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Plasma Levels of C-Type Lectin REG3α and Gut Damage in People With Human Immunodeficiency Virus

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Background. Regenerating islet-derived protein 3α (REG3α) is an antimicrobial peptide secreted by intestinal Paneth cells. Circulating REG3α has been identified as a gut damage marker in inflammatory bowel diseases. People living with human immunodeficiency virus (PWH) on antiretroviral therapy (ART) present with an abnormal intestinal landscape leading to microbial translocation, persistent inflammation, and development of non-AIDS comorbidities. Herein, we assessed REG3α as a marker of gut damage in PWH.

Methods. Plasma from 169 adult PWH, including 30 elite controllers (ECs), and 30 human immunodeficiency virus (HIV)–uninfected controls were assessed. REG3α plasma levels were compared with HIV disease progression, epithelial gut damage, microbial translocation, and immune activation markers.

Results. Cross-sectionally, REG3α levels were elevated in untreated and ART-treated PWH compared with controls. ECs also had elevated REG3α levels compared to controls. Longitudinally, REG3α levels increased in PWH without ART and decreased in those who initiated ART. REG3α levels were inversely associated with CD4 T-cell count and CD4:CD8 ratio, while positively correlated with HIV viral load in untreated participants, and with fungal product translocation and inflammatory markers in all PWH.

Conclusions. Plasma REG3α levels were elevated in PWH, including ECs. The gut inflammatory marker REG3α may be used to evaluate therapeutic interventions and predict non-AIDS comorbidity risks in PWH.

Keywords. HIV; gut damage; REG3α; microbial translocation; inflammation.

Human immunodeficiency virus (HIV) infection is characterized by a rapid decline in mucosal CD4 T-cell count, early epithelial gut damage, and subsequent translocation of microbial products into the systemic circulation [1]. Epithelial gut damage and microbial translocation have been linked with inflammation, HIV disease progression, and occurrence of non-AIDS comorbidities such as cardiovascular and fatty liver diseases, neurocognitive dysfunctions, and cancer in people living with HIV (PWH) under antiretroviral therapy (ART) [2, 3].

In the absence of ART, the majority of PWH, called progressors, have an abnormal gastrointestinal landscape characterized by villous atrophy, crypt hyperplasia, loosened tight junctions, gastrointestinal inflammation, and increased intestinal permeability [4–7]. Despite long-term ART, damage to the gut mucosa persists in PWH [8–10]. However, elite controllers (ECs), a rare subset of PWH who maintain undetectable viral load (VL) without ART, present with lower levels of gut mucosal damage compared with progressors [11].

The mechanism behind persistent gut damage in PWH is not fully understood [12]. Epithelial gut damage has been observed to appear prior to immune changes in simian immunodeficiency virus (SIV)–infected rhesus macaques [7]. Deterioration of the gastrointestinal landscape in PWH and SIV-infected rhesus macaques has been shown to cause translocation of bacterial and fungal products contributing to chronic immune activation and the development of non-AIDS comorbidities [1, 12–15]. Thus, understanding the underlying mechanisms of gut damage in PWH may help to develop novel therapeutic strategies to

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reduce systemic immune activation and subsequent development of non-AIDS comorbidities in PWH on ART.

Markers of gut barrier integrity are commonly used in clinical research, as assessing the entire gut epithelium by endoscopy remains difficult [16]. Blood markers of microbial translocation such as lipopolysaccharide (LPS) and soluble CD14 (sCD14) are used as indirect measures of gut epithelium integrity. Circulating intestinal fatty acid binding protein (I-FABP), an intracellular protein constitutively expressed in enterocytes, is commonly used. Upon intestinal cell death, I-FABP is released into the mucosa and subsequently translocates into the blood in inflammatory bowel diseases (IBDs) [17, 18]. In PWH, circulating levels of I-FABP were found to be elevated in HIV progressors but not in ECs [11, 19]. However, some studies found an increase in I-FABP levels after ART initiation, which does not mirror the decrease of microbial translocation and inflammation observed after ART initiation [19–21]. Moreover, we and others have shown that circulating I-FABP levels are not associated with some markers of microbial translocation and inflammation in ART-treated PWH [15, 22].

Immunoglobulin A (IgA), antimicrobial peptides, the mucus layer, and tight junctions establish a barrier preventing translocation of commensal bacteria and pathogens into systemic circulation. Regenerating islet-derived protein 3α (REG3α), also called HIP (hepatocarcinoma-intestine-pancreas) or PAP (pancreatitis-associated protein), is a C-type lectin antimicrobial peptide constitutively secreted in the gut lumen by Paneth cells [23]. REG3α is selectively produced in the small intestine upon bacterial colonization as its homolog REG3γ is absent in germ-free mice [24]. Upon intestinal stress, REG3α production is increased to help contain bacterial infection by binding to peptidoglycan and killing gram-positive bacteria [23, 24]. Upon loss of gut barrier integrity, REG3α can cross the epithelium, translocate into the lamina propria, and enter the systemic circulation [25]. Hence, circulating levels of REG3α are considered to be a marker of gut damage during enteropathies such as Crohn and celiac diseases, ulcerative colitis, and graft-vs-host disease (GVHD) [25–27].

Herein, we investigated whether the gut damage marker REG3α was elevated in the plasma of PWH. We compared REG3α levels in PWH in early and chronic phases of HIV infection, in participants who initiated ART or remained untreated, as well as in ECs. We also assessed the association between plasma levels of REG3α and markers of HIV disease progression, microbial translocation, and inflammation.

