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Plasma and Immunoglobulin G Galactosylation Associate with HIV Persistence During Antiretroviral Therapy

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Abstract

Global antibody glycosylation is dynamic and plays critical roles in shaping different immunological outcomes and direct antibody functionality during HIV infection. However, the relevance of global antibody or plasma glycosylation patterns to HIV persistence after antiretroviral therapy (ART) has not been characterized. First, we compared glycomes of total plasma and isolated immunoglobulin G (IgG) from HIV+ ART-suppressed, HIV+ viremic, and HIV-negative individuals. Second, in ART-suppressed individuals, we examined the associations between glycomes and 1) levels of cell-associated HIV DNA and RNA in PBMCs and isolated CD4+ T cells, 2) CD4 count and CD4%, and 3) expression of CD4+ T-cell activation markers. HIV infection is associated with persistent alterations in the IgG glycome including decreased levels of di-sialylated glycans, which is associated with a lower anti-inflammatory activity, and increased levels of fucosylated glycans, which is associated with lower antibody-dependent cell-mediated cytotoxicity (ADCC). We also show that levels of certain mono- and di-galactosylated nonfucosylated glycomic traits (A2G1, A2G2, and A2BG2), which have been reported to be associated with higher ADCC and higher anti-inflammatory activities, exhibit significant negative

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AUTHORSHIP CONTRIBUTIONS:

M.A-M. conceived the study. S.V., I.T.A., F.C., A.A., E.P., L.J.M., and M.A-M. designed and carried out experiments. I.T.A., A.K., A.L., R.C.K., L.J.M., G.L., and M.A-M. analyzed and interpreted data. K.L., and K.M. selected study subjects and provided samples. M.A-M. drafted the manuscript and all authors edited the final version of the manuscript.

CONFLICT OF INTEREST DISCLOSURE

GL declares he is a founder and owner of Genos Ltd, biotech company that specializes in glycan analysis and has several patents in the field. I.T.A is an employee of Genos Ltd.

correlations with levels of cell-associated total HIV DNA and HIV RNA in ART-suppressed individuals. Finally, levels of certain circulating anti-inflammatory glycans are associated with higher levels of CD4 T cells and lower levels of T cell activation. Our findings represent the first proof-of-concept evidence that glycomic alterations, known to be associated with differential states of inflammation and ADCC activities, are also associated with levels of HIV persistence in the setting of ART suppression.

Keywords

HIV latency; IgG glycosylation; plasma glycosylation; sialylation; fucosylation

INTRODUCTION

Antiretroviral therapy (ART) has dramatically reduced morbidity and mortality in HIV+ individuals [1]. However, ART requires lifelong administration and does not eradicate HIV. Persistent HIV infection continues to cause immune activation, chronic inflammation, and damage to multiple organs [2–4]. The chronic immune activation and inflammation likely feedback to contribute to the persistence of the HIV reservoir [5], and they increase the incidence of non-AIDS, inflammatory-related illnesses [6–9]. This vicious cycle between HIV persistence and immune activation/inflammation has been suggested by many reports [10–13]. However, the physiological processes underlying this cycle remain poorly understood and likely involve multifactorial mechanisms including possible on-going HIV production, Cytomegalovirus (CMV) infection, loss of regulatory T cells (Tregs), microbial translocation, and other factors [14–18]. Identifying novel host determinants governing this cycle *in vivo* is an important step in developing effective strategies to cure HIV infection and/or prevent the development of inflammation-associated HIV comorbidities.

Recent advances in the glycobiology field show that host glycosylation and glycan-lectin signaling play critical roles in immune responses [19] and in cell-cell [20] and cell-pathogen interactions [21]. Altered glycosylation is documented in many diseases including cancer [22–25], autoimmune [26, 27], and infectious diseases [28, 29]. Altered serum cell-free glycan structures have been identified as biomarkers for cancer [22–25] and various cellular processes [26, 27]. Glycans can enter the circulation from tissues through active secretion or leakage, but antibody glycosylation also plays a critical role in the impact of circulating glycans on the immune system [30].

Glycosylation can alter the antibody capacity to interact with classical and non-classical Fc receptors, which defines the antibody functionality in inducing antibody-dependent cell-mediated cytotoxicity (ADCC), antibody-dependent cellular phagocytosis (ADCP), complement dependent cytotoxicity (CDC), and anti-inflammatory activities [31–34]. Higher levels of antibody galactosylation and lower levels of fucosylation are associated with a higher ADCC function of antibodies [35–37]. Antibody sialylation and galactosylation are linked to strong anti-inflammatory responses [38–41]; however, the exact mechanism underlies this effect is not clear. It has been suggested that it could be related to either switching the antibody's binding from classic Fc receptors to non-classical Fc

receptors [42, 43], or binding to sialic-acid-binding proteins (siglecs) on the surface of monocytes and macrophages, thus initiating an inhibitory signal that leads to an anti-inflammatory response through inhibition of TLR4 signal transduction [44–47].

HIV infection has been associated with certain changes in the antibody glycosylation, mainly higher levels of agalactosylated antibodies [28, 48]. Changes in global and antigen-specific antibody glycosylation have been associated with a differential activity of anti-HIV antibodies to control HIV infection [28]. However, as yet, there are no data linking antibody glycosylation to the HIV latent reservoir during suppressive ART. In this study, we hypothesized that the ability of antibody glycosylation to impact several immunological responses, including ADCC and immune activation/inflammation, play a role in defining HIV reservoir size during ART. To address this hypothesis, we examined relationships between the glycomes of plasma and total immunoglobulin G (IgG) and nucleic-acid measures of HIV persistence during ART.

METHODS

Study population

Fresh PBMCs and plasma from HIV-infected ART-suppressed (viral load < 20 copies/ml), HIV-infected unsuppressed (viral load > 50 copies/ml), and HIV-negative individuals were included in the analysis (Supplementary Table 1) and obtained from Philadelphia FIGHT and The Wistar Institute. Written informed consent was provided by all patients and donors recruited, and the protocols used were approved by the Institutional Review Boards of the University of Pennsylvania and The Wistar Institute. Plasma RNA viral load, CD4+ T cell counts, and CD4% were measured at all patient visits.

T-Cell Immunophenotyping

Markers of T-cell activation (HLA-DR, CD69, and CD25) that have been associated with HIV latency [49–53], were measured using flow cytometry. Briefly, freshly isolated PBMCs were stained with LIVE/DEAD® Fixable Aqua Dead Cell Stain Kit (Invitrogen) and then stained with the following fluorescently-conjugated monoclonal antibodies: CD3 BV421 (clone UCHT1), CD4 PE-Cy7 (clone RPA-T4), CD25 BV605 (clone BC96), HLA-DR FITC (clone L243) from Biolegend. CD69 PE (clone FN50) from BD Biosciences. Stained cells were fixed in PBS with 1% paraformaldehyde, and stored in the dark at 4°C until acquisition. All phenotyping data were collected on BD LSR II (BD Biosciences). Data were analyzed using FlowJo software (Treestar).

