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Human T_h17 Cells Lack HIV-Inhibitory RNases and Are Highly Permissive to Productive HIV Infection

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ABSTRACT

Human immunodeficiency virus (HIV) infects and depletes $CD4^+$ T cells, but subsets of $CD4^+$ T cells vary in their susceptibility and permissiveness to infection. For example, HIV preferentially depletes interleukin-17 (IL-17)-producing T helper 17 (T_h17) cells and T follicular helper (T_{fh}) cells. The preferential loss of T_h17 cells during the acute phase of infection impairs the integrity of the gut mucosal barrier, which drives chronic immune activation—a key determinant of disease progression. The preferential loss of T_h17 cells has been attributed to high CD4, CCR5, and CXCR4 expression. Here, we show that T_h17 cells also exhibit heightened permissiveness to productive HIV infection. Primary human $CD4^+$ T cells were sorted, activated under T_h17- or T_h0-polarizing conditions and infected, and then analyzed by flow cytometry. T_h17-polarizing cytokines increased HIV infection, and HIV infection was disproportionately higher among T_h17 cells than among $IL\text{-}17^-$ or gamma interferon-positive ($IFN\text{-}\gamma^+$) cells, even upon infection with a replication-defective HIV vector with a pseudotype envelope. Further, T_h17-polarized cells produced more viral capsid protein. Our data also reveal that T_h17-polarized cells have diminished expression of RNase A superfamily proteins, and we report for the first time that RNase 6 inhibits HIV. Thus, our findings link T_h17 polarization to increased HIV replication.

IMPORTANCE

Our study compares the intracellular replicative capacities of several different HIV isolates among different T cell subsets, providing a link between the differentiation of T_h17 cells and HIV replication. T_h17 cells are of key importance in mucosal integrity and in the immune response to certain pathogens. Based on our findings and the work of others, we propose a model in which HIV replication is favored by the intracellular environment of two $CD4^+$ T cell subsets that share several requirements for their differentiation: T_h17 and T_{fh} cells. Characterizing cells that support high levels of viral replication (rather than becoming latently infected or undergoing cell death) informs the search for new therapeutics aimed at manipulating intracellular signaling pathways and/or transcriptional factors that affect HIV replication.

Recent advances in the field of T helper cell development have shed new light on how human immunodeficiency virus (HIV) pathogenesis causes AIDS.

The rapid and preferential loss of T_h17 cells—so named for their secretion of interleukin-17 (IL-17)—from the gut-associated lymphoid tissue (GALT) during acute HIV infection represents a critical aspect of HIV immunopathology (1). Recent studies link the HIV-induced preferential depletion of T_h17 (and T_h17-like) cells to AIDS-associated opportunistic infections, gut mucosal barrier perturbation, and chronic immune activation (2, 3).

Pathogenic and nonpathogenic primate models differ in their loss of T_h17 cells, and these differences suggest a central role of T_h17 cell loss in driving HIV pathogenesis. For example, in simian immunodeficiency virus (SIV)-infected macaques, the peak and set point viral loads are restricted by the initial size of the T_h17 compartment (4), and a higher initial T_h17/T_h1 ratio at mucosal sites predicts a more rapid disease progression to AIDS (5). Further, the SIV-induced loss of the gut T_h17 compartment is associated with mucosal damage and the translocation/dissemination of the enteric pathogen *Salmonella enterica* serovar Typhimurium (2, 6). In contrast, sooty mangabeys, which do not progress to AIDS, maintain healthy mucosal function and levels of T_h17 cells following SIV infection (1, 2). HIV-induced T_h17 cell depletion thus facilitates the mucosal damage and subsequent chronic immune dysregulation associated with progression to AIDS.

T_h17 cells bridge innate and adaptive immune signaling at mucosal surfaces, and their preferential loss during acute HIV infection undermines mucosal immunity via multiple mechanisms. T_h17 cells are enriched within mucosal tissues, especially in the GALT, which is a major site of HIV replication (1, 7). T_h17 cells require several cytokines for their differentiation, including IL-1β, IL-6, and IL-23, which are expressed at high levels during HIV infection (8–16). T_h17 cells, like other GALT effector/memory T cells, express high levels of HIV receptors, thus conferring their susceptibility to infection (17).

