Link between Interleukin-23 and Anti-CD4 Autoantibody Production in Antiretroviral-Treated HIV-Infected Individuals

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ABSTRACT Potential mechanisms of poor CD4⁺ T cell reconstitution after viral suppression with antiretroviral therapy (ART) in HIV disease have been extensively investigated. We recently discovered that anti-CD4 autoantibody plays a role in impaired CD4⁺ T cell recovery from ART in HIV-infected individuals with viral suppression, which accounts for a mechanism specific for CD4⁺ T cell depletion. However, the mechanism of pathological anti-CD4 autoantibody production in treated HIV disease remains unknown. Here, we report that seasonal influenza vaccination induced IgG anti-CD4 autoantibodies, predominantly of the IgG3 subclass, in some virus-suppressed ART-treated HIV⁺ subjects. To explore the mechanism of anti-CD4 antibody production in this population, we performed and analyzed gene profiles in isolated B cells using a gene microarray and 32 plasma cytokines. Notably, both gene expression and multiple cytokine analyses showed that interleukin 23 (IL-23), at the prevaccination plasma level, was the key cytokine linked to IgG anti-CD4 antibody production in response to immunization *in vivo*. Exogenous recombinant IL-23 (rIL-23) increased autoreactive IgG binding on CD4⁺ T cells from HIV⁺ subjects *in vitro*. Results from this study may reveal a role of IL-23 in anti-CD4 autoantibody production in treated HIV.

IMPORTANCE In our published studies (Z. Luo et al., *J Leukoc Biol* 102:1481–1486, 2017, <https://doi.org/10.1189/jlb.5A0617-219R>; Z. Luo et al., *J Infect Dis* 216:82–91, 2017, <https://doi.org/10.1093/infdis/jix223>), we determined that pathological anti-CD4 IgGs from immunologic nonresponders on virally suppressive ART (CD4 cell counts < 350 cells/ μ l) mediated CD4⁺ T cell death via antibody-mediated cytotoxicity (ADCC), which plays a role in poor CD4⁺ T cell recovery from ART. Up to 25% of HIV-infected individuals are nonresponders and demonstrate increased morbidity and mortality. However, the mechanism of anti-CD4 autoantibody production in treated HIV remains unknown. In this study, we report that IL-23 may be the key cytokine to promote anti-CD4 autoantibody production after immunization in ART-treated HIV-infected individuals.

KEYWORDS . IL-23, anti-CD4 autoantibody, HIV, human immunodeficiency virus

In most HIV-infected individuals, antiretroviral therapy (ART) successfully suppresses HIV replication, restores CD4⁺ T cell counts in the circulation, and decreased morbidity and mortality. However, some patients fail to restore CD4⁺ T cell counts despite extended ART (1, 2). Among these HIV-infected individuals, the risks for neurologic

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dysfunction, cardiovascular disease, malignancy, and liver disease increase (3–5). Potential mechanisms for poor CD4⁺ T cell reconstitution are not fully understood, with possible explanations including low-nadir CD4⁺ T cells, persistent immune activation, thymic insufficiency, lymphatic fibrosis, and gut mucosal dysfunction that leads to microbial translocation and inflammation (6–9). Our recent study reveals that IgG anti-CD4 autoantibodies from immunologic nonresponders on virally suppressive ART (CD4 cell counts < 350 cells/ μ l) induced NK cell activation and antibody-dependent NK cell-mediated cytotoxicity (ADCC) against primary CD4⁺ T cells (10, 11).

HIV-associated anti-CD4 autoantibody production may stem from various mechanisms: molecular mimicry, viral replication and stimulation, polyclonal B lymphocyte activation, and dysregulation of B/T lymphocyte interaction (12, 13). HIV-infected individuals who received long-term ART continue to have increased B cell activation, autoreactive antibody production, and residual elevated systemic microbial translocation (14). Influenza vaccine-induced B cell activation was directly correlated with plasma levels of microbial translocation (15). A previous study showed that anti-lymphocyte antibodies (ALA) were induced by seasonal influenza vaccination in HIV-1 patients (16). These results suggest that B cell perturbations occur in ART-treated HIV-infected subjects.

Studies in animals and humans have confirmed that Th17 cells, chemokines, and cytokines contribute to human systemic and organ-specific autoantibody production and autoimmune diseases (17, 18). To investigate anti-CD4 autoantibody production in ART-treated HIV-infected individuals, we measured anti-CD4 antibodies before and after influenza vaccine immunization, assessed gene expression patterns by microarray analysis, and analyzed multiplex cytokines.

RESULTS

Increased anti-CD4 antibody production after influenza immunization in HIV infection. Influenza vaccination not only induced influenza virus-specific IgG, IgA, and IgM (14) but also increased IgA, IgG, and IgM anti-CD4 antibodies in some HIV-infected individuals but not in healthy controls (Fig. 1A). To identify IgG subclasses in response to vaccination, IgG1 to IgG4 anti-CD4 antibodies were evaluated. On day 0 (D0), higher levels of IgG1 and IgG3 anti-CD4 antibodies, but not IgG2 and IgG4 antibodies, were found in HIV-infected individuals than in healthy controls (Fig. 1B). After influenza vaccination, levels of IgG3 anti-CD4 antibodies increased significantly in HIV-infected individuals but not in healthy controls, while IgG1, IgG2, and IgG4 anti-CD4 antibody levels did not change (Fig. 1B).

To determine whether IgG3 anti-CD4 antibodies were related to anti-influenza virus antibodies, we assessed influenza virus antigen-specific IgG antibody subtypes from IgG1 to IgG4. IgG1 and IgG3 influenza virus antigen-specific IgGs were increased after vaccination in both healthy controls and HIV⁺ subjects (Fig. 1C). Although IgG3 influenza virus antigen-specific antibodies increased significantly, the increase was not correlated with plasma levels of IgG3 anti-CD4 antibody in HIV⁺ subjects ($P = 0.867$, $r = -0.036$). Furthermore, we observed direct correlations between IgM anti-influenza virus antibody level (but not IgG or IgA) and levels of IgM, IgG, IgA, and IgG3 anti-CD4 autoantibodies (Fig. 1D). We also investigated the association between plasma anti-CD4 IgG levels on day 7 postvaccination and the percentages of cycling CD4⁺ T cells (a marker of CD4⁺ T cell activation in HIV disease [19]). A higher percent Ki-67 expression in CD4⁺ T cells was seen in HIV-infected individuals than in healthy controls before and after vaccination (Fig. 1E). Intriguingly, a direct correlation was observed between Ki-67-expressing CD4⁺ T cells on day 7 and anti-CD4 antibody levels on day 7 postvaccination ($P = 0.007$, $r = 0.523$) (Fig. 1F). This result suggests a link between CD4⁺ T cell activation and anti-CD4 autoantibody production in response to vaccination in HIV disease.

Correlations between plasma anti-CD4 autoantibody levels and the percentages of apoptotic CD4⁺ T cells postvaccination. Cell apoptosis is related to activation. Next, we evaluated the association between anti-CD4 IgGs and markers related to cell

FIG 1 Plasma anti-CD4 antibody levels pre- and postvaccination. Plasma anti-CD4 IgG levels were measured on D0, D7, and D14 postvaccination. (A) Median plasma levels of IgG, IgA, and IgM anti-CD4 antibodies in healthy controls and HIV-infected individuals before (D0) and after (D14) vaccination. (B) Median plasma levels of IgG anti-CD4 antibody subtypes (IgG1 to IgG4) in healthy controls and HIV-infected individuals before (D0) and after (D14) vaccination. (C) Median plasma levels of IgG1, IgG2, IgG3, and IgG4 anti-influenza vaccine antibodies in healthy controls and HIV-infected individuals before (D0) and after (D14) vaccination. (D) Direct correlations between IgM but not IgG or IgA anti-influenza vaccine antibodies and all three types or one subtype of anti-CD4 autoantibodies on D14 postvaccination. Statistically significant differences are highlighted in gray; no highlighting indicates no statistically significant difference. (E) Median percentages of Ki-67⁺ CD4⁺ T cells in healthy controls and HIV-infected individuals before (D0) and after (D7) vaccination. (F) Correlation between plasma levels of IgG anti-CD4 antibodies (D7) and the percentages of Ki-67⁺ in CD4⁺ T cells (D7) in HIV-infected subjects. Wilcoxon matched-pair signed-rank tests, one-way analysis of variance (ANOVA) followed by Dunnett's multiple-comparison tests, and Spearman correlation tests were used. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

