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MAJOR ARTICLE

Plasma Human Immunodeficiency Virus 1 Soluble Glycoprotein 120 Association With Correlates of Immune Dysfunction and Inflammation in Antiretroviral Therapy–Treated Individuals With Undetectable Viremia

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Background. Chronic inflammation persists in some people living with human immunodeficiency virus (HIV) during antiretroviral therapy and is associated with premature aging. The glycoprotein 120 (gp120) subunit of HIV-1 envelope sheds and can be detected in plasma, showing immunomodulatory properties even in the absence of detectable viremia. We evaluated whether plasma soluble gp120 (sgp120) and a family of gp120-specific anti–cluster A antibodies, linked to CD4 depletion in vitro, contribute to chronic inflammation, immune dysfunction, and subclinical cardiovascular disease in participants of the Canadian HIV and Aging Cohort Study with undetectable viremia.

Methods. Cross-sectional assessment of sgp120 and anti–cluster A antibodies was performed in 386 individuals from the cohort. Their association with proinflammatory cytokines and subclinical coronary artery disease was assessed using linear regression models.

Results. High levels of sgp120 and anti–cluster A antibodies were inversely correlated with $CD4^+$ T cell count and CD4/CD8 ratio. The presence of sgp120 was associated with increased levels of interleukin 6. In participants with detectable atherosclerotic plaque and detectable sgp120, anti–cluster A antibodies and their combination with sgp120 levels correlated positively with the total volume of atherosclerotic plaques.

Conclusions. This study showed that sgp120 may act as a pan toxin causing immune dysfunction and sustained inflammation in a subset of people living with HIV, contributing to the development of premature comorbid conditions.

Keywords. HIV-1; soluble gp120; anti–cluster A antibodies; chronic inflammation; cardiovascular diseases.

While antiretroviral therapy (ART) efficiently inhibits viral replication, people living with human immunodeficiency virus (HIV; PLWH) have a 15-year gap in comorbidity-free years [1]. The underlying causes for this gap are numerous and include the presence of residual immune dysfunction and chronic antigenic stimulation by HIV, which contributes to a state of sustained inflammation [2]. Persistent immune dysfunction has also been associated with the immunological nonresponse that many PLWH experience [3–7]. These individuals, despite efficient viral control, have failure to restore circulating $CD4^+$ T cells and have persistent immune activation, possibly leading to increased comorbid conditions.

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The HIV-1 envelope (Env) glycoprotein, in particular the glycoprotein (gp) 120 domain of the gp120/gp41 heterodimer, besides its role in viral entry, has been shown to exhibit a variety of immunoregulatory activities that could contribute to HIV-1 pathogenesis and immune dysfunction. Owing to its noncovalent interaction with gp41, gp120 can be spontaneously released from the surface of virions and infected cells [8–10]. Consequently, variable amounts of soluble gp120 (sgp120) can be detected in plasma and tissues from PLWH, even during ART [7, 11, 12]. In several studies, sgp120 has been associated with HIV-1–induced immune dysfunction [4–7]. It has also been reported to exert proinflammatory activities, as binding of gp120 to CD4 on the surface of monocytes, macrophages, T cells and dendritic cells has been found to induce the production of cytokines, including interleukin 6 (IL-6), interleukin 10 and IL-1β, interferon α and γ, and tumor necrosis factor (TNF) α) [4, 13–15].

Notably, gp120 shed from productively infected cells has been shown to interact with CD4 present on uninfected bystander $CD4^+$ T cells [15, 16]. This interaction leads to exposure of CD4-induced Env epitopes and sensitization of uninfected bystander $CD4^+$ T cells to antibody-dependent cellular cytotoxicity (ADCC) mediated by HIV-positive plasma [16, 17]. Within HIV-positive plasma, antibodies targeting conserved CD4-induced gp120 cluster A epitopes have been shown to elicit potent ADCC activity against sgp120-coated cells [15, 18]. Antibodies recognizing the gp120 inner-domain cluster A region have been found to be responsible for most of the ADCC activity exhibited by chronically HIV-1–infected individuals, provided that the epitopes recognized by them are exposed [19].

Together, these observations suggest that sgp120 is associated with chronic inflammation, causing persistent antigen stimulation even in the context of prolonged ART and undetectable viremia in plasma, and a potential contributor to an increased risk of comorbid conditions. In the current study, we investigated whether the presence of sgp120 and ADCC-mediating antibodies, specifically anti–cluster A antibodies, were associated with correlates of immune dysfunction, chronic inflammation, and subclinical cardiovascular disease (CVD). To this end, we optimized an enzyme-linked immunosorbent assay (ELISA)–based assay to measure sgp120 in plasma samples from 386 PLWH with long-term ART treatment and undetectable viremia from the Canadian HIV and Aging Cohort Study (CHACS) [20].

METHODS

Ethics Statement

This research project was reviewed and approved by the Centre de Recherche du CHUM (CRCHUM) Research Ethics Board (Ethics Committee approval nos. 22.173, 11.063, and 11.062). Written informed consent was obtained from all study participants, and the research adhered to the standards indicated by the Declaration of Helsinki.

Study Design

We designed a cross-sectional study, nested within the CHACS. The specific objectives of the study were to quantify sgp120 in plasma of participants and study the relationship of sgp120 and anti–cluster A antibodies to correlates of immune dysfunction, inflammation, and subclinical CVD. Longitudinal samples from 9 sgp120-positive participants were also analyzed.

