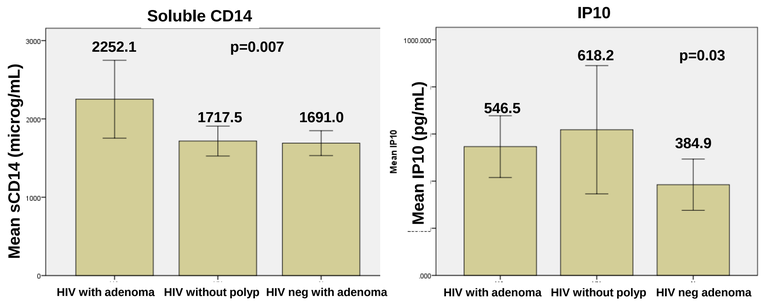


Results

Table 1. Study Population

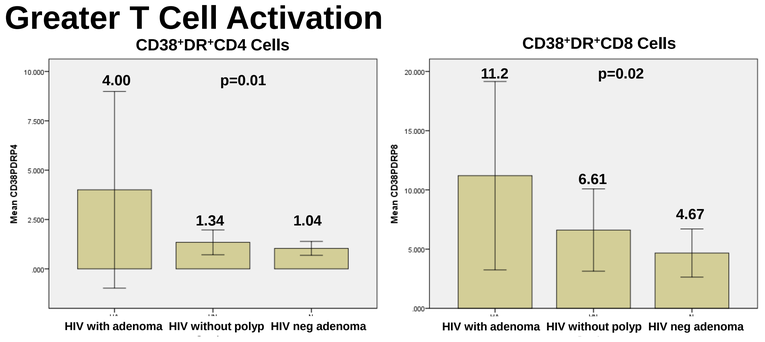
	HIV Negative with Adenomas	HIV Positive without Polyps	HIV Positive with Adenomas	p-value
Patients (n)	10	13	10	N/A
Gender (Male)	8	8	6	0.65
Age (Years)	57.4	54.8	62.1	0.85
BMI (kg/m2)	30.6	25.4	26.5	0.005*
Race (Black)	7	6	7	0.26
Ethnicity (Hispanic)	8	2	6	0.0035*
Indication (Screening)	2	11	7	0.0055*
Aspirin use	2	2	4	0.47
NSAID use	1	3	3	0.67
Diabetes	3	0	3	0.09
Dyslipidemia	4	8	8	0.18
Smoking (current)	0	5	6	0.19
Polyp burden (>2)	0	N/A	4	0.91
Polyp size (>1cm)	2	N/A	1	0.39
Viral load (copies/mL)	N/A	690.1	47.9	0.0003*
Absolute CD4	N/A	723.5	874.1	0.47
Absolute CD8	N/A	796.6	1125.9	0.05

Figure 1. HIV Patients with Adenomas Have Greater Plasma Biomarkers of Immune Activation



- **HIV patients with adenomas had elevated plasma sCD14 markers, as compared with HIV patients without adenomas (p=0.0076) and HIV negative patients with adenomas (p=0.0079).**
- **Lipopolysaccharide (LPS) levels were similar among groups (mean 191.6, 174.6, and 179,6; p=NS) for HA, HN, and non-HIV patients, respectively.**

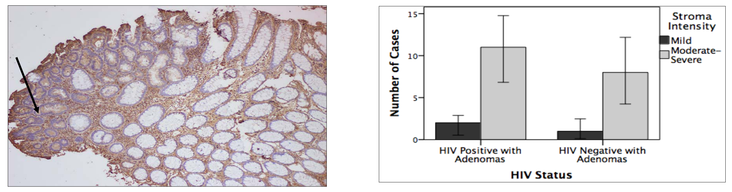
Figure 2. HIV Patients with Adenomas Have Greater T Cell Activation



- **HIV patients with adenomas had evidence for enhanced immune activation, with greater CD38+DR+ CD4 and CD8 cells than non-HIV patients with adenomas (p=0.017 and p=0.022, respectively). HIV patients will adenomas also demonstrated enhanced T-cell activation when compared to HIV patients without adenomas (p=0.010).**

Results

Figure 3. TLR4 Staining Strong in the Stromal Compartment of Adenomas in HIV Pos and Neg



- **Moderately high cytoplasmic and membranous staining for TLR4 in dysplastic/adenomatous glands (black arrow). H&E stain 100x (Left panel)**
- **Mean TLR4 stroma intensity in HIV+ colon adenomas vs. HIV – colon adenomas were 2.92 and 3.22, respectively (p-value 0.309) (Right panel)**

Ongoing Microbiome Analysis

- **Plasma Samples**
- **Assess for genomic evidence of bacterial translocation**
- **Sample isolation, library preparation, and 16S rRNA gene sequencing with Illumina MiSeq platform completed for all samples**
- **Plasma microbiome analysis and sequence processing underway**
  - **Hierarchical clustering of samples**
  - **Operational Taxonomic Unit selection**
  - **Diversity metrics**
    - **Alpha (within sample)**
    - **Beta (sample-to-sample)**
  - **Ordination and clustering**
- **Full analysis expected May 2017**

Summary and Conclusions

- **Virally suppressed HIV patients with colon adenomas have evidence for persistent systemic immune activation**
  - **T-cell immune activation, in particular, is evident in these populations (CD38+DR+ CD4 and CD8 cells)**
- **Soluble CD14 is elevated in the plasma of HIV patients with adenomas**
  - **Plasma LPS level not elevated in HIV patients with adenomas**
- **TLR4, the cell-surface receptor for LPS, intensely stains in the stromal compartment of adenomas in both HIV positive and negative patients**
- **Microbiome analysis will confirm whether there is genomic evidence for bacterial translocation associated with the presence of adenomas in patients with HIV**

References

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Acknowledgments

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