qPCR quantification of cellular HIV-1 DNA and RNA

CD4+ T cells were isolated from PBMCs using EasySep Human CD4+ T cell enrichment kit (Stemcell Technologies, Vancouver, British Columbia, Canada). Cellular RNA and DNA from total unfractionated PBMCs and isolated CD4+ T cells were purified using the AllPrep DNA/RNA kit (Qiagen, Ventura CA) as specified by the manufacturer, quantified using a Nanodrop (ND-1000) spectrophotometer and normalized to cell equivalents by qPCR using human genomic TERT for DNA and RPLP0 expression for RNA (Life Technologies, Grand Island NY). Total cellular HIV-1 DNA and RNA was quantified with a qPCR TaqMan assay

using LTR-specific primers F522-43 (5' GCC TCA ATA AAG CTT GCC TTG A 3'; HXB2522–543) and R626-43 (5' GGG CGC CAC TGC TAG AGA 3'; 626–643) coupled with a FAM-BQ probe (5' CCA GAG TCA CAC AAC AGA CGG GCA CA 3) using the QuantStudio 6 Flex Real-Time PCR System (Applied Biosystems). Cell-associated HIV-1 DNA copy number was determined using a reaction volume of 20 µl with 10 µl of 2× TaqMan Universal Master Mix II including UNG (Life Technologies), 4 pmol of each primer, 4 pmol of probe, and 5 µl of DNA. Cycling conditions were 50°C for 2 min, 95°C for 10 min, then 60 cycles of 95°C for 15s and 59°C for 1 min. Cell-associated HIV-1 RNA copy number was determined in a reaction volume of 20 µl with 10 µl of 2× TaqMan RNA to Ct 1 Step kit (Life Technologies), 4 pmol of each primer, 4pmol of probe, 0.5 µl reverse transcriptase, and 5µl of RNA. Cycling conditions were 48°C for 20 min, 95°C for 10 min, then 60 cycles of 95°C for 15s and 59°C for 1 min. For HIV-1 DNA measurements, external quantitation standards were prepared from pNL4-3 in a background of HIV-1 negative human cellular DNA, calibrated to the Virology Quality Assurance (VQA, NIH Division of AIDS) cellular DNA quantitation standards. For HIV RNA measurements, external quantitation standards were prepared from full-length NL4-3 virion RNA followed by copy number determination using the Abbott RealTime assay (Abbott Diagnostics, Des Plains Ill) and calibrated to VQA HIV-1 RNA standards. Patient specimens were assayed with up to 800 ng total cellular RNA or DNA in replicate reaction wells and copy number determined by extrapolation against a 7-point standard curve (1–10,000 cps) performed in triplicate.

IgG isolation from plasma

Plasma samples have been incubated for 30 minutes at 56 °C to inactivate HIV and then stored at –20 °C prior *N*-glycan analysis. Total IgG from plasma was isolated using CIM[®] Protein G 96-well plate (BIA Separations, Ajdovš in a, Slovenia), and vacuum manifold (Pall Corporation, Port Washington, NY, USA). All steps during the isolation procedure were performed at 380 mm Hg, except for plasma sample application and IgG elution (around 200 mm Hg). All buffers were filtered through 0.2 µm PES filters (Nalgene Thermo Fischer Scientific, Waltham, ME, USA). Plasma samples (100 µL) were centrifuged for 3 minutes at 12 100 *g*, pipetted to the previously designated wells of a collection plate and diluted with 1× PBS, pH 7.4 in the ratio 1:7. All diluted plasma samples were filtered through 0.45 µm and 0.2 µm AcroPrep GHP filter plates (Pall Corporation) using vacuum manifold (around 380 mm Hg) and immediately applied to preconditioned Protein G plate as was previously described [54]. Protein G plate was then washed and IgG eluted with 0.1 mol L^{–1} formic acid and immediately neutralized with ammonium bicarbonate to pH 7.0. Protein G plate was regenerated and stored at 4 °C. The volume of 300 µL from each elution fraction was dried in a vacuum centrifuge with vacuum concentrator Savant SC210A, refrigerated vapor trap Savant RVT400 and vacuum pump OFP400 (Thermo Scientific) for subsequent *N*-glycan analysis.

N-glycan release, labeling, and analysis by ultra-performance liquid chromatography

N-glycans from isolated IgG and plasma samples (10 µL) were released with PNGase F, labeled with 2-aminobenzamide and excess of reagents removed by clean-up using hydrophilic interaction liquid chromatography solid phase extraction (HILIC-SPE), as previously described [54, 55]. Eluates were stored at –20 °C until the ultra-performance

liquid chromatography (UPLC) analysis. Fluorescently labeled and purified *N*-glycans were separated by HILIC-UPLC using Acquity UPLC instrument (Waters, Milford, MA, USA) as previously described [54, 55]. IgG *N*-glycan samples were all separated into 24 peaks [30] and total plasma *N*-glycans into 39 peaks [56]. The amount of *N*-glycans in each chromatographic peak was expressed as a percentage of total integrated area (% Area). From these directly measured IgG and total plasma *N*-glycans, additional glycan traits have been derived to assess changes in the amount of glycan classes that represent structurally similar glycan species (Supplementary Table 2 and 3).

Statistical analyses—Data were analyzed using Prism 7.0 (GraphPad Software, La Jolla, CA, USA). Nonparametric two-tailed Mann-Whitney U-test was used for estimating significance of glycan abundance difference between pairs of patient groups and False Discovery Rate (FDR) for correction for multiple testing was done using Storey et. al. procedure [57] $p < 0.05$ and $FDR < 20\%$ results were considered significant. Associations between glycan abundance and other patient measurements and characteristics was done using Spearman's rank correlation with $p < 0.05$ used as a significance threshold.

RESULTS

HIV infection is associated with persistent alterations in the IgG glycome

The glycomes of isolated IgG from HIV-negative controls, HIV+ ART-suppressed individuals (viral load < 20 copies/ml), and HIV+ unsuppressed individuals (viral load = > 50 copies/ml) (Supplementary Table 1) were profiled using UPLC. IgG *N*-glycans were separated into 24 chromatographic peaks [30] (Supplementary Figure 1A). Each peak represents a glycan trait. Certain IgG glycan traits were combined into categories, glycan structures that contain galactose (G0 = agalactosylated, G1 = mono-galactosylated, G2 = di-galactosylated) or that contain sialic acid (S = sialylated), fucose (F = fucosylated), or a bisecting GlcNAc (B = bisected) (Supplementary Table 2). Levels of di-sialylated glycans in total IgG glycome were significantly reduced in HIV+ unsuppressed individuals ($p = 0.002$, median = 11.2%, IQR = 1.4), and HIV+ ART-suppressed individuals ($p = 0.0006$, median = 12.4%, IQR = 1.2), when compared to HIV-negative controls (median = 15.7%, IQR = 2.4) (Figure 1A, B). Out of IgG di-sialylated glycan structures, in particular levels of A2G2S2 glycan trait (glycan structure with two sialic acids, two galactoses, and no core fucose or bisecting GlcNAc) was significantly reduced in the IgG glycomes of HIV+ unsuppressed individuals ($p = 0.002$, median = 5.6%, IQR = 2), and HIV+ ART-suppressed individuals ($p = 0.0003$, median = 8.2%, IQR = 1.2), when compared to HIV-negative controls (median = 10.8%, IQR = 3) (Figure 1A, C). Levels of IgG fucosylated glycans were significantly induced in HIV+ unsuppressed individuals ($p = 0.015$, median = 89.4%, IQR = 2.5), and trending in HIV+ ART-suppressed individuals ($p = 0.059$, median = 86.4%, IQR = 2.7), when compared to HIV-negative controls (median = 83.2%, IQR = 3.2) (Figure 1A, D). Finally, as reported before [28, 48], levels of agalactosylated glycans were induced in HIV+ unsuppressed individuals (median = 34.1%, IQR = 6.1), when compared to HIV+ ART-suppressed individuals ($p = 0.0002$, median = 19.7%, IQR = 7.5) and trending when compared to HIV-negative controls ($p = 0.06$, median = 19.5%, IQR = 9.2) (Figure 1A, E). These results confirm some previously reported data and report new IgG glycomic

alterations that associate with HIV infection and persistent despite suppressive ART. In particular, decreased levels of IgG sialylated glycans, which is associated with a pro-inflammatory activity [38–40], and increased levels of IgG fucosylated glycans, which is associated with lower ADCC and higher inflammation [35–37, 58, 59], regardless of the ART-suppression status.