Study Population

The CHACS protocol has been described elsewhere [20]. Briefly, it is a prospective cohort study and biobank, ongoing since 2012 in 10 clinical sites across Canada. Inclusion criteria are to be ≥40 years old or to have lived with HIV for ≥15 years and have a life expectancy of >1 year at enrollment. Participants with no known CVD and a 10-year Framingham risk score of overt CVD ranging from 5% to 20%, no allergy to contrast medium, and no renal failure, were invited to participate in the cardiovascular imaging substudy, in which they underwent computed tomography coronary angiography (CCTA). Participants were selected for the present study if they were recruited into the Montréal and Québec CHACS study sites and had undetectable viral loads. For all participants, data on sociodemographic characteristics, HIV disease history, and traditional cardiovascular risk factors are available through the CHACS study database.

CCTA Protocol

CCTA was performed as described elsewhere [21–23]. A detailed description is provided in the Supplementary Materials.

ELISA Protocol

The sandwich ELISA was described elsewhere, with modifications [7, 15]. A detailed description is provided in the Supplementary Materials.

Protein Production and Purification

Production and purification of monomeric soluble HIV-1_{YU2} gp120 was described elsewhere [15, 24]. A detailed description is provided in the Supplementary Materials.

Multiplex Measurements of Soluble Inflammatory Markers

Customized Human Magnetic Luminex Assays (R&D Systems) were used to measure soluble inflammatory markers in plasma samples (see Supplementary Table 4 for complete analyte list). A detailed description is provided in the Supplementary Materials.

Table 1. Characteristics of Participants in the Canadian HIV and Aging Cohort Study With Undetectable Human Immunodeficiency Virus Viremia, and participants to subgroups

Characteristic	Study Participants, No. (%)[a]				
	Total Study Population (n = 386)	Cardiovascular Imaging Substudy (n = 145)	*P* Value[b]	Multiplex Substudy Participants, No. (%)[a] (n = 157)	*P* Value[b]
Age, mean (SD), years	55.8 (7.6)	56.6 (6.5)	.14	56.6 (6.6)	.07
Male sex	347 (90.0)	134 (92.4)	.23	144 (91.7)	.39
Ethnicity					
White	317 (82.1)	123 (84.8)	.39	131 (83.4)	.29
Black	32 (8.3)	12 (8.2)		15 (9.6)	
Other	37 (9.6)	10 (6.9)		11 (7.0)	
Duration of HIV infection, median (IQR), years	18.3 (12–25)	18.3 (12–25)	.27	19.0 (14–25)	.52
Nadir $CD4^+$ cell count, median (IQR), cells/mL	210 (120–310)	200 (90–300)	.30	195 (100–300)	.11
Nadir $CD4^+$ cell count <200/mL	155 (49.1)	67 (52.8)		74 (54.4%)	
Absolute $CD4^+$ cell count, mean (SD), cells/mL	615 (250)	620 (255)	.27	617 (251)	.50
Absolute $CD8^+$ cell count, mean (SD), cells	763 (376)	796 (382)	.10	809 (408)	.07
CD4/CD8 ratio, mean (SD)	0.96 (0.53)	0.96 (0.47)	.92	0.93 (0.49)	.35
Duration of ART, mean (SD), years	13.9 (6.7)	14.1 (6.8)	.58	14.1 (6.9)	.58
Smoking status					
Never smoker	128 (33.5)	42 (29.4)	.22	48 (31.0)	.44
Past or present smoker	254 (66.5)	101 (70.6)		107 (69.0)	
Intravenous drug use	43 (11.2)	14 (9.6)	.51	15 (9.5)	.42
Alcohol abuse	31 (8.0)	11 (7.6)	.84	11 (7.0)	.57
High blood pressure	113 (29.7)	45 (31.5)	.56	51 (32.7)	.31
Diabetes mellitus	37 (9.6)	15 (10.4)	.71	18 (11.5)	.30
Framingham risk score, median (IQR)	9 (5–16)	9 (6–13)	.72	9 (6–13)	.58
Log anti–cluster A antibodies, mean (SD), RU	2.4 (1.4)	2.2 (1.2)	.22	2.3 (1.2)	.71
sgp120					
Undetectable	279 (72.3)	80 (55.2)	<.001	83 (52.9)	<.001
Low	68 (17.6)	40 (27.6)		48 (30.6)	
High	39 (10.1)	25 (17.2)		26 (16.56)	

Abbreviations: ART, antiretroviral therapy; gp120, glycoprotein 120; HIV, human immunodeficiency virus; IQR, interquartile range; RU, relative units; SD, standard deviation.

[a]Data represent no. (%) of participants unless otherwise specified.

[b]*P* values were obtained using Fisher exact test for categorical variables and k-sample equality of median test for continuous variables, and they represent comparisons between subcohort participants and nonparticipants.

Measurement of HIV DNA

HIV DNA was measured as described elsewhere [25]. A detailed description is provided in the Supplementary Materials.

Statistical Analysis

Appropriate descriptive statistics were used for normally and nonnormally distributed variables, and variables were log-transformed, as needed. A detailed description of the statistical analysis performed is provided in the Supplementary Materials.

RESULTS

Plasma from 386 participants was available for measurements of sgp120 and anti–cluster A antibodies, 157 of whom also had soluble inflammatory markers measured using a multiplex platform. Of the 386, 145 took part in the cardiovascular imaging substudies. The baseline characteristics of all participants and those in each subgroup are summarized in Table 1. There were no differences in terms of sociodemographic characteristics, immune parameters, or traditional cardiovascular risk factors between the total study group and the 2 subgroups.

