Gut mucosal dysfunction through impaired pattern recognition receptor expression and gut microbiota changes in chronic SIV infection

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Running title: SIV infection dampens pattern recognition receptor-cytokine signaling

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Supplemental Materials.

Table 1. Experimental Groups

	Uninfected	Acute	Chronic	Therapy	1A11 Acute	1A11 Chronic
N =	9	11	15	5	5	4
Infection	None	SIVmac251	SIVmac251	SIVmac251	SIVmac1A11	SIVmac1A11
Stage	N/A	Acute	Chronic	Chronic	Acute	Chronic
Duration (weeks)	N/A	1 – 2	10 – 34	28 – 40	1 - 2	8 - 23
Therapy	None	None	None	PMPA and FTC, initiated 1 week pi	None	None
Viral Loads						
Plasma[1]	N/A	$2.8x10^6$ ($3.0x10^5$ – $5.3x10^6$)	$1.2x10^5$ (UD – $8.5x10^5$)	$1.5x10^2$ (UD – $4.5x10^2$)	ND	ND
Jejunum[2]	N/A	$8.9 x 10^5$ ($1.3x10^3$ – $4.5x10^6$)	$3.6x10^4$ ($4.5x10^1$ – $1.7x10^5$)	$2.3x10^3$ ($1.7x10^3$ – $3.2 x 10^3$)	$2.14x10^3$ ($1.1x10^2$ – $9.3x10^3$)	$1.1x10^2$ (UD – $3.0x10^2$)
CD4+ T cells[3]						
Peripheral	53.7 (44.1 – 61.7)	35.4 (21.0 – 48.1)	34.0 (2.7 – 67.3)	48.4 (32.0 – 57.7)	ND	ND
Jejunum	36.0 (25.1 – 54.4)	7.2 (1.0 – 17.5)	6.7 (0.3 – 30.6)	40.6 (36.0 – 45.0)	ND	ND

Abbreviations: N/A – Not applicable; ND – Not Done; PMPA - Tenofovir disoproxil fumarate; FTC – emtricitabine; pi – post-infection; UD – Undetectable
[1]Mean SIV RNA copies/ml (range)
[2]Mean SIV RNA copies/µg total RNA (range)
[3]Mean percent CD4+ T cells (range)

Supplement Table 1. Experimental groups. Virologic and immunologic parameters are shown for all animals used in the TLR and cytokine analysis, including those that have been previously published[1].

Supplemental Table 2. Forward and reverse barcode names and sequences that correspond to the sequenced from jejunum samples.

Sample ID	BarcodeSequence	ForwardName	ReverseBarcode	ReverseName
43	AACCAGTC	F1	CAATTGGT	R5
44	AACGCTAA	F2	CAATTGGT	R5
45	AAGACTAC	F3	CAATTGGT	R5
46	AATCGATA	F4	CAATTGGT	R5
109	ACCAATTG	F5	CAATTGGT	R5
110	ACTGAAGT	F6	CAATTGGT	R5
112	ATTGCCGC	F7	CAATTGGT	R5
113	CAACCTTA	F8	CAATTGGT	R5
115	CCTAATAA	F9	CAATTGGT	R5
116	CCTCTGAT	F10	CAATTGGT	R5
117	CGGTCGAG	F11	CAATTGGT	R5
118	CTAATGGC	F12	CAATTGGT	R5
119	CTCATGCG	F13	CAATTGGT	R5
120	GAACGGAG	F14	CAATTGGT	R5
122	GCCTACGC	F15	CAATTGGT	R5
126	GGAGGCTG	F17	CAATTGGT	R5
127	GGATGCCA	F18	CAATTGGT	R5
128	GGATTAGG	F19	CAATTGGT	R5
129	GTTGGCCG	F20	CAATTGGT	R5
130	TATTAACT	F21	CAATTGGT	R5
182	TGACTGCT	F22	CAATTGGT	R5
185	TGGCGATT	F23	CAATTGGT	R5
242	TTCAGCGA	F24	CAATTGGT	R5
311	CGGTCGAG	F11	ACTTCAGT	R6
371	GGATTAGG	F19	ACTTCAGT	R6
400	TTGGCTAT	F25	ACTTCAGT	R6
402	AACCAGTC	F1	GCGGCAAT	R7
403	AACGCTAA	F2	GCGGCAAT	R7
432	AAGACTAC	F3	GCGGCAAT	R7
434	ACCAATTG	F5	GCGGCAAT	R7
30327.2	ACTGAAGT	F6	GCGGCAAT	R7
343	ATTGCCGC	F7	GCGGCAAT	R7

