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HIV-associated mucosal gene expression: region-specific alterations

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Abstract

Objective—Despite the use of HAART to control HIV, systemic immune activation and inflammation persists with the consequence of developing serious non-AIDS events. The mechanisms that contribute to persistent systemic immune activation have not been well defined. The intestine is the major source of “sterile” inflammation and plays a critical role in immune function; thus, we sought to determine whether intestinal gene expression was altered in virally controlled HIV-infected individuals.

Design and methods—Gene expression was compared in biopsy samples collected from HIV-uninfected and HIV-infected individuals from the ileum, right colon (ascending colon), and left colon (sigmoid). Affymetrix gene arrays were performed on tissues and pathway analyses were conducted. Gene expression was correlated with systemic markers of intestinal barrier dysfunction and inflammation and intestinal microbiota composition.

Results—Genes involved in cellular immune response, cytokine signaling, pathogen-influenced signaling, humoral immune response, apoptosis, intracellular and second messenger signaling, cancer, organismal growth and development, and proliferation and development were upregulated in the intestine of HIV-infected individuals with differences observed in the ileum, right, and left colon. Gene expression in the ileum primarily correlated with systemic markers of inflammation

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Conflicts of interest

The authors have no conflicts of interest to disclose.

(e.g., *IL7R*, *IL2*, and *TLR2* with serum TNF) whereas expression in the colon correlated with the microbiota community (e.g., *IFNG*, *IL1B*, and *CD3G* with Bacteroides).

Conclusion—These data demonstrate persistent, proinflammatory changes in the intestinal mucosa of virally suppressed HIV-infected individuals. These changes in intestinal gene expression may be the consequence of or contribute to barrier dysfunction and intestinal dysbiosis observed in HIV.

Keywords

gut leakiness; HIV; immune function; inflammation; intestine gene expression; microbiota

Introduction

The gastrointestinal tract is a major target for HIV infection leading to significant CD4⁺ T-cell loss and increased levels of inflammatory mediators [1]. Despite the use of HAART to control viral loads, systemic immune activation and inflammation persists resulting in serious non-AIDS events including, cardiovascular disease, neurocognitive decline, and cancer [2,3]. The mechanisms that contribute to persistent systemic immune activation and inflammation have not been well defined. Data suggest that microbial translocation products coming from the gastrointestinal tract (e.g., lipopolysaccharide, LPS) may be a major contributor to the persistent stimulation of the innate immune system leading to enhanced production of inflammatory mediators, especially IL6 [4,5]. Increased levels of systemic IL6 are correlated with augmented risk of serious non-AIDS events in virally suppressed HIV-infected individuals; thus, a better understanding of the mechanisms leading to persistent stimulation of the innate immune system may direct therapeutic strategies.

Alterations in the intestinal microbiome and mucosal immune system responses to these alterations are likely driving the systemic inflammation observed in HIV-infected individuals. There is increasing evidence that changes in the composition (dysbiosis) and decreased diversity of the host intestinal microbiome may contribute to the pathogenesis of a number of inflammatory-mediated diseases such as inflammatory bowel disease, metabolic disease, and liver disorder associated with chronic alcohol consumption [6–8]. Recently, several publications have demonstrated intestinal dysbiosis in HIV-infected individuals, independent of antiretroviral therapy status [9–13]. Intestinal dysbiosis can impact intestinal barrier function, and indeed, intestinal hyper-permeability is evident in HIV-infected individuals allowing proinflammatory bacteria/bacterial products into the systemic circulation [11,14,15]. Despite these findings, there have not been extensive studies evaluating the relationship between host innate and adaptive immune changes with epithelial barrier function, host microbiome, and systemic inflammatory and microbial translocation markers in HIV-infected individuals.

The current study was undertaken to begin to fill this gap in our knowledge by comparing changes in expression of immune and epithelial barrier host genes in the gastrointestinal tract (ileum, right colon, and left colon) in virally controlled, treated HIV-infected individual and HIV-uninfected matched controls. We also correlated changes in the gastrointestinal tract microbiome with systemic markers of inflammation and microbial translocation.

Materials and methods

Study participants

Colonic biopsy samples collected during colonoscopy (terminal ileum, right colon (ascending colon, above the cecum), left colon (sigmoid)) were obtained from the tissue bank at Rush University Medical Center, Division of Gastroenterology after obtaining study approval from the Rush University Medical Center institutional review board. All individuals gave written and verbal informed consent prior to tissue collection under the tissue bank institutional review board at Rush University. Samples from HIV-infected individuals were matched with healthy control tissue such that there were no significant differences between HIV-infected and healthy control individuals with regard to age, sex, or race. The CD4⁺ cell counts and viral loads for HIV-infected study participants have been reported previously [9]. Important for our study were as follows: all individuals were chronically infected with HIV and receiving care from a physician, all HIV-infected individuals were on HAART, and [13] 15 of 18 HIV-infected individuals had viral loads that were below the detection level of the assay. Subject data are shown in Table 1.