METHODS

Study Design

A total of 169 adult PWH were enrolled from the Montreal Primary HIV Infection Study, from patients followed at the Chronic Viral Illness Service (CVIS) at the McGill University Health Centre (MUHC), from the Canadian HIV and Aging Cohort (CHACS) [28] and from the Canadian cohort of HIV-Infected Slow Progressors. PWH were categorized into those in early HIV infection (n = 51), defined as being within 6 months of the estimated date of HIV acquisition determined using the Department of Health and Human Services–National Institutes of Health Acute HIV Infection and Early Diagnosis Research Program guidelines [29, 30], or those in chronic HIV infection who were either untreated (n = 22) or ART-treated (n = 66). Samples from 30 ECs maintaining plasma viremia <1.7 $\log_{10}$ copies/mL and CD4 T-cell count >200 cells/μL in the absence of ART were analyzed. Groups of PWH were compared to 30 HIV-uninfected controls who were mostly partners of PWH, recruited from the CVIS at the MUHC and the CHACS (Supplementary Figure 1). We prospectively followed 22 PWH for 2 years. Ten participants were followed from the early phase of the infection before and after at least 1 year on ART, while 12 ART-naive persons with early HIV infection were followed and remained without ART (Table 1, Supplementary Figure 1). All participants were fasting at the time of blood collection. Participants did not present with any acute condition or history of IBD. To account for potential confounders, we recorded renal/pancreatic/liver functions, serum lipid levels, viral coinfections, and the usage of antibiotics. Blood samples were collected to perform clinical measurements. Plasma and peripheral blood mononuclear cells were isolated and stored at −80°C and in liquid nitrogen, respectively, until used.

Clinical Laboratory Measurements

Plasma HIV type 1 (HIV-1) p24 antigen/antibody and a confirmatory Western blot test diagnosed HIV infection as previously reported [15]. Quantification of plasma VL was done using the Abbott RealTime HIV-1 assay (Abbott Laboratories). Total immunoglobulin G (IgG), immunoglobulin M (IgM), and IgA levels were measured in serum using an Olympus AU58000 (Beckman Coulter). CD4 and CD8 T-cell counts were measured using 4-color flow cytometry.

Markers of Epithelial Gut Damage, Microbial Translocation, Inflammation, and Global B-Cell Activation

REG3α and I-FABP were quantified in plasma using an enzyme-linked immunosorbent assay (ELISA) kit (R&D Systems and Hycult Biotech, respectively). Anti-cytomegalovirus (CMV) IgG concentrations were measured using the anti-CMV IgG enzyme immunoassay test kit (GenWay Biotech). LPS was quantified using a human LPS ELISA kit (Cusabio). sCD14 was quantified by immunoassay (Quantikine, R&D Systems). Plasma (1→3)-β-D-glucan level (β-D-glucan) was measured by the Fungitell *Limulus* Amebocyte Lysate assay (Associates of Cape Cod). Plasma levels of interleukin 6 (IL-6), interleukin 8 (IL-8), and tumor necrosis factor (TNF)–α were measured by MSD multiplexes

Table 1. Participant Characteristics

Characteristic	Early HIV Infection (n = 51)	Chronic HIV Infection (n = 88)		ECs (n = 30)	Controls (n = 30)
		ART Naive (n = 22)	ART Treated (n = 66)		
Age, y					
Median	34	38	55	43	58
IQR	28–44	33–50	47–61	36–50	52–61
Sex, %					
Women	3	23	11	27	27
Men	97	77	89	73	73
CD4 count, cells/μL					
Median	460	220	595	576	821
IQR	310–640	35–345	416–700	498–745	519–1022
Range	210–1680	3–489	54–1251	290–1090	281–1173
CD8 count, cells/μL					
Median	810	770	720	660	373
IQR	620–1040	407–1147	552–968	433–955	273–536
Range	279–2590	54–1425	140–1475	260–1560	188–843
CD4:CD8 ratio					
Median	0.57	0.19	0.77	0.86	2.08
IQR	0.39–0.8	0.06–0.43	0.54–1.12	0.69–1.24	1.22–3.01
Viral load, $\log_{10}$ copies/mL					
Median	4.5	5.1	<1.7	<1.7	NA
IQR	3.8–5.0	4.4–5.5	1.7-1.7	NA	
Range	1.3–7.5	3.9–5.9	1.6–1.7	NA	
Time to initiation of ART, y					
Median	NA	NA	3.0	NA	NA
IQR			0.6–7.0		
Range			0–23.8		
ART duration, y					
Median	NA	NA	13.6	NA	NA
IQR			1.6–17.8		
Range			0.2–25.4		

Abbreviations: ART, antiretroviral therapy; ECs, elite controllers; HIV, human immunodeficiency virus; IQR, interquartile range; NA, not applicable.

(MesoScale Discovery) [31]. Interleukin 22 (IL-22) levels were quantified by ELISA (R&D Systems). Kynurenine and tryptophan plasma levels were measured using an automated online solid-phase extraction liquid chromatographic tandem mass spectrometric method [10]. The kynurenine-to-tryptophan ratio was calculated as a measure of indoleamine-2,3-dioxygenase (IDO-1) enzyme activity. All measurements were done in duplicate as previously reported [31]. Percentage of activated CD4 and CD8 T-cells was determined by flow cytometry analysis of the coexpression of HLA-DR and CD38 [15].

Statistical Analyses

Statistical analyses were conducted using GraphPad Prism 6.0 software. Comparisons were conducted using nonparametric Mann–Whitney *U* test and Kruskal–Wallis test with Dunn correction for multiple variables. Correlations were performed using a nonparametric Spearman test. An α level of 5% was used for statistical significance. Multivariate analysis was performed using IBM SPSS 24.0 software.

Ethical Considerations

All study participants provided written consent for enrollment and ethical approval was obtained from the MUHC and the Centre Hospitalier de l'Université de Montréal research ethics boards. The study was conducted in accordance with the Declaration of Helsinki.

