GUT BARRIER PROTECTANT INTESTINAL ALKALINE PHOSPHATASE IS REDUCED IN PEOPLE WITH HIV

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BACKGROUND

Microbial Translocation in People with HIV (PWH)

- Intestinal microbial translocation is increased among PWH and leads to systemic immune activation and inflammation[1]
- Mechanisms of impaired GI mucosal barrier function need to be further elucidated in PWH

Intestinal Alkaline Phosphatase as an Intestinal Mucosal Barrier Protectant

- Intestinal alkaline phosphatase (IAP) is an intestinal brush border enzyme that is important for maintaining healthy GI barrier function[2]
- IAP promotes intestinal homeostasis by regulating the pH of the gut lumen via bicarbonate secretion, dephosphorylating ATP/UDP released from damaged cells, and by detoxifying lipopolysaccharides (LPS)[2,3]
- IAP dephosphorylates the lipid moiety of LPS, rendering it unable to induce proinflammatory responses through TLR4 and subsequent MyD88-dependent signaling pathways in the gut[3]
- IAP is reduced in diseases in which intestinal dysbiosis has been implicated, such as inflammatory bowel disease and DM2[4]
- High IAP levels appears to be protective against DM2 in human studies[5]
- In mice, oral IAP supplementation can prevent and reverse metabolic syndrome[6]
- To our knowledge, IAP has not yet been investigated in PWH

OBJECTIVE

Central Question: Does the gut protectant IAP differ between people with HIV and healthy controls?

We hypothesized that IAP activity in the terminal ileum would be reduced in people with HIV compared to non-HIV controls.

METHODS

Study Design and Participants

- Observational study of 30 PWH and 6 participants without HIV in Boston, MA
- All participants had no known gastrointestinal disease
- PWH were treated with stable ART > 6 months and had suppressed HIV RNA

Measurement of IAP and IL-1β from Intestinal Fluid

- Participants underwent intestinal fluid collection via aspiration in the lumen of the terminal ileum as part of colonoscopy
- IAP activity was assessed by mixing luminal fluid with phosphatase assay reagent containing p-nitrophenyl phosphate followed by determining optical density at 405 nm
- Intestinal fluid IL-1β levels were assessed by ELISA
- IAP and IL-1β were both normalized to total protein in the intestinal fluid

Blood Biomarkers

- We assessed CD4+ T-cell count, HIV RNA, HbA1c, and CRP via venous blood draw

^{18}FDG PET Assessment of Carotid Arterial Inflammation

- Patients underwent ^{18}F-FDG PET imaging to assess arterial inflammation. PET imaging was performed 120 minutes after administration of 10 mCi of ^{18}F-FDG
- Bilateral carotid arterial inflammation was measured using the ratio of FDG uptake in the carotid arterial wall/blood background, as the target to background ratio (TBR)

Statistical Analysis

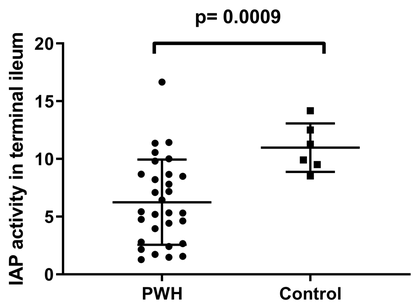
- Comparisons between two groups were analyzed using student's t-test
- Spearman correlation coefficients were assessed for the relationship between the continuous variables IAP activity and carotid TBR among all individuals
- Relationships of IL-1β (in the terminal ileum) with CRP and hemoglobin A1c (both measured in blood) was assessed among PWH using Pearson correlation coefficients

RESULTS

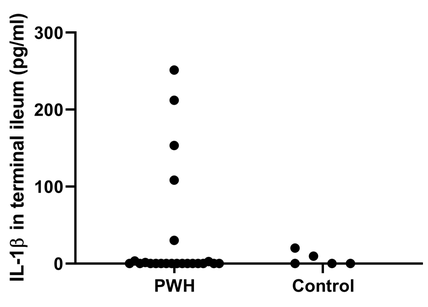
Clinical Characteristics of PWH and Uninfected Controls