Assessment of gene expression

Gene expression analysis was performed via an Affymetrix (Santa Clara, California, USA) custom QuantiGene 55 Plex assay. The 55 genes measured in this study included five housekeeping genes [hypoxanthine phosphoribosyltransferase (*HPRT1*), glyceraldehyde 3-phosphate dehydrogenase (*GAPDH*), ribosomal protein, large, P0 (*RPLP0*), beta-actin (*ACTB*), and succinate dehydrogenase complex, subunit A (*SDHA*)] and 50 genes of interest (Table 2). Samples were prepared as tissue homogenates using Affymetrix lysis buffer and processed according to the manufacturer's instructions and mRNA levels were determined using a Luminex-based custom multiplex bead array (Affymetrix).

Background values were subtracted from the raw intensity readings for each sample. Values that were less than or equal to 0 were set to 1 (floor effect), and all values were log 2 transformed. These values were then normalized to the average of two housekeeping genes. Selection of two housekeeping genes was based on a scree plot of eigenvalues with two variables being consistent with an inflection break in the scree plot; thus, *ACTB* and *GAPDH* were selected [16]. Some gene expression data were below the level of background; thus, genes that did not have more than 50% of the samples in the detectable range (above background) were excluded based on unreliable gene expression data. Based on these criteria the following genes could not be assessed and were removed from all analyses: CD3 antigen (*CD3C*), toll-like receptor 9 (*TLR9*), tight junction protein 1 (*TJP1*, *ZO1*), tight junction protein 2 (*TJP2*, *ZO2*), Claudin 2 (*CLDN2*), Claudin 3 (*CLDN3*), transforming growth factor, beta 1 (*TGFB1*), chemokine (C-X-C motif) ligand 12 (*CXCL12*), thymic stromal lymphopoietin (*TSLP*), and toll interacting protein (*TOLLIP*). Likewise, patient samples that did not have at least 50% of genes in the detectable range (above background) were excluded based on unreliable gene expression data. Based on these criteria, samples from one healthy control (ileum) and 12 HIV-infected individuals (four ileum, four right colon, and four left colon) were not included in the analysis. Samples were analyzed via Significance Analysis of Microarrays (SAM) software add-on for Microsoft Excel to select

biologically significant differences in gene expression between groups. The criteria selected for SAM analysis were median false discovery rate 0.03% or less and change more than 1.5-fold.

Pathway analysis was conducted using Ingenuity Pathway Analysis (IPA 8.8; Ingenuity Systems, Inc., Redwood City, California, USA). Significance of Canonical Pathways is calculated by Fisher's exact test and Downstream Effects Analysis identifies functions that are expected to increase or decrease based on expected casual effects between genes and functions using a z score algorithm.

Markers of intestinal barrier function and intestinal hyperpermeability

Serum intestinal fatty acid binding protein (IFABP) is a validated marker of apoptosis-mediated cell death and an indicator of intestinal barrier function [17,18]. Serum samples from healthy controls and HIV-infected individuals were analyzed for IFABP using an Elisa kit according to manufactures instructions (Hycult Biotech Inc, Plymouth Meeting, Pennsylvania, USA). Serum LPS binding protein (LBP) is a protein involved in the acute phase response to LPS and is a marker of intestinal leakiness [19,20]. Serum samples from healthy controls and HIV-infected individuals were analyzed for LBP according to manufacture instructions (Hycult Biotech).

IFABP was analyzed using a nonparametric Mann–Whitney test as group variances were significantly different, and LBP was analyzed using an independent t test using GraphPad Prism version 5.0 (GraphPad Software, San Diego, California, USA).

Microbiota analysis and selection of taxon

These methods have been described in our previous publication [9]. In brief, DNA was extracted using a commercially available kit and adequacy of DNA content was verified with flurometric quantitation and samples with inadequate DNA content were not sequenced. 5'GAGTTTGATCNTGGCTCAG3' forward primer and 5'GNTTTACNGCGGCKGCTG3' reverse primers were used to pyrosequence the 16S rDNA on a 454 GS FLX platform, with barcoding, using titanium kits. Custom C# and python scripts and python scripts in the Quantitative Insights Into Microbial Ecology (QIIME) software pipeline (VirtualBox versions 1.5 and 1.6) were used to process the sequencing files. The sequence outputs were filtered for low-quality sequences (defined as any sequences that are <200 bp or >1000 bp, sequences with any nucleotide mismatches to either the barcode or primer, sequences with homopolymer runs >6, sequences with an average quality score of <25, sequences with ambiguous bases >6) and were truncated at the reverse primer. Sequences were de-noised using USEARCH, and chimera checked with UCHIME and Chimera Slayer. Operational taxonomic units were picked using uclust at 97% similarity, and representative sequences were generated. Sequences were aligned with PyNAST and taxonomy assignment was performed in Qiime 1.6VB against the Qiime 1.6 version of Greengenes database using the RDP classifier at an 80% bootstrap value threshold (see [9] and references therein). Indicator species analysis was conducted on logtransformed data to determine between group differences (i.e., Control vs. HIV-infected).