RESULTS

Study Participant Characteristics

Participants had a median age of 48 (interquartile range [IQR], 36–56) years, and 87.1% were male. Untreated PWH had a lower CD4 T-cell count with a median of 480 (IQR, 321–658) cells/μL, whereas CD4 T-cell count was higher in those receiving ART (552 [IQR, 410–691] cells/μL). Conversely, untreated PWH had a higher CD8 T-cell count (772 [IQR, 611–1073] cells/μL) than

those receiving ART (727 [IQR, 552–953] cells/μL). Median $\log_{10}$ VL per mL of plasma for ART-naive early and chronically HIV-infected groups was 4.5 (IQR, 3.8–5.0) and 5.1 (IQR, 4.4–5.5), respectively. PWH receiving ART for a median of 13.6 (IQR, 1.6–17.8) years had suppressed viremia of <50 (1.7 $\log_{10}$) copies/mL (Table 1).

Plasma REG3α Levels Were Elevated in HIV-Infected Participants and Decreased With ART

Cross-sectional analysis showed higher plasma levels of REG3α during early infection (mean 1938 ± standard deviation 374 pg/mL), untreated chronic infection (3084 ± 293 pg/mL), and ART-treated PWH (2441 ± 630 pg/mL) compared to controls (715 ± 243 pg/mL) ($P < .0001$ for all). Interestingly, REG3α levels were also elevated in ECs (1442 ± 270 pg/mL) compared with controls ($P = .048$). REG3α was higher in untreated chronic than untreated early HIV infection ($P < .0001$). Such values were also lower in treated chronic ($P = .027$) compared with untreated chronic HIV infection (Figure 1*A*). Longitudinal assessment of 12 early HIV-infected PWH not receiving ART showed an increase in REG3α levels from a median of 1878 ± 357 pg/mL to 2074 ± 328 pg/mL over a 24-month interval (Figure 1*C*; $P = .032$). One participant had a decrease in plasma REG3α. For this participant, CD4 count increased (719 then 881 cells/μL), and CD4:CD8 ratio remained elevated (0.83 vs 0.69) despite similar viremia at the 2 timepoints (4.15 vs 4.25 $\log_{10}$ copies/mL). This participant may have had a slower T-cell depletion and lower gut damage than other untreated participants [32, 33], mimicking the SIV-infection tolerance of sooty mangabeys [34, 35]. Conversely, 10 participants with early HIV infection who initiated ART during follow-up had decreased REG3α levels after 24 months (1952 ± 385 vs 1622 ± 318 pg/mL; $P = .049$) (Figure 1*D*). One participant had an increase in REG3α levels after 2 years on ART. This participant had an undetectable viremia (<1.7 $\log_{10}$ copies/mL) at the time of the second sampling and his CD4 count was increased (289 vs 557 cells/μL). However, we did not collected information on potential diarrhea, colitis, or any digestive illness for this participant at the second timepoint.

Nonparametric analyses showed that neither duration of ART nor the time to ART initiation had an influence on REG3α levels in ART-treated, chronically HIV infected participants