Measuring sgp120 in Plasma From PLWH With Undetectable Viremia

Based on a previous iteration [7], we optimized an ELISA to measure sgp120 in plasma (Supplementary Figure 1*A*). In this assay, we use a gp120 inner-domain–specific antibody (C11) that targets the highly conserved N-termini and 8-stranded β-sandwich structure of gp120 formed at the late stage of HIV-1 entry [26]. This epitope is therefore buried on the trimeric Env present on virions or infected cells but exposed on sgp120 [16, 26, 27]. The novelty of this assay is the use of the broadly neutralizing CD4 binding site (CD4BS) N6 antibody as a readout, which does not compete for C11 binding. This antibody was reported to target up to 98% of global HIV-1 isolates [28]. Using our assay, a strong linearity ($r = 0.9836$; $P < .001$) over a 500-fold range was observed between the signal obtained and the quantity of purified recombinant monomeric soluble

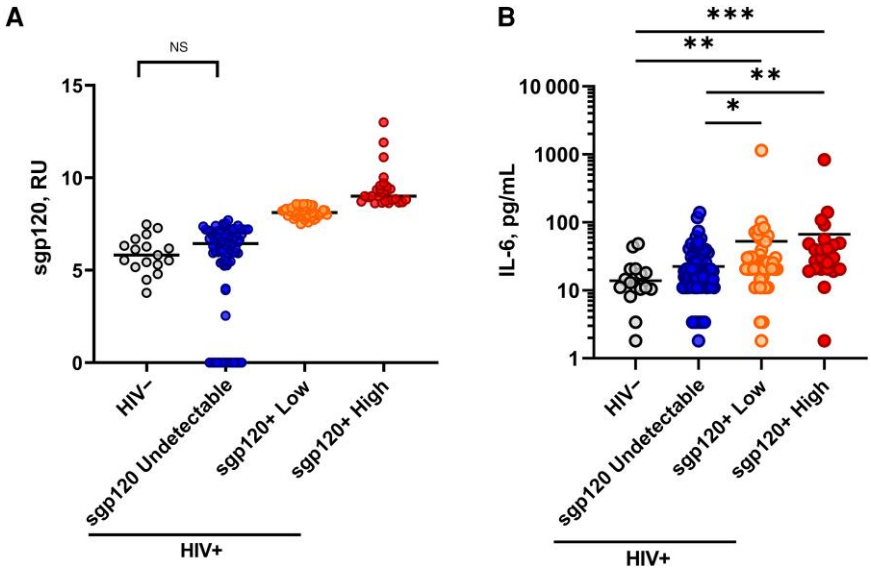

Figure 1. Detection of soluble glycoprotein 120 (sgp120) in people living with human immunodeficiency virus (HIV) with undetectable viremia is associated with inflammation. Representative stratification is shown for 157 people living with HIV, based on levels of sgp120 (*A*) and interleukin 6 (IL-6) (*B*). Statistical analysis was performed using Mann-Whitney *U* tests. $*P < .05$; $**P < .01$; $***P < .001$. Abbreviations: HIV–, HIV negative; HIV+, HIV positive; NS, not significant; RU, relative units.

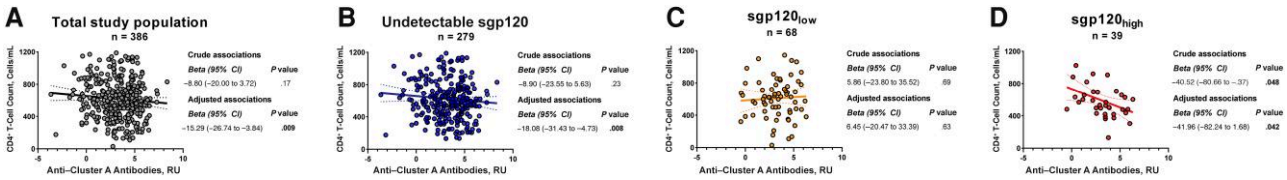

Figure 2. Anti–cluster A antibodies are inversely correlated with $CD4^+$ T-cell counts in people living with human immunodeficiency virus (PLWH) presenting with high levels of soluble glycoprotein 120 (sgp120). Correlations between $CD4^+$ T-cell counts and anti–cluster A antibody levels are shown for 386 PLWH, stratified by sgp120 levels. Correlations are shown for the total study population (*A*) and for PLWH with undetectable sgp120 (*B*), low levels of sgp120 ($sgp120_{low}$) (*C*), or high levels of sgp120 ($sgp120_{high}$) (*D*). Levels of anti–cluster A antibodies were log_2 transformed. Univariable and multivariable linear regressions were performed, with the beta parameters representing the mean predicted change in absolute $CD4^+$ cell counts for each 1-log_2 increase in titers of anti–cluster A antibodies. Multivariable models are adjusted for age, sex, ethnicity, smoking status, duration of antiretroviral therapy, nadir $CD4^+$ cell counts, and levels of anti–CD4 binding site antibodies. Abbreviations: CI, confidence interval; RU, relative units.

HIV-1_{YU2} gp120 used (Supplementary Figure 1*B*). This assay enabled us to measure sgp120 in plasma samples from PLWH with undetectable viral loads compared with uninfected individuals (Figure 1*A*). We further stratified PLWH into 3 subgroups based on the positivity threshold established with the uninfected plasma samples: (1) undetectable sgp120, (2) low levels of sgp120, and (3) high levels of sgp120 (Figure 1*A*). Of the 386 plasma samples analyzed, 72.3% (n = 279) had undetectable sgp120, 17.6% (n = 68) had low levels, and 10.1% (n = 39) had high levels (Table 1).