In our previous publication, 10 taxon were identified as being significantly increased in HIV-infected individuals including p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Prevotellaceae; g_Prevotella ($P < 0.00$) and 20 taxon were significantly decreased including p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Roseburia ($P = 0.02$) and p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides ($P < 0.00$) [9].

Correlations of gene array data with systemic markers and microbiota

Gene array data were correlated with IFABP, LBP, sCD14, serum IL6, serum, TNF, serum lipoteichoic acid (LTA), and mucosal-associated Roseburia, Bacteroides, and Prevotella to determine whether gene expression was correlated with changes in intestinal barrier function, intestinal hyperpermeability, systemic markers of inflammation, or mucosal-associated microbiota. Correlations were conducted using GraphPad Prism version 5.0 (GraphPad Software).

Results

Subject/sample characteristics

A total of 33 individuals (18 healthy controls, 15 HIV-infected individuals) were included in the analysis of RNA expression with a total of 90 biopsy samples being collected (Table 1). Out of the 90 biopsy samples, 67 samples were used for the analysis with the remaining 23 samples being excluded due to low gene expression levels. A total of 19 ileum biopsies (11 control, 8 HIV), 31 right colon biopsies (17 control, 14 HIV), and 27 left colon biopsies (18 control, 9 HIV) were included in the analysis. There were no differences in age, sex, BMI, or race between healthy control individuals and HIV-infected individuals (Table 1).

Gene expression pattern in HIV-infected individuals

SAM analysis of all samples (combined ileum, right colon, and left colon) identified 20 genes that were significantly upregulated in HIV-infected individuals compared with healthy controls (1.5-fold change in expression and false discovery rate < 0.03 ; Table 2).

Upregulated genes included genes regulating and involved in immune system function (*IL1B*, *IL2*, *IL6*, *IL7R*, *IL8*, *IL17A*, *IL22*, *IFNG*, *IFNB1*, *CD3G*, *CCL2*, *CCL5*, *CCR5*, *TLR2*, *TLR4*, *TLR5*, *TLR7*, *IRAK2*) and genes regulating the expression of cell-cell junction proteins (*DSG3* and *CLDN1*) (Table 2) indicating that persistent inflammatory responses and innate immune activation are found in the gastrointestinal tract of HIV-infected individuals, even when on suppressive antiretroviral therapy.

IPA 8.8 revealed the top 15 Canonical Pathways that were impacted in the HIV-infected gastrointestinal samples (Table 3): altered T-cell and B-cell signaling in rheumatoid arthritis; role of pattern recognition receptors in recognition of bacteria and viruses; communication between innate and adaptive immune cells; triggering receptor expressed on myeloid cells 1 (TREM1) signaling; role of cytokines in mediating communication between immune cells; role of macrophages, fibroblasts, and endothelial cells in rheumatoid arthritis; role of hypercytokinemia/ hyperchemokinemias in the pathogenesis of influenza; toll-like receptor signaling; differential regulation of cytokine production in macrophages and T-helper cells

by IL17A and IL17F; differential regulation of cytokine production in intestinal epithelial cells by IL17A and IL17F; high-mobility group protein B1 (HMGB1) signaling; glucocorticoid receptor signaling; colorectal cancer metastasis signaling; crosstalk between dendritic cells and natural killer cells; and NF- κ B signaling. These canonical pathways fall under the signaling pathway categories of cellular immune response, pathogen-influenced signaling, cytokine signaling, humoral immune response, cellular stress and injury, and organismal growth and development.

A downstream effects analysis predicts the effect of changes in gene expression on biological processes and diseases based on expected causal effects between genes and function derived from the literature compiled in the Ingenuity Knowledge Base. Diseases and functions highly implicated were in the categories of inflammatory response (cell-mediated response, inflammatory response, Th1 immune response), cell-to-cell signaling (e.g. activation of leukocytes, activation of myeloid cells, response of mononuclear leukocytes), cellular development (maturation of leukocytes, maturation of phagocytes), and infectious disease (bacterial infection) (Supplementary Table 1, <http://links.lww.com/QAD/A636>).