FIG 2 Plasma anti-CD4 antibody levels and their correlations with apoptotic CD4⁺ T cells. Annexin V binding on CD4⁺ T cells were tested on D7 postvaccination. (A) Correlation between plasma levels of IgG anti-CD4 antibodies (D0) and peripheral CD4⁺ T cell counts (D0) in HIV-infected subjects. (B) Correlation between plasma levels of IgG anti-CD4 antibodies (D7) and apoptosis of circulating follicular helper CD4⁺ T (Tfh; D7) cells (CD3⁺ CD4⁺ CXCR5⁺ CXCR3[−] PD1⁺) in HIV-infected subjects. (C) Correlation between plasma levels of IgG3 anti-CD4 antibodies (D7) and apoptosis of circulating peripheral CD4⁺ T cells (D7) in HIV-infected subjects. (D) Direct correlations between vaccine-mediated plasma IgG3 anti-CD4 antibody production and the percentages of apoptosis in each CD4⁺ T cell subtype. Spearman correlation tests were used.

apoptosis. Consistent with our previous study (11), baseline CD4⁺ T cell counts were inversely correlated with plasma levels of IgG anti-CD4 antibodies in HIV-infected individuals (Fig. 2A). In our previous study, we found that IgG anti-CD4 autoantibody mediated CD4⁺ T cell death via ADCC; those anti-CD4 IgGs were purified from plasma samples of patients with CD4⁺ T cell counts below 350 cells/ μ l (11). To determine the interplay between influenza virus antigen-induced IgG anti-CD4 antibody and peripheral CD4⁺ T cell depletion, we measured apoptotic CD4⁺ T cells postvaccination. We also measured apoptosis of circulating T follicular helper (Tfh) cells (CD3⁺ CD4⁺ CXCR5⁺ CXCR3[−] PD1⁺) (20) and observed a direct correlation of plasma anti-CD4 IgG levels on D7 with the percentages of apoptotic circulating Tfh CD4⁺ T cells on D7 postvaccination in HIV-infected individuals (Fig. 2B). Furthermore, IgG3 anti-CD4 antibody levels were directly correlated with the proportions of apoptotic CD4⁺ T cells in the total population (Fig. 2C) and subsets (Fig. 2D) of CD4⁺ T cells postvaccination.

B cell gene “signatures” associated with anti-CD4 antibody production in HIV.

To study the mechanism of anti-CD4 antibody production in HIV-infected subjects after vaccination, we examined the gene expression profiles of total B cells from HIV-infected subjects with high levels of IgG anti-CD4 antibodies (≥ 2 -fold change of anti-CD4 IgG at D14 versus D0) and low IgG anti-CD4 antibodies (< 2 -fold change of anti-CD4 IgG at D14 versus D0). The functional and pathway analyses were performed using gene ontology (GO) to identify genes with significantly different expression ($P < 0.05$) in individuals with high versus low levels of anti-CD4 antibodies. Figure 3A represents annotated networks of interacting proteins with significant gene expression differences identified by the GO biological process ($P < 0.05$). Intriguingly, IgG anti-CD4 antibody expression was associated with differentially expressed genes linked to chemotaxis; inflammatory responses; response to HIV; CD4⁺ T cell differentiation, activation, and regulation; and NK cell activation (Fig. 3A).

Next, KEGG (Kyoto Encyclopedia of Genes and Genomes) biological pathways were used to annotate and characterize genes expressed differently in the two groups. A selected list of biological pathways enriched with transcripts was significantly altered

FIG 3 The unique gene expression profile at baseline is associated with anti-CD4 antibody production following influenza vaccination. Gene expression was analyzed in isolated B cells by microarray to compare HIV⁺ high-anti-CD4 antibody induction (≥ 2 -fold change of anti-CD4 IgG at D14/D0) and HIV⁺ low-anti-CD4 antibody induction (< 2 -fold change of anti-CD4 IgG at D14/D0) by vaccination. (A) Clustering of gene ontology (GO) term functions and pathways differentially expressed in HIV⁺ subjects with high levels of anti-CD4 antibodies compared to those with low levels of anti-CD4 antibodies ($P < 0.05$). (B) Representative immunological pathways enriched in coherently changed genes in HIV⁺ subjects with high levels of anti-CD4 antibodies compared to those with low levels of anti-CD4 antibodies. (C) Gene function network relevant to anti-CD4 antibody production.

TABLE 1 Baseline plasma levels of multiple cytokines from healthy controls and two HIV⁺ study groups

Cytokine	Level (pg/ml) in group (mean $\pm$ SD)			P value (low vs. high) ^a
	Healthy	HIV ⁺ , low anti-CD4	HIV ⁺ , high anti-CD4	
IL-1 α	0.334 $\pm$ 0.227	0.265 $\pm$ 0.233	0.360 $\pm$ 0.275	0.172
IL-1 β	0.114 $\pm$ 0.084	0.200 $\pm$ 0.337	0.148 $\pm$ 0.097	0.519
IL-2	0.078 $\pm$ 0.027	0.091 $\pm$ 0.069	0.094 $\pm$ 0.08	0.236
IL-5	0.645 $\pm$ 0.323	0.868 $\pm$ 0.452	0.947 $\pm$ 0.449	0.155
IL-6	0.708 $\pm$ 0.445	1.069 $\pm$ 1.526	1.903 $\pm$ 2.271	0.131
IL-7	11.512 $\pm$ 7.778	9.172 $\pm$ 6.821	8.079 $\pm$ 6.41	0.669
IL-8	5.393 $\pm$ 3.763	6.452 $\pm$ 8.226	8.692 $\pm$ 13.167	0.427
IL-10	0.307 $\pm$ 0.230	0.230 $\pm$ 0.369	0.373 $\pm$ 0.601	0.327
IL-12/IL-23 p40	123.712 $\pm$ 60.051	126.755 $\pm$ 102.05	202.529 $\pm$ 138.536	0.037
IL-12 p70	0.133 $\pm$ 0.110	0.089 $\pm$ 0.091	0.14 $\pm$ 0.088	0.060
IL-13	0.422 $\pm$ 0.286	0.425 $\pm$ 0.417	0.545 $\pm$ 0.534	0.261
IL-15	1.908 $\pm$ 0.412	2.192 $\pm$ 0.566	2.374 $\pm$ 0.493	0.203
IL-16	323.789 $\pm$ 255.482	254.815 $\pm$ 309.702	381.439 $\pm$ 425.733	0.223
IL-17A	1.839 $\pm$ 0.887	1.758 $\pm$ 0.848	2.353 $\pm$ 1.045	0.030
IL-21	1.133 $\pm$ 0.492	0.873 $\pm$ 0.550	0.917 $\pm$ 0.511	0.970
IL-22	0.530 $\pm$ 0.680	0.402 $\pm$ 0.339	0.368 $\pm$ 0.263	0.590
IL-23	0.700 $\pm$ 0.386	0.581 $\pm$ 0.295	0.923 $\pm$ 0.236	0.009
IL-27	1258.012 $\pm$ 422.044	963.689 $\pm$ 357.116	1047.864 $\pm$ 431.705	0.574
Eotaxin	121.135 $\pm$ 51.229	170.700 $\pm$ 95.155	149.816 $\pm$ 33.373	0.277
Eotaxin-3	17.976 $\pm$ 6.023	19.539 $\pm$ 8.470	16.812 $\pm$ 7.211	0.261
GM-CSF	0.207 $\pm$ 0.072	0.189 $\pm$ 0.073	0.185 $\pm$ 0.052	0.953
IFN- γ	3.667 $\pm$ 1.733	5.909 $\pm$ 8.254	12.336 $\pm$ 12.547	0.025
IP-10	245.292 $\pm$ 168.977	453.205 $\pm$ 536.611	771.884 $\pm$ 812.516	0.111
MCP-1	72.172 $\pm$ 17.388	84.009 $\pm$ 26.312	88.197 $\pm$ 24.003	0.605
MDC	945.846 $\pm$ 265.322	1210.458 $\pm$ 320.234	1238.153 $\pm$ 266.056	0.803
MIP-1 α	16.978 $\pm$ 8.750	28.69 $\pm$ 24.339	31.423 $\pm$ 23.256	0.619
MIP-1 β	63.492 $\pm$ 26.155	71.424 $\pm$ 43.196	62.231 $\pm$ 26.948	0.386
MIP-3 α	5.202 $\pm$ 4.601	5.890 $\pm$ 4.684	4.979 $\pm$ 2.884	0.452
TARC	243.862 $\pm$ 166.004	287.018 $\pm$ 221.33	232.386 $\pm$ 207.842	0.434
TNF- α	1.887 $\pm$ 0.413	2.210 $\pm$ 0.740	2.895 $\pm$ 0.695	0.003
TNF- β	0.428 $\pm$ 0.088	0.365 $\pm$ 0.125	0.405 $\pm$ 0.119	0.181
VEGF	40.319 $\pm$ 29.924	55.303 $\pm$ 49.031	57.483 $\pm$ 55.184	0.806