Plasma glycomic alterations associated with HIV infection

The glycomes of plasma (Figure 2) from the same individuals as above were profiled using UPLC. Total plasma *N*-glycans were chromatographically separated into 39 peaks [56] (Supplementary Figure 1B). Each peak represents a glycan trait. Additionally, structurally similar glycan species have been combined into derived traits to assess changes in the levels of galactosylation, sialylation, fucosylation, bisecting GlcNAc and branching (Figure 2A, Supplementary Table 3). Levels of several plasma glycan structures were modulated in HIV + unsuppressed individuals when compared to both HIV+ ART-suppressed and HIV-negative controls, suggesting reversible changes when measured at the total plasma level. Levels of plasma bisecting GlcNAc, agalactosylated, core-fucosylated, neutral (no-sialic-acid) glycan structures were induced in HIV+ unsuppressed individuals when compared to both HIV+ ART-suppressed and HIV-negative controls ($p<0.05$) (Figure 2A–E). Levels of plasma di-sialylated and di-galactosylated glycan structures were reduced in HIV+ unsuppressed individuals when compared to both HIV+ ART-suppressed and HIV-negative controls ($p<0.05$) (Figure 2A, E–G).

Levels of glycomic traits in isolated IgG and total plasma glycomes correlate with nucleic acid measures of HIV persistence during ART

To test the hypothesis that glycomic alterations in plasma or IgG can affect HIV reservoir size during suppressive ART, we examined relationships between nucleic acid measures of the latent reservoir and both the glycomes of isolated IgG and total plasma. Levels of cell-associated HIV total DNA and HIV RNA were measured in unfractionated PBMCs and negatively-selected peripheral blood CD4+ T cells from HIV+ ART-suppressed individuals within the above-mentioned cohort. Levels of total HIV DNA and cell-associated HIV RNA in both unfractionated PBMC and isolated CD4+ were negatively correlated with levels of three glycan traits in isolated IgG (Figure 3) and three glycan traits in total plasma (Figure 4). All these glycan structures are similar in that they are mono- or di-galactosylated, with no sialic acid or core fucose, and two of these three glycan structures are the same in isolated IgG and total plasma (A2G2 and A2BG2).

Levels of A2G1 (monogalactosylated glycan structure with no sialic acid or core fucose) in total IgG glycome inversely correlated with levels of total HIV DNA in unfractionated PBMC ($p=0.0026$, $\rho = -0.755$) and isolated CD4+ T cells ($p=0.002$, $\rho = -0.768$), and inversely correlated with levels of cell-associated HIV RNA in unfractionated PBMC ($p=0.05$, $\rho = -0.53$) and isolated CD4+ T cells ($p=0.017$, $\rho = -0.634$) (Figure 3A). Levels of A2G2 (di-galactosylated glycan structure with no sialic acid or core fucose) in total IgG glycome inversely correlated with levels of total HIV DNA in unfractionated PBMC ($p=0.0055$, $\rho = -0.713$) and isolated CD4+ T cells ($p=0.02$, $\rho = -0.61$), and inversely correlated with levels of cell-associated HIV RNA in unfractionated PBMC

($p=0.02$, $\rho = -0.61$) and isolated CD4+ T cells ($p=0.036$, $\rho = -0.57$) (Figure 3B). Levels of A2BG2 (di-galactosylated structure with bisecting GlcNAc, with no sialic acid or core fucose) in total IgG glycome inversely correlated with levels of total HIV DNA in unfractionated PBMC ($p=0.027$, $\rho = -0.6$) and isolated CD4+ T cells ($p=0.05$, $\rho = -0.54$), and inversely correlated with levels of cell-associated HIV RNA in unfractionated PBMC ($p=0.025$, $\rho = -0.6$) and isolated CD4+ T cells ($p=0.044$, $\rho = -0.55$) (Figure 3C).

Levels of A2[6]BG1 (monogalactosylated structure with bisecting GlcNAc, with no sialic acid or core fucose) in total plasma glycome inversely correlated with levels of total HIV DNA in unfractionated PBMC ($p=0.018$, $\rho = -0.63$) and isolated CD4+ T cells ($p=0.002$, $\rho = -0.758$), and inversely correlated with levels of cell-associated HIV RNA in isolated CD4+ T cells ($p=0.009$, $\rho = -0.68$) (Figure 4A). Levels of A2G2 (di-galactosylated glycan structure, with no sialic acid or core fucose) in total plasma glycome inversely correlated with levels of total HIV DNA in unfractionated PBMC ($p=0.05$, $\rho = -0.53$) and isolated CD4+ T cells ($p=0.0077$, $\rho = -0.69$), and inversely correlated with levels of cell-associated HIV RNA in unfractionated PBMC ($p=0.048$, $\rho = -0.54$) and isolated CD4+ T cells ($p=0.04$, $\rho = -0.56$) (Figure 4B). Levels of A2BG2 (di-galactosylated glycan structure with bisecting GlcNAc, with no sialic acid or core fucose) in total plasma glycome inversely correlated with levels of total HIV DNA in unfractionated PBMC ($p=0.019$, $\rho = -0.63$) and isolated CD4+ T cells ($p=0.0018$, $\rho = -0.77$), and inversely correlated with levels of cell-associated HIV RNA in unfractionated PBMC ($p=0.02$, $\rho = -0.6$) and isolated CD4+ T cells ($p=0.007$, $\rho = -0.7$) (Figure 4C).

Interestingly, the levels of A2G2 were higher in the IgG glycomes of HIV+ ART-suppressed individuals (median = 1%, IQR = 0.9) when compared to HIV-negative controls ($p=0.01$, median = 0.6%, IQR = 0.3), and trend higher when compared to HIV+ unsuppressed individuals ($p = 0.06$, median = 0.6%, IQR = 0.6) (Figure 5). To test if age played a role in the observed relationships, we examined the correlations between the expression of these glycan traits and age. No correlation was detected between age and levels of A2G1, A2G2, A2BG2 in isolated IgG glycome or levels of A2[6]BG1, A2G2, and A2BG2 in total plasma glycome (Supplementary Figure 2). In addition, all significant glycan associations with HIV DNA/RNA loads were tested using linear regression with both glycan abundance and age as factors, and none of the results lost significance indicating independence of glycan association from age.

Levels of circulating anti-inflammatory glycans associate with higher levels of CD4 count and lower levels of T cell activation

Multiparametric flow cytometry was used to assess the percentage of three activation markers that have been associated with HIV latency [49–53], HLA-DR, CD69, and CD25 on CD4+ T cells. The percentage of sialylated glycans (FA2BG2S1 in IgG; FA3G3S3 and A4G4S3 in total plasma) and galactosylated glycans (A2BG2 in total plasma), glycans associated with anti-inflammatory activities [38–41], exhibited significant positive correlations with CD4 count, CD4%, and inverse correlations with the expression of several CD4+ T cell activation markers in HIV+ ART-suppressed individuals (Figure 6). In particular, levels of A4G4S3 in total plasma correlated with CD4 count ($p= 0.03$, $\rho= 0.57$),

CD4% ($p = 0.01$, $\rho = 0.67$), and inversely correlated with the percentage of CD4+ T cells expressing the HLA-DR late activation marker ($p = 0.01$, $\rho = -0.66$) or the intermediate or late CD25 activation marker ($p = 0.025$, $\rho = -0.6$) (Figure 6A–D). Levels of FA3G3S3 in total plasma positively correlated with CD4% ($p = 0.045$, $\rho = 0.55$), and inversely correlated with the percentage of CD4+ T cells expressing the CD25 activation marker ($p = 0.038$, $\rho = -0.56$) (Figure 6E–F). Levels of FA2BG2S in IgG glycome positively correlated with CD4 count ($p = 0.001$, $\rho = 0.8$) (Figure 6G). Levels of A2BG2 in total plasma inversely correlated with the percentage of CD4+ T cells expressing the CD69 early activation marker ($p = 0.045$, $\rho = -0.55$) (Figure 6H).