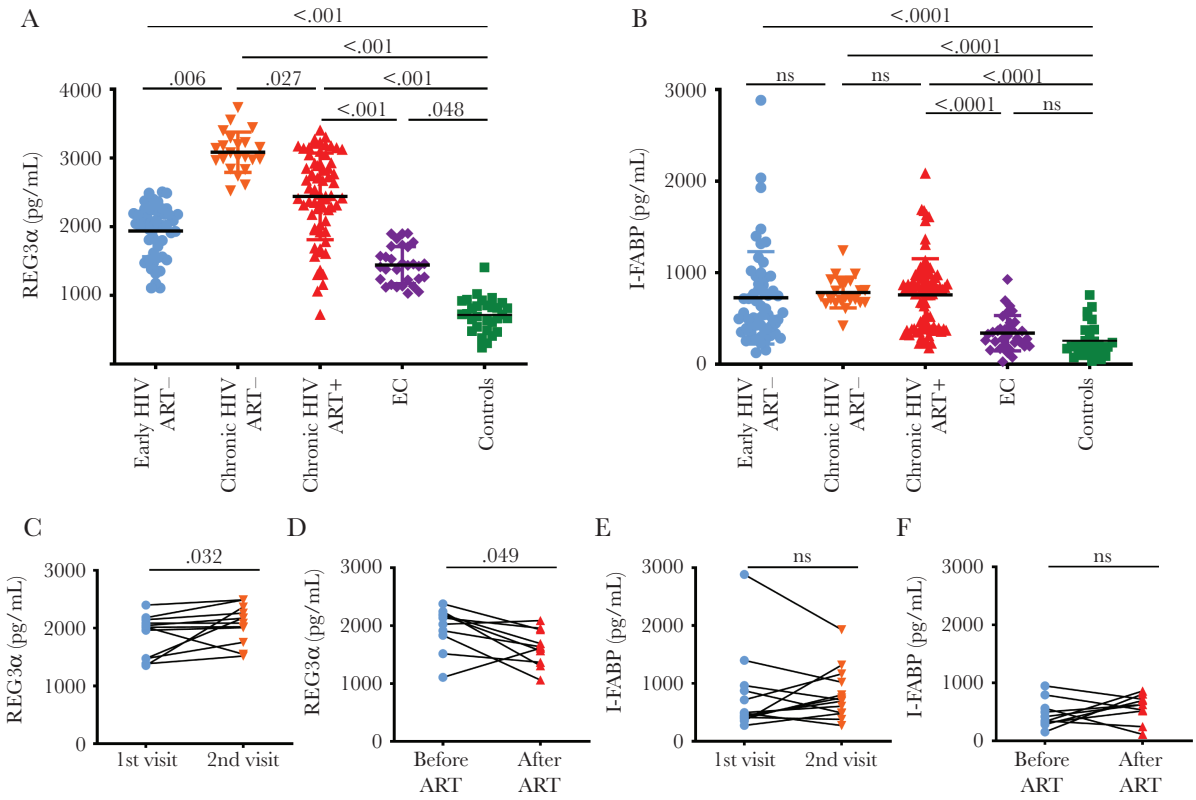

Figure 1. Plasma levels of regenerating islet-derived protein 3α (REG3α) were elevated over the course of human immunodeficiency virus (HIV) infection. *A*, Plasma REG3α levels during early and chronic infection compared to elite controllers (ECs) and uninfected controls. Early HIV without antiretroviral therapy (ART$^-$) (n = 51), chronic HIV without ART (n = 22), chronic HIV with ART (ART$^+$; n = 66), ECs (n = 30), and controls (n = 30), Kruskal–Wallis test. *B*, Plasma intestinal fatty acid binding protein (I-FABP) levels in early HIV infection without ART (n = 56), chronic HIV without ART (n = 22), chronic HIV with ART (n = 71), ECs (n = 30), and controls (n = 30), Kruskal–Wallis test. Longitudinal analysis showed that plasma levels of REG3α (*C*) but not I-FABP levels (*E*) increased over 24 months in people living with HIV (PWH) without ART (n = 12), Wilcoxon test. Longitudinal analysis showed that plasma levels of REG3α (*D*) but not I-FABP (*F*) decreased in PWH after 24 months on ART (n = 10), Wilcoxon test. ns, not significant.

(r = –0.07, P = .65 and r = 0.02, P = .89, respectively; data not shown). Multivariate analysis showed that elevated REG3α levels among PWH were independent of sex, age, and CD4 and CD8 T-cell counts (data not shown). There was no association between REG3α levels and serum levels of the pancreatic enzymes lipase and amylase (data not shown).

Plasma levels of I-FABP were elevated in early, chronic ART-naive and chronic ART-treated PWH compared to controls (P < .0001 for all 3 comparisons) (Figure 1*B*). ECs did not have elevated plasma levels of I-FABP compared with controls (P > .99). As opposed to REG3α, no differences in plasma levels of I-FABP were observed between the different HIV-infected groups, including early, chronic ART-naive and chronic ART-treated PWH. Prospective analysis demonstrated that plasma levels of I-FABP did not change significantly after 2 years in PWH who initiated ART and those who did not (Figure 1*E* and 1F).

Plasma Levels of REG3α Correlated With Markers of HIV Disease Progression

REG3α levels correlated positively with HIV plasma VL (r = 0.29, P = .013) (Figure 2*A*) in both untreated early and chronic HIV infection. Plasma levels of REG3α also inversely correlated with CD4 T-cell count (r = –0.28, P = .0003) (Figure 2*B*) and CD4:CD8 ratio (r = –0.29, P = .0002) in PWH (Figure 2*C*). No correlations were observed between REG3α levels and CD8 T-cell count (r = –0.006, P = .94) (Table 2). Plasma levels of I-FABP did not correlate with these markers (Table 2). REG3α plasma levels were also associated with total plasma IgG and with anti-CMV IgG titer in PWH (r = 0.24, P = .03) (Table 2 and Figure 2*D*, respectively).

REG3α Levels Were Associated With Markers of Epithelial Gut Damage and Microbial Translocation

Plasma REG3α levels were weakly correlated with plasma levels of I-FABP in PWH (r = 0.17, P = .029) (Figure 3*A*). Loss of barrier integrity is implicated in the translocation of microbial products from the gut lumen into the blood [1, 12, 14, 15]. Indeed, plasma levels of LPS, a validated marker of bacterial translocation [1], correlated with REG3α levels in HIV progressors (r = 0.24, P = .005) (Figure 3*B*), but not in ART-treated chronic PWH only (Table 3). In addition, REG3α levels correlated with sCD14, a marker of myeloid cell activation following LPS stimulation (r = 0.31, P = .0009; Figure 3*C*). Plasma levels of β-D-glucan, a marker of fungal translocation [15], also correlated with REG3α levels (r = 0.19, P = .028) (Table