^aStatistically significant differences in cytokine levels between the HIV⁺ high-anti-CD4 antibody induction group and the HIV⁺ low-anti-CD4 antibody group are highlighted in gray. Mann-Whitney's *U* test (unpaired) was used.

by IgG anti-CD4 antibody production among HIV-infected individuals (Fig. 3B). These pathways included the carbon metabolism signaling pathway, the interleukin 17 (IL-17) signaling pathway, the chemokine signaling pathway, the cytokine-cytokine receptor interaction signal pathway, and the oxidative phosphorylation pathway (Fig. 3B). The genes that showed differences in these pathways were identified, and CluePedia (21), CentiScape, and MCODE (22) were performed to assess protein-to-protein function and interaction. Interaction network decomposition analysis was also performed. The network indicated that some cytokines and proteins (such as those encoded by *IL-23A*, *JAK2*, *MAPK3*, *CXCL8*, and *CXCL9*) may play major roles in anti-CD4 antibody production (Fig. 3C).

Plasma IL-23 level is correlated with anti-CD4 antibody production in HIV-infected subjects. To study the etiological role of inflammatory processes on anti-CD4 antibody production, multiplex cytokine analyses were performed on D0, including Th17 cytokines, chemokines, and proinflammatory cytokines from plasma. Cytokines in each sample and comparisons between HIV-infected subjects and healthy controls are shown (Table 1). Among the 32 cytokines listed in Materials and Methods, IL-12/IL-23 p40, IL-17A, IL-23, gamma interferon (IFN- γ), and tumor necrosis factor alpha (TNF- α) were significantly increased in plasma from HIV-infected subjects with high anti-CD4 antibody production compared to those with low anti-CD4 antibody production following vaccination (Fig. 4A). Next, we examined the association of cytokines on D0 with IgG and IgG3 anti-CD4 antibody production, and baseline plasma IL-23 level

FIG 4 The direct correlation between baseline IL-23 and anti-CD4 IgG production following vaccination in HIV⁺ subjects and IL-23-associated cytokine network. (A and B) *In vivo* studies. Thirty-two cytokines were tested in plasma on D0 (baseline) prevaccination. (A) The median plasma levels of IL-17A, IL-12/IL-23 p40, IL-23, TNF- α , and IFN- γ are shown, as they represented the most increased cytokines in the HIV⁺ high-anti-CD4 IgG group compared to the HIV⁺ low-anti-CD4 IgG group. (B) The plasma levels of IL-23 were directly correlated with IgG anti-CD4 antibody production on D14 in HIV⁺ subjects. (C) *In vitro*, PBMCs from HIV⁺ subjects on D7 postvaccination were stimulated with anti-CD3/anti-CD28 antibodies in medium, in the presence of TNF- α (1 ng/ml), or in the presence of 0.1 and 1 μ g/ml of IL-23 for 5 days and restimulated with PMA and ionomycin for 4 h. The median percentages of IL-17A⁺, IFN- γ ⁺, and TNF- α ⁺ in CD4⁺ T cells and relative to medium control are shown. One-way ANOVA followed by Dunnett's multiple-comparison tests and Spearman correlation tests were used. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

was correlated with both IgG and IgG3 anti-CD4 antibody levels before and after influenza immunization (Fig. 4B; Table 2). Baseline plasma levels of IL-12/IL-23 p40 ($P = 0.06$) and IFN- γ ($P = 0.057$) tended to correlate with plasma IgG anti-CD4 antibody levels, but the correlation did not achieve statistical significance (Table 2).

To assess if there was an interaction between IL-23 and the other inflammatory cytokines, IL-17A, IFN- γ , and TNF- α , which were increased in HIV⁺ subjects at baseline, we cultured PBMCs from HIV-infected subjects with recombinant IL-23 (rIL-23) *in vitro*. PBMCs were stimulated with anti-CD3/anti-CD28 antibodies in the presence or absence of rIL-23. Consistent with a previous study (23), upon stimulation with phorbol myristate acetate (PMA) and ionomycin, a small number of CD4⁺ T cells expressed IL-17A by day 5 (Fig. 4C). Additionally, rIL-23 increased IFN- γ and TNF- α expression in CD4⁺ T

TABLE 2 Direct correlations between plasma IL-23 level with anti-CD4 autoantibodies on D0 and D14 postvaccination^a

Antibody and day	IL-23		TNF- α		IL-12/IL-23 p40		IL-17A		IFN- γ	
	P	r	P	r	P	r	P	r	P	r
Anti-CD4 IgG										
D0	0.032	0.421	0.802	0.052	0.061	0.372	0.795	0.054	0.057	0.378
D14	0.014	0.484	0.247	0.236	0.067	0.365	0.962	0.010	0.072	0.359
Anti-CD4 IgG3										
D0	0.064	0.369	0.711	0.076	0.755	−0.064	0.820	−0.047	0.909	−0.024
D14	0.004	0.551	0.134	0.302	0.833	−0.044	0.909	0.023	0.529	−0.129

^aStatistically significant differences are highlighted in gray. The Spearman correlation test was used. *r*, correlation coefficient.

cells (Fig. 4C). These results suggest that IL-23 in HIV⁺ subjects may partially drive increases in IL-17A, IFN- γ , and TNF- α , which is consistent with *in vivo* cytokine patterns observed in the high-anti-CD4 antibody group (Fig. 4A).

Exogenous IL-23 induced autoantibodies against CD4⁺ T cells *in vitro*. To detect the activity of IL-23 on B cells, we cultured PBMC with or without IL-23 or in the presence of IL-23 neutralization antibody for 48 h. The phenotypes on B cells were assessed by flow cytometry. The markers were tested, including CD27, CD38, CD86, and CD138 for B cell activation and subsets, as well as annexin V for B cell apoptosis. CD27 was used as a memory B cell marker, CD38 and CD86 were used as B cell activation markers, and CD138 was used as a plasma B cell marker. The percentage of CD138⁺ cells in B cells was increased and the percentage of apoptotic B cells was decreased in response to rIL-23 (Fig. 5A). However, there was no difference in the percentage of CD27, CD38, or CD86 in response to rIL-23 (Fig. 5B). To verify the effect of IL-23 on anti-CD4 autoantibody production, we cultured PBMCs from HIV-infected subjects on D7 after vaccination *in vitro* in the presence or absence of rIL-23 and a neutralizing antibody against IL-23. The neutralizing antibody against IL-23 was used to prevent IL-23-mediated spontaneous anti-CD4 IgG secretion from B cells in those HIV⁺ subjects. Unexpectedly, we did not detect anti-CD4 IgGs in the cell culture supernatants in response to rIL-23; however, IgG autoantibodies were detected bound to the surface of CD4⁺ T cells but not to the surface of CD3⁺ CD4[−] cells (likely CD8⁺ T cells) in response to dose-dependent rIL-23 on day 10 (Fig. 5C and D and data not shown). This is consistent with the results from our previous study that purified anti-CD4 IgGs from plasma samples of ART-treated HIV⁺ subjects bound to CD4⁺ T cell surface and induced CD4⁺ T cell activation *in vitro* (11). The neutralizing antibody against IL-23 slightly reduced the percentage of IgG binding to CD4⁺ T cells compared to those in medium alone but did not reach a significant difference (Fig. 5C and D). Moreover, no difference was observed in total IgG or IgM production in the culture supernatants after treatment with rIL-23 (Fig. 5E).

DISCUSSION

Our published studies (10, 11, 24) were the first to determine that pathological anti-CD4 IgGs from immunologic nonresponders on virally suppressive ART (CD4 cell counts < 350 cells/ μ l) mediated CD4⁺ T cell death via ADCC, which plays a role in poor CD4⁺ T cell recovery from ART. Up to 25% of HIV-infected individuals are nonresponders (25) and demonstrate increased morbidity and mortality (4, 26–28). The effect of anti-CD4 autoantibody on CD4⁺ T cell depletion in HIV was recently confirmed by Lisco et al. (29). In this study, we show that plasma levels of anti-CD4 IgG increased following influenza vaccination in some ART-treated HIV-infected individuals and that the increase was directly correlated with baseline IL-23 levels as well as apoptosis of both total CD4⁺ T cells and CD4⁺ Tfh cells.