Association of Anti–Cluster A Antibody Levels With Correlates of Immune Dysfunction

We have previously reported that the release of sgp120 from infected cells sensitizes uninfected bystander $CD4^+$ T cells to ADCC mediated by HIV-positive plasma in vitro, but whether this happens in PLWH remains unclear [15–17]. We therefore measured the levels of anti–cluster A antibodies using an engineered stabilized gp120 inner-domain protein (ID2) exposing only the cluster A region [29, 30]. Figure 2 and Supplementary Table 1 present the associations between anti–cluster A antibodies and absolute $CD4^+$ cell counts or the CD4/CD8 ratio (Supplementary Figure 1). In the total study population, after adjustment for potential confounders (including age, sex, ethnicity, smoking status, nadir $CD4^+$ cell count, duration of ART, and levels of anti-CD4BS antibodies), each 1-log_2 increase in anti-cluster A antibody levels was associated with a mean predicted decrease in $CD4^+$ cell count of -15.3×10^6/mL (95% confidence interval [CI], -26.7×10^6/mL to -3.8×10^6/mL; $P = .009$) (Figure 2*A*).

On stratification of the 386 PLWH by sgp120 levels, we observed that the mean predicted change in $CD4^+$ cell counts

varies by stratum of sgp120. For those with sgp120 below detection levels, each increase of 1-$\log_2$ in anti–cluster A antibodies is associated with a mean predicted decrease in absolute $CD4^+$ cell count of -18.1×10^6/mL ($P = .008$) (Figure 2*B*), while the mean predicted decline for participants with high levels of sgp120 is -42.0×10^6/mL ($P = .04$) (Figure 2*D*). However, the *P* value for the interaction was 0.11 (Supplementary Table 1). A similar dynamic is observed with the CD4/CD8 ratio (presented in Supplementary Figure 2 and Supplementary Table 1); within the total study population, each 1-$\log_2$ increase in the levels of anti–cluster A antibodies is associated with a mean projected decrease in the CD4/CD8 ratio of −0.06 (95% CI, −0.08 to −0.03; $P < .001$) (Supplementary Figure 2*A*).

The magnitude of this association was more pronounced in the subgroup with high levels of sgp120, in which the mean predicted decline in CD4/CD8 ratio was −0.13 (95% CI, −0.22 to −0.04; $P = .004$) (Supplementary Figure 2*D*). However, this difference between the groups was not statistically significant (the *P* value for interaction between sgp120 levels and anti–cluster A antibodies was .21) (Supplementary Table 1). Of note, no association was observed between anti–cluster A antibodies and $CD4^+$ T-cell count or CD4/CD8 ratio in individuals presenting with low levels of sgp120 (Figure 2*C*, Supplementary Figure 2*C*, and Supplementary Table 1). We also measured antibodies targeting the CD4BS, using the gp120 resurfaced stabilized core 3, a previously described probe exposing the CD4BS [31]. We found that only 66 of 386 individuals (17%) had detectable levels of CD4BS antibodies; from which 44 had undetectable sgp120 levels, 13 had low sgp120 levels, and 9 had high sgp120 levels. No associations between CD4BS antibody levels and $CD4^+$ T-cell counts were observed.

Association Between sgp120 and Proinflammatory Markers

The HIV-1 gp120 is a pleiotropic molecule beyond its key role in viral entry [4, 7, 14, 15]. The presence of sgp120 was associated with increased levels of proinflammatory markers in the plasma of early and acute PLWH [7]. To test whether this observation could be extended to PLWH receiving long-term ART treatment, we performed multiplex measurements of various soluble markers associated with chronic inflammation (Supplementary Table 4) in a subset of 157 PLWH. Our results show that plasma levels of IL-6 are significantly higher in people with low or high levels of sgp120 than in the sgp120-undetectable group and uninfected controls (Figure 1*B*). Interestingly, we observed a positive correlation between sgp120 and TNF-α levels in the group with high sgp120 levels (Supplementary Table 3; $r = 0.410$, $P = .04$). Figure 3 shows the correlations between all inflammatory biomarkers measured by multiplex platform, as a function of detectable levels of sgp120. Edge bundling correlation plots show the effect of sgp120 on the network of associations between laboratory and clinical markers. While the undetectable group has weak associations among the parameters measured (Figure 3*A*), the presence of low (Figure 3*B*) or high (Figure 3*C*) levels of sgp120 intensifies the network of associations. Notably, the inverse association between soluble CD14 with lymphocyte counts and CD4/CD8 ratios is strengthened in the presence of sgp120. These results suggest that sgp120 acts as an effect modifier, modulating the associations among the different biomarkers analyzed in this study.