DISCUSSION

Levels of anti-HIV-1 antibodies can reflect the degree of HIV persistence and low-level viral replication [60–62], yet little is known about the IgG and plasma glycomic determinants of persistent HIV and reservoir size after ART *in vivo*. Here, for the first time, we report novel plasma and IgG glycomic alterations that are associated with suppressed HIV infection as well as with CD4 T cell lymphocyte levels of HIV DNA and RNA during suppressive antiretroviral therapy.

Given the recent paradigm on the critical roles of plasma and IgG glycosylation in modulating several immunological functions and the documented role of anti-HIV-1 antibody glycosylation in controlling HIV infection, we hypothesized that altered plasma and IgG glycosylation during HIV infection plays an important role in determining HIV reservoir size during suppressive therapy. Our data revealed that certain IgG and plasma glycomic alterations associate with HIV infection, some of them are persistent despite suppressive ART, while some are reversible. Induction of agalactosylated glycan in total IgG glycome was previously associated with HIV infection and inflammation and was confirmed in our study [28, 48]. In addition, we revealed certain persistent HIV-associated glycomic alterations that have been reported to be associated with inflammation (a loss of disialylated glycans and an increase of fucosylated glycans) and reduction of ADCC activity (an increase of fucosylated glycans) [35–40, 58, 59]. These glycomic dysregulations may underlie chronic inflammation and immunological dysfunction during suppressed HIV infection thus contributing to the pathogenesis of HIV-associated comorbidities.

Our data also revealed that a few select glycomic traits, mono- and di-galactosylated glycan structures with no sialic acid or core fucose, glycans structures, in both total plasma and isolated IgG exhibit significant negative correlations with levels of HIV DNA and cell-associated HIV RNA in unfractionated PBMCs and isolated CD4+ T cells. The biological roles of galactosylated IgG have been characterized, providing key insights into the mechanisms underlying our observations. Of interest, IgG galactosylation drives better ADCC, ADCP, and CDC in monoclonal antibodies [35, 36, 63]. Antibody galactosylation has also been associated with an anti-inflammatory activity, by promoting the association between Fc γ RIIB (CD32b) and dectin-1 [41]. While antibody glycosylation is programmed in an antigen-specific manner [64], future experiments will be necessary to determine if higher galactosylation of anti-HIV-1 antibodies correlate with better ADCC, ADCP, and/or CDC activity against HIV-infected cells during suppressive therapy. Concurrently,

galactosylated antibodies are expected to promote an anti-inflammatory state decreasing immune activation and chronic inflammation during ART. Lower activation can contribute to a reduction of persistent HIV reservoir by preventing the potential HIV on-going replication in tissues during ART [5]. Future *in vitro* and animal-model experiments will be needed to gauge the direct effects of these glycan structures on HIV persistence. It also remains to be determined how host cellular and circulating glycomic features can refine current known immune associations of persistent HIV, including associations with surface expression of activation markers and immune negative checkpoints (PD-1, TIGIT, and LAG-3) [65–67], and expression of select anti-HIV-1 host restriction factors [68]. A better understanding of host and viral factors driving the formation and maintenance of the latent HIV reservoir is critical to the development of effective curative strategies.

Our secondary objective was to assess the relationship between the plasma and IgG glycomes and cellular activation in the setting of ART. Cell-based measurements of viral persistence are consistently associated with markers of immune activation [65]. In line with our hypothesis, levels of glycans known to promote anti-inflammatory activity (sialylated and galactosylated glycan structures) in the plasma and isolated IgG exhibited positive correlations with CD4 count and CD4% and significant negative correlations with the percentage of CD4+ T cells expressing markers of T-cell activation. Our data, therefore, suggest that the documented anti-inflammatory roles of certain circulating glycans may impact immune activation and chronic inflammation during therapy. These anti-inflammatory features of circulating glycans may be pertinent to the development of interventions to attenuate HIV-associated inflammation and immune activation during suppressed ART.

Our study does have limitations. First, it is documented that genetics [30, 69], diet [30, 69], age [70, 71], and gender [71], impact antibody glycosylation. While we did not detect an influence of age on our main results, a larger sample size will be needed to explore the full extent of the association between circulating glycans and HIV persistence adjusting for all factors that can influence antibody glycosylation. Second, as antibody glycosylation is programmed in an antigen-specific manner [64], detailed experiments to gauge the impact each anti-HIV specific antibody glycosylation on HIV persistence will be needed. Third, our data were obtained from blood; given the enrichment of the HIV reservoir in tissues [72, 73], it will be needed to analyze the role of circulating glycans in maintaining HIV persistence in tissues. Lastly, our data describe cross-sectional samples from chronically-infected adults; thereby there is a need to also analyze longitudinal changes, acute infection, and both pediatric and older aged cohorts in the future. In addition, in this study, we used nucleic acid-based measures of HIV reservoir size. Currently, there is no gold standard method to measure the size of HIV reservoir [74, 75]. The quantitative viral outgrowth assay underestimates the size of HIV reservoir [74–76], while the nucleic acid-based measures overestimate it, as only a portion of latently infected cells is able to reactivate and produce replication-competent virus [77–79]. However, recent reports indicated that levels of HIV DNA and RNA during ART are predictors of viral rebound upon therapy interruption [80]. Therefore, these nucleic acid measures have a biological value.

In summary, our findings represent the first proof-of-concept evidence of plasma and IgG glycomic alterations *in vivo* that may modulate HIV persistence in the setting of suppression. Although it is difficult to unequivocally demonstrate a causal relationship between plasma and IgG glycomes and HIV reservoir during ART, the robust literature on the role of antibody glycosylation in modulating certain immune functions that are compatible with our findings and hypotheses, suggests that these glycomic alterations should be further explored as biomarkers of HIV persistence and also explored within the context of the several HIV-eradication strategies. Advances in glycan-based technologies may lead us to untangle the association between HIV and the host immune environment during ART, laying the groundwork for discovering novel glycan-based interventions for curing HIV infection or preventing inflammation-associated comorbidities.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

ADCC	antibody-dependent cell-mediated cytotoxicity
ADCP	antibody-dependent cellular phagocytosis
ART	antiretroviral therapy
CDC	complement dependent cytotoxicity
CMV	Cytomegalovirus
IgG	immunoglobulin G
Tregs	regulatory T cells

References

1. Wandeler G, Johnson LF, Egger M. Trends in life expectancy of HIV-positive adults on antiretroviral therapy across the globe: comparisons with general population. *Current opinion in HIV and AIDS*. 2016; 11:492–500. [PubMed: 27254748]
2. Biggar RJ, Chaturvedi AK, Goedert JJ, Engels EA, Study HACM. AIDS-related cancer and severity of immunosuppression in persons with AIDS. *Journal of the National Cancer Institute*. 2007; 99:962–72. [PubMed: 17565153]