Our previous study showed that IgG anti-CD4 antibodies inversely correlate with CD4⁺ T cell counts in ART-treated HIV-infected individuals (11). Additionally, anti-CD4 IgG antibodies purified from plasma of immunologic nonresponders mediated ADCC to induce CD4⁺ T cell cytolysis and apoptosis (11). In the current study, anti-CD4 IgG3

FIG 5 Exogenous IL-23 induces autoantibodies against CD4⁺ T cells *in vitro*. PBMCs from HIV⁺ subjects on D7 postvaccination were stimulated with medium, 0.1 and 1 µg/ml of IL-23, or neutralizing antibody against IL-23 for 2 days or 10 days. (A) Median percentages of apoptotic B cells and CD138⁺ B cells. (B) Median percentages of CD27, CD38, and CD86 expression on B cells. (C) IgG binding on the surfaces of CD4⁺ T cells, shown as dot plots from a representative donor, in CD3⁺ CD4⁺ and CD3⁺ CD4⁻ T cells. (D) Median percentages of IgG on CD3⁺ CD4⁺ or CD3⁺ CD4⁻ T cells. (E) The median levels of total IgG and IgM were evaluated in cell culture supernatants by ELISA. One-way ANOVA followed by Tukey's multiple-comparison test. *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$; ****, $P < 0.0001$.

antibody production following vaccination was significantly correlated with apoptosis of total CD4⁺ T cells and subtypes of CD4⁺ T cells. A previous report showed that, even against the same antigen epitopes, IgG subclasses (IgG1 to IgG4) differed in their functions because of their distinct affinities for Fc receptors (30). In this study, the primary IgG subtype that increased following influenza vaccination was anti-CD4 IgG3. Compared to other IgG subclasses, IgG3 antibodies have the most elongated and most flexible hinge region, showing high affinity for FcγRI, FcγRIIa, and FcγRIIb (31). A previous study revealed that the early induction of anti-chikungunya virus IgG3 antibodies is strongly associated with clearance efficiency to the virus (32). In the RV144 HIV vaccine study, vaccine-induced IgG3 against HIV Env was correlated with medium to high levels of ADCC activity (33). These results suggest that IgG3 anti-CD4 antibodies are likely functional, providing a rationale for further studies to determine whether the IgG3 anti-CD4 antibody results in CD4⁺ T cell apoptosis and depletion. In this study, although plasma anti-CD4 antibody level was directly correlated with the percentage of apoptotic of CD4⁺ T cells, we did not observe the decline of CD4⁺ T cells count on day 7 after vaccination. However, this study tested only influenza vaccine-mediated anti-CD4 IgG after one-time administration; the exposure of a trigger for developing a pathological autoantibody may need to be repeated multiple times and take years (34). We hypothesize that in these patients, antigen stimulation may increase low-affinity and inefficient antibody-producing B cells, as reported from previous studies (35), which can serve as

progenitor cells for further somatic hypermutation and affinity maturation and eventually become functional antibody-secreting B cells (36).

The immune activation induced by vaccination or antigen (either transient or persistent) may promote anti-CD4 autoantibody production in some HIV patients undergoing virus-suppressive ART. Transient increases in HIV replication have been observed in HIV patients following seasonal influenza vaccination in several studies (37, 38). Whether influenza virus antigen-mediated anti-CD4 autoantibodies play a role in HIV reservoir activation deserves further investigation. In this study, all patients and controls had received multiple cycles of seasonal influenza vaccination, including the cycle of the previous year. Thus, the baseline level of the current vaccination represented 1 year from the last vaccination in all subjects. Upon the 2013-2014 seasonal vaccination, the levels of anti-CD4 antibodies (IgG, IgG3, IgA, and IgM) were directly correlated with levels of influenza virus antigen-specific IgM but not with levels of IgG or IgA. The IgM production following vaccination may be generated by polyreactive low-affinity IgM⁺ CD27⁺ B cells after activation and rapid differentiation (39). However, the question of whether the synergistic effect of influenza virus antigens and additional immune stimulation causes anti-CD4 autoantibody production in HIV⁺ individuals with a high baseline level of IL-23 warrants further investigation. After long-term ART and viral suppression, HIV may still actively replicate in the lymph nodes and tissues in some HIV⁺ individuals (40). CD4⁺ T cell death driven by HIV replication in the lymph nodes may result in the release of CD4 self-antigens or HIV gp120-bound CD4 proteins, which serve as antigenic drivers for inducing anti-CD4 antibodies (7). Furthermore, a high level of apoptotic Tfh cells could exacerbate this pathological activity.

Based on analysis of differential expression genes (DEGs) from HIV-infected subjects with high versus low anti-CD4 autoantibody production after vaccination, we found that DEGs affect the inflammatory response; the response to HIV; CD4⁺ T cell differentiation, activation, and regulation; and NK cell activation. Our recent study showed that purified anti-CD4 antibody from the plasma of HIV⁺ subjects induced NK cell activation *in vitro* (11, 41), supporting the link between NK cell activation and anti-CD4 autoantibody production. The multiplex cytokines from plasma and gene expression results showed that IL-23 levels were directly correlated with further anti-CD4 antibody production in response to vaccination. IL-23 is a heterodimeric cytokine composed of an IL-12/IL-23 p40 subunit and an IL-23 p19 subunit. We found that IL-12/IL-23 p40 was significantly increased in plasma from HIV-infected subjects with high levels of anti-CD4 antibodies compared to those with low levels of anti-CD4 antibodies. The *in vitro* study confirmed that IL-23 could induce autoantibodies bound to CD4⁺ T cells in peripheral blood mononuclear cells (PBMCs) from patients. IL-23 has previously been shown to drive a pathogenic CD4⁺ T cell population responsible for the induction of autoimmunity in mice (23), suggesting the onset of autoimmune disease via the IL-23-TH17 cell-dependent pathway (42). Although some other cytokines, such as IL-17A, IFN- γ , and TNF- α , showed noteworthy differences, the *in vitro* experiments confirmed that IL-23 could induce these cytokines. Again, IL-23 may be the central factor with a cytokine network for autoantibody induction in treated HIV.

IL-23 is active at mucosal surfaces and is produced by the CD8a⁻ CD11b⁻ CD11c⁺ dendritic cells in the lamina propria of the small intestine (43), suggesting that IL-23 is produced in the intestinal mucosa where anti-CD4 production is mediated. Previous studies showed that T helper type 17 (TH17) cells and other immune cells (e.g., $\gamma\delta$ T cells) respond to IL-23, which plays a role in autoimmunity (44, 45). Although extensive studies have shown the function of IL-23 in T cells, little is known about its effect on B cells. In a previous study, IL-23 had a synergistic effect, with TH17 cells and TH17 cell-derived cytokines promoting a shift of B cells from secreting noninflammatory antibodies to secreting autoantibodies that had proinflammatory activity and triggered the autoimmune disease (42). In another study, mice with the IL-23 p19 subunit deficiency (p19^{-/-}) could not reach normal levels of T cell-dependent antibody responses, but they were able to reach normal T cell-independent B cell responses (46), suggesting a

TABLE 3 Clinical characteristics

Characteristic	Value for group ^a			P value (two HIV ⁺ groups) ^b
	HIV [−] (n = 16)	HIV ⁺ , low anti-CD4 (n = 17)	HIV ⁺ , high anti-CD4 (n = 9)	
Sex (male/female)	5/11	12/5	5/4	0.67
Age (yr)	38 (32–52)	43 (28–49)	47 (36–57)	0.17
CD4 ⁺ T cell counts (cells/ μ l)	710 (429–936)	658 (438–751)	421 (249–600)	0.02
Nadir CD4 ⁺ T cell counts		396 (226–490)	212 (108–270)	0.03
Yrs of ART		6 (4–6)	4.5 (3–6)	0.33

^aData are medians (interquartile ranges).^bFisher's exact test and Mann-Whitney's *U* test (unpaired) were used.

role of IL-23 in T cell-dependent immune responses. In the current study, we did not find differences in total IgG or IgM production in the culture supernatants after treatment with rIL-23 *in vitro* (Fig. 5E). Therefore, the increased level of autoantibodies against CD4⁺ T cells by rIL-23 (Fig. 5C and D) may not be due to nonspecific proliferation and development of total B cells.