	PWH n=30	Uninfected Controls n=6	P Value
Age, years	53.1±9.1	46.8±11.3	0.25
Male, %	83.3	100	0.16
Race and ethnicity			0.62
White, %	53.3	66.7	
Black, %	40	33.3	
BMI, kg/m^2	27.3±4.3	26.9±4.2	0.83
HbA1c (%)	5.5±0.3	5.5±0.4	0.86
CD4+ T cells, cells/mm^3	729±234		
Undetectable viral load, %	86.7%		
CRP, mg/L	1.86±1.45	1.12±0.91	0.13
IAP Activity (IAP/μg) in terminal ileum	6.25±3.69	10.98±2.10	**0.0009**
Intestinal fluid IL-1β (pg/mg protein)	23.2±57.4	5.97±8.96	0.19

Levels of IAP and IL-1β in People with HIV and Uninfected Controls

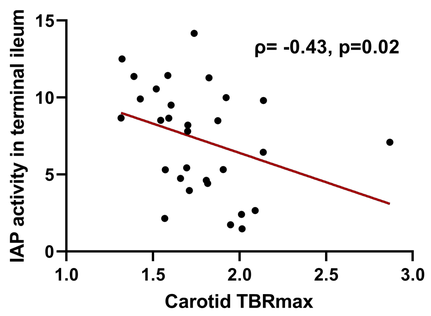


Terminal Ileum IAP activity is lower in PWH compared to people without HIV. Height of the bar represents the mean and error bar represents the standard deviation.



IL-1β trended to be higher in the terminal ileum of PWH compared to people without HIV, p=0.09.

IAP Levels are Inversely Associated with Carotid FDG Uptake



IL-1β in the Terminal Ileum is Associated with Inflammation and Hemoglobin A1c

	Relationships with IL-1β	
	r	P Value
CRP	0.64	0.001
A1c	0.45	0.03

CRP, C-Reactive Protein; A1c, hemoglobin A1c.

CONCLUSIONS

- IAP, a brush border enzyme which detoxifies LPS and reduces intestinal inflammation, was found to be significantly lower in the terminal ileum of PWH compared to uninfected controls
- In contrast, proinflammatory IL-1β in the terminal ileum trended to be higher in PWH
- IAP activity was inversely associated with carotid arterial inflammation as measured by carotid FDG uptake, suggesting that IAP may mediate an atheroprotective effect via enhancement of intestinal barrier function
- IL-1β (as measured in intestinal fluid) was associated with systemic marker of inflammation, CRP, and with hemoglobin A1c in PWH, which may suggest that heightened intestinal inflammation may play a role in systemic inflammation and glucose dysregulation in PWH
- A potential mechanism our findings suggest is that reduced IAP among PWH may decrease the intestinal barrier's ability to detoxify LPS in the gut, leading to heightened microbial translocation and systemic inflammation
- IAP activity may provide additional insight into the pathogenesis of intestinal barrier dysfunction, systemic immune activation, and its associated comorbidities in PWH including atherosclerotic cardiovascular disease

LIMITATIONS

- Our sample size was small and findings need to be confirmed in a larger cohort
- The cross-sectional nature of our study precludes the assessment of causality

FUTURE DIRECTIONS

- Histopathological assessment of the intestinal mucosa, immunological studies using flow cytometry and serum biomarkers of microbial translocation and inflammation are under investigation to better understand precise mechanisms involving IAP and GI epithelial integrity in PWH
- Therapeutic use of exogenous intestinal alkaline phosphatase has shown beneficial effects in reversing metabolic syndrome in animal models and could represent a novel therapeutic strategy in the context of intestinal barrier damage in PWH
- Future studies to target intestinal inflammation in PWH are needed to assess effects on glucose metabolism and immune activation

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