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Biomarkers of cellular stress do not associate with sCD14 in progressive HIV and SIV infections.

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Background

- Damage to the stuctural and/or immunological barrier of the gastrointestinal (GI) tract can lead to microbial translocation.
- Microbes or microbial components that translocate from the GI lumen then can directly stimulate the systemic immune system and contribute to inflammation.
- Microbial translocation is an important phenomenon in many diseases, including HIV and SIV infection.
- To date direct measurements of microbial products are difficult for several reasons which include:
  1. lack of appropriate assays to measure multiple microbial antigens simultaneously;
  2. confounding factors that influence assays used to detect microbial products; and
  3. biological mechanisms which are involved in clearing microbial antigens from circulation.

Study Outline and Methods

- We measured, by ELISA, plasma levels of:

Innate immune responses to microbial products

sCD14 - produced by myeloid cells after stimulation by LPS. sCD14 is one of the most commonly measured surrogate biomarkers of microbial translocation.

Cell-death associated proteins

High mobility group box 1 (HMGB1) - a protein produced during cellullar stress that may play a role in chronic inflammation. HMGB1 also binds to the CD14/TLR4 signaling complex.

Receptor for advanced glycation endproducts (RAGE) - a receptor that shares common ligands and signaling pathways with the CD14/TLR4 complex.

Biomarkers associated with damage to the GI tract

Intestinal fatty acid binding protein (IFABP) - a protein released during epithelial damage.

Zonulin - a protein that functions to maintain intracellular junctions.

Study Cohort – Humans

ID	Timepoint	Plasma Viremia[a]	CD4+ count[b]
1	HIV- ARV-	6,810	0
	HIV+ ARV+ (4 yrs)	<40	542
2	HIV- ARV-	26,202	45
	HIV+ ARV+ (4 yrs)	<40	510
3	HIV- ARV-	266,478	35
	HIV+ ARV+ (4 yrs)	<50	298
4	HIV- ARV-	28,432	33
	HIV+ ARV+ (4 yrs)	<40	571
5	HIV- ARV-	6,842	12
	HIV+ ARV+ (4 yrs)	<50	322
6	HIV- ARV-	116,716	44
	HIV+ ARV+ (4 yrs)	<40	241
7	HIV- ARV-	<50	82
	HIV+ ARV+ (4 yrs)	<40	353
8	HIV- ARV-	601,301	5
	HIV+ ARV+ (4 yrs)	<40	347
9	HIV- ARV-	240,966	43
	HIV+ ARV+ (4 yrs)	<40	759
10	HIV- ARV-	231,414	3
	HIV+ ARV+ (4 yrs)	<40	547
11	HIV- ARV-	25,831	16
	HIV+ ARV+ (4 yrs)	80	172
12	HIV- ARV-	28,342	6
	HIV+ ARV+ (4 yrs)	<40	605
13	HIV- ARV-	186,674	231
	HIV+ ARV+ (4 yrs)	<40	195
14	HIV- ARV-	379,425	7
	HIV+ ARV+ (4 yrs)	<40	588
15	HIV- ARV-	149,655	45
	HIV+ ARV+ (4 yrs)	<40	238
16	HIV- ARV-	145,610	18
	HIV+ ARV+ (4 yrs)	<40	149
17	HIV- ARV-	10,603	12
	HIV+ ARV+ (4 yrs)	<50	167
18	HIV- ARV-	<50	18
	HIV+ ARV+ (4 yrs)	<40	352
19	HIV- ARV-	9,251	74
	HIV+ ARV+ (4 yrs)	<40	291
20	HIV- ARV-	142,379	11
	HIV+ ARV+ (4 yrs)	<40	368
21	HIV- ARV-	128,742	12
	HIV+ ARV+ (4 yrs)	<50	258
22	HIV- ARV-	212,286	74
	HIV+ ARV+ (4 yrs)	<50	644
23	HIV- ARV-	339,630	12
	HIV+ ARV+ (4 yrs)	<40	630
24	HIV- ARV-	71,873	6
	HIV+ ARV+ (4 yrs)	<40	503
25	HIV- ARV-	609,435	124
	HIV+ ARV+ (4 yrs)	<40	212
26	HIV- ARV-	45,764	24
	HIV+ ARV+ (4 yrs)	<40	361
27	HIV- ARV-	43,907	12
	HIV+ ARV+ (4 yrs)	<40	349
28	HIV- ARV-	15,269	12
	HIV+ ARV+ (4 yrs)	<40	347
29	HIV- ARV-	15,000	362
	HIV+ ARV+ (4 yrs)	<50	457
30	HIV- ARV-	10,600	182
	HIV+ ARV+ (1 yr)	<400	400
31	HIV- ARV-	4,620	444
	HIV+ ARV+ (1 yr)	<50	291
32	HIV- ARV-	7,870	372
	HIV+ ARV+ (1 yr)	<50	319
33	HIV- ARV-	216,000	113
	HIV+ ARV+ (1 yr)	<48	323
34	HIV- ARV-	17,700	45
	HIV+ ARV+ (1 yr)	ND	ND