The high plasma concentrations of anti-CD4 IgG ranged from ~100 to 1,000 ng/ml in the HIV⁺ subjects (Fig. 1A), suggesting that the number of precursor anti-CD4 IgG-producing B cells is very low in the circulation. The percentages of IgG binding on CD4⁺ T cells in the blood from HIV-infected subjects *ex vivo* were lower than 0.5%, as shown in our previous study (10). From our previous studies (10, 11), anti-CD4-IgG-mediated effects in HIV are most likely exhibited in the lymph nodes or tissues, where levels of cell-to-cell interactions and the concentration of antibodies are much higher than in the peripheral blood. Thus, due to the small population of anti-CD4 IgG-producing B cells in the PBMCs and the limited ability of antigen-specific B cells to survive *in vitro*, it may be difficult to detect anti-CD4 IgGs in the cell culture supernatants. Nonetheless, we have revealed that purified anti-CD4 IgGs from plasma samples of HIV⁺ immune nonresponders bound to the CD4⁺ T cell surface and induced CD4⁺ T cell activation *in vitro* (11). Therefore, the IL-23-mediated increases in autoantibodies bound to the CD4⁺ T cell surface (Fig. 5C and D) may indicate anti-CD4 autoantibodies, because autoantibody binding was low or undetectable on the CD3⁺ CD4[−] cells (likely CD8⁺ T cells) (Fig. 5C and D).

Antagonists targeting IL-23 have been applied to several autoimmune diseases. Eight therapeutic agents blocking IL-12/IL-23 p40 or IL-23 p19 individually were used or tested in the clinic for immune-mediated diseases. For example, the IL-12/IL-23 p40 inhibitors ustekinumab and briakinumab have been used to treat psoriasis and psoriatic arthritis; the IL-23 p19 inhibitors tildrakizumab, guselkumab, BI-655066, AMG 139, LY3074828, and LY2525623 are undergoing clinical trials for treatment of psoriasis, Crohn's disease, ankylosing spondylitis, and rheumatoid arthritis (47). However, IL-23 is required for critical neutrophilic responses to bacterial challenge in mice (48, 49); IL-23 knockout results in susceptibility to fungal challenge in mice (50). Thus, blocking the IL-23 signaling pathway may lead to profound immune suppression and perturbations in antibody production and may increase the susceptibility of HIV⁺ patients to opportunistic infection and cancer (51).

In summary, the seasonal influenza vaccination induced IgG anti-CD4 antibodies in some ART-treated HIV-infected subjects, and IgG3 was the predominant subtype. Preexisting increased plasma IL-23 levels may facilitate anti-CD4 autoantibody generation. However, the other cytokines known to regulate B and T cells, such as IL-17A, IFN- γ , and TNF- α , may also regulate this process, which is worth further investigation. One limitation of the study is the small sample size of immunologic nonresponders; thus, vaccine-mediated anti-CD4 IgG production and function in immunologic nonresponders deserve further investigation. Based on the amino acid sequences and conformational structures of influenza vaccination antigens and CD4 protein, we think the anti-CD4

antibody most likely was not generated from the antigen mimic or the cross-reactive effect but was generated from bystander B cell activation. Notably, increases in anti-CD4 IgM level following vaccination were greater than those of IgG (Fig. 1A). In general, IgM is considered less antigen specific than IgG. The increase in anti-CD4 IgM levels in HIV⁺ subjects is consistent with the nonspecific effect of B cell activation following influenza vaccination. Nonetheless, seasonal influenza vaccination is the most effective method of preventing influenza virus infection in both healthy people and HIV⁺ individuals.

MATERIALS AND METHODS

Study subjects. Sixteen healthy controls and 26 HIV-infected ART-treated aviremic subjects were studied. HIV-infected subjects had had ART for at least 2 years with undetectable viral loads (plasma HIV RNA < 50 copies/ml). All participants had received the 2013-2014 influenza vaccination (Fluvirin; Glaxo-Smith Kline), as shown in our previous studies (14, 52). The clinical characteristics of the participants are shown in Table 3. Briefly, blood samples were collected on day 0 (D0) before vaccination, days 7 to 10 (D7) postvaccination, and days 14 to 21 (D14) postvaccination. All participants had received the influenza vaccine in the previous year. The Medical University of South Carolina (Pro00020606) approved this study; all participants provided written informed consent.

Flow cytometry. Whole blood was collected in EDTA-containing tubes for immediate plasma isolation and cryopreservation at -80°C without prior thawing until analysis. PBMCs were isolated from whole blood over a Ficoll-Paque gradient and used for annexin V assays. The percentage and absolute count of CD4⁺ T cells and the percentage of Tfh cells were determined using fresh whole blood samples. For surface staining, antibodies were incubated with either blood or PBMCs at room temperature for 15 min. After surface staining of the whole blood, red cells were lysed, and the cells were washed and analyzed by flow cytometry. The fluorochrome-labeled monoclonal antibodies used in this study included the following: anti-human CD3 (OKT3), anti-human CD4 (RPA-T4), anti-human CD8 (RPA-T8), anti-human CD27 (M-T271), anti-human CD45RA (HI100), anti-human CXCR5 (RF8B2), anti-human CXCR3 (G025H7), anti-human PD1 (MIH4), annexin V (BD Pharmingen, San Jose, CA), and isotype control antibodies (BD Pharmingen). No annexin V staining was used as a negative control for the apoptosis gating strategy. All others were gated based on isotypes. Cells were collected in a Guava 8HT flow cytometer (Millipore, Billerica, MA), and data were analyzed by FlowJo software (version 10.0.8).

ELISA for plasma levels of anti-CD4 autoantibody and influenza virus-specific antibody. For enzyme-linked immunosorbent assay (ELISA), microtiter plates were coated with either $1\text{ }\mu\text{g}/100\text{ }\mu\text{l}$ /well soluble CD4 protein (sCD4; Progenics, Tarrytown, NY) or a 1:20 dilution of the 2013-2014 seasonal influenza virus antigens at 4°C overnight. After washing, diluted plasma was added to each well for 1 h at room temperature. After washing, horseradish peroxidase-labeled goat anti-human IgG, IgM, or IgA (KPL, Gaithersburg, MD) or IgG1 to -4 subtypes (Thermo Fisher Scientific, Waltham, MA) were added to the microtiter wells and cultured for 60 min. Next, 2- and 2'-azino-di(3-ethylbenzthiazoline-6-sulfonate) (ABTS) substrate solutions were used to detect binding. Absorbance was measured at 405 nm. Anti-CD4 IgG antibody concentrations were calculated in duplicate using the human monoclonal anti-CD4 antibody zanolimumab (HuMax-CD4; Genmab) to establish a standard curve (0.05 to 10 ng/ml).

Gene expression analysis by microarray. Total B cells were isolated in 5×10^7 PBMCs using a B cell isolation kit (Miltenyi Biotec, Bergisch Gladbach, Germany; purity > 97%). Total RNA was extracted from 2×10^6 B cells using the RNeasy Micro kit following the manufacturer's manual (Qiagen, Hilden, Germany), quantitated using a Nanodrop 2000 instrument (Nanodrop, Waltham, MA), and qualified with Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA) before Affymetrix GeneChip assays were performed. An Affymetrix human GeneChip U133 Plus 2.0 array (Affymetrix, Santa Clara, CA) was used for the RNA hybridization and labeling assay according to the manufacturer's protocol. The analysis of the scanned images and signal values for each probe set were obtained with GeneChip operating software (GCOS; Affymetrix). All microarray data analyses and statistical analyses were performed in R (<http://www.r-project.org/>). Normalization was performed with the RMA algorithm, which included background adjustment and quantile normalization. The Limma (53) package was used to identify differentially expressed genes in comparisons of HIV⁺ subjects with high anti-CD4 antibody production with HIV⁺ subjects with low anti-CD4 production in response to vaccination. Empirical Bayes moderation and Benjamini and Hochberg false-discovery rate (FDR) corrections for multiple tests were employed. The selection criteria of differentially expressed genes were defined as a change of >1.2-fold and a *P* value of <0.05. Gene ontology (GO) analysis, pathway enrichment analysis, protein-to-protein interactions of different expression genes, and weighted composite functional interaction network construction were completed using the Cytoscape (<http://www.cytoscape.org/>) plug-in with ClueGO (v2.3.5) and CluePedia (v1.3.5).