3. Zucchetto A, Virdone S, Taborelli M, Grande E, Camoni L, Pappagallo M, Regine V, Grippo F, Polesel J, Dal Maso L, Suligoi B, Frova L, Serraino D. Non-AIDS-Defining Cancer Mortality: Emerging Patterns in the Late HAART Era. *Journal of acquired immune deficiency syndromes*. 2016; 73:190–6. [PubMed: 27116048]
4. Rodger AJ, Lodwick R, Schechter M, Deeks S, Amin J, Gilson R, Paredes R, Bakowska E, Engsig FN, Phillips A, Insight Smart ESG. Mortality in well controlled HIV in the continuous antiretroviral therapy arms of the SMART and ESPRIT trials compared with the general population. *Aids*. 2013; 27:973–9. [PubMed: 23698063]
5. Katlama C, Deeks SG, Autran B, Martinez-Picado J, van Lunzen J, Rouzioux C, Miller M, Vella S, Schmitz JE, Ahlers J, Richman DD, Sekaly RP. Barriers to a cure for HIV: new ways to target and eradicate HIV-1 reservoirs. *Lancet*. 2013; 381:2109–17. [PubMed: 23541541]
6. Grinspoon S, Carr A. Cardiovascular risk and body-fat abnormalities in HIV-infected adults. *N Engl J Med*. 2005; 352:48–62. [PubMed: 15635112]
7. Hunt PW, Cao HL, Muzoora C, Ssewanyana I, Bennett J, Emenyonu N, Kembabazi A, Neilands TB, Bangsberg DR, Deeks SG, Martin JN. Impact of CD8+ T-cell activation on CD4+ T-cell recovery and mortality in HIV-infected Ugandans initiating antiretroviral therapy. *Aids*. 2011; 25:2123–31. [PubMed: 21881481]
8. Lyons JL, Uno H, Ancuta P, Kamat A, Moore DJ, Singer EJ, Morgello S, Gabuzda D. Plasma sCD14 is a biomarker associated with impaired neurocognitive test performance in attention and learning domains in HIV infection. *J Acquir Immune Defic Syndr*. 2011; 57:371–9. [PubMed: 21646912]
9. Sandler NG, Wand H, Roque A, Law M, Nason MC, Nixon DE, Pedersen C, Ruxrungtham K, Lewin SR, Emery S, Neaton JD, Brenchley JM, Deeks SG, Sereti I, Douek DC, Group ISS. Plasma levels of soluble CD14 independently predict mortality in HIV infection. *The Journal of infectious diseases*. 2011; 203:780–90. [PubMed: 21252259]
10. Chan W, Sviridov D, Dart AM. HIV, atherosclerosis and inflammation: implications for treatment. *Journal of HIV therapy*. 2009; 14:61–8. [PubMed: 20218241]
11. Deeks SG. Immune dysfunction, inflammation, and accelerated aging in patients on antiretroviral therapy. *Topics in HIV medicine : a publication of the International AIDS Society, USA*. 2009; 17:118–23.
12. Klatt NR, Chomont N, Douek DC, Deeks SG. Immune activation and HIV persistence: implications for curative approaches to HIV infection. *Immunological reviews*. 2013; 254:326–42. [PubMed: 23772629]
13. Smith D. HIV as an inflammatory disease. *Journal of HIV therapy*. 2009; 14:50–1. [PubMed: 20218238]
14. Brenchley JM, Price DA, Schacker TW, Asher TE, Silvestri G, Rao S, Kazzaz Z, Bornstein E, Lambotte O, Altmann D, Blazar BR, Rodriguez B, Teixeira-Johnson L, Landay A, Martin JN, Hecht FM, Picker LJ, Lederman MM, Deeks SG, Douek DC. Microbial translocation is a cause of systemic immune activation in chronic HIV infection. *Nat Med*. 2006; 12:1365–71. [PubMed: 17115046]
15. Jiang W, Lederman MM, Hunt P, Sieg SF, Haley K, Rodriguez B, Landay A, Martin J, Sinclair E, Asher AI, Deeks SG, Douek DC, Brenchley JM. Plasma levels of bacterial DNA correlate with immune activation and the magnitude of immune restoration in persons with antiretroviral-treated HIV infection. *J Infect Dis*. 2009; 199:1177–85. [PubMed: 19265479]
16. Kovacs A, Al-Harhi L, Christensen S, Mack W, Cohen M, Landay A. CD8(+) T cell activation in women coinfecting with human immunodeficiency virus type 1 and hepatitis C virus. *J Infect Dis*. 2008; 197:1402–7. [PubMed: 18444798]
17. Ostrowski SR, Katzenstein TL, Pedersen BK, Gerstoft J, Ullum H. Residual viraemia in HIV-1-infected patients with plasma viral load <or=20 copies/ml is associated with increased blood levels of soluble immune activation markers. *Scand J Immunol*. 2008; 68:652–60. [PubMed: 19055701]
18. Sedaghat AR, Siliciano RF, Wilke CO. Low-level HIV-1 replication and the dynamics of the resting CD4+ T cell reservoir for HIV-1 in the setting of HAART. *BMC Infect Dis*. 2008; 8:2. [PubMed: 18171475]

19. Barrera C, Espejo R, Reyes VE. Differential glycosylation of MHC class II molecules on gastric epithelial cells: implications in local immune responses. *Human immunology*. 2002; 63:384–93. [PubMed: 11975982]
20. de Freitas Junior JC, Silva Bdu R, de Souza WF, de Araujo WM, Abdelhay ES, Morgado-Diaz JA. Inhibition of N-linked glycosylation by tunicamycin induces E-cadherin-mediated cell-cell adhesion and inhibits cell proliferation in undifferentiated human colon cancer cells. *Cancer chemotherapy and pharmacology*. 2011; 68:227–38. [PubMed: 20927523]
21. Dwek RA, Butters TD, Platt FM, Zitzmann N. Targeting glycosylation as a therapeutic approach. *Nature reviews. Drug discovery*. 2002; 1:65–75. [PubMed: 12119611]
22. Meany DL, Zhang Z, Sokoll LJ, Zhang H, Chan DW. Glycoproteomics for prostate cancer detection: changes in serum PSA glycosylation patterns. *Journal of proteome research*. 2009; 8:613–9. [PubMed: 19035787]
23. Misonou Y, Shida K, Korekane H, Seki Y, Noura S, Ohue M, Miyamoto Y. Comprehensive clinico-glycomic study of 16 colorectal cancer specimens: elucidation of aberrant glycosylation and its mechanistic causes in colorectal cancer cells. *Journal of proteome research*. 2009; 8:2990–3005. [PubMed: 19292502]
24. An HJ, Lebrilla CB. A glycomics approach to the discovery of potential cancer biomarkers. *Methods in molecular biology*. 2010; 600:199–213. [PubMed: 19882130]
25. de Leoz ML, An HJ, Kronewitter S, Kim J, Beecroft S, Vinall R, Miyamoto S, de Vere White R, Lam KS, Lebrilla C. Glycomic approach for potential biomarkers on prostate cancer: profiling of N-linked glycans in human sera and pRNS cell lines. *Disease markers*. 2008; 25:243–58. [PubMed: 19126968]
26. Durand G, Seta N. Protein glycosylation and diseases: blood and urinary oligosaccharides as markers for diagnosis and therapeutic monitoring. *Clinical chemistry*. 2000; 46:795–805. [PubMed: 10839767]
27. Moremen KW, Tiemeyer M, Nairn AV. Vertebrate protein glycosylation: diversity, synthesis and function. *Nature reviews. Molecular cell biology*. 2012; 13:448–62. [PubMed: 22722607]
28. Ackerman ME, Crispin M, Yu X, Baruah K, Boesch AW, Harvey DJ, Dugast AS, Heizen EL, Ercan A, Choi I, Streeck H, Nigrovic PA, Bailey-Kellogg C, Scanlan C, Alter G. Natural variation in Fc glycosylation of HIV-specific antibodies impacts antiviral activity. *The Journal of clinical investigation*. 2013; 123:2183–92. [PubMed: 23563315]
29. Pasek M, Duk M, Podbielska M, Sokolik R, Szechinski J, Lisowska E, Krotkiewski H. Galactosylation of IgG from rheumatoid arthritis (RA) patients--changes during therapy. *Glycoconjugate journal*. 2006; 23:463–71. [PubMed: 17006638]
30. Pucic M, Knezevic A, Vidic J, Adamczyk B, Novokmet M, Polasek O, Gornik O, Supraha-Goreta S, Wormald MR, Redzic I, Campbell H, Wright A, Hastie ND, Wilson JF, Rudan I, Wuhrer M, Rudd PM, Josic D, Lauc G. High throughput isolation and glycosylation analysis of IgG-variability and heritability of the IgG glycome in three isolated human populations. *Molecular & cellular proteomics : MCP*. 2011; 10 M111 010090.
31. Goede V, Fischer K, Busch R, Engelke A, Eichhorst B, Wendtner CM, Chagorova T, de la Serna J, Dilhuydy MS, Illmer T, Opat S, Owen CJ, Samoylova O, Kreuzer KA, Stilgenbauer S, Dohner H, Langerak AW, Ritgen M, Kneba M, Asikanius E, Humphrey K, Wenger M, Hallek M. Obinutuzumab plus chlorambucil in patients with CLL and coexisting conditions. *The New England journal of medicine*. 2014; 370:1101–10. [PubMed: 24401022]
32. Junttila TT, Parsons K, Olsson C, Lu Y, Xin Y, Theriault J, Crocker L, Pabon O, Baginski T, Meng G, Totpal K, Kelley RF, Sliwkowski MX. Superior in vivo efficacy of afucosylated trastuzumab in the treatment of HER2-amplified breast cancer. *Cancer research*. 2010; 70:4481–9. [PubMed: 20484044]
33. Scott AM, Wolchok JD, Old LJ. Antibody therapy of cancer. *Nature reviews. Cancer*. 2012; 12:278–87.
34. Sondermann P, Szymkowski DE. Harnessing Fc receptor biology in the design of therapeutic antibodies. *Current opinion in immunology*. 2016; 40:78–87. [PubMed: 27038127]