Study Cohort - Pigtail Macaques

ID	Timepoint	Plasma Viremia[a]	CD4+ count[b]
PT98P030	Pre-SIV	0	975
	Acute SIV	1,736	ND
	Chronic SIV	621	372
PTA0P039	Pre-SIV	0	1,144
	Acute SIV	1,767,348	230
	Chronic SIV	19,161	45
PT99P052	Pre-SIV	0	1,597
	Acute SIV	331,131	ND
	Chronic SIV	643,458	30
PT99P029	Pre-SIV	0	765
	Acute SIV	475,587	355
	Chronic SIV	5,394,895	8
PT98P005	Pre-SIV	0	765
	Acute SIV	323,594	203
	Chronic SIV	7,625	33
PT99P030	Pre-SIV	0	842
	Acute SIV	103558	369
	Chronic SIV	6,600	372
PTA0P007	Pre-SIV	0	653
	Acute SIV	125,440	199
	Chronic SIV	43,311	31
PTA1P012	Pre-SIV	0	1,347
	Acute SIV	355,277	387
	Chronic SIV	201,583	160
PTA0P012	Pre-SIV	0	690
	Acute SIV	2,403,815	154
	Chronic SIV	226,244	37

Study Cohort - Rhesus macaques

ID	Timepoint	Plasma Viremia[a]	CD4+ count[b]
RHCL4C	Pre-SIV	0	150
	Acute SIV	873,043	219
	Chronic SIV	840,000	47
RHCL7P	Pre-SIV	0	886
	Acute SIV	612,776	1,812
	Chronic SIV	560,000	ND
RHCL86	Pre-SIV	0	1,037
	Acute SIV	1,471,247	251
	Chronic SIV	1,100,000	91
RHDCBC	Pre-SIV	0	446
	Acute SIV	133,266	454
	Chronic SIV	1,200,000	ND
RHDCJWA	Pre-SIV	0	730
	Acute SIV	556,021	195
	Chronic SIV	700,000	ND
RHDCKJ	Pre-SIV	0	956
	Acute SIV	627,870	853
	Chronic SIV	5,300,000	192
RHDCVF	Pre-SIV	0	410
	Acute SIV	178,242	160
	Chronic SIV	7,200,000	268
RHDU1A	Pre-SIV	0	339
	Acute SIV	557,837	182
	Chronic SIV	2,200,000	ND
RHDU20	Pre-SIV	0	747
	Acute SIV	709,493	304
	Chronic SIV	67,000	ND
RHDU2C	Pre-SIV	0	454
	Acute SIV	12,688	154
	Chronic SIV	470,000	ND
RHDU2W	Pre-SIV	0	297
	Acute SIV	66,799	165
	Chronic SIV	920,000	93

[a] Plasma viral load (copies/mL)
[b] CD4+ T cell count (cells/ul blood)
ND = not done

Results

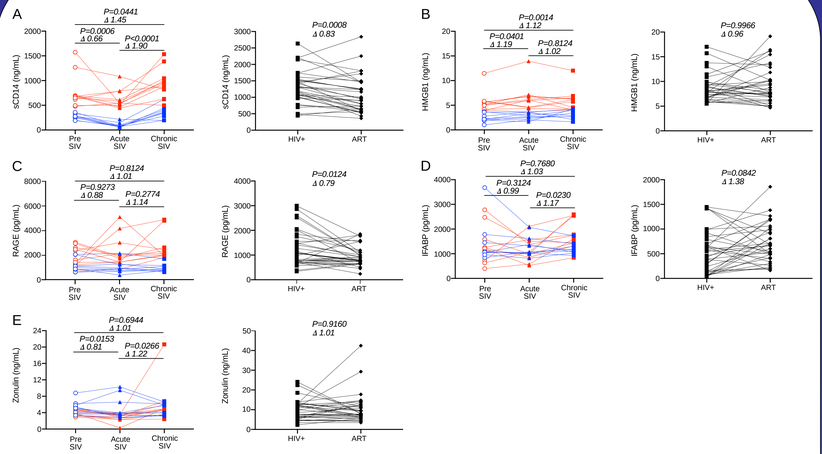

Fig. 1. Plasma biomarker levels in NHPs during SIV infection and in HIV-infected humans following ART.
(A-E) Plasma levels of biomarker analytes in NHPs (n=20, graphs on the left, with PTs in blue and RMs in red) pre-SIV infection, during acute-SIV infection, and during chronic infection; and HIV-infected humans (n=34, graphs on the right) pre- and post-ART treatment for (A) sCD14, (B) HMGB1, (C) RAGE, (D) IFABP, and (E) Zonulin. Lines connect the same study participant over time. Wilcoxon t tests were used to determine each P-value. Δ values represent the mean fold-change between timepoints.