Gene expression patterns: ileum vs. right colon vs. left colon

Analysis of each region separately (ileum, right colon, left colon) shows overlapping but distinct changes associated with each region. Gene expression values in each region are shown in Supplementary Table 2, <http://links.lww.com/QAD/A636> with similarities and differences highlighted in Fig. 1. Most genes [14] were significantly upregulated throughout the intestine indicating a large proportion of genes are similarly regulated independent of region. Upregulation of the toll-like receptors in HIV-infected individuals was limited to the colon, wherein most of intestinal bacteria and viruses reside, with *TLR5* increased in the right colon of HIV-infected samples (2.28-fold), and *TLR2* and *TLR7* increased in both the right and left colon (*TLR2*: 2.04 and 1.89; *TLR7*: 1.72 and 1.89, respectively). Some of the differences in gene expression by site may be due to changes in inflammatory cell infiltration; thus, we compared *CD3G* expression in both healthy control individuals and HIV-infected individuals and found that *CD3G* expression was statistically indistinguishable in each region of the intestine (Supplementary Figure 1, <http://links.lww.com/QAD/A636>, one-way analysis of variance (ANOVA): healthy controls $P = 0.071$ and HIV-infected individuals $P = 0.899$); thus, the changes we observed in gene expression between regions of the intestine seem to be the result of a gene-specific changes rather than a consequence of changes in the overall number of immune cells present.

Analysis of the top Canonical Pathways affected by HIV revealed that four categories were shared between all three regions of the intestine: role of pattern recognition receptors in recognition of bacteria and viruses, communication between innate and adaptive immune cells, TREM signaling, and role of cytokines in mediating communication between immune cells. Rounding out the top five pathways for each site were toll-like receptor signaling in the ileum, differential regulation of cytokine production in macrophages and T-helper cells by IL17A and IL17F in the right colon, and HMGB1 signaling in the left colon (Supplementary Table 3, <http://links.lww.com/QAD/A636>).

Systemic markers of intestinal leakiness and inflammation

HIV-infected individuals had evidence of increased intestinal permeability compared with HIV-uninfected controls. IFABP was not statistically different between HIV-infected individuals (1461 ± 220.2 , $n = 18$) and healthy controls (1510 ± 385.8 , $n = 19$) ($P = 0.323$, Fig. 2); however, HIV-infected individuals (8184 ± 1721 , $n = 18$) had significantly lower serum LBP than healthy controls ($13\,600 \pm 1700$, $n = 20$) (t test $P = 0.032$, $t(36) = 2.236$), indicative of increased gut permeability to LPS and increased exposure of liver to gut-derived LPS. Despite this change in permeability, neither LBP nor IFABP correlated with gene expression in the intestine (Supplementary Table 4, <http://links.lww.com/QAD/A636>). We previously reported that LTA (a component of the cell wall of Gram-positive bacteria) is increased in HIV-infected individuals [9]. In the current study, we found that LTA correlated with only CCL5 in the right colon ($R = 0.46$, $P = 0.04$, Supplementary Table 4, <http://links.lww.com/QAD/A636>).

Intestinal leakiness is associated with intestinal and systemic inflammation; thus, we correlated serum sCD14, IL6, and TNF with gene expression in the intestine. sCD14 was increased in the HIV-infected individuals; however, neither sCD14 nor serum IL6 correlated with any gene (Supplementary Table 4, <http://links.lww.com/QAD/A636>). Serum TNF negatively correlated with eight genes, mostly in the ileum, the primary site of gastrointestinal lymphoid tissue (*IL17A* $R = -0.70$, $P = 0.03$; *IL2* $R = -0.69$, $P = 0.04$; *IL22* $R = -0.72$, $P = 0.03$; *IL8* $R = -0.69$, $P = 0.04$; *IL6* $R = -0.72$, $P = 0.03$; *CCL2* $R = -0.72$, $P = 0.03$; *TLR2* $R = -0.69$, $P = 0.04$), with one significant correlation in the left colon (*IL7R* $R = -0.51$, $P = 0.04$) (Supplementary Table 4, <http://links.lww.com/QAD/A636>).

The intestinal microbiome

We previously demonstrated that the intestinal microbiome is altered in HIV-infected individuals compared with healthy controls [9]. Thus, we sought to determine whether changes in the intestinal microbiota correlate with gene expression in the intestine. We selected three bacterial taxa that are significantly different in healthy control and HIV-infected individuals including Prevotella (p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Prevotellaceae; g_Prevotella; increased in HIV), Bacteroides (p_Bacteroidetes; c_Bacteroidia; o_Bacteroidales; f_Bacteroidaceae; g_Bacteroides; decreased in HIV), and butyrate producing Roseburia (p_Firmicutes; c_Clostridia; o_Clostridiales; f_Lachnospiraceae; g_Roseburia; decreased in HIV) [9]. Prevotella was not correlated with any gene and Roseburia correlated only with *IRAK2* ($R = -0.42$, $P = 0.049$) in HIV-infected individuals. Bacteroides significantly correlated with a number of genes including: *CD3G* ($R = -0.39$, $P = 0.004$), *IL1B* ($R = -0.42$, $P = 0.002$), *IFNG* ($R = -0.29$, $P = 0.03$), *CCR5* ($R = -0.28$, $P = 0.04$), *CCL5* ($R = -0.37$, $P = 0.01$), *IL7R* ($R = -0.30$, $P = 0.03$), *IL2* ($R = -0.27$, $P = 0.049$), and *IRAK2* ($R = -0.38$, $P = 0.004$) when control and HIV-infected individuals were pooled (ileum, right, and left colon combined). *TLR7* correlated with Bacteroides ($R = 0.42$, $P = 0.045$) only in HIV-infected individuals (ileum, right colon, and left colon). The majority of the microbiota reside in the colon and when evaluated by region and indeed, the majority of the Bacteroides correlations were in the colon with *CD3G* ($R = -0.55$, $P = 0.007$), *IL1B* ($R = -0.56$, $P = 0.006$), *CCL5* ($R = -0.51$, $P = 0.01$), *IL7R* ($R =$