Association of sgp120 and Anti-Cluster A Antibodies with the Size of Coronary Artery Plaque in Participants With Subclinical CVD and Detectable sgp120

In the subgroup of 145 participants with available cardiovascular imaging, 97 (67%) presented with ≥1 coronary artery plaque measurable on CCTA, defining subclinical CVD. Levels of sgp120 (adjusted odds ratio, 1.04 [95% CI, 0.96–1.12]), anti–cluster A antibodies (1.01 [0.82–1.24), or a score defined by multiplying the levels of sgp120 and anti–cluster A antibodies (1.03 [0.96–1.1]) were not associated with the presence or absence of CVD. However, among the 46 individuals with detectable subclinical CVD and detectable sgp120, we found an association between the size of the coronary artery plaques and anti–cluster A antibodies ($P = .01$), as well as of the multiplicative score of anti–cluster A antibodies with levels of sgp120 ($P = .006$) (Figure 4 and Supplementary Table 2), after adjusting for potential confounders (age, sex, hypertension, diabetes mellitus, lipid levels, smoking status, alcohol use disorder, intravenous drug use, and duration of ART). We repeated those analyses using the low attenuation fraction of coronary artery plaque volume (a marker of high-risk plaque) as opposed to the total volume of coronary artery plaque, and the associations were unchanged (Supplementary Table 2).

Longitudinal Analysis of sgp120

We analyzed plasma samples from 9 sgp120-positive PLWH in the CHACS cohort with no viremia. These individuals had 3 sample visits ranging from 1 to 5 years after the baseline assessment. We measured sgp120, anti–cluster A antibodies, and 3 inflammatory markers (IL-6, TNF-α, and soluble CD163 [sCD163]). We found that sgp120 levels are dynamic (Figure 5*A*). Interestingly, IL-6, TNF-α and sCD163 followed a similar pattern to that of sgp120 in most participants (Figure 5*B*–5*D*). While this does not prove causality, it suggests an association among these markers and sgp120. Further supporting our hypothesis that anti–cluster A antibodies are negatively associated with $CD4^+$ T-cell counts, we observed opposite trends between anti–cluster A Abs and $CD4^+$ T-cell counts in 6 of these 9 participants (Figure 6*A* and 6*B*).

DISCUSSION

In this cross-sectional study, nested within a large observational cohort, we explored whether sgp120 could be associated with

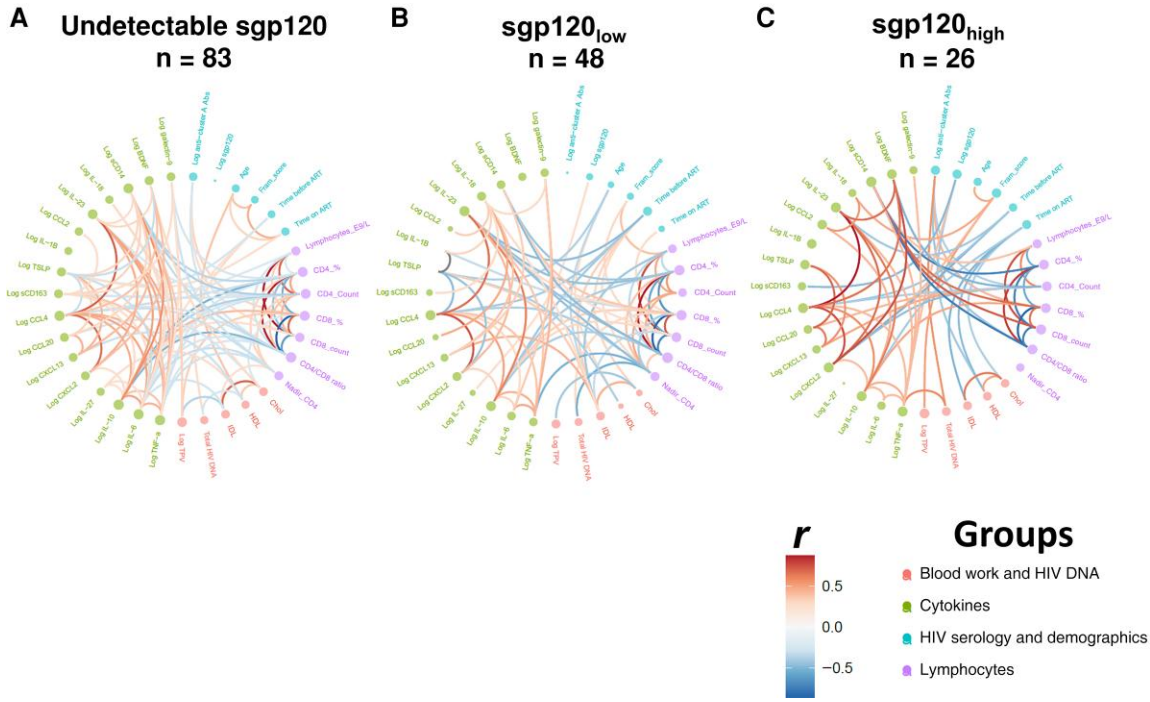

Figure 3. Soluble glycoprotein 120 (sgp120) acts as an "effect modifier" of the associations between clinical and laboratory markers. Its presence modifies the network of associations between clinical and laboratory parameters in people living with human immunodeficiency virus (HIV) with undetectable viremia on stratification by undetectable sgp120 (*A*), low levels of sgp120 ($sgp120_{low}$) (*B*), or high levels of sgp120 ($sgp120_{high}$) (*C*). Spearman rank correlations were computed and graphed as edge bundling correlation plots, using program R v. 4.1.2. Statistical tests were 2 sided, and differences were considered significant at $P < .05$. Edges are only shown if $P < .05$, and nodes are sized according to the connecting edges' *r* values. Nodes are color coded according to the grouping of variables. Abbreviations: Abs, antibodies; ART, antiretroviral-therapy; BDNF, brain-derived neurotrophic factor; Chol, cholesterol; Fram score, Framingham risk score; HDL, high-density lipoproteins; IL-1β, interleukin 1β; LDL, low-density lipoproteins; sCD14, soluble CD14; sCD163, soluble CD163; TNF, tumor necrosis factor; TPV, total atherosclerotic plaque volume; TSLP, thymic stromal lymphopoietin.

