

# Mucosal Cytokines are Increased Up To 75-fold in HAART-treated Subjects with Detectable Plasma Viral Loads Compared to Those with Fully Suppressed Viremia.

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# Abstract

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**Background.** The gut mucosa physiologically expresses levels of chemokines and cytokines that maintain low-level inflammation. We have demonstrated that HIV infection is associated with increased mucosal inflammation and that mucosal mononuclear cells display an enhanced vulnerability to HIV infection. Increased expression of pro-inflammatory cytokine mRNA has been demonstrated in the mucosa of HIV infected patients. These cytokines upregulate HIV replication in infected cells, contribute to apoptosis (in vitro) and recruit additional target cells to the area, facilitating viral replication (in vivo). We sought to determine if mucosal cytokine activity differs in suppressed versus unsuppressed subjects on HAART, providing additional targets for adjunctive therapy. **Methods.** Banked recto-sigmoid biopsies from 15 HIV sero-positive men on HAART with CD4 counts >100 cells/mm<sup>3</sup> were retrospectively evaluated for mucosal cytokine mRNA activity. Six subjects had undetectable plasma HIV-1 RNA (<50 copies/ml) and undetectable mucosal viral load; 9 subjects had detectable HIV-1 RNA plasma viral load (mean 4.5 log; range 3.75-5.51 log) and high mucosal HIV-1 viral load (mean 4.1 log; range 3.7-5.01 log). To quantify mucosal cytokine mRNA, total RNA was isolated from 3 pooled biopsies and amplified using primers specific for b-actin, IL-1b, IL-2, IL-6, IL-10, IL-12, TNF-a, g-INF, and RANTES using real time PCR. Results were normalized for b-actin content and expressed as an average ratio of high:low mucosal viral load subjects. **Results.** Subjects with detectable plasma and mucosal HIV-1 RNA express significantly increased levels of mucosal mRNA for g-INF (75-fold; p=0.001), RANTES (25-fold; p=0.004), TNF-a (14-fold; p=0.01), IL-2 (19.5-fold; p=0.001), IL-1b (9-fold; p=0.02), IL-10 (9-fold; p=0.004), when compared to subjects with undetectable plasma and low mucosal HIV RNA. No significant differences were observed in the expression levels of IL-12 or IL-6. Duration of infection, duration of HAART and CD4 levels were not significantly different. Plasma phenotypic resistance profiles are pending. **Conclusions.** Mucosal cytokine profiles are significantly increased in subjects with detectable plasma and high mucosal viral burden compared to those effectively suppressed in plasma. Increases do not simply reflect a change in Th1/Th2 ratios but were detected in pro-inflammatory (TNF-a, IL-1) and Th1 (IL-2, g-Inf) as well as Th2 (IL-10) and chemokine (RANTES) mRNA. As both study populations on HAART were clinically well at time of sample collection, many years (mean=12) following infection, these data may identify a sub-group with mucosal hyper-reactivity that correlates with incomplete responsiveness to HAART and identifies new adjunctive targets for therapy as well as mechanisms for mucosal apoptosis.

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# Introduction

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- Mucosal surfaces are continually exposed to antigens and thereby need to maintain a state of active immune surveillance.
- Mucosal mononuclear cells display an enhanced vulnerability to HIV infection.
- Untreated HIV infection is associated with significant mucosal inflammation and the increased expression of pro-inflammatory cytokine mRNA.
- These cytokines are potent inducers of HIV-1 replication, contribute to apoptosis, and recruit additional target cells to the area.
- We sought to determine the cytokine profiles expressed in the gut mucosa of subjects with and without ongoing viral replication with the hypothesis that they would be increased in settings of high viral burden.