35. Thomann M, Reckermann K, Reusch D, Prasser J, Tejada ML. Fc-galactosylation modulates antibody-dependent cellular cytotoxicity of therapeutic antibodies. *Molecular immunology*. 2016; 73:69–75. [PubMed: 27058641]
36. Chung AW, Crispin M, Pritchard L, Robinson H, Gorny MK, Yu X, Bailey-Kellogg C, Ackerman ME, Scanlan C, Zolla-Pazner S, Alter G. Identification of antibody glycosylation structures that predict monoclonal antibody Fc-effector function. *Aids*. 2014; 28:2523–30. [PubMed: 25160934]
37. Shields RL, Lai J, Keck R, O'Connell LY, Hong K, Meng YG, Weikert SH, Presta LG. Lack of fucose on human IgG1 N-linked oligosaccharide improves binding to human FcγRIII and antibody-dependent cellular toxicity. *The Journal of biological chemistry*. 2002; 277:26733–40. [PubMed: 11986321]
38. Anthony RM, Nimmerjahn F, Ashline DJ, Reinhold VN, Paulson JC, Ravetch JV. Recapitulation of IVIG anti-inflammatory activity with a recombinant IgG Fc. *Science*. 2008; 320:373–6. [PubMed: 18420934]
39. Kaneko Y, Nimmerjahn F, Ravetch JV. Anti-inflammatory activity of immunoglobulin G resulting from Fc sialylation. *Science*. 2006; 313:670–3. [PubMed: 16888140]
40. Washburn N, Schwab I, Ortiz D, Bhatnagar N, Lansing JC, Medeiros A, Tyler S, Mekala D, Cochran E, Sarvaiya H, Garofalo K, Meccariello R, Meador JW 3rd, Rutitzky L, Schultes BC, Ling L, Avery W, Nimmerjahn F, Manning AM, Kaundinya GV, Bosques CJ. Controlled tetra-Fc sialylation of IVIg results in a drug candidate with consistent enhanced anti-inflammatory activity. *Proceedings of the National Academy of Sciences of the United States of America*. 2015; 112:E1297–306. [PubMed: 25733881]
41. Karsten CM, Pandey MK, Figge J, Kilchenstein R, Taylor PR, Rosas M, McDonald JU, Orr SJ, Berger M, Petzold D, Blanchard V, Winkler A, Hess C, Reid DM, Majoul IV, Strait RT, Harris NL, Kohl G, Wex E, Ludwig R, Zillikens D, Nimmerjahn F, Finkelman FD, Brown GD, Ehlers M, Kohl J. Anti-inflammatory activity of IgG1 mediated by Fc galactosylation and association of FcγRIIb and dectin-1. *Nature medicine*. 2012; 18:1401–6.
42. Anthony RM, Kobayashi T, Wermeling F, Ravetch JV. Intravenous gammaglobulin suppresses inflammation through a novel T(H)2 pathway. *Nature*. 2011; 475:110–3. [PubMed: 21685887]
43. Sondermann P, Pincetic A, Maamary J, Lammens K, Ravetch JV. General mechanism for modulating immunoglobulin effector function. *Proceedings of the National Academy of Sciences of the United States of America*. 2013; 110:9868–72. [PubMed: 23697368]
44. Haga CL, Ehrhardt GR, Boohaker RJ, Davis RS, Cooper MD. Fc receptor-like 5 inhibits B cell activation via SHP-1 tyrosine phosphatase recruitment. *Proceedings of the National Academy of Sciences of the United States of America*. 2007; 104:9770–5. [PubMed: 17522256]
45. Massoud AH, Yona M, Xue D, Chouiali F, Alturahi H, Ablona A, Mourad W, Piccirillo CA, Mazer BD. Dendritic cell immunoreceptor: a novel receptor for intravenous immunoglobulin mediates induction of regulatory T cells. *The Journal of allergy and clinical immunology*. 2014; 133:853–63. e5. [PubMed: 24210883]
46. Seite JF, Cornec D, Renaudineau Y, Youinou P, Mageed RA, Hillion S. IVIg modulates BCR signaling through CD22 and promotes apoptosis in mature human B lymphocytes. *Blood*. 2010; 116:1698–704. [PubMed: 20516366]
47. Varki A, Gagneux P. Multifarious roles of sialic acids in immunity. *Annals of the New York Academy of Sciences*. 2012; 1253:16–36. [PubMed: 22524423]
48. Moore JS, Wu X, Kulhavy R, Tomana M, Novak J, Moldoveanu Z, Brown R, Goepfert PA, Mestecky J. Increased levels of galactose-deficient IgG in sera of HIV-1-infected individuals. *Aids*. 2005; 19:381–9. [PubMed: 15750391]
49. Bullen CK, Laird GM, Durand CM, Siliciano JD, Siliciano RF. New ex vivo approaches distinguish effective and ineffective single agents for reversing HIV-1 latency in vivo. *Nat Med*. 2014; 20:425–9. [PubMed: 24658076]
50. Chun TW, Carruth L, Finzi D, Shen X, DiGiuseppe JA, Taylor H, Hermankova M, Chadwick K, Margolick J, Quinn TC, Kuo YH, Brookmeyer R, Zeiger MA, Barditch-Crovo P, Siliciano RF. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997; 387:183–8. [PubMed: 9144289]