T follicular helper (T_{fh}) cells share many characteristics with T_h17 cells, including their utilization of signal transducer and activator of transcription 3 (STAT3) and interferon-regulated factor

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4 (IRF4) activity and their expression of IL-21 (18, 19). There are several notable differences between T_h17 and T_{fh} cells: T_{fh} cells express their own master transcription factor, Bcl6, and the T_h17-destabilizing transcription factor c-Maf (20). T_{fh} cells also express the chemokine receptors CXCR5 and CCR7, which promote T_{fh} homing to germinal centers. Although T_{fh} cells constitute a major site of viral production during HIV infection (21), they do not express CCR5 (22). Nonetheless, both cell types are preferentially infected during acute HIV infection, and the resulting, combined loss of IL-21-producing T_h17 and T_{fh} cells during HIV infection stifles B cell development (23). Thus, the depletion of IL-17- and IL-21-expressing cells could represent a central mechanism by which HIV disrupts mucosal immunity during the early stages of infection and promotes opportunistic infections at mucosal sites that are associated with chronic immune activation and disease progression.

Despite effective viral suppression with combined antiretroviral therapy and the heightened *in vivo* availability of T_h17-polarizing cytokines and antigens following HIV infection, the T_h17 compartment often fails to reconstitute to preinfection levels in the gut and cervical mucosae (3, 24). The early loss of the enterocyte-proliferative and antimicrobial peptide-inducing cytokines produced by T_h17 cells impairs the integrity of the gut mucosal barrier, driving microbial translocation and chronic immune activation—key determinants of disease progression to AIDS (25, 26).

The mechanisms underlying the preferential loss of T_h17 cells *in vivo* during HIV infection remain unclear.

Prior studies examining the susceptibility of T_h17 cells to HIV infection focused on entry-level factors such as HIV receptor expression. T_h17 cells reportedly lack the expression of the HIV-inhibitory chemokine MIP-1β and also express high levels of the HIV-binding proteins α4β7 integrin, CD4, and CXCR4 (17). T_h17 cells are enriched in CCR6 expression, and $CCR6^+$ cells express significantly higher levels of the HIV coreceptor CCR5 than do $CCR6^-$ cells (27, 28).

However, even upon successful viral entry, $CD4^+$ T cells vary in ability to become productively infected. Differences in the intracellular environment ultimately determine whether an HIV-infected cell will die, become latently infected, or become a factory for the production of viral progeny. Interestingly, $CCR6^+$ and T_h17-polarized cells also showed higher rates of infection when infected with replication-defective, pseudotyped HIV vectors in a vesicular stomatitis virus glycoprotein (VSV-G) envelope, suggesting that postentry mechanisms may also contribute to the preferential loss of T_h17 cells (27–29). Using surface markers to identify T_h17 cells rather than intracellular cytokine staining may overestimate the frequency of T_h17 cells and result in the inclusion of cells that do not express IL-17 (30).

Here, we demonstrate that T_h17 cells are highly permissive to HIV infection and replication and that T_h17-polarizing cytokines enhance viral replication *in vitro*. We find that T_h17-polarized cells express significantly lower levels of HIV-inhibitory RNases than T_h0-polarized cells. Our findings support the concept that T_h17 cells could be a major source of viral production during acute HIV infection, and we propose that common features of T_h17 cells and some T_{fh} cells may help explain their proclivity to become major sources of viral production during acute HIV infection (31–33).

MATERIALS AND METHODS

$CD4^+$ T cell isolation and CCR6 sorting. $CD4^+$ T lymphocytes were isolated from human peripheral blood mononuclear cells by negative selection using the Easy Sep $CD4^+$ human T cell enrichment kit, according to the manufacturer's protocol (Stem Cell Technologies Inc., Vancouver, BC, Canada). To enrich for T_h17 cells, $CD4^+$ T cells were sometimes stained with an anti-CCR6 antibody (clone 11A9; BD Bioscience, San Jose, CA) and then sorted by fluorescently activated cell sorting (FACS).

Cell culture conditions. Cells were maintained at 37°C and 5% CO_2 in sterile-filtered RPMI medium supplemented with 10% human serum (Gemini Bio-Products, Sacramento, CA), penicillin-streptomycin-gentamicin, L-glutamine minimal essential medium (MEM) amino acids, and sodium pyruvate (Life Technologies, Grand Island, NY).

For phytohemagglutinin (PHA) activation, total CD4 T cells were cultured in complete medium containing PHA (2.5 μg/ml; Sigma-Aldrich, St. Louis, MO) and IL-2 (10 ng/ml; R&D Systems, Minneapolis, MN) for 3 days and then washed and cultured overnight in complete medium containing only IL-2 prior to HIV infection. After HIV infection (described below), cells were washed in phosphate-buffered saline (PBS) and then resuspended in medium containing IL-2 (10 ng/ml) or the T_h17-polarizing cytokines IL-1β (10 ng/ml), IL-6 (10 ng/ml), transforming growth factor β (TGF-β) (2 ng/ml), and IL-23 (10 ng/ml; Peprotech, Rocky Hill, NJ).