-0.49 , $P = 0.02$), *IRAK2* ($R = -0.44$, $P = 0.03$) in the right colon and *IL1B* ($R = -0.45$, $P = 0.04$) in the left colon (Supplemental Table 5, <http://links.lww.com/QAD/A636>).

Discussion

We evaluated gene expression in the gastrointestinal tract of HIV-infected individuals compared with healthy controls. Proinflammatory pathways (e.g. *IL1B*, *IL6*, *IFNG*) and innate immune signaling genes (e.g. *TLR2*, *TLR4*, *TLR5*, *TLR7*, *TLR8*) were increased in tissue from HAART-suppressed HIV-infected individuals. We also demonstrated upregulation of intestinal barrier genes *CLDN1* and a decrease in *CLDN4* in HIV-infected individuals, changes that are consistent with persistent loss of gut epithelial barrier function as well as a concurrent decrease in serum levels of LBP in HIV-infected individuals indicative of leaky gut. Previous studies evaluating gene expression in HIV-infected individuals have focused on peripheral blood and lymph node tissue with only a limited number of reports evaluating gene expression in gastrointestinal tract tissue. These limited studies have demonstrated elevated inflammatory gene expression in jejunum tissue from simian immunodeficiency virus (SIV)-infected rhesus macaques [21] and HIV-1-infected patients [22]. Our study is unique in that we were able to assess regional differences in gene expression between biopsies collected from the ileum, right colon (ascending colon), and left colon (sigmoid) in healthy control and HIV-infected individuals.

We observed significant overlap between each site in the intestine; however, there were important differences. There was a significant upregulation of *IL23A* (an important stimulator of Th17 signaling) in the left colon suggesting a Th17 inflammatory effect of HIV in the colon. Th17 is also involved in the pathogenesis of inflammatory bowel disease, which is known to have a high degree of mucosal inflammation, intestinal barrier dysfunction, and dysbiosis [23,24]; thus, the Th17 profile suggests a high degree of inflammation. Indeed, gene expression in the gastrointestinal tract correlated with systemic inflammation. Serum TNF levels were correlated to innate and adaptive immune function and inflammatory markers in the ileum including *IL17A*, *IL2*, *IL22*, *IL8*, *IL6*, *CCL2*, *TLR2*. This finding may reflect the fact that the majority of lymphoid tissue is localized to the ileum as compared with the colon whereas only *IL7R* in the left colon correlated with serum TNF. Several of the top canonical pathways affected in the HIV-infected intestine were those associated with rheumatoid arthritis. This observation again indicates persistent inflammation is a feature of the HIV-infected intestine.

With the exception of *TLR4*, changes in TLR expression were limited to the colon, a finding that is consistent with microbiota being found in greater abundance in the colon relative to the small intestine. TLRs are important for maintaining intestinal epithelial homeostasis, and TLR receptor expression is impacted by the microbiota [25,26]. It is not surprising then that altered TLR expression has been observed in diseases that are known to have intestinal dysbiosis, suggesting they may be an exaggerated host defense to intestinal microbiota. For example, inflammatory bowel disease is associated with altered expression of *TLR3* and *TLR4* [27]. Intestinal dysbiosis in HIV-infected individuals, reported by our group and others [9–13], may have impacted TLR expression or vice versa as there is a significant amount of crosstalk that occurs between the intestinal microbiota and the host [28].