51. Finzi D, Blankson J, Siliciano JD, Margolick JB, Chadwick K, Pierson T, Smith K, Lisiewicz J, Lori F, Flexner C, Quinn TC, Chaisson RE, Rosenberg E, Walker B, Gange S, Gallant J, Siliciano RF. Latent infection of CD4⁺ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med.* 1999; 5:512–7. [PubMed: 10229227]
52. Finzi D, Hermankova M, Pierson T, Carruth LM, Buck C, Chaisson RE, Quinn TC, Chadwick K, Margolick J, Brookmeyer R, Gallant J, Markowitz M, Ho DD, Richman DD, Siliciano RF. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science.* 1997; 278:1295–300. [PubMed: 9360927]
53. Siliciano JD, Kajdas J, Finzi D, Quinn TC, Chadwick K, Margolick JB, Kovacs C, Gange SJ, Siliciano RF. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4⁺ T cells. *Nat Med.* 2003; 9:727–8. [PubMed: 12754504]
54. Trbojevic-Akmacic I, Ugrina I, Lauc G. Comparative Analysis and Validation of Different Steps in Glycomics Studies. *Methods in enzymology.* 2017; 586:37–55. [PubMed: 28137572]
55. Akmacic IT, Ugrina I, Stambuk J, Gudelj I, Vuckovic F, Lauc G, Pucic-Bakovic M. High-throughput glycomics: optimization of sample preparation. *Biochemistry. Biokhimiia.* 2015; 80:934–42. [PubMed: 26542006]
56. Gudelj I, Baciarello M, Ugrina I, De Gregori M, Napolioni V, Ingelmo PM, Bugada D, De Gregori S, Derek L, Pucic-Bakovic M, Novokmet M, Gornik O, Saccani Jotti G, Meschi T, Lauc G, Allegri M. Changes in total plasma and serum N-glycome composition and patient-controlled analgesia after major abdominal surgery. *Scientific reports.* 2016; 6:31234. [PubMed: 27501865]
57. Jannot AS, Ehret G, Perneger T. $P < 5 \times 10^{-8}$ has emerged as a standard of statistical significance for genome-wide association studies. *J Clin Epidemiol.* 2015; 68:460–5. [PubMed: 25666886]
58. Li J, Hsu HC, Ding Y, Li H, Wu Q, Yang P, Luo B, Rowse AL, Spalding DM, Bridges SL Jr, Mountz JD. Inhibition of fucosylation reshapes inflammatory macrophages and suppresses type II collagen-induced arthritis. *Arthritis Rheumatol.* 2014; 66:2368–79. [PubMed: 24838610]
59. Soliman NA, Keshk WA, Shoheib ZS, Ashour DS, Shamloula MM. Inflammation, oxidative stress and L-fucose as indispensable participants in schistosomiasis-associated colonic dysplasia. *Asian Pacific journal of cancer prevention : APJCP.* 2014; 15:1125–31. [PubMed: 24606429]
60. Keating SM, Pilcher CD, Jain V, Lebedeva M, Hampton D, Abdel-Mohsen M, Deng X, Murphy G, Welte A, Facente SN, Hecht F, Deeks SG, Pillai SK, Busch MP. HIV Antibody Level as a Marker of HIV Persistence and Low-Level Viral Replication. *The Journal of infectious diseases.* 2017; 216:72–81. [PubMed: 28498985]
61. Burbelo PD, Bayat A, Rhodes CS, Hoh R, Martin JN, Fromentin R, Chomont N, Hutter G, Kovacs JA, Deeks SG. HIV antibody characterization as a method to quantify reservoir size during curative interventions. *J Infect Dis.* 2014; 209:1613–7. [PubMed: 24286982]
62. Yukl SA, Boritz E, Busch M, Bentsen C, Chun TW, Douek D, Eisele E, Haase A, Ho YC, Hutter G, Justement JS, Keating S, Lee TH, Li P, Murray D, Palmer S, Pilcher C, Pillai S, Price RW, Rothenberger M, Schacker T, Siliciano J, Siliciano R, Sinclair E, Strain M, Wong J, Richman D, Deeks SG. Challenges in detecting HIV persistence during potentially curative interventions: a study of the Berlin patient. *PLoS pathogens.* 2013; 9:e1003347. [PubMed: 23671416]
63. Heyl KA, Karsten CM, Slevogt H. Galectin-3 binds highly galactosylated IgG1 and is crucial for the IgG1 complex mediated inhibition of C5aReceptor induced immune responses. *Biochemical and biophysical research communications.* 2016; 479:86–90. [PubMed: 27620493]
64. Mahan AE, Jennewein MF, Suscovich T, Dionne K, Tedesco J, Chung AW, Streeck H, Pau M, Schuitemaker H, Francis D, Fast P, Laufer D, Walker BD, Baden L, Barouch DH, Alter G. Antigen-Specific Antibody Glycosylation Is Regulated via Vaccination. *PLoS pathogens.* 2016; 12:e1005456. [PubMed: 26982805]
65. Hatano H, Jain V, Hunt PW, Lee TH, Sinclair E, Do TD, Hoh R, Martin JN, McCune JM, Hecht F, Busch MP, Deeks SG. Cell-based measures of viral persistence are associated with immune activation and programmed cell death protein 1 (PD-1)-expressing CD4⁺ T cells. *The Journal of infectious diseases.* 2013; 208:50–6. [PubMed: 23089590]
66. Chew GM, Fujita T, Webb GM, Burwitz BJ, Wu HL, Reed JS, Hammond KB, Clayton KL, Ishii N, Abdel-Mohsen M, Liegler T, Mitchell BI, Hecht FM, Ostrowski M, Shikuma CM, Hansen SG, Maurer M, Korman AJ, Deeks SG, Sacha JB, Ndhlovu LC. TIGIT Marks Exhausted T Cells,