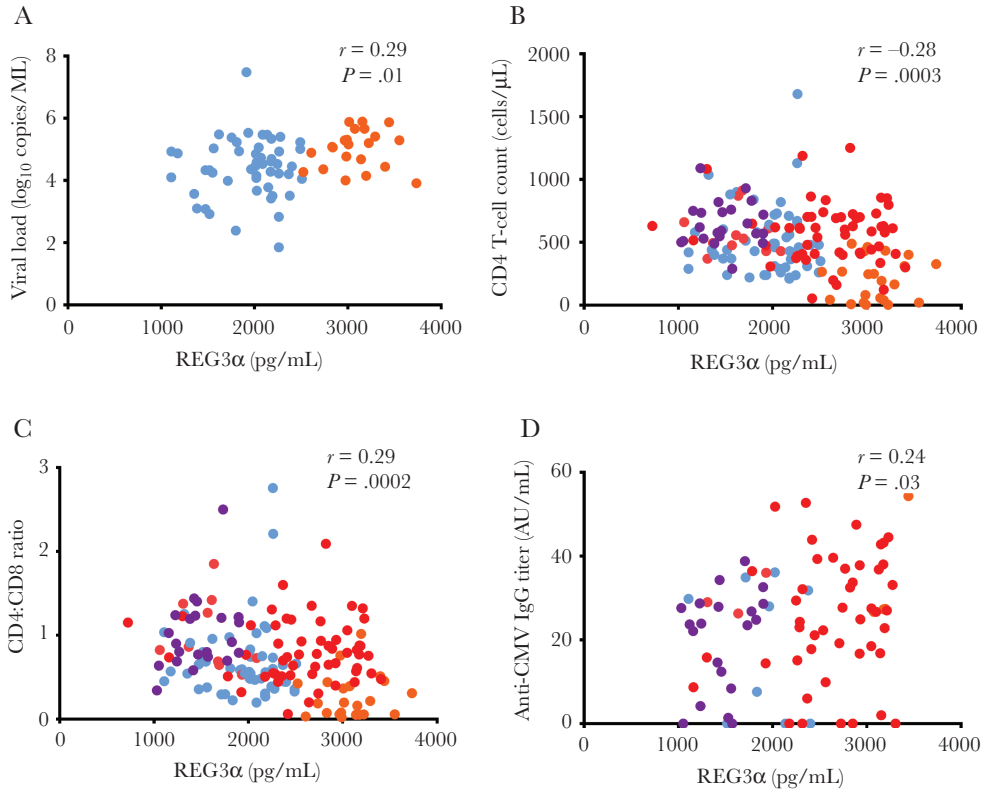

Figure 2. Plasma levels of regenerating islet-derived protein 3α (REG3α) correlated with markers of disease progression. *A*, Plasma REG3α levels correlated with human immunodeficiency virus (HIV) viral load in participants with early HIV and chronic HIV without ART (n = 72). *B*, Plasma REG3α inversely correlated with CD4 T-cell count (*B*) and CD4:CD8 ratio (*C*) in most participants (n = 161). *D*, Plasma REG3α was associated with anti-cytomegalovirus (CMV) immunoglobulin G (IgG) titer in people living with HIV (n = 82). Spearman test was used for correlations. Light blue indicates early HIV infection, orange indicates chronic HIV infection without ART, red indicates chronic HIV infection with ART, and purple indicates elite controllers.

Table 2. Correlation Between Plasma Levels of Regenerating Islet-Derived Protein 3α or Intestinal Fatty Acid Binding Protein and Markers of Disease Progression, Microbial Translocation, and Inflammation in People Living With Human Immunodeficiency Virus (HIV) in Early and Chronic HIV Infection

	Correlation With Plasma Levels in PWH	
Parameter	REG3α	I-FABP
CD4 T-cell count	***r* = –0.28**	*r* = –0.07
	***P* = .0003**	*P* = .39
	n = 161	n = 160
CD8 T-cell count	*r* = –0.006	*r* = 0.11
	P = .94	*P* = .16
	n = 161	n = 160
CD4:CD8 ratio	***r* = –0.29**	*r* = –0.35
	***P* = .0002**	*P* = .66
	n = 161	n = 160
Viral load	***r* = 0.29**	*r* = 0.0015
	***P* = .0134**	*P* = .99
	n = 72	n = 72
LPS	***r* = 0.24**	*r* = 0.047
	***P* = .005**	*P* = .56
	n = 139	n = 160
β-D-glucan	***r* = 0.19**	*r* = 0.11
	***P* = .03**	*P* = .17
	n = 139	n = 160
Soluble CD14	***r* = 0.37**	*r* = –0.0012
	***P* = .0013**	*P* = .99
	n = 74	n = 83
Tryptophan	***r* = –0.41**	*r* = –0.031
	***P* = .0001**	*P* = .78
	n = 81	n = 87
Kynurenine	***r* = 0.30**	*r* = 0.12
	***P* = .0056**	*P* = .26
	n = 81	n = 88
Kynurenine/tryptophan ratio	***r* = 0.39**	*r* = 0.12
	***P* = .0003**	*P* = .28
	n = 81	n = 87
IL-6	***r* = 0.53**	*r* = 0.11
	***P* < .0001**	*P* = .17
	n = 139	n = 160
IL-8	***r* = 0.51**	***r* = 0.17**
	***P* < .0001**	***P* = .035**
	n = 190	**n = 160**
TNF-α	*r* = –0.006	*r* = 0.13
	P = .94	*P* = .10
	n = 139	n = 160
Total IgG	***r* = 0.44**	*r* = 0.16
	***P* = .0019**	*P* = .27
	n = 47	n = 49
Total IgM	*r* = 0.2	*r* = –0.081
	P = .18	*P* = .58
	n = 47	n = 49
Total IgA	*r* = –0.12	*r* = 0.005
	P = .43	*P* = .97
	n = 50	n = 52

Values in boldface indicate *P* values <.05.

Abbreviations: β-D-glucan (1→3)-β-D-glucan; I-FABP, intestinal fatty acid binding protein; IgA, immunoglobulin A; IgG, immunoglobulin G; IgM, immunoglobulin M; IL, interleukin; LPS, lipopolysaccharide; PWH, people living with human immunodeficiency virus; REG3α, regenerating islet-derived protein 3α; TNF, tumor necrosis factor.

2), including in ART-treated chronic PWH (Table 3). Plasma levels of I-FABP did not correlate with these markers of microbial translocation (Table 2), with the exception of β-D-glucan plasma levels among ART-treated people living with chronic HIV (Table 3).