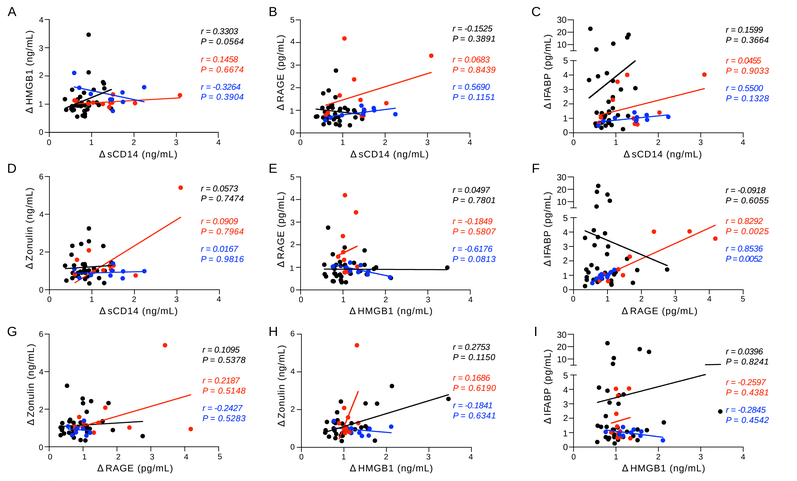

Fig. 2. Plasma biomarker associations.
(A-I) Correlational analyses between plasma biomarkers in humans (black), RMs (red), and PTs (blue). Δ values represent the change in biomarker levels from either pre- to chronic infection (NHPs) or pre-to post-ART (humans). All associations were analyzed using the Spearman test.

Results

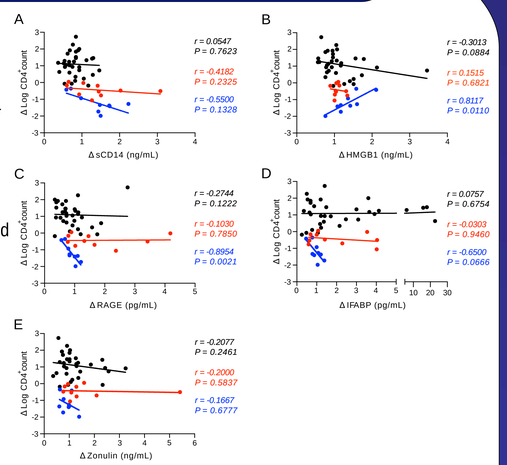

Fig. 3. Plasma biomarker associations with $CD4^+$ T cell frequencies.
(A-E) Correlational analyses between $\Delta CD4^+$ T cell count and Δ plasma biomarkers in humans (black), RMs (red), and PTs (blue) for (A) sCD14, (B) HMGB1, (C) RAGE, (D) IFABP, and (E) Zonulin. The $\Delta CD4^+$ T cell count represents (log(chronic SIV-infected $CD4^+$ count/pre-SIV infection $CD4^+$ count for NHPs and (log(ART timepoint $CD4^+$ count/chronic HIV^+ART^- $CD4^+$count). All associations were analyzed using the Spearman rank test.

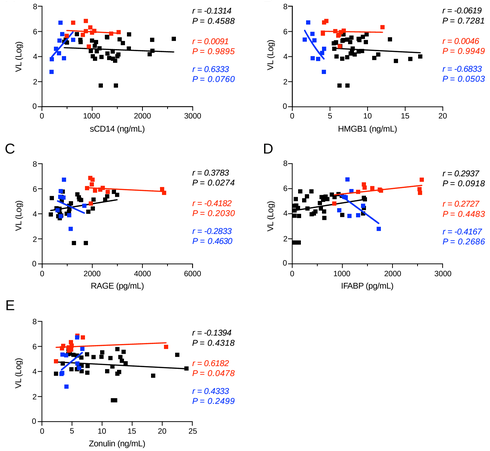

Fig. 4. Plasma biomarker associations with plasma viral load.
(A-E) Correlational analyses between Δ SIV or HIV plasma viral load and plasma biomarkers at chronic timepoints in humans (black), RMs (red), and PTs (blue) for (A) sCD14, (B) HMGB1, (C) RAGE), (D) IFABP, and (E) Zonulin. All associations were analyzed using the Spearman rank test.

Conclusions

- Our data suggest that HMGB1 and RAGE are not associated with elevated levels of sCD14 observed in HIV-infected individuals and SIV-infected NHPs and, thus, elevated sCD14 is unlikely to be attributed to cellular death in these cases.
- We also do not find evidence that markers of intestinal epithelial damage correlate with sCD14, suggesting that these epithelial markers themselves are not adequate biomarkers of microbial translocation.