Plasma levels of 32 cytokines. Plasma levels of the following 32 cytokines were measured: interleukin 1 α (IL-1 α), IL-1 β , IL-2, IL-5, IL-6, IL-7, IL-8, IL-10, IL-12/IL-23 p40, IL-12 p70, IL-13, IL-15, IL-16, IL-17A, IL-21, IL-22, IL-23, IL-27, eotaxin, eotaxin-3, granulocyte-macrophage colony-stimulating factor (GM-CSF), gamma interferon (IFN- γ), IFN- γ -induced protein 10 (IP-10), monocyte chemoattractant protein-1 (MCP-1), macrophage-derived chemokine (MDC), macrophage inflammatory protein 1 α (MIP-1 α), MIP-1 β , MIP-3 α , thymus and activation-regulated chemokine (TARC), tumor necrosis factor alpha (TNF- α), TNF- β , and vascular endothelial growth factor (VEGF). All levels were measured simultaneously using a human TH17/cytokine/chemokine ProInflam kit (Meso Scale Diagnostics, Rockville, MD) according to the manufacturer's instructions.

Cytokine induction by exogenous IL-23 *in vitro*. PBMCs from ART-treated HIV-infected subjects were thawed and cultured with anti-CD3/anti-CD28 antibodies (10 μ g/ml) in the presence of TNF- α (1 μ g/ml) or rIL-23 in RPMI 1640 supplemented with 10% (vol/vol) fetal bovine serum (FBS) and 50 μ g/ml penicillin/streptomycin. The final concentrations of rIL-23 were 0 μ g/ml (medium control), 0.1 μ g/ml, and 1 μ g/ml. By day 5, PBMCs were restimulated with a leukocyte activation cocktail (BD, San Jose, CA) at 2 μ l/ml, which contains PMA, ionomycin, and brefeldin A (BFA). After culturing at 37°C for 4 h, PBMCs were collected and washed with PBS. Aqua blue (Life Technologies, Carlsbad, CA) was used at 4°C for 20 min to exclude dead cells. Next, an antibody cocktail containing anti-human CD3 (OKT3), anti-human CD4 (RPA-T4), anti-human CD27 (M-T271), and fluorescein isothiocyanate (FITC)-conjugated anti-human CD45RA (HI100) was used for surface staining. After washing and permeabilization, cells were intracellularly stained with anti-human IL-17A, anti-human TNF- α , and anti-human IFN- γ . After washing, cells were collected and analyzed using a BD FACSVerse flow cytometer (BD, San Jose, CA), and data were analyzed using FlowJo software (version 10.0.8).

Autoantibody production in response to IL-23 stimulation *in vitro*. Frozen PBMCs from HIV⁺ subjects on D7 postvaccination were thawed and cultured with rIL-23 or IL-23 neutralization antibody (MT155; Mabtech, Cincinnati, OH) in complete RPMI medium (cRPMI), consisting of RPMI 1640 supplemented with 15% (vol/vol) FBS, 50 μ g/ml penicillin/streptomycin, and 1 mM sodium pyruvate. The final concentrations of rIL-23 were 0 μ g/ml (medium control), 0.1 μ g/ml, and 1 μ g/ml; that of the IL-23 neutralization antibody was 10 μ g/ml. By day 7, half the culture medium was replaced with fresh medium that contained rIL-23 or IL-23 neutralization antibody in order to keep cells viable. By day 10, cultured supernatant was collected to detect anti-CD4 IgGs, total IgG or IgM, and cultured cells were collected to detect IgG autoantibodies which were bound to CD3⁺ CD4⁺ T cells. Aqua blue was used to exclude dead cells. Autoantibody binding on CD4⁺ T cells was surface stained with anti-human IgG (G18-145) at 4°C for 30 min.

Data availability. Microarray data are available in GEO (Gene Expression Omnibus) under accession number [GSE109837](https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE109837).

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Z.L. and W.J. conceived of and directed the project. Z.L., M.L., and Z.M. analyzed data. Z.L. and M.L. wrote the manuscript. Z.L. and L.M. performed laboratory sample testing. F.M., G.D., S.L.H., D.S., H.L., P.J., and W.J. were involved in critically revising the manuscript.

We declare no competing financial or nonfinancial interests.

REFERENCES

- Guihot A, Bourgarit A, Carcelain G, Autran B. 2011. Immune reconstitution after a decade of combined antiretroviral therapies for human immunodeficiency virus. *Trends Immunol* 32:131–137. <https://doi.org/10.1016/j.it.2010.12.002>.
- Kelley CF, Kitchen CMR, Hunt PW, Rodriguez B, Hecht FM, Kitahata M, Crane HM, Willig J, Mugavero M, Saag M, Martin JN, Deeks SG. 2009. Incomplete peripheral CD4⁺ cell count restoration in HIV-infected patients receiving long-term antiretroviral treatment. *Clin Infect Dis* 48:787–794. <https://doi.org/10.1086/597093>.
- Battegay M, Nüesch R, Hirschel B, Kaufmann GR. 2006. Immunological recovery and antiretroviral therapy in HIV-1 infection. *Lancet Infect Dis* 6:280–287. [https://doi.org/10.1016/S1473-3099\(06\)70463-7](https://doi.org/10.1016/S1473-3099(06)70463-7).
- Baker JV, Peng G, Rapkin J, Abrams DI, Silverberg MJ, MacArthur RD, Cavert WP, Henry WK, Neaton JD. 2008. CD4⁺ count and risk of non-AIDS diseases following initial treatment for HIV infection. *AIDS* 22:841–848. <https://doi.org/10.1097/QAD.0b013e3282f7cb76>.
- Lapadula G, Cozzi-Lepri A, Marchetti G, Antinori A, Chiodera A, Nicastrì E, Parruti G, Galli M, Gori A, Monforte AA. 2013. Risk of clinical progression among patients with immunological nonresponse despite virological suppression after combination antiretroviral treatment. *AIDS* 27:769–779. <https://doi.org/10.1097/QAD.0b013e32835cb747>.
- Diaz A, Alos L, Leon A, Mozos A, Caballero M, Martinez A, Plana M, Gallart T, Gil C, Leal M, Gatell JM, Garcia F, Study Group of Lymphoid Tissue Immunopathogenesis in HIV. 2010. Factors associated with collagen deposition in lymphoid tissue in long-term treated HIV-infected patients. *AIDS* 24:2029–2039. <https://doi.org/10.1097/QAD.0b013e32833c3268>.
- Lederman MM, Calabrese L, Funderburg NT, Clagett B, Medvik K, Bonilla H, Gripshover B, Salata RA, Taege A, Lisgaris M, McComsey GA, Kirchner E, Baum J, Shive C, Asaad R, Kalayjian RC, Sieg SF, Rodriguez B. 2011. Immunologic failure despite suppressive antiretroviral therapy is related to activation and turnover of memory CD4 cells. *J Infect Dis* 204:1217–1226. <https://doi.org/10.1093/infdis/jir507>.
- Hunt PW, Martin JN, Sinclair E, Bredt B, Hagos E, Lampiris H, Deeks SG. 2003. T cell activation is associated with lower CD4⁺ T cell gains in human immunodeficiency virus-infected patients with sustained viral