# Experimental Design

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- Rectal biopsies collected (standard site of 30cm) from 15 HIV positive subjects on HAART with either undetectable plasma and mucosal HIV RNA or detectable plasma and mucosal HIV RNA and healthy controls.
- Quantify the relative expression of mucosal cytokine mRNA for IL-1 $\beta$ , IL-2, IL-6, IL-10, IL-12, TNF- $\alpha$ ,  $\gamma$ -INF, RANTES. Results are normalized for  $\beta$ -actin content and expressed in terms of fold-difference between groups.

# Patient characteristics

Age	Sex	CDC class	CD4	pvl(log10)	mvl(log10)	Yrs HIV	Meds
54	M	C2	1047	<50	<10	11	Hydroxyrea.Invirase.Neviraprine.Stavudine
40	M	A3	394	<50	<10	11	Lamivudine.Delaviridine.Zidovudine.
38	M	A2	379	<50	<10	15	Lamivudine.Invirase.Ritonivir.Nevirapine.Stavudine
45	M	B1	1100	<50	<10	10	Nelfinavir, Zidovudine.
50	M	A3	130	<50	<10	18	Nevirapine, Lamivudine Stavudine, Indinavir
49	M	C3	243	<50	<10	5	Lamivudine.Nevirapine.Nelfinavir.
46	M	C3	115	<50	<10	6	Lamivudine.Abacavir.Nevirapine.Nelfinavir.
50	M	B2	NA	NA	4.21	4	Hydroxyrea.Invirase.Ritonivir.Nevirapine.Nelfinivir.
55	M	B3	629	3.73	4.14	10	Lamivudine.Zidovudine.
34	M	C1	612	4.14	3.79	10	Lamivudine.Nelfinivir.Stavudine.
35	M	C3	171	4.21	3.91	12	Lamivudine.Invirase.Ritonivir.Nevirapine.Stavudine
40	M	C3	177	5.47	3.86	11	Lamivudine.Agenerase.Ritonivir.Zidovudine.
35	M	A2	414	4.19	4.47	7	Lamivudine.Zidovudine.Nevirapine.
38	M	A2	210	NA	4.99	8	Lamivudine,Stavudine, Effarenz
50	M	B3	248	4.47	3.83	16	Lamivudine.Invirase.Stavudine.

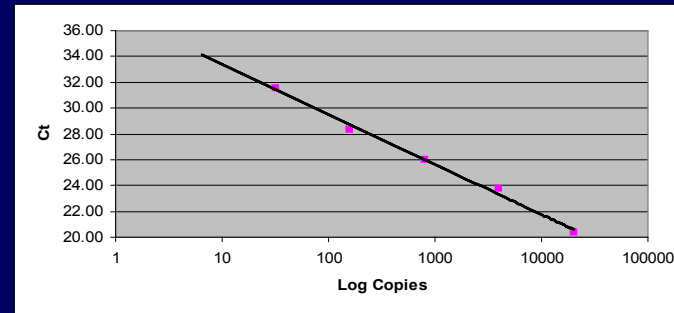
# Relative Quantitation of Tissue Cytokine RNA by Real Time PCR