Gene expression in the intestine may impact important biological functions one of which is intestinal barrier integrity and the inflammatory processes that can be initiated by barrier dysfunction, particularly in the presence of intestinal dysbiosis. Of the three tight junction markers that were included in the array, two were significantly altered in HIV-infected individuals, with the directionality of these changes being consistent with augmented intestinal hyperpermeability (i.e. increased *CLDN1* in all regions and decreased *CLDN4* in the left colon; Supplemental Table 2, <http://links.lww.com/QAD/A636>). Thus, our data do demonstrate changes in mRNA expression that is consistent with leakiness; however, these changes in tight junctional proteins did not correlate with systemic markers of bacterial translocation (i.e., serum LBP or serum LTA). mRNA levels do not necessarily always correlate with protein levels and protein levels may be inappropriately trafficked as has been observed in inflammatory bowel disease wherein *CLDN1* mRNA and protein are increased but is not trafficked to the cell surface wherein it can be functional [29]. Other studies have observed changes in the expression of tight junction and adherins junction genes associated with intestinal inflammation and, despite a lack of significant correlations, our studies are in line with these previous reports with *CLDN1* and *CLDN4* being significantly affected in a direction that is consistent with intestinal barrier dysfunction [30,31]. Future studies evaluating protein levels (i.e. Western blotting) and protein localization (i.e., immunohistochemistry) will be required to determine the contribution of specific tight junction components to HIV-infected associated intestinal barrier dysfunction. Another possibility is that serum markers of gut leakiness may correlate with the expression of tight junction components that were not included in our analysis. Lastly, it should be noted that serum IFABP is a marker of intestinal epithelial cell-associated intestinal leakiness (i.e. intestinal epithelial cell apoptosis) and since epithelial cell-associated intestinal leakiness and tight junctional associated hyperpermeability do not necessarily occur together then lack of significant correlations is not a surprise.

Changes in gene expression may be the consequence of an altered microbiome or vice versa. We have demonstrated significant intestinal dysbiosis among HIV-infected individuals all of whom were receiving HAART compared with controls [9]. We focused our current analysis on specific bacterial taxa that are significantly affected in HIV-infected individuals: Roseburia (a butyrate producing bacteria that has anti-inflammatory properties, reduced in HIV), Bacteroides (a beneficial commensal bacteria, reduced in HIV), and Prevotella (a potentially pathogenic bacteria, increased in HIV). We found only a limited number of correlations between the bacterial taxa and host gene expression with the vast majority of significant correlations being with Bacteroides. Bacteroides correlated with *CD3G*, *IL1B*, *IFNG*, *CCR5*, *IL7R*, *IL2*, and *IRAK2* when HIV-uninfected and HIV-infected individuals were combined, an effect that was limited to gene expression in the colon as well as a negative correlation observed with Bacteroides and *TLR7* in only HIV-infected individuals. The genes included those associated with inflammation (e.g. *IL1B*, *IFNG*) and innate immune signaling (e.g. *IRAK*, *TLR7*). Bacteroides and other short-chain fatty acid producing bacteria can potentially change gene expression through histone deacetylase inhibitor (HDACi) activity [32] that may account for the changes observed in our study. There have been limited studies in the HIV field linking changes in gastrointestinal tract microbiome (and their metabolites) with host gene expression, barrier integrity, and inflammatory

processes [12,15,33] and the link between these metabolic changes and inflammation and innate immune sensing genes require further study.

We demonstrated significant upregulation of inflammatory and innate signaling gene expression in the gastrointestinal tract of HAART-suppressed HIV-infected individuals compared with HIV-uninfected controls. Individuals included in this study were all HAART-treated; thus, it is difficult to determine what impact HAART may have had on gene expression. HAART has been shown to alter gene expression in peripheral blood mononuclear cells (PBMC), lymphatic tissue, and brain [34–39]; however, without inclusion of a non-HAART-treated control group it is difficult to determine what impact HAART may have had in our study especially as the impact of HAART on gene expression seems to be tissue/compartment-specific. We found persistent changes in gene expression of gut barrier functional markers (*CLDN1*, *CLDN4*) suggesting that persistent gut leakiness and translocation of microbial products may be contributing to the ongoing stimulation of innate immune cells observed in HIV. Although no tight junction gene correlated with serum markers of gut leakiness (LBP or LTA), the significant change in LBP and concurrent changes in gene expression of tight junction genes in HIV-infected individuals suggest additional mechanisms and/or tight junction components may be contributing to barrier dysfunction. The most significant changes of *TLR* gene expression were in the colon (where most of the host microbiome resides) and changes in immune-related gut were in the ileum (where most of the gut immune system resides). Significant correlations between serum markers/gene expression and the microbiota/gene expression were almost exclusively observed when HIV-uninfected and HIV-infected individuals were pooled together (with the exceptions of *IRAK2* and Roseburia and *TLR7* and Bacteroides). This observation limits the HIV-specific conclusions we can draw and is likely the consequence of a limited sample size and yet despite this limitation, important information can be gathered from these correlations. As we better characterize the relationship between systemic markers of intestinal barrier integrity and inflammation, microbiota, and mucosal gene expression, we can use noninvasive markers of blood or stool to predict gene expression in the intestine.