- suppression during antiretroviral therapy. *J Infect Dis* 187:1534–1543. <https://doi.org/10.1086/374786>.
9. Teixeira L, Valdez H, McCune JM, Koup RA, Badley AD, Hellerstein MK, Napolitano LA, Douek DC, Mbisa G, Deeks S, Harris JM, Barbour JD, Gross BH, Francis IR, Halvorsen R, Asaad R, Lederman MM. 2001. Poor CD4 T cell restoration after suppression of HIV-1 replication may reflect lower thymic function. *AIDS* 15:1749–1756. <https://doi.org/10.1097/00002030-200109280-00002>.
 10. Luo Z, Zhou Z, Ogunrinde E, Zhang T, Li Z, Martin L, Wan Z, Wu H, Qin Z, Ou T, Zhang J, Ma L, Liao G, Heath S, Huang L, Jiang W. 2017. The effect of plasma auto-IgGs on CD4+ T cell apoptosis and recovery in HIV-infected patients under antiretroviral therapy. *J Leukoc Biol* 102:1481–1486. <https://doi.org/10.1189/jlb.5A0617-219R>.
 11. Luo Z, Li Z, Martin L, Wan Z, Meissner EG, Espinosa E, Wu H, Yu X, Fu P, Westerink MAJ, Kilby JM, Wu J, Huang L, Heath SL, Li Z, Jiang W. 2017. Pathological role of anti-CD4 antibodies in HIV-infected immunologic non-responders under viral suppressive antiretroviral therapy. *J Infect Dis* 216:82–91. <https://doi.org/10.1093/infdis/jix223>.
 12. Nangaku M, Couser WG. 2005. Mechanisms of immune-deposit formation and the mediation of immune renal injury. *Clin Exp Nephrol* 9:183–191. <https://doi.org/10.1007/s10157-005-0357-8>.
 13. Rawlings DJ, Metzler G, Wray-Dutra M, Jackson SW. 2017. Altered B cell signalling in autoimmunity. *Nat Rev Immunol* 17:421–436. <https://doi.org/10.1038/nri.2017.24>.
 14. Luo Z, Ma L, Zhang L, Martin L, Wan Z, Warth S, Kilby A, Gao Y, Bhargava P, Li Z, Wu H, Meissner EG, Li Z, Kilby JM, Liao G, Jiang W. 2016. Key differences in B cell activation patterns and immune correlates among treated HIV-infected patients versus healthy controls following influenza vaccination. *Vaccine* 34:1945–1955. <https://doi.org/10.1016/j.vaccine.2015.12.038>.
 15. Luo Z, Li M, Wu Y, Meng Z, Martin L, Zhang L, Ogunrinde E, Zhou Z, Qin S, Wan Z, Westerink MAJ, Warth S, Liu H, Jin P, Stronck D, Li Q-Z, Wang E, Wu X, Heath SL, Li Z, Alekseyenko AV, Jiang W. 2019. Systemic translocation of *Staphylococcus* drives autoantibody production in HIV disease. *Microbiome* 7:25. <https://doi.org/10.1186/s40168-019-0646-1>.
 16. Cagigi A, Rinaldi S, Santilli V, Mora N, C Manno E, Cotugno N, Zangari P, Aquilani A, Guzzo I, Dello Strologo L, Rossi P, Palma P. 2013. Premature ageing of the immune system relates to increased anti-lymphocyte antibodies (ALA) after an immunization in HIV-1-infected and kidney-transplanted patients. *Clin Exp Immunol* 174:274–280. <https://doi.org/10.1111/cei.12173>.
 17. Tabarkiewicz J, Pogoda K, Karczmarczyk A, Pozarowski P, Giannopoulos K. 2015. The role of IL-17 and Th17 lymphocytes in autoimmune diseases. *Arch Immunol Ther Exp (Warsz)* 63:435–449. <https://doi.org/10.1007/s00005-015-0344-z>.
 18. Gerard C, Rollins BJ. 2001. Chemokines and disease. *Nat Immunol* 2:108–115. <https://doi.org/10.1038/84209>.
 19. Jiang W, Younes SA, Funderburg NT, Mudd JC, Espinosa E, Davenport MP, Babineau DC, Siegf SF, Lederman MM. 2014. Cycling memory CD4+ T cells in HIV disease have a diverse T cell receptor repertoire and a phenotype consistent with bystander activation. *J Virol* 88:5369–5380. <https://doi.org/10.1128/JVI.00017-14>.
 20. Locci M, Havenar-Daughton C, Landais E, Wu J, Kroenke MA, Arlehamn CL, Su LF, Cubas R, Davis MM, Sette A, Haddad EK, Pognard P, Crotty S, International AIDS Vaccine Initiative Protocol C Principal Investigators. 2013. Human circulating PD-1+ CXCR3– CXCR5+ memory Tfh cells are highly functional and correlate with broadly neutralizing HIV antibody responses. *Immunity* 39:758–769. <https://doi.org/10.1016/j.immuni.2013.08.031>.
 21. Bindea G, Galon J, Mlecnik B. 2013. CluePedia Cytoscape plugin: pathway insights using integrated experimental and in silico data. *Bioinformatics* 29:661–663. <https://doi.org/10.1093/bioinformatics/btt019>.
 22. Bader GD, Hogue CW. 2003. An automated method for finding molecular complexes in large protein interaction networks. *BMC Bioinformatics* 4:2. <https://doi.org/10.1186/1471-2105-4-2>.
 23. Langrish CL, Chen Y, Blumenschein WM, Mattson J, Basham B, Sedgwick JD, McClanahan T, Kastelein RA, Cua DJ. 2005. IL-23 drives a pathogenic T cell population that induces autoimmune inflammation. *J Exp Med* 201:233–240. <https://doi.org/10.1084/jem.20041257>.
 24. Jiang W, Luo Z, Martin L, Wan Z, Fu P, Wagner A, Ling B, Heath S, Haque A, McRae-Clark A. 2018. Drug use is associated with anti-CD4 IgG-mediated CD4+ T cell death and poor CD4+ T cell recovery in viral-suppressive HIV-infected individuals under antiretroviral therapy. *Curr HIV Res* 16:143–150. <https://doi.org/10.2174/1570162X16666180703151208>.
 25. Robbins GK, Spritzler JG, Chan ES, Asmuth DM, Gandhi RT, Rodriguez BA, Skowron G, Skolnik PR, Shafer RW, Pollard RB, Team ACTG. 2009. Incomplete reconstitution of T cell subsets on combination antiretroviral therapy in the AIDS Clinical Trials Group protocol 384. *Clin Infect Dis* 48:350–361. <https://doi.org/10.1086/595888>.
 26. Lewden C, Chene G, Morlat P, Raffi F, Dupon M, Dellamonica P, Pellegrin JL, Katlama C, Dabis F, Leport C, Agence Nationale de Recherches sur le Sida et les Hepatitis Virales COA-CSG, Agence Nationale de Recherches sur le Sida et les Hepatitis Virales COASG. 2007. HIV-infected adults with a CD4 cell count greater than 500 cells/mm3 on long-term combination antiretroviral therapy reach same mortality rates as the general population. *J Acquir Immune Defic Syndr* 46:72–77. <https://doi.org/10.1097/QAI.0b013e318134257a>.
 27. Gutierrez F, Padilla S, Masia M, Iribarren JA, Moreno S, Viciano P, Hernandez-Quero J, Aleman R, Vidal F, Salavert M, Blanco JR, Leal M, Dronda F, Perez Hoyos S, del Amo J, Co RM. 2008. Patients' characteristics and clinical implications of suboptimal CD4 T-cell gains after 1 year of successful antiretroviral therapy. *Curr HIV Res* 6:100–107. <https://doi.org/10.2174/157016208783885038>.
 28. Lapadula G, Chatenoud L, Gori A, Castelli F, Di Giambenedetto S, Fabbiani M, Maggiolo F, Foca E, Ladisa N, Sighinolfi L, Di Pietro M, Pan A, Torti C, Italian MC. 2015. Risk of severe non AIDS events is increased among patients unable to increase their CD4+ T-cell counts >200+/mm3 despite effective HAART. *PLoS One* 10:e0124741. <https://doi.org/10.1371/journal.pone.0124741>.
 29. Lisco A, Wong CS, Lage SL, Levy I, Brophy J, Lennox J, Manion M, Anderson MV, Mejia Y, Grivas C, Mystakelis H, Burbelo PD, Perez-Diez A, Rupert A, Martens CA, Anzick SL, Morse C, Chan S, Deleage C, Sereti I. 2019. Identification of rare HIV-1-infected patients with extreme CD4+ T cell decline despite ART-mediated viral suppression. *JCI Insight* 4:e127113. <https://doi.org/10.1172/jci.insight.127113>.
 30. Ravetch JV, Bolland S. 2001. IgG Fc receptors. *Annu Rev Immunol* 19:275–290. <https://doi.org/10.1146/annurev.immunol.19.1.275>.
 31. Caaveiro JM, Kiyoshi M, Tsumoto K. 2015. Structural analysis of Fc/FcγR complexes: a blueprint for antibody design. *Immunol Rev* 268:201–221. <https://doi.org/10.1111/imr.12365>.
 32. Kam Y-W, Simarmata D, Chow A, Her Z, Teng T-S, Ong EK, Rénia L, Leo Y-S, Ng LF. 2012. Early appearance of neutralizing immunoglobulin G3 antibodies is associated with chikungunya virus clearance and long-term clinical protection. *J Infect Dis* 205:1147–1154. <https://doi.org/10.1093/infdis/jis033>.
 33. Yates NL, Liao H-X, Fong Y, deCamp A, Vandergrift NA, Williams WT, Alam SM, Ferrari G, Yang Z-Y, Seaton KE, Berman PW, Alpert MD, Evans DT, O'Connell RJ, Francis D, Sinangil F, Lee C, Nitayaphan S, Serks-Ngarm S, Kaewkungwal J, Pitisuttithum P, Tartaglia J, Pinter A, Zolla-Pazner S, Gilbert PB, Nabel GJ, Michael NL, Kim JH, Montefiori DC, Haynes BF, Tomaras GD. 2014. Vaccine-induced Env V1-V2 IgG3 correlates with lower HIV-1 infection risk and declines soon after vaccination. *Sci Transl Med* 6:228ra39. <https://doi.org/10.1126/scitranslmed.3007730>.
 34. Munroe ME, Lu R, Zhao YD, Fife DA, Robertson JM, Guthridge JM, Niewold TB, Tsokos GC, Keith MP, Harley JB, James JA. 2016. Altered type II interferon precedes autoantibody accrual and elevated type I interferon activity prior to systemic lupus erythematosus classification. *Ann Rheum Dis* 75:2014–2021. <https://doi.org/10.1136/annrheumdis-2015-208140>.
 35. De Silva NS, Klein U. 2015. Dynamics of B cells in germinal centres. *Nat Rev Immunol* 15:137–148. <https://doi.org/10.1038/nri3804>.
 36. Wu X, Zhang Z, Schramm CA, Joyce MG, Kwon YD, Zhou T, Sheng Z, Zhang B, O'Dell S, McKee K, Georgiev IS, Chuang G-Y, Longo NS, Lynch RM, Saunders KO, Soto C, Srivatsan S, Yang Y, Bailer RT, Louder MK, Mulliken JC, Connors M, Kwong PD, Mascola JR, Shapiro L, NISC Comparative Sequencing Program. 2015. Maturation and diversity of the VRC01-antibody lineage over 15 years of chronic HIV-1 infection. *Cell* 161:470–485. <https://doi.org/10.1016/j.cell.2015.03.004>.
 37. Rosok B, Voltersvik P, Bjerknes R, Axelsson M, Haaheim LR, Asjo B. 1996. Dynamics of HIV-1 replication following influenza vaccination of HIV+ individuals. *Clin Exp Immunol* 104:203–207. <https://doi.org/10.1046/j.1365-2249.1996.25732.x>.
 38. Gunthard HF, Wong JK, Spina CA, Ignacio C, Kwok S, Christopherson C, Hwang J, Haubrich R, Havlir D, Richman DD. 2000. Effect of influenza vaccination on viral replication and immune response in persons infected with human immunodeficiency virus receiving potent antiretroviral therapy. *J Infect Dis* 181:522–531. <https://doi.org/10.1086/315260>.
 39. Weller S, Braun MC, Tan BK, Rosenwald A, Cordier C, Conley ME, Plebani A, Kumararatne DS, Bonnet D, Tournilhac O, Tchernia G, Steiniger B,