Internal control PCR  
with b-actin control  
kit

RNA Extraction  
Reverse  
transcription

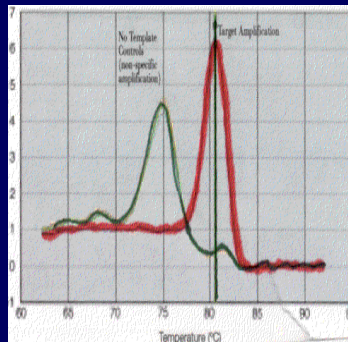
Cytokine pcr  
with Sybr  
Green

Standards  
human  
colon RNA

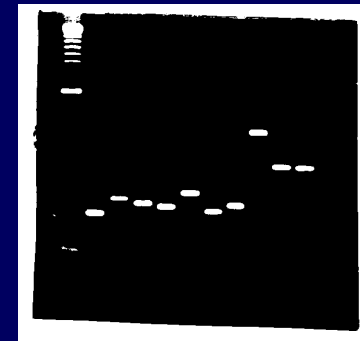


Standards  
PHA/LPS  
Stimulated  
PBMC

## Product verification



IL-1  
IL-2  
IL-6  
IL-10  
IL-12  
Tnf  
Inf-g  
RANTES



$(\text{Cytokine RNA copies} / \text{b-actin RNA copies}) * 10^6$

# Results

## Mucosal cytokine expression in fully suppressed subjects.

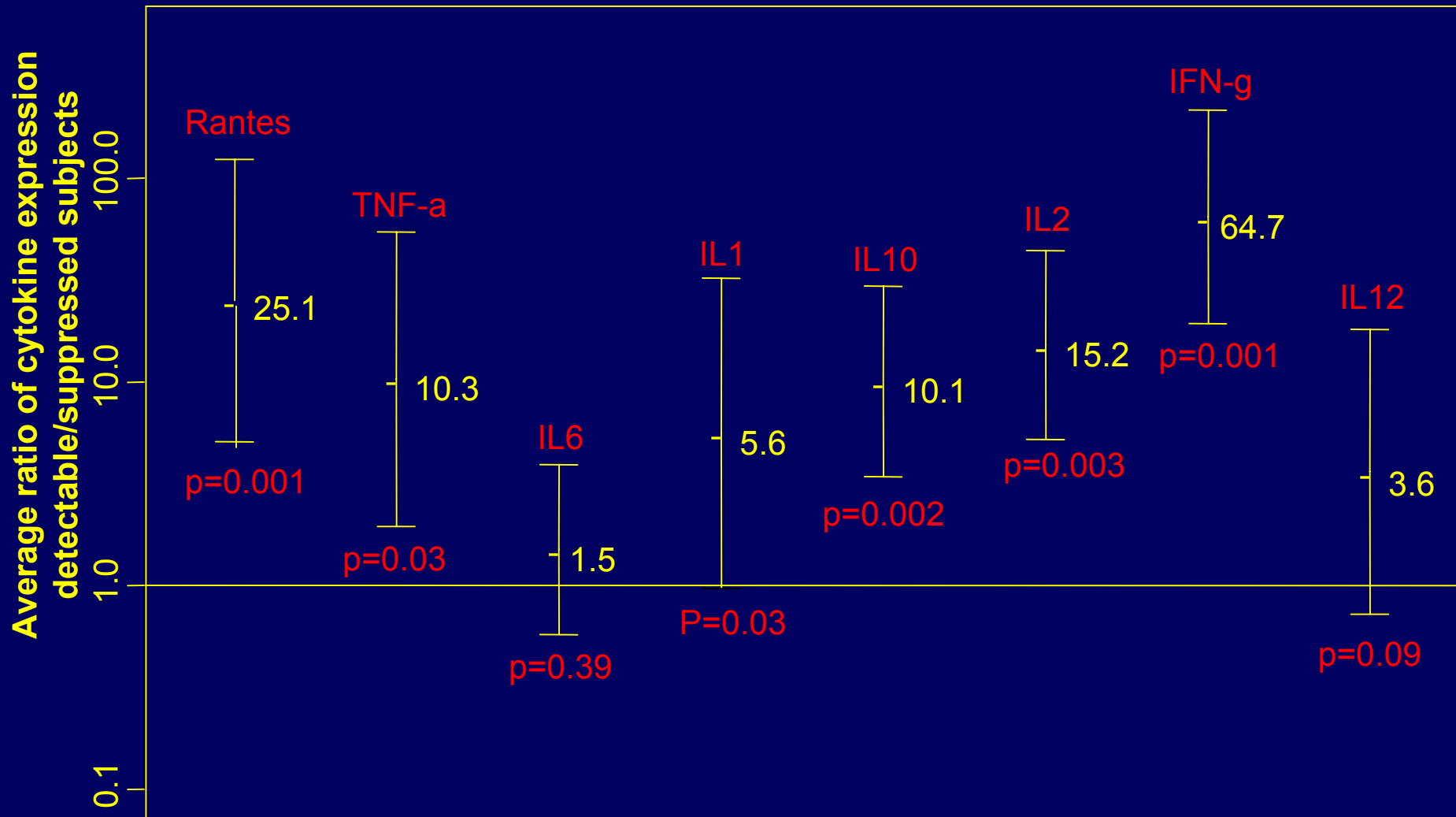
	mRNA copies per 10 <sup>6</sup> copies of b-actin mRNA							
Patient ID	RANTES	Tnf-alpha	IL-6	IL-1	IL-10	IL2	Inf-gamma	IL-12
136	1249	1757	1568	78	267	68	63	150
142	7467	NA	8143	889	1099	223	344	8341
148	1406	55432	NA	20574	2013	1161	387	5676
154	436	12940	997	234	131	84	86	2047
170	1781	54871	1983	375	425	94	166	314
178	2401	2872	4584	131	326	94	65	920
199	1024	8273	2440	57	119	54	52	1317
<b>mean</b>	<b>2252</b>	<b>22691</b>	<b>3286</b>	<b>3191</b>	<b>626</b>	<b>254</b>	<b>166</b>	<b>2681</b>
<b>St Dev</b>	<b>2379</b>	<b>25462</b>	<b>2679</b>	<b>7671</b>	<b>696</b>	<b>404</b>	<b>142</b>	<b>3119</b>