Conclusion

Further studies will be required to determine the relationship between mucosal gene expression, the intestinal microbiota, and systemic markers of inflammation. This important information may direct therapeutic interventions aimed at altering the microbiota to help normalize the mucosal environment.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Fig. 1. Gene expression in HIV-infected individuals shows similar features in the ileum, right colon, and left colon; however, some distinguishing features found in the colon

Biopsies were collected from healthy control individuals and HIV-infected samples and subsequently analyzed via Significance Analysis of Microarrays analysis with a significant difference being defined as a greater than 1.5-fold change in gene expression and a false discovery rate of less than 0.03 [(+) indicates the gene was increased in HIV and (-) indicates the gene was decreased in HIV].

Fig. 2. HIV-infected individuals have evidence of intestinal barrier dysfunction

IFABP was not significantly altered in HIV-infected (HIV+) individuals compared with healthy controls (HIV-) (Mann-Whitney test, $P = 0.987$); however, LBP was significantly decreased in HIV-infected individuals indicating lipopolysaccharide exposure (Student's t test, $P = 0.001$). IFABP, intestinal fatty acid binding protein; LBP, lipopolysaccharide binding protein.

Table 1

Subject demographics.

	Healthy controls (<i>n</i> = 18)	HIV-infected individuals (<i>n</i> = 18)	Statistics
Age (years)	54.0 (34.0–66.0)	51.5 (40.0–59.0)	0.223 ^a
Sex (men/women)	15/3	15/3	1.000 ^b
BMI	29.8 (21.7–42.0)	27.3 (17.9–52.3)	0.327 ^a
Race			–
African American	8	7	
Asian	1	1	
White	3	4	
Hispanic	6	6	
CD4 ⁺	n/a	428.1 (106–948)	–
Viral load	–	326.5 (0–4604)	–
Ileum biopsy samples	12	12	–
Right colon biopsy samples	17	18	–
Left colon biopsy samples	18	13	–

^aIndependent samples *t* test.^bFisher's exact test.

Table 2

Significance Analysis of Microarrays analysis of biopsy gene expression from ileum, right, and left colon between healthy control and HIV-infected individuals.

Gene symbol	Gene name	Fold change
IL1B	Interleukin-1, beta	4.07 ^a
IL17A	Interleukin-17A	3.50 ^a
CD3G	T-cell receptor T3 gamma chain	3.49 ^a
IFNG	Interferon gamma	3.30 ^a
TLR4	Toll-like receptor 4	3.05 ^a
IL7R	Interleukin-7 receptor subunit alpha	2.99 ^a
IL22	Interleukin-22	2.99 ^a
IL2	Interleukin-2	2.84 ^a
IFNB1	Interferon beta	2.84 ^a
CCR5	C-C chemokine receptor type 5	2.69 ^a
IL8	Interleukin-8 (CXCL8)	2.69 ^a
DSG3	Desmoglein-3	2.65 ^a
IL6	Interleukin-6	2.56 ^a
CCL5	C-C motif chemokine 5	2.42 ^a
CLDN1	Claudin 1	2.38 ^a
CCL2	Chemokine (C-C motif) ligand 2	2.27 ^a
IRAK2	Interleukin-1 receptor-associated kinase	2.23 ^a
TLR5	Toll-like receptor 5	2.20 ^a
TLR2	Toll-like receptor 2	2.14 ^a
TLR7	Toll-like receptor 7	1.97 ^a
IL23A	Interleukin 23, alpha subunit p19	1.89
IL10	Interleukin-10	1.78
TLR8	Toll-like receptor 8	1.76
CCR2	Chemokine (C-C motif) receptor 2	1.70
TLR1	Toll-like receptor 1	1.60
IFNA1	Interferon, alpha 1	1.49
TRAF6	TNF receptor-associated factor 6, E3 ubiquitin protein ligase	1.48
CCL4	Chemokine (C-C motif) ligand 4	1.44
F11R	F11 receptor	1.24
OCLN	Occludin	1.20
IL7	Interleukin-7	1.15
IRAK1	Interleukin-1 receptor-associated kinase 1	1.10
DSP	desmoplakin	1.07

Gene symbol	Gene name	Fold change
CD3E	CD3e molecule, epsilon (CD3-TCR complex)	1.07
IRF7	Interferon regulatory factor 7	0.97
TLR3	Toll-like receptor 3	0.84
CXCR4	Chemokine (C-X-C motif) receptor 4	0.84
CDH1	Cadherin 1, type 1, E-cadherin	0.74
IRF1	Interferon regulatory factor 1	0.72
CLDN4	Claudin 4	0.66

^a Significant difference between healthy controls and HIV-infected individuals (>1.5-fold change and false discovery rate <0.03). Values greater than 1 indicate the gene is increased in HIV and values less than 1 indicate the gene is decreased in HIV.

Table 3

Top canonical pathway analysis of genes significantly impacted in tissue biopsies from HIV-infected individuals.