$CCR6^+$- or $CCR6^-$-sorted cells from each donor were activated with antibodies to CD3 (plate bound; 5 μg/ml) and CD28 (soluble; 1 μg/ml; eBioscience, San Diego, CA) for 3 days in the presence of IL-1β and IL-6 (5 ng/ml each), TGF-β (1 ng/ml), and IL-23 (20 ng/ml; Peprotech, Rocky Hill, NJ). Following activation, cells were washed and then resuspended in fresh medium containing only IL-2 (2 ng/ml) and IL-23 (10 ng/ml). Cells from all polarizing conditions were maintained in IL-2/IL-23 medium after HIV infection.

Virus preparation. HIV-1_{BaL} stocks were prepared by infecting human macrophages in complete RPMI 1640 medium supplemented with 20% heat-inactivated fetal bovine serum (FBS; Gemini Bio-Products, Sacramento, CA). HIV-1_{IIIB} was produced in PM1 cells (AIDS Reagent Program; catalog no. 3038) in IL-2-containing, complete RPMI 1640 medium. The transmitted-founder isolate HIV-1_{AD17}, which was also produced in PM1 cells, was kindly provided by George Shaw (34, 35). Pseudotyped virions were generated by Fugene (Promega, Fitchburg, WI) cotransfection of 293T cells with two plasmids: one expressing an envelope-deficient HIV backbone obtained through the NIH AIDS Reagent Program (catalog no. 4692; Division of AIDS, NIAID, NIH) and the other expressing an amphotropic murine leukemia virus (AMLV) envelope. Supernatants were analyzed by p24 enzyme-linked immunosorbent assay (ELISA) and stored at −80°C.

HIV infections. Equal numbers of PHA-activated and CCR6-sorted cells were infected with HIV_{BaL}, HIV_{IIIB}, and HIV_{AD17} for 2.5 h and then washed and resuspended in medium containing IL-2 or T_h17-polarizing cytokines (PHA activated) or IL-2 and IL-23 (CCR6-sorted, prepolarized cells), as indicated in Results. For replication-defective, pseudotype virus infections, 500 μl of supernatant virus (p24, 130 ng/ml) was added per million cells in 1.5 ml of the indicated medium, and cells were then analyzed by flow cytometry 4 days postinfection.

Flow cytometry staining and analysis. Four to 6 days after infection (unless otherwise noted), cells from each condition were stimulated with phorbol myristate acetate (PMA; 50 ng/ml), ionomycin (500 ng/ml; Sigma-Aldrich), and BD GolgiPlug (BD Bioscience) for 5 h. To assess viability, cells were stained with Live/Dead Aqua (Life Technologies) according to the manufacturer's instructions, immediately prior to extracellular marker staining and fixation. For intracellular staining, cells were permeabilized according to the BD Perm/Wash protocol; then stained for p24 (Beckman Coulter; clone KC57), IL-17A, and gamma interferon (IFN-γ) (eBioscience and BioLegend, respectively); and then washed prior to analysis.

Compensation and data collection were achieved using BD Comp-

Beads (BD Bioscience) and BD FACSDiva software version 6.0 (BD Bioscience). Data analysis and flow cytometry plots were generated using FlowJo software version 9.7.2 (FlowJo, LLC, Ashland OR). Sorting and data collection were performed at the University of Maryland—Baltimore flow cytometry core facilities at the Institute of Human Virology and at the Greenebaum Cancer Center.

RNA purification and microarray. Total RNA was extracted from cell lysates using Qiagen Quickspin columns and DNase treatment, according to the manufacturer's protocol (Qiagen Inc., Valencia, CA). RNA quality assessment and microarray analysis were performed by the University of Maryland Greenebaum Cancer Center Translational Genomics core facility, using Affymetrix HuGene 2.0 chips (Affymetrix Inc., Santa Clara, CA). Normalized gene expression estimates were obtained with the Frozen Robust Multiarray Analysis (fRMA) method (36). A generalized linear model approach, coupled with empirical Bayes standard error shrinkage and including coefficients for data heterogeneity as derived from surrogate variable analysis (SVA) (37), was used for identifying differentially expressed genes in T_h17-polarized cells relative to T_h0-polarized cells. Correction for multiple testing was performed using the Benjamini-Hochberg method. The identification of pathways and biological processes differentially expressed was performed using gene set enrichment analysis (GSEA) (38) and analysis of functional annotation (AFA) as previously described (39–41).