- Staudt LM, Casanova J-L, Reynaud C-A, Weill J-C. 2004. Human blood IgM “memory” B cells are circulating splenic marginal zone B cells harboring a prediversified immunoglobulin repertoire. *Blood* 104:3647–3654. <https://doi.org/10.1182/blood-2004-01-0346>.
40. Lorenzo-Redondo R, Fryer HR, Bedford T, Kim E-Y, Archer J, Pond SLK, Chung Y-S, Penugonda S, Chipman J, Fletcher CV, Schacker TW, Malim MH, Rambaut A, Haase AT, McLean AR, Wolinsky SM. 2016. Persistent HIV-1 replication maintains the tissue reservoir during therapy. *Nature* 530:51–56. <https://doi.org/10.1038/nature16933>.
 41. Luo Z, Li Z, Martin L, Hu Z, Wu H, Wan Z, Kilby M, Heath SL, Huang L, Jiang W. 2017. Increased natural killer cell activation in HIV-infected immunologic non-responders correlates with CD4⁺ T cell recovery after antiretroviral therapy and viral suppression. *PLoS One* 12:e0167640. <https://doi.org/10.1371/journal.pone.0167640>.
 42. Pfeifle R, Rothe T, Ipseiz N, Scherer HU, Culemann S, Harre U, Ackermann JA, Seefried M, Kleyer A, Uderhardt S, Haug B, Hueber AJ, Daum P, Heidkamp GF, Ge C, Böhm S, Lux A, Schuh W, Magorivska I, Nandakumar KS, Lönnblom E, Becker C, Dudziak D, Wührer M, Rombouts Y, Koeleman CA, Toes R, Winkler TH, Holmdahl R, Herrmann M, Blüml S, Nimmerjahn F, Schett G, Krönke G. 2017. Regulation of autoantibody activity by the IL-23-TH17 axis determines the onset of autoimmune disease. *Nat Immunol* 18:104–113. <https://doi.org/10.1038/ni.3579>.
 43. Becker C, Wirtz S, Blessing M, Pirhonen J, Strand D, Bechthold O, Frick J, Galle PR, Autenrieth I, Neurath MF. 2003. Constitutive p40 promoter activation and IL-23 production in the terminal ileum mediated by dendritic cells. *J Clin Invest* 112:693–706. <https://doi.org/10.1172/JCI17464>.
 44. Cua DJ, Tato CM. 2010. Innate IL-17-producing cells: the sentinels of the immune system. *Nat Rev Immunol* 10:479–489. <https://doi.org/10.1038/nri2800>.
 45. Sutton CE, Lalor SJ, Sweeney CM, Brereton CF, Lavelle EC, Mills KH. 2009. Interleukin-1 and IL-23 induce innate IL-17 production from $\gamma\delta$ T cells, amplifying Th17 responses and autoimmunity. *Immunity* 31:331–341. <https://doi.org/10.1016/j.immuni.2009.08.001>.
 46. Ghilardi N, Kljavin N, Chen Q, Lucas S, Gurney AL, De Sauvage FJ. 2004. Compromised humoral and delayed-type hypersensitivity responses in IL-23-deficient mice. *J Immunol* 172:2827–2833. <https://doi.org/10.4049/jimmunol.172.5.2827>.
 47. Teng MW, Bowman EP, McElwee JJ, Smyth MJ, Casanova J-L, Cooper AM, Cua DJ. 2015. IL-12 and IL-23 cytokines: from discovery to targeted therapies for immune-mediated inflammatory diseases. *Nat Med* 21:719–729. <https://doi.org/10.1038/nm.3895>.
 48. Godínez I, Raffatellu M, Chu H, Paixão TA, Haneda T, Santos RL, Bevins CL, Tsolis RM, Bäuml AJ. 2009. Interleukin-23 orchestrates mucosal responses to *Salmonella enterica* serotype Typhimurium in the intestine. *Infect Immun* 77:387–398. <https://doi.org/10.1128/IAI.00933-08>.
 49. Huppler AR, Conti HR, Hernández-Santos N, Darville T, Biswas PS, Gaffen SL. 2014. Role of neutrophils in IL-17-dependent immunity to mucosal candidiasis. *J Immunol* 192:1745–1752. <https://doi.org/10.4049/jimmunol.1302265>.
 50. Kagami S, Rizzo HL, Kurtz SE, Miller LS, Blauvelt A. 2010. IL-23 and IL-17A, but not IL-12 and IL-22, are required for optimal skin host defense against *Candida albicans*. *J Immunol* 185:5453–5462. <https://doi.org/10.4049/jimmunol.1001153>.
 51. Teng MW, Vesely MD, Duret H, McLaughlin N, Towne JE, Schreiber RD, Smyth MJ. 2012. Opposing roles for IL-23 and IL-12 in maintaining occult cancer in an equilibrium state. *Cancer Res* 72:3987–3996. <https://doi.org/10.1158/0008-5472.CAN-12-1337>.
 52. Luo Z, Ogunrinde E, Li M, Zhang L, Martin L, Zhou Z, Hu Z, Zhang T, Li Z, Zhang J, Su B, Zhang T, Wu H, Ma L, Liao G, Eckard AR, Westerink MAJ, Heath SL, Jiang W. 2019. Increased influenza-specific antibody avidity in HIV-infected women compared with HIV-infected men on antiretroviral therapy. *AIDS* 33:33–44. <https://doi.org/10.1097/QAD.0000000000002022>.
 53. Smyth GK, Speed T. 2003. Normalization of cDNA microarray data. *Methods* 31:265–273. [https://doi.org/10.1016/s1046-2023\(03\)00155-5](https://doi.org/10.1016/s1046-2023(03)00155-5).