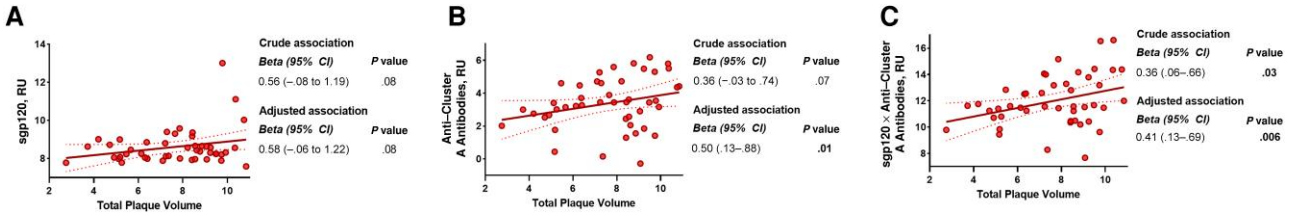

Figure 4. The combination of soluble glycoprotein 120 (sgp120) levels and anti–cluster A antibodies correlates positively with subclinical cardiovascular disease. Associations are displayed between the size of coronary artery plaque volume and sgp120 levels (*A*), anti–cluster A antibodies (*B*), and the multiplicative combination of both (*C*) in people living with human immunodeficiency virus who are positive for sgp120 and have detectable subclinical cardiovascular disease. Values for sgp120, anti–cluster A antibodies, and total plaque volume (mm^3) were log_2 transformed. Univariable and multivariable linear regressions were performed, with the beta parameters representing the mean predicted change in log total plaque volume for each 1-log_2 increase in the exposure. Multivariable models are adjusted for age, sex, smoking status, low- or high-density lipoproteins, diabetes mellitus, hypertension, alcohol use disorder, intravenous drug use, and duration of antiretroviral therapy. Abbreviations: CI, confidence interval; RU, relative units.

chronic inflammation and CVD in PLWH receiving long-term ART treatment. We demonstrated that sgp120 was detectable in the plasma of 107 of 386 participants (28%) with undetectable viremia and that these participants had more pronounced immune dysfunction associated with the presence of anti–cluster A antibodies, as well as distinct soluble inflammatory marker profiles. Longitudinal samples enabled us to evaluate sgp120 levels over time. We found that sgp120 levels are modulated over time and overall are positively associated with IL-6, TNF-α, and sCD163 but negatively associated with $CD4^+$ T-cell counts. In addition, for those with detectable coronary atherosclerosis, anti–cluster A antibodies and their combination with sgp120 were associated with a significant increase in the size of coronary atherosclerotic plaques. Taken together,