REG3α Levels Correlated With Markers of Systemic Immune Activation

As gut permeability allows for increased microbial translocation, intestinal damage has been associated with increased inflammation in PWH [1, 15]. In our study participants, plasma levels of IL-6 and IL-8, which have been linked with the risk of developing non-AIDS comorbidities [2], were strongly correlated with plasma levels of REG3α in early and chronically HIV-infected participants (r = 0.50, P < .0001 [IL-6] and r = 0.18, P < .02 [IL-8]) (Figure 4A and 4B). Such correlations were maintained in ART-treated chronic PWH (Table 3) Plasma levels of CXCL13, a marker of immune activation in PWH, were also associated with REG3α (r = 0.16, P = .04; data not shown) [31]. Conversely, plasma levels of TNF-α were not associated with plasma levels of REG3α (Table 2). A trend was observed between plasma levels of REG3α and IL-22 (r = 0.21, P = .06; data not shown), a cytokine involved in the immune response against bacterial pathogens in epithelial cells [36]. Plasma levels of REG3α also correlated with the innate activation marker IDO-1, whose enzymatic activity was measured by the kynurenine-to-tryptophan ratio in plasma of PWH in both early and chronic HIV infection (r = 0.35, P = .0009; Figure 4C). The association of REG3α levels with markers of inflammation and IDO-1 activity was independent of sex, age, and CD4 and CD8 T-cell counts. Importantly, such correlations were not detected with plasma levels of I-FABP (Table 2).

Last, we observed that plasma levels of REG3α levels were associated with the percentage of activated HLA-DR$^+$CD38$^+$CD4 (r = 0.46, P = .048; Supplementary Figure 1*A*) and CD8 T-cells (r = 0.66, P = .002; Supplementary Figure 1*B*). Conversely, plasma levels of I-FABP were not associated with percentage of activated CD4 (r = 0.20, P = .39; data not shown) nor CD8 T cells (r = 0.04, P = .87; data not shown).

DISCUSSION

REG3α has been previously validated as a marker of gut damage in IBDs and GVHD as it is solely produced in the intestine [25–27, 37]. As PWH present with intestinal abnormalities even after long-term ART, we assessed their plasma levels of REG3α. To our knowledge, we are the first to report elevated circulating REG3α in untreated and ART-treated PWH. Plasma levels of REG3α were increased in PWH in chronic infection compared to those in early infection. Initiation of ART was associated with a decrease without normalization of plasma levels of REG3α. In contrast to I-FABP, plasma levels of REG3α were significantly higher in ECs compared with controls. Furthermore, plasma levels of REG3α were associated with markers of HIV disease

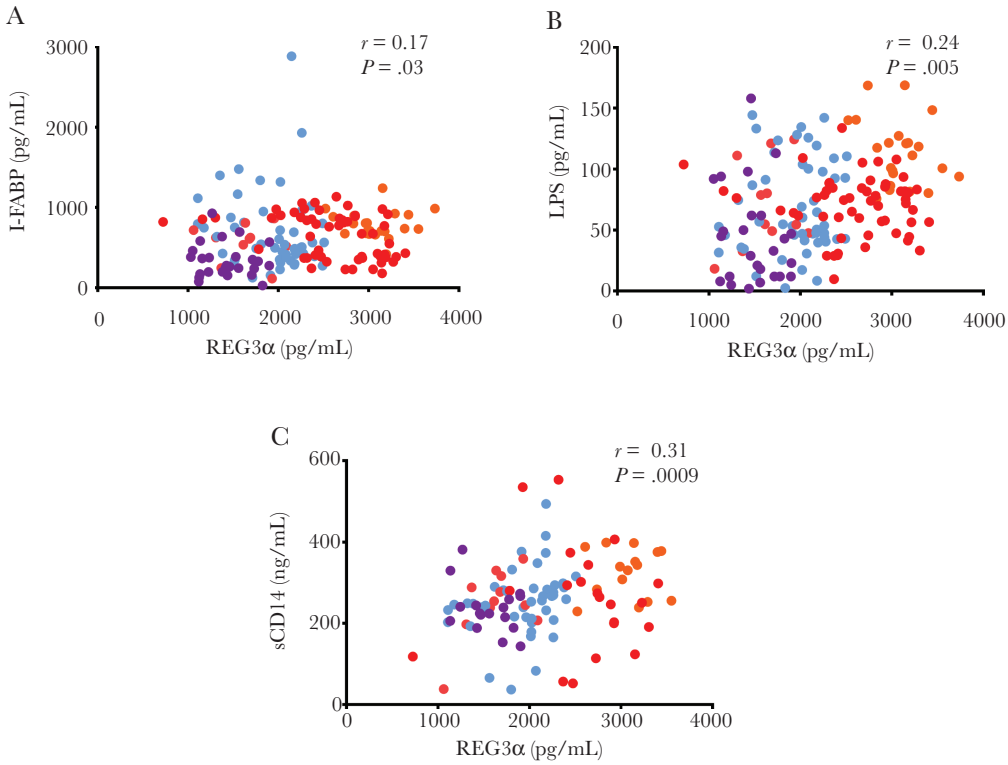

Figure 3. Plasma levels of regenerating islet-derived protein 3α (REG3α) were associated with markers of epithelial gut damage and microbial translocation. *A*, Plasma levels of REG3α correlated with plasma intestinal fatty acid binding protein (I-FABP) levels in all people living with human immunodeficiency virus (PWH; n = 165). *B*, Plasma levels of REG3α were correlated with plasma levels of lipopolysaccharide (LPS; n = 163). *C*, Plasma levels of REG3α correlated with plasma levels of soluble CD14 (sCD14) in PWH (n = 109). Spearman test was used for correlations. Light blue indicates early human immunodeficiency virus (HIV) infection, orange indicates chronic HIV infection without antiretroviral therapy (ART), red indicates chronic HIV infection with ART, and purple indicates elite controllers.

progression and microbial translocation in untreated PWH, and with markers of systemic inflammation and T-cell activation in all PWH.