- Correlates with Disease Progression, and Serves as a Target for Immune Restoration in HIV and SIV Infection. *PLoS pathogens*. 2016; 12:e1005349. [PubMed: 26741490]
67. Fromentin R, Bakeman W, Lawani MB, Khoury G, Hartogensis W, DaFonseca S, Killian M, Epling L, Hoh R, Sinclair E, Hecht FM, Bacchetti P, Deeks SG, Lewin SR, Sekaly RP, Chomont N. CD4+ T Cells Expressing PD-1, TIGIT and LAG-3 Contribute to HIV Persistence during ART. *PLoS pathogens*. 2016; 12:e1005761. [PubMed: 27415008]
 68. Abdel-Mohsen M, Wang C, Strain MC, Lada SM, Deng X, Cockerham LR, Pilcher CD, Hecht FM, Liegler T, Richman DD, Deeks SG, Pillai SK. Select host restriction factors are associated with HIV persistence during antiretroviral therapy. *AIDS*. 2015; 29:411–20. [PubMed: 25602681]
 69. Knezevic A, Polasek O, Gornik O, Rudan I, Campbell H, Hayward C, Wright A, Kolcic I, O'Donoghue N, Bones J, Rudd PM, Lauc G. Variability, heritability and environmental determinants of human plasma N-glycome. *Journal of proteome research*. 2009; 8:694–701. [PubMed: 19035662]
 70. Kristic J, Vuckovic F, Menni C, Klaric L, Keser T, Beceheli I, Pucic-Bakovic M, Novokmet M, Mangino M, Thaqi K, Rudan P, Novokmet N, Sarac J, Missoni S, Kolcic I, Polasek O, Rudan I, Campbell H, Hayward C, Aulchenko Y, Valdes A, Wilson JF, Gornik O, Primorac D, Zoldos V, Spector T, Lauc G. Glycans are a novel biomarker of chronological and biological ages. *J Gerontol A Biol Sci Med Sci*. 2014; 69:779–89. [PubMed: 24325898]
 71. Chen G, Wang Y, Qiu L, Qin X, Liu H, Wang X, Wang Y, Song G, Li F, Guo Y, Li F, Guo S, Li Z. Human IgG Fc-glycosylation profiling reveals associations with age, sex, female sex hormones and thyroid cancer. *J Proteomics*. 2012; 75:2824–34. [PubMed: 22365975]
 72. Estes JD, Kityo C, Ssali F, Swainson L, Makamdop KN, Del Prete GQ, Deeks SG, Luci PA, Chipman JG, Beilman GJ, Hoskuldsson T, Khoruts A, Anderson J, Deleage C, Jasurda J, Schmidt TE, Hafertepe M, Callisto SP, Pearson H, Reimann T, Schuster J, Schoephoerster J, Southern P, Perkey K, Shang L, Wietgreffe SW, Fletcher CV, Lifson JD, Douek DC, McCune JM, Haase AT, Schacker TW. Defining total-body AIDS-virus burden with implications for curative strategies. *Nature medicine*. 2017; 23:1271–1276.
 73. Fukazawa Y, Lum R, Okoye AA, Park H, Matsuda K, Bae JY, Hagen SI, Shoemaker R, Deleage C, Lucero C, Morcock D, Swanson T, Legasse AW, Axthelm MK, Hesselgesser J, Gelezianas R, Hirsch VM, Edlefsen PT, Piatak M Jr, Estes JD, Lifson JD, Picker LJ. B cell follicle sanctuary permits persistent productive simian immunodeficiency virus infection in elite controllers. *Nature medicine*. 2015; 21:132–9.
 74. Hodel F, Patxot M, Snaka T, Ciuffi A. HIV-1 latent reservoir: size matters. *Future virology*. 2016; 11:785–794. [PubMed: 28757894]
 75. Bruner KM, Hosmane NN, Siliciano RF. Towards an HIV-1 cure: measuring the latent reservoir. *Trends in microbiology*. 2015; 23:192–203. [PubMed: 25747663]
 76. Ho YC, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DI, Lai J, Blankson JN, Siliciano JD, Siliciano RF. Replication-competent noninduced proviruses in the latent reservoir increase barrier to HIV-1 cure. *Cell*. 2013; 155:540–51. [PubMed: 24243014]
 77. Ho Y-C, Shan L, Hosmane NN, Wang J, Laskey SB, Rosenbloom DS, Lai J, Blankson JN, Siliciano JD, Siliciano RF. Replication-Competent Noninduced Proviruses in the Latent Reservoir Increase Barrier to HIV-1 Cure. *Cell*. 2013; 155:540–541. [PubMed: 24243014]
 78. Eriksson S, Graf EH, Dahl V, Strain MC, Yukl SA, Lysenko ES, Bosch RJ, Lai J, Chioma S, Emad F, Abdel-Mohsen M, Hoh R, Hecht F, Hunt P, Somsouk M, Wong J, Johnston R, Siliciano RF, Richman DD, O'Doherty U, Palmer S, Deeks SG, Siliciano JD. Comparative analysis of measures of viral reservoirs in HIV-1 eradication studies. *PLoS pathogens*. 2013; 9:e1003174. [PubMed: 23459007]
 79. Laird GM, Eisele EE, Rabi SA, Lai J, Chioma S, Blankson JN, Siliciano JD, Siliciano RF. Rapid quantification of the latent reservoir for HIV-1 using a viral outgrowth assay. *PLoS Pathog*. 2013; 9:e1003398. [PubMed: 23737751]
 80. Li JZ, Etemad B, Ahmed H, Aga E, Bosch RJ, Mellors JW, Kuritzkes DR, Lederman MM, Para M, Gandhi RT. The size of the expressed HIV reservoir predicts timing of viral rebound after treatment interruption. *AIDS*. 2016; 30:343–53. [PubMed: 26588174]

Figure 1. HIV-associated glycomic alterations in total IgG glycome

(A) Heatmap describing alterations in total IgG glycome associated with unsuppressed and suppressed HIV infection. Red = higher levels and blue = lower levels. S2 = percentage of di-sialylated structures, F = percentage of all fucosylated structures, G0 = percentage of agalactosylated structures, G2 = percentage of di-galactosylated structures, S1 = percentage of monosialylated structures, G1 = percentage of monogalactosylated structures, and B = percentage of structures with bisecting GlcNAc. (B) Percentage of di-sialylated glycan structures in total IgG glycome. (C) Percentage of A2G2S2 glycan trait in total IgG glycome. (D) Percentage of fucosylated glycan structures in total IgG glycome. (E)

Percentage of agalactosylated glycan structures in total IgG glycome. All statistical comparisons were performed using two-tailed non-parametric Mann–Whitney t test. Error bars represent median and interquartile range (IQR).

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Figure 2. HIV-associated glycomic alterations in total plasma glycome

(A) Heatmap describing alterations in total plasma glycome associated with unsuppressed and suppressed HIV infection. Red = higher levels and blue = lower levels. S0 = neutral glycan structures, S1 = monosialylated structures, S2 = di-sialylated structures, S3 = trisialylated structures, S4 = tetrasialylated structures, G0 = agalactosylated structures, G1 = monogalactosylated structures, G2 = di-galactosylated structures, G3 = trigalactosylated structures, G4 = tetragalactosylated structures, LB = low branched (monoantennary and diantennary) structures, HB = high branched (triantennary and tetraantennary), B = structures with bisecting GlcNAc, FUC-A = antennary fucosylated structures, and FUC-C =

core fucosylated structures. **(B)** Percentage of structures with bisecting GlcNAc in total plasma glycome. **(C)** Percentage of agalactosylated glycan structures in total plasma glycome. **(D)** Percentage of core-fucosylated glycan structures in total plasma glycome. **(E)** Percentage of neutral glycan structures in total plasma glycome. **(F)** Percentage of di-sialylated glycan structures in total plasma glycome. **(G)** Percentage of di-galactosylated glycan structures in total plasma glycome. All statistical comparisons were performed using two-tailed non-parametric Mann–Whitney t test. Error bars represent median and interquartile range (IQR).

Figure 3. Levels of certain glycomic traits in isolated IgG glycomes correlate with HIV persistence during ART

Correlations between unfractionated PBMC and CD4+ T cell-associated HIV DNA and RNA and (A) percentage of A2G1, (B) percentage of A2G2, and (C) percentage of A2BG2, in total IgG glycomes of HIV+ ART-suppressed individuals. P-values were obtained using two-tailed non-parametric Spearman's rank tests.

Figure 4. Levels of certain glycomic traits in total plasma glycomes correlate with HIV persistence during ART

Correlations between unfractionated PBMC and CD4+ T cell-associated HIV DNA and RNA and (A) percentage of A2[6]BG1, (B) percentage of A2G2, and (C) percentage of A2BG2, in total plasma glycomes of HIV+ ART-suppressed individuals. P-values were obtained using two-tailed non-parametric Spearman's rank tests.

Figure 5. Percentage of A2G2 glycan trait in total IgG glycome is higher in ART-suppressed individuals

Percentage of A2G2 glycan trait in total IgG glycome. Statistical comparisons were performed using non-parametric two-tailed Mann–Whitney t test. Error bars represent median and interquartile range (IQR).

Figure 6. Levels of certain circulating anti-inflammatory glycans associate with higher levels of CD4 count and lower levels of T cell activation

Correlations between: (A–D) levels of A4G4S4 glycan trait in total plasma glycomes of HIV + ART-suppressed individuals and (A) CD4 count, (B) CD4%, (C) percentage of CD4+ T cells expressing the HLA-DR late activation marker, and (D) percentage of CD4+ T cells expressing the intermediate or late CD25 activation marker. (E–F) Levels of FA3G3S3 glycan trait in total plasma glycomes of HIV+ ART-suppressed individuals and (E) CD4%, and (F) percentage of CD4+ T cells expressing the intermediate or late CD25 activation marker. (G) Levels of FA2BG2S1 glycan trait in total IgG glycomes of HIV+ ART-suppressed individuals and CD4 count. (H) Levels of A2BG2 glycan trait in total IgG glycome of HIV+ ART-suppressed individuals and percentage of CD4+ T cells expressing the CD69 early activation marker. P-values were obtained using two-tailed non-parametric Spearman's rank tests.