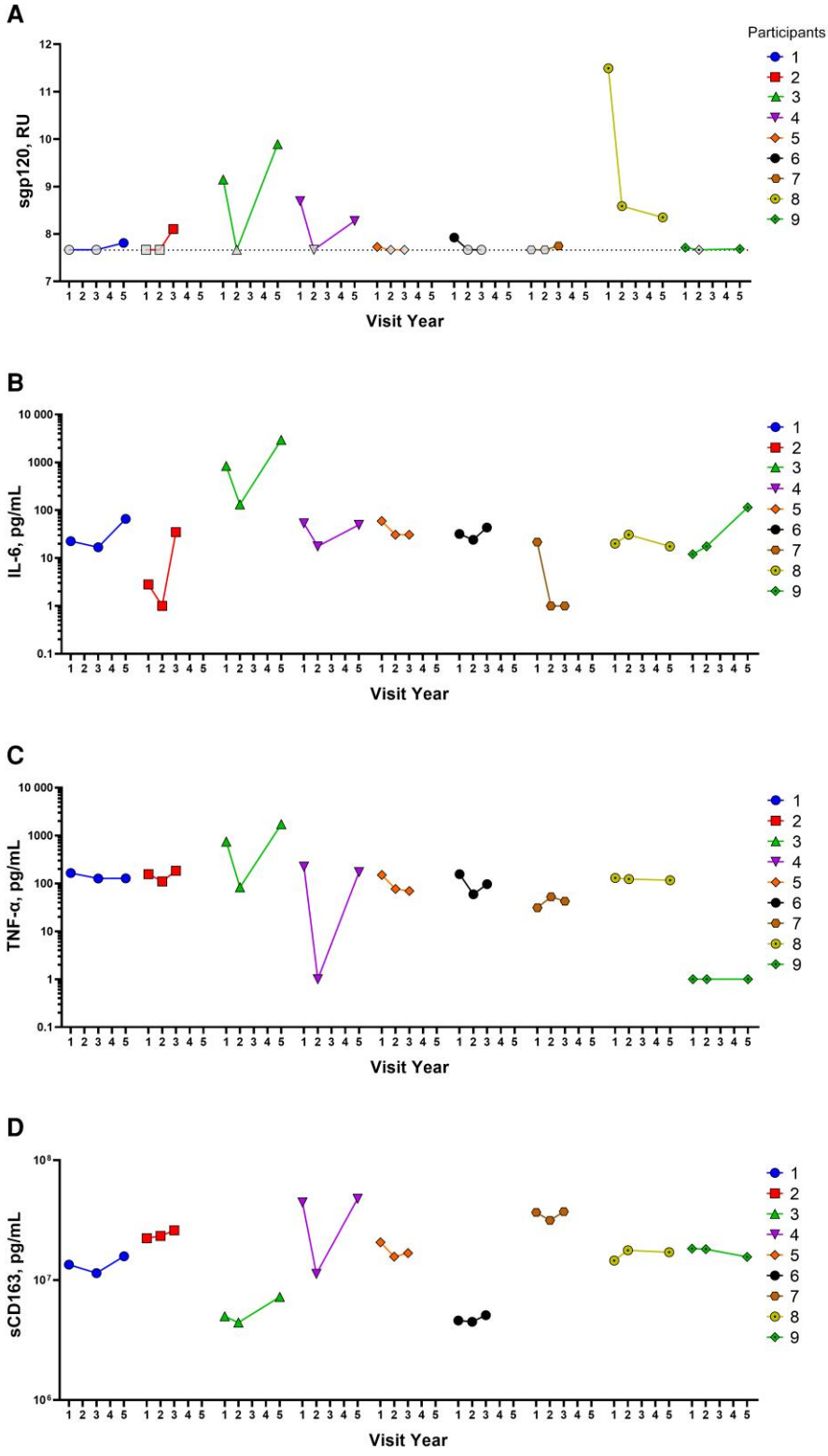

Figure 5. Longitudinal analysis of soluble glycoprotein 120 (sgp120) and some inflammatory markers. Levels of sgp120 (*A*), interleukin 6 (IL-6) (*B*), tumor necrosis factor (TNF) α (*C*), and soluble CD163 (sCD163) (*D*) were measured over time for 9 participants with detectable levels of sgp120. Dashed line in *A* represents seropositivity threshold established in plasma samples from uninfected participants. Abbreviation: RU, relative units.

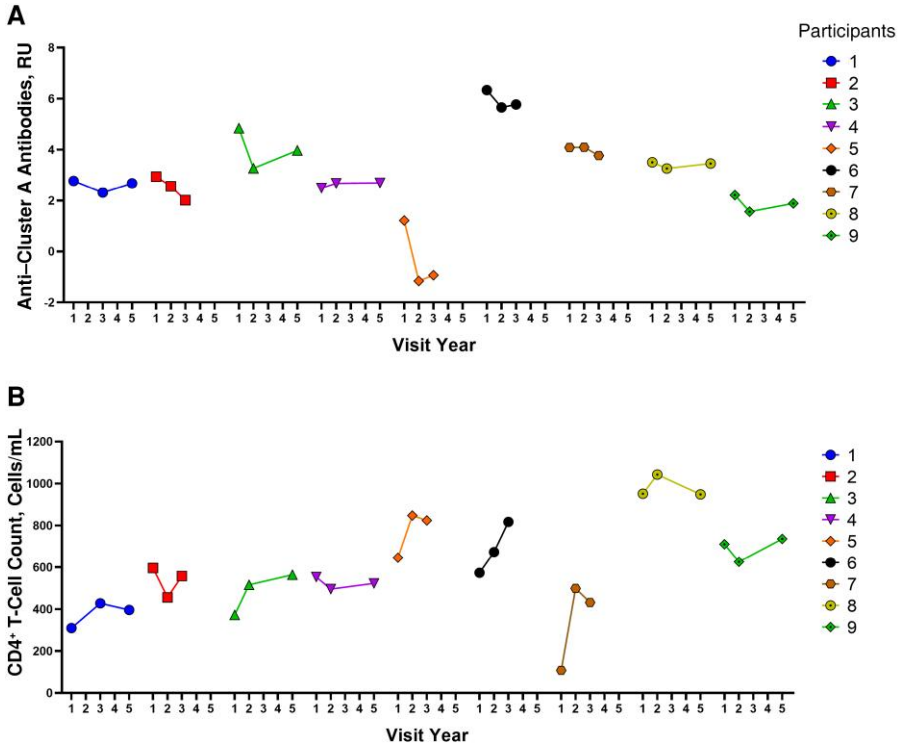

Figure 6. Longitudinal analysis of anti–cluster A antibodies and $CD4^+$ T-cell counts. Levels of anti–cluster A antibodies (*A*) and $CD4^+$ T-cell counts (*B*) were measured over time for 9 participants with detectable levels of soluble glycoprotein 120 (sgp120). Abbreviation: RU, relative units.

these results suggest that gp120 might represent a targetable pathway to decrease immune dysfunction, chronic inflammation, and CVD risk in PLWH.

We optimized an ELISA to detect sgp120 using C11, a monoclonal antibody recognizing a highly conserved gp120 region that is buried at the Env trimer interface and gets exposed on dissociation of the gp120 from gp41 [26], thus recognizing sgp120. We used the N6 broadly neutralizing antibody known to recognize 98% of global HIV-1 strains [28] as a readout. The use of a detection antibody targeting the CD4BS enables the detection of sgp120 which has the potential of binding the CD4 receptor whose interaction has been associated with immune activation and inflammation in vitro [4, 14, 15]. While sgp120 alone was not significantly associated with most clinical parameters measured, stratification of 386 PLWH based on sgp120 levels unveiled a striking inverse correlation between the levels of anti–cluster A antibodies with $CD4^+$ cell count and CD4/CD8 ratio. These associations remained after adjustment for potential confounders (including age, sex, ethnicity, smoking status, nadir CD4, duration of ART, and levels of anti-CD4BS antibodies). This in vivo association is consistent with previously well-established in vitro evidence showing that binding of sgp120 to uninfected bystander $CD4^+$ T cells leads to the death of these cells via ADCC on recognition by HIV-positive plasma [15–17].