# Results

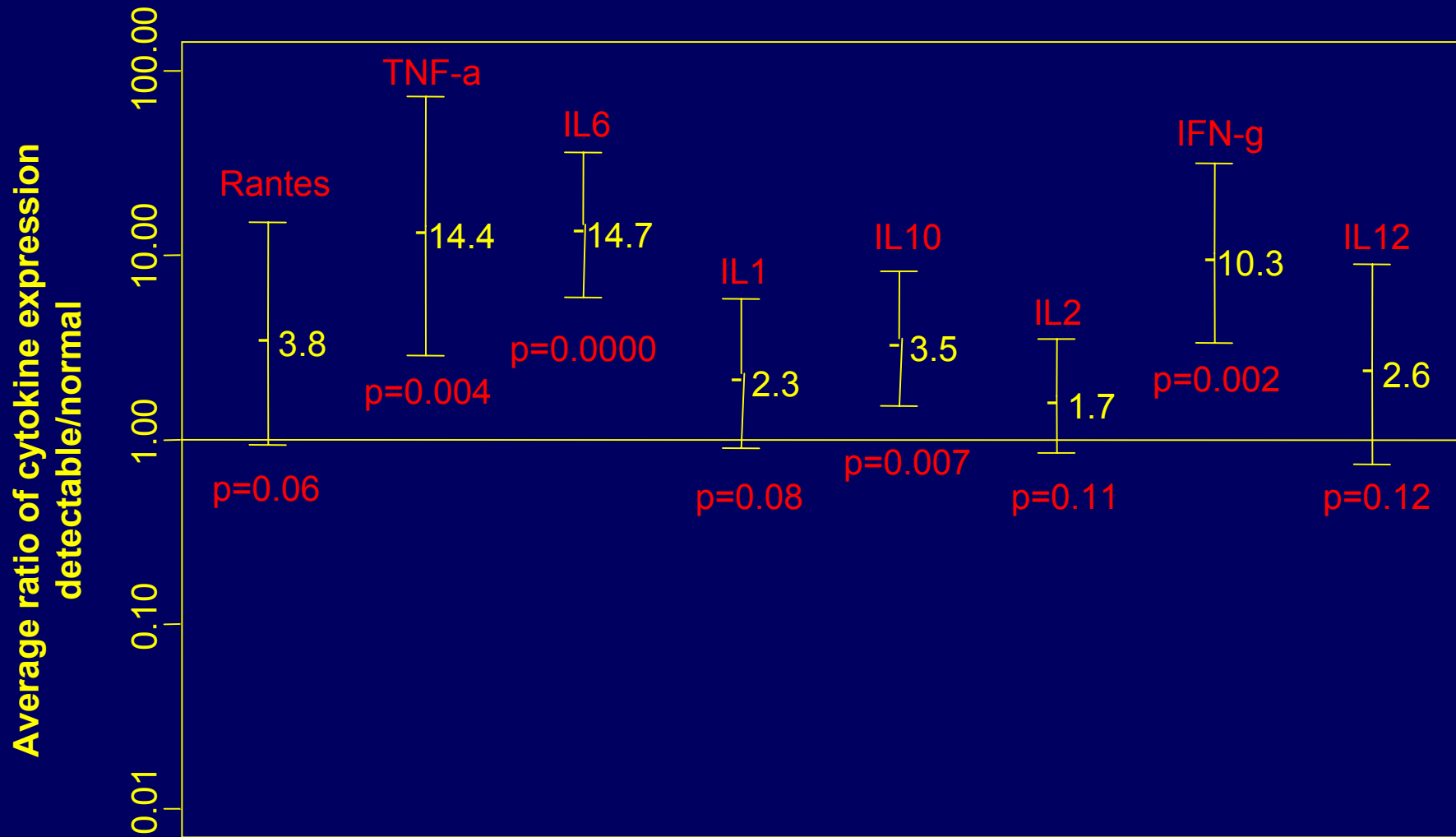
## Mucosal cytokine expression in subjects with detectable viral burden.