REG3α is a member of a family of antimicrobial peptides secreted by Paneth cells in the crypts of the intestinal epithelium. Gut antimicrobial peptides keep pathogens and commensal microbes at bay from the mucosa [38]. REG3α acts as a C-type lectin by binding to peptidoglycan from gram-positive bacteria, forming a pore that in turn kills bacteria [39]. REG3α is exclusively secreted into the intestinal lumen and thus its presence in the lamina propria is a marker of gut damage, allowing for its translocation into the blood. Hence, elevated plasma REG3α levels are an indicator of gut epithelium integrity. Increased gut damage and circulating levels of REG3α were found in people with Crohn disease, ulcerative colitis, and celiac disease, but not in people with irritable bowel syndrome [25].

As PWH, including those on long-term ART, have increased gut damage, they also experience increased microbial translocation and chronic inflammation. Because routine access to gut tissue samples remains challenging [16], methods of assessing gut damage in PWH rely on plasma/serum level markers. Existing markers such as soluble suppressor of tumorigenicity 2 assesses the degree of inflammation in any type of epithelium and were only elevated during the early phase of the infection in PWH [8]. Research studies commonly utilize circulating I-FABP as a measure of enterocyte cell death and turnover [11, 18]. Although circulating I-FABP is a reliable measure of the level of enterocyte damage, it yields little information concerning the degree of intestinal permeability prior to or after enterocyte lysis. In contrast, during homeostasis, REG3α is constitutively secreted into the gut lumen with very small quantities translocating into systemic circulation. However, upon gut damage, REG3α translocates into systemic circulation. Thus, circulating REG3α levels reflect the degree of gut damage independent of enterocyte cell death.

Plasma levels of REG3α were more elevated in chronic vs early HIV infection. Moreover, our longitudinal analyses demonstrated an increase of REG3α levels in the absence of ART. These findings are consistent with our knowledge of the degree of gut damage in PWH. In contrast, no significant differences in I-FABP levels were detected between the early and chronic phase of HIV infection in our cross-sectional nor in the longitudinal analysis. Measuring gut damage with REG3α compared to I-FABP levels allows for a better identification of participants in early or chronic HIV infection as well as ECs from controls. In addition, stronger correlations were

Table 3. Correlation Between Plasma Levels of Regenerating Islet-Derived Protein 3α or Intestinal Fatty Acid Binding Protein and Markers of Disease Progression, Microbial Translocation, and Inflammation in Antiretroviral Therapy–Treated People Living With Human Immunodeficiency Virus in Chronic Infection

	Correlation With Plasma Levels in ART-Treated PWH With Chronic Infection	
Parameter	REG3α	I-FABP
CD4 T-cell count	*r* = –0.015 *P* = .90 n = 66	*r* = –0.009 *P* = .94 n = 66
CD8 T-cell count	*r* = –0.022 *P* = .86 n = 66	*r* = –0.062 *P* = .62 n = 66
CD4:CD8 ratio	*r* = –0.069 *P* = .58 n = 66	*r* = 0.016 *P* = .89 n = 66
LPS	*r* = –0.035 *P* = .78 n = 66	*r* = –0.039 *P* = .75 n = 66
β-D-glucan	***r* = 0.25** ***P* = .04** **n = 66**	***r* = 0.39** ***P* = .0011** **n = 66**
Soluble CD14	*r* = –0.22 *P* = .48 n = 12	*r* = 0.035 *P* = .92 n = 12
Tryptophan	***r* = –0.46** ***P* = .02** **n = 24**	*r* = –0.33 *P* = .12 n = 24
Kynurenine	*r* = 0.76 *P* = .73 n = 24	***r* = 0.48** ***P* = .017** **n = 24**
Kynurenine/tryptophan ratio	***r* = 0.4** ***P* = .05** **n = 24**	***r* = 0.56** ***P* = .0045** **n = 24**
IL-6	***r* = 0.29** ***P* = .01** **n = 66**	*r* = 0.044 *P* = .72 n = 66
IL-8	***r* = 0.32** ***P* = .008** **n = 66**	*r* = 0.22 *P* = .06 n = 66
TNF-α	*r* = –0.035 *P* = .78 n = 66	*r* = 0.085 *P* = .50 n = 66

Values in boldface indicate *P* values <.05.

Abbreviations: β-D-glucan (1→3)-β-D-glucan; ART, antiretroviral therapy; I-FABP, intestinal fatty acid binding protein; IL, interleukin; LPS, lipopolysaccharide; PWH, people living with human immunodeficiency virus; REG3α, regenerating islet-derived protein 3α; TNF, tumor necrosis factor.

detected between plasma levels of REG3α with most validated markers of disease progression, microbial translocation, and inflammation.

This article is the first to report circulating levels of REG3α in ECs to be significantly higher than controls while remaining lower than HIV progressors. This was expected as ECs display reduced epithelial gut damage compared with HIV-infected progressors [11]. However, persistence of chronic inflammation and elevated plasma levels of sCD14 suggest that, similar to HIV-infected progressors, ECs also present with chronic microbial translocation and inflammation [40]. Indeed, increased gut damage may explain the higher frequency of non-AIDS comorbidities like cardiovascular diseases in ECs compared to controls [41].

We and others have previously shown an association between loss of barrier integrity, gut damage, and translocation of bacterial and fungal products into the systemic circulation [1, 12, 14, 15]. Indeed, study findings demonstrated that circulating levels of REG3α correlated with plasma levels of LPS and β-D-glucan levels in PWH. As increased gut damage allows the passage of microbial products into the circulation, it also contributes to systemic inflammation and immune activation. We observed that REG3α correlated with plasma levels of proinflammatory cytokines such as IL-6 and IL-8, and the percentage of activated CD4 and CD8 T cells. REG3α levels were also associated with total IgG but not IgM or IgA plasma levels, suggesting a link between gut damage and B-cell activation. Moreover, IDO-1 activity, measured by plasma tryptophan-to-kynurenine ratio, was strongly correlated with REG3α levels. As IDO-1 activity is linked with dysbiosis and microbial translocation during HIV infection, this further strengthens the association between REG3α and gut inflammation in PWH [10, 42]. IDO-1 activity is a marker of activated innate immune cells such as monocytes and macrophages and has been shown to predict cardiovascular diseases [43]. Moreover, anti-CMV IgG titer was associated with inflammation and mortality in HIV-uninfected population [44]. In line with our previous findings showing an association between CMV coinfection and elevated gut damage in PWH [45, 46], we found an association between plasma REG3α and anti-CMV IgG titer.