Gene symbol	P	Upregulated	Downregulated	Unchanged
Altered T-cell and B-cell signaling in rheumatoid arthritis (cellular immune response, disease-specific pathways)	2.67E-27	IFNG, IL2, IL6, IL22, IL17A, IL1B, TLR2, TLR4, TLR5, TLR7	–	IL10, IL23A, TLR1, TLR8
Role of PRRs in recognition of bacteria and viruses (cellular immune response and pathogen-influenced signaling)	2.74E-27	CCL5, IL8, IFNB1, IFNG, IL2, IL6, IL17A, IL1B, TLR2, TLR4, TLR5, TLR7	–	IL10, TLR1, TLR8
Communication between innate and adaptive immune cells (cellular Immune response)	4.43E-27	CCL5, IL8, IFNB1, IFNG, IL2, IL6, IL1B, TLR2, TLR4, TLR5, TLR7	–	IL10, TLR1, TLR8
TREM1 signaling (cellular immune response and cytokine signaling)	6.53E-21	CCL2, IL8, IL6, IL1B, TLR2, TLR4, TLR5, TLR7	–	IL10, TLR1, TLR8
Role of cytokines in mediating communication b/w immune cells (cellular immune response, cytokine signaling, humoral immune response)	6.12E-20	IL8, IFNB1, IFNG, IL2, IL6, IL22, IL17A, IL1B	–	IL10, IL23A
Role of macrophages, fibroblasts, and endothelial cells in rheumatoid arthritis (disease-specific pathways)	1.36E-19	CCL2, CCL5, IL8, IL6, IL17A, IL1B, IRAK2, TLR2, TLR4, TLR5, TLR7	–	IL10, TLR1, TLR8
Role of hypercytokinemia/hyperchemokines in the pathogenesis of influenza (disease-specific pathways, pathogen-influenced signaling)	1.83E-18	CCL2, CCL5, CCR5, IL8, IFNB1, IFNG, IL6, IL17A, IL1B	–	–
Toll-like receptor signaling (cellular immune response, humoral immune response, pathogen-influenced signaling, apoptosis)	3.43E-14	IL1B, IRAK2, TLR2, TLR4, TLR5, TLR7	–	TLR1, TLR8
Differential regulation of cytokine production in macrophages and T-helper cells by IL17A & IL17F (cytokine signaling)	4.73E-14	CCL2, CCL5, IL6, IL17A, IL1B	–	IL10
Differential regulation of cytokine production in intestinal epithelial cells by IL17A & IL17F (cytokine signaling)	2.56E-13	CCL2, CCL5, IFNG, IL17A, IL1B	–	IL10
HMGB1 signaling (cellular immune response, cellular stress and injury, cytokine signaling, humoral immune response)	1.84E-12	CCL2, IL8, IFNG, IL2, IL6, IL17A, IL1B, TLR4	–	–
Hepatic fibrosis/hepatic stellate cell activation (disease-specific pathways, ingenuity toxicity list pathways)	1.89E-12	CCL2, CCL5, CCR5, IL8, IFNG, IL6, IL1B, TLR4	–	IL10
Hepatic cholestasis (disease-specific pathways, ingenuity toxicity list pathways)	2.10E-11	IL8, IFNG, IL2, IL6, IL17A, IL1B, IRAK2, TLR4	–	–
Glucocorticoid receptor signaling (intracellular and second messenger signaling)	2.37E-11	CCL2, CCL5, CD3G, IL8, IFNG, IL2, IL6, IL1B	–	IL10
Colorectal cancer metastasis signaling (cancer, disease-specific pathways)	4.24E-10	IFNG, IL6, TLR2, TLR4, TLR5, TLR7	–	TLR1, TLR8
Crosstalk b/w dendritic cells and NK cells (cellular immune response)	1.39E-09	IFNB1, IFNG, IL2, IL6, TLR4, TLR7	–	–

Gene symbol	P	Upregulated	Downregulated	Unchanged
NF- κ B signaling (cellular immune response, cytokine signaling, humoral immune response, ingenuity toxicity list pathway, organismal growth and development)	1.81E-09	IL1B, TLR2, TLR4, TLR5, TLR7	-	TLR1, TLR8
Dendritic cell maturation (cellular immune response, cytokine signaling, pathogen-influenced signaling)	2.30E-09	IFNB1, IL6, IL1B, TLR2, TLR4	-	IL10, IL23A
MSP-RON signaling pathway (cellular immune response)	3.26E-09	CCL2, CCR2, IFNG, TLR2, TLR4	-	-
Hematopoiesis from pluripotent stem cells (cellular growth, proliferation and development, organismal growth and development)	5.57E-09	CD3G, IL8, IL2, IL6	-	IL10

The Signaling Pathway Categories that each canonical pathway falls under is indicated in parentheses.