We also observed a significant increase in IL-6 in individuals presenting detectable levels of sgp120. On one hand, plasma levels of IL-6 have been reported to be independently associated with frailty [32] as well as serious non-AIDS conditions or death among PLWH with undetectable viremia [33]. On the other hand, in vitro studies demonstrated that sgp120 binding to the CD4 receptor or CCR5/CXCR4 coreceptors on immune cells induces the liberation of inflammatory markers [4, 14, 15]. Together, these data suggest that sgp120 could contribute to the development of comorbid conditions by inducing proinflammatory cytokines in vivo. While we cannot establish a direct causal relationship between plasma sgp120 and the variation in inflammatory markers or the $CD4^+$ T-cell count, our longitudinal samples strongly suggest an association between sgp120 and these markers over time.

Anti–cluster A antibodies, present in all PLWH [34, 35], and sgp120 were shown to eliminate uninfected bystander $CD4^+$ T cells by ADCC in vitro. Here we extend these results by showing an association with $CD4^+$ T-cell counts in a subset of PLWH (those presenting high levels of sgp120). Furthermore, the combination of these 2 parameters was associated with the size of atherosclerotic plaques, which defines subclinical CVD [20, 23], and this association remained after adjustment for potential confounding factors (age, sex, hypertension, diabetes mellitus, lipid levels, smoking status, alcohol use disorder, intravenous drug use, and duration of ART). This suggests that

the levels of sgp120 and anti–cluster A antibodies could contribute to the development of CVD, which could be linked to depletion of uninfected bystander $CD4^+$ cells, as it was shown that a low CD4/CD8 ratio was predictive of the presence of CVD in PLWH receiving ART [36, 37].

Notably, we propose that sgp120 acts as an "effect modifier," potentially driving immune dysfunction, chronic inflammation, and the development of premature comorbid conditions. Thus, small molecules targeting gp120 could potentially "detoxify" its immunomodulatory effect and alleviate the development of premature comorbid conditions. One such molecule is the recently Food and Drug Administration–approved small-molecule gp120 inhibitor fostemsavir, a prodrug of temsavir (TMR), a novel attachment inhibitor that can prevent Env-CD4 interaction (BMS-663068, GSK3684934, Rukobia) and is currently used in combination with other ART for adults with multidrug-resistant HIV-1 [38, 39]. Intriguingly, many PLWH receiving fostemsavir exhibit clinical benefits that go beyond its capacity to decrease viral loads below detection levels [38, 39]. Our group has recently shown that TMR reduces the release of gp120 and that shed gp120 produced from TMR-treated cells has decreased capacity to interact with uninfected bystander $CD4^+$ T cells, protecting these cells from ADCC responses and sgp120-mediated cytokine burst in human PBMCs [15]. Whether the use of TMR in treated PLWH presenting high levels of sgp120 could help alleviate sgp120-mediated chronic inflammation and immune dysfunction is yet to be shown.

The source of circulating sgp120 in long-term ART-treated PLWH with undetectable viral loads is not well understood. Although ART suppresses viral replication, it does not prevent proviral gene expression from the viral reservoir [40]. As such, a fraction of cells harboring intact or defective proviruses that persist during ART have been found to be transcriptionally competent, leading to the production of viral proteins and activation of the immune system [41, 42]. Interestingly, defective proviruses able to produce *env* transcripts have been found in a subset of PLWH receiving ART [43], raising the possibility that translation-competent proviruses could contribute to the production and release of sgp120, even during ART.

Accordingly, we did observe a significant association between the levels of HIV DNA and the combination of sgp120 and anti–cluster A antibodies (Figure 3 and Supplementary Table 3), although sgp120 alone was not significantly associated. Whether this is due to the limited number of individuals with detectable sgp120 in our cohort remains to be determined. Alternatively, the lack of association between sgp120 alone and the reservoir might be due to the fact that measures of HIV reservoir were performed in the blood and not in the tissues, which may be a preferential site for persistence of the active reservoir [44]. Finally, it has been demonstrated that the size of the viral reservoir in PLWH receiving ART was associated with subclinical CVD [25, 45]; whether this association is linked to the persistence of sgp120 in those individuals remains to be determined. Future studies are needed to elucidate the mechanism by which sgp120 persists over time and whether it may represent a potential therapeutic target to reduce the risk of early-onset comorbid conditions for PLWH.

Limitations of our study include its cross-sectional design, the underrepresentation of women, the reliance on subgroups in whom data was available for CVD assessment, and limited power for analysis of the sgp120 strata. While reverse causality is unlikely (CVD could not cause the presence of sgp120), a causal relationship cannot be inferred from this design, and replication of findings in independent populations is needed.

In conclusion, in this longitudinal and cross-sectional study, nested within a large cohort study of adults aging with HIV infection with undetectable viral load, we demonstrate that sgp120 is detectable in about one-third of participants, and that along with levels of anti–cluster A antibodies, it modulates immune and inflammation profiles. This may represent a novel treatment target for PLWH, to address the risk of inflammation-related comorbid conditions.

Supplementary Data

Supplementary materials are available at *The Journal of Infectious Diseases* online (http://jid.oxfordjournals.org/). Supplementary materials consist of data provided by the author that are published to benefit the reader. The posted materials are not copyedited. The contents of all supplementary data are the sole responsibility of the authors. Questions or messages regarding errors should be addressed to the author.

Notes

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Data availability. Data and reagents are available on request.

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