	mRNA copies per 106 copies of $\beta$ -actin							
Patient ID	RANTES	Tnf-alpha	IL-6	IL-1	IL-10	IL2	Inf-gamma	IL-12
281	201289	818187	NA	4746	14157	4890	37725	3133
365	90144	24052	4994	1460	5859	2119	10695	1331
375	7833	101345	NA	NA	NA	NA	NA	18463
376	126757	223711	1496	6597	3851	1217	938	6949
387	24387	16258	11027	1343	1272	3959	4689	14348
405	44514	495843	2042	1607	1850	526	5306	na
410	116978	510748	3252	940	3605	1737	8706	10794
416	143692	42770	5967	1720	4929	2881	23389	553
372	752	35271	NA	NA	NA	NA	NA	NA
mean	84038	252021	4796	2631	5075	2475	13064	7939
St Dev	69027	289128	3496	2159	4314	1541	13018	6859

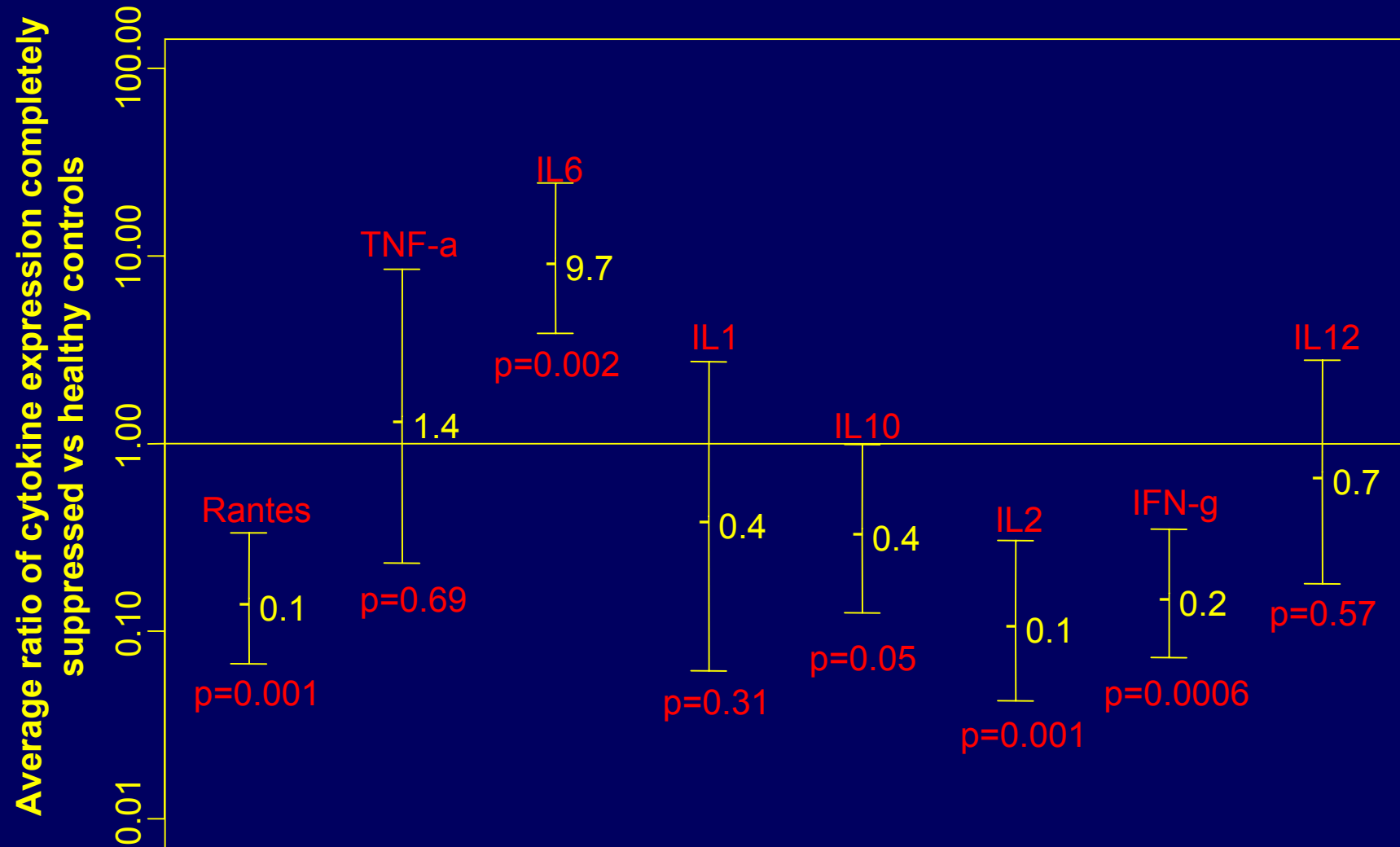
# Th1, Th2 and Pro-inflammatory Cytokine mRNA are increased in the mucosa of subjects with detectable vs completely suppressed viral burden



# Th1 Th2 and pro-inflammatory cytokine Mrna are increased in the mucosa of subjects with detectable viral burden vs healthy controls



# Th1, Th2 and pro-inflammatory cytokine Mrna are decreased in the mucosa of completely suppressed subjects vs healthy controls



# Results

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- Subjects with detectable plasma and mucosal HIV RNA express significantly higher levels of mucosal mRNA for  $\gamma$ -INF, RANTES, TNF- $\alpha$ , IL-2, IL-1 $\beta$  and IL-10 than those with undetectable plasma and mucosal HIV RNA.
- No significant difference was observed in the expression level of mucosal mRNA for IL-12 or IL6 between the two groups studied.

# Conclusions

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- Persistent viral replication is associated with the increased expression of diverse cytokine mRNA in the gut mucosa.
- Increases occur in not only in pro-inflammatory cytokines ( $\text{Tnf-}\alpha$ ,  $\text{IL-1}\beta$ ) but also in Th1 ( $\text{IL-2}$ ,  $\gamma\text{-INF}$ ) and Th2 cytokines ( $\text{IL-10}$ ) and chemokines ( $\text{RANTES}$ )
- To the degree that these cytokine profiles reflect inflammatory responses, higher mucosal viral burden is associated with increased mucosal inflammation.