Methods:

Gut Microbiota analysis:

DNA was isolated from the jejunum tissue samples of rhesus macaques using the MoBio power soil kit (MoBio) according to the manufacturer's instructions. A nested PCR protocol was used to amplify and barcode the V4 domain of the 16S rRNA gene. Briefly, 1µL of DNA template and primers 27F (AGAGTTTGATCMTGGCTCAG) and 1492R (GGTTACCTTGTTACGACTT) were used to amplify a large portion of the 16S rDNA gene. One microliter of the resulting amplicon was combined with primers 515F:

(**AATGATACGGCGACCACCGAGATCTACAC**NNNNNNNNTATGGTAATT*GT*GTGCCAGCMGCCGCGGTAA)

and 806R:

(**CAAGCAGAAGACGGCATACGAGAT**NNNNNNNNAGTCAGTCAG*CC*GGACTACHVGGGTWTCTAAT)

and used to amplify the V4 domain. Each primer contained a unique 8 nt barcode (N) a primer pad (underlined), a two base linker (italicized) and the Illumina adaptor sequences (bold text). A unique combination of these primers was used for barcode each sample (see supplemental table 2).

PCR mixtures contained 1 Unit Kapa2G Robust Hot Start Polymerase (Kapa Biosystems), 1.5 mM MgCl2, and 10 pmol of each primer, and 1ul of template DNA. The first PCR step cycling conditions were an initial incubation at 95°C for 5 min, followed by 25 cycles of 95°C for 30 s, 50°C for 30 s, and 72°C for 90 s and a final extension of 72°C for 7 min. The second PCR conditions were an initial incubation at 95°C for 3 min, followed by 25 cycles of 95°C for 45 s, 50°C for 60 s, and 72°C for 90 s and a final extension of 72°C for 10 min. The final PCR product was

quantified on the Qubit instrument using the Qubit high sensitivity DNA kit (Invitrogen) and individual amplicon libraries were pooled together at the same concentration. Two-hundred microliters of the pooled libraries were run on a 1% agarose gel at 100v for 45 min and a band of ~384 bp (amplicons plus adaptors sequences) was extracted and purified using the QIAquick gel extraction kit (Qiagen) and processed for Illumina paired-end library preparation, cluster generation, and 250-bp paired-end sequencing on an Illumina MiSeq instrument (Genome Center DNA Technologies Core).

Sequencing reads were trimmed of their barcodes, demultiplexed and combined using custom Perl scripts and aligned to the Greengenes (release 13_5) database using Qiime with the default parameters. The resulting Biom tables were filtered to remove low abundance OTUs using the filter_otus_from_otu_table.py Qiime script as previously described[2, 3]. The resulting OTUs tables were used for downstream analysis.

Statistical analysis. Statistical analyses of qRT-PCR data were performed using GraphPad Prism (San Diego, CA). Statistical differences between groups were analyzed using ANOVA with Tukey post-test on normalized Ct values. Correlations were determined by calculating Spearman correlation coefficients using normalized Ct values. Differences in microbial abundance were analyzed using a two-tailed Mann-Whitney test. Correlations and correlation heatmaps were performed in R.

1. Verhoeven D, Sankaran S, Silvey M, Dandekar S. Antiviral therapy during primary simian immunodeficiency virus infection fails to prevent acute loss of CD4+ T cells in gut mucosa but enhances their rapid restoration through central memory T cells. *Journal of virology* 2008; **82**(8)**:** 4016-4027.

2. Bokulich NA, Subramanian S, Faith JJ, Gevers D, Gordon JI, Knight R *et al.* Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. *Nature methods* 2013; **10**(1)**:** 57-59.

3. Caporaso JG, Kuczynski J, Stombaugh J, Bittinger K, Bushman FD, Costello EK *et al.* QIIME allows analysis of high-throughput community sequencing data. *Nature methods* 2010; **7**(5)**:** 335-336.