Conversely to plasma levels of I-FABP, REG3α were associated with levels of CD4 and CD8 T-cell activation, which have been reported to be a predictor of HIV disease progression independently of plasma VL [2].

We acknowledge that our study presents some limitations as we did not assess gut microbiota composition in our study participants. Elevated REG3α production in the gut lumen would also be associated with shifts in microbiota composition resulting from the killing of certain bacteria as seen in a mouse model of colitis [47]. However, the causative role of REG3α in microbiota modification in PWH has yet to be explored. In addition, although we accounted for several factors such as usage of antibiotics and CMV coinfection, we did not collect information on alcohol consumption in our participants, which might play a role in gut damage. In this study, we did not assess the predictive value of REG3α elevation for the risk of development of non-AIDS comorbidities, as clinical outcomes were rare. Last, gut tissue expression of REG3α needs to be studied to confirm its role and value as a marker of gut damage in PWH [37].

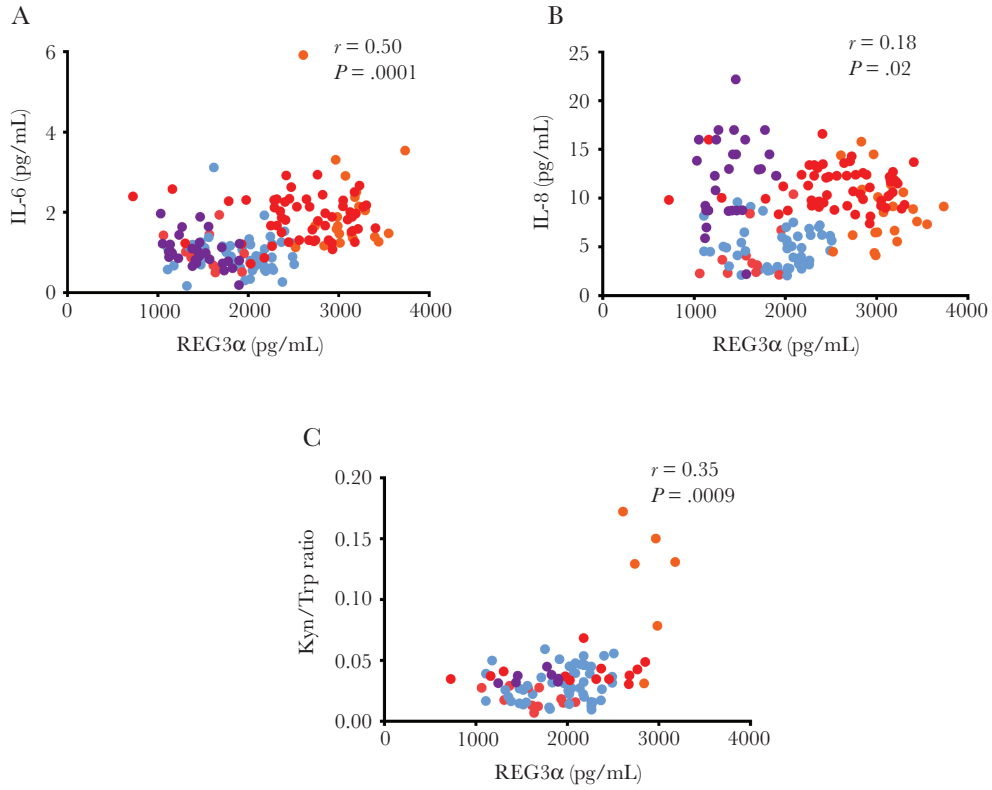

Figure 4. Plasma levels of regenerating islet-derived protein 3α (REG3α) were associated with markers of myeloid and lymphoid activation. Plasma levels of REG3α were correlated with plasma levels of interleukin 6 (IL-6; *A*) and interleukin 8 (IL-8; *B*) (n = 168). Plasma levels of REG3α correlated with indoleamine-2,3-dioxygenase (IDO-1) activity as REG3α levels were positively associated with the kynurenine-to-tryptophan (Kyn/Trp) ratio (*C*) in a subset of people living with human immunodeficiency virus (HIV) (n = 88). Spearman test was used for correlations. Light blue indicates early HIV infection, orange indicates chronic HIV infection without antiretroviral therapy (ART), red indicates chronic HIV infection with ART, and purple indicates elite controllers.

CONCLUSIONS

Compared to I-FABP, REG3α plasma levels were able to identify participants in early or chronic HIV infection as well as those receiving or not receiving ART, and ECs. In addition, REG3α presented stronger correlations with several validated makers of HIV disease progression and inflammation.

As gut damage and microbial translocation are associated with inflammation and non-AIDS comorbidities [2, 48], robust markers of epithelial gut damage are warranted to provide better care for PWH. We showed that plasma levels of the C-type lectin REG3α were elevated in PWH. Measuring REG3α levels may contribute to the assessment of the risk of developing non-AIDS comorbidities in PWH and provide a useful marker to evaluate therapeutic interventions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online. Consisting of data provided by the authors to benefit the reader, the posted materials are not copyedited and are the sole responsibility of the authors, so questions or comments should be addressed to the corresponding author.

Notes

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