Immunoblot protein analysis. $CCR6^+$ or $CCR6^-$ cells were activated for 3 days using anti-CD3/CD28 as described above, under T_h0- or T_h17-polarizing conditions, and then maintained in medium containing IL-2 or IL-23 for an additional 3 days. Cells were then washed in phosphate-buffered saline (PBS) and pelleted, then lysed in RIPA buffer containing an EDTA-free protease and phosphatase inhibitor cocktail (Sigma-Aldrich), flash-frozen, and stored at −80°C. Equal amounts of total protein were boiled for 10 min in the presence of 4× dithiothreitol (DTT) loading buffer and loading dye and then loaded into 1% polyacrylamide gels (Life Technologies). Protein from the gel was transferred to polyvinylidene difluoride (PVDF) membranes (Life Technologies), blocked with a 5% (wt/vol) solution of powdered milk, washed, and then incubated at 4°C overnight in the presence of anti-human RNase antibodies (Abnova Inc., Taipei, Taiwan). Bound RNase antibodies were probed with anti-mouse or anti-rabbit horseradish peroxidase (HRP)-linked secondary antibodies, depending on the source of the primary antibody. As a loading control, blots were also probed with an HRP-conjugated antibody to glyceraldehyde 3-phosphate dehydrogenase (GAPDH) (ThermoFisher Scientific Inc., Rockville, MD).

p24 ELISA. Four days postinfection, infected cells were pelleted by centrifugation at 300 × *g* for 7 min, and supernatant was then collected and frozen at −80°C. Several dilutions of each supernatant were diluted into 1× lysis buffer in a 96-well plate, in triplicate. The Institute of Human Virology Core Facility (University of Maryland—Baltimore, Baltimore, MD) performed ELISA using commercially available p24 ELISA plates (PerkinElmer Inc., Boston, MA). Measured p24 values were normalized to a standard curve of known concentrations and were used only if they fell within the detectable range.

Luciferase assays. TZM-BL cells (AIDS Reagent Program; catalog no. 8129) were seeded at 5,000 cells/well in a 96-well plate (Sigma-Aldrich) and incubated overnight at 37°C (5% CO_2) in Dulbecco's modified Eagle's medium (DMEM) containing penicillin-streptomycin and L-glutamine (Life Technologies) and 10% fetal bovine serum (FBS) (Gemini Bio-Products). Thawed supernatant from sorted, polarized, infected T cells was added to the preseeded TZM-BL cells such that the final p24 concentration was 5 ng/ml. As controls, an equivalent volume of donor- and polarization-matched supernatants from uninfected cells was added. Each condition was done in triplicate, and samples were then incubated at 37°C (5% CO_2) for 48 h. Finally, the TZM-BL cells were washed and lysed in Steady-Glo luciferase detection buffer (Promega), according to the manufacturer's instructions. Luciferase activity was measured in a Veritas luminometer (Promega). The p24-specific luciferase activity was evaluated by subtracting the relative light units (RLU) of the uninfected supernatant-treated cells from the RLU of the condition-matched, infected supernatants.

Statistical analyses and figure generation. Raw experimental data were analyzed, graphed, and rendered using Microsoft Excel (Microsoft Inc., Redmond, WA), GraphPad Prism (GraphPad Inc., La Jolla, CA), and Microsoft PowerPoint software. Depending on whether we were analyzing data from different conditions or different donors, paired and unpaired Student *t* tests were used to measure significant differences among normally distributed data sets.

RESULTS

***In vitro* HIV infection of PHA-activated T cells expands rapidly upon the addition of T_h17-polarizing cytokines.** T_h17-polarizing cytokines IL-1β and IL-6 have been reported to increase HIV infection *in vitro* (42, 43). Another recent *in vitro* study suggested that T_h17-polarizing cytokines, in the presence of antiretroviral therapy, could replenish the T_h17 cells lost during HIV infection, but the authors did not show the effects of the T_h17-polarizing cytokines on infection in the absence of antiretroviral treatment (44). Further, their T_h17-polarizing cytokines were added in addition to IL-2. IL-2 not only increases HIV infection *in vitro* more than the T_h1- or T_h2-polarizing cytokines IL-12 and IL-4 but also inhibits IL-17 expression and promotes the plasticity of T_h17 cells (45, 46). Therefore, we set out to compare the dynamics of T cell function and HIV infection in the presence of IL-2 or T_h17-polarizing cytokines, hypothesizing that T_h17 polarization would increase the percentage of infected cells.

First, we compared the kinetics of HIV_{BAL} infection when IL-2 (10 ng/ml) or the T_h17-polarizing cytokines IL-1β, IL-6, and IL-23 (all 10 ng/ml), and TGF-β (2 ng/ml) were added after HIV infection (Fig. 1A). Cells from two donors were analyzed 3, 5, and 7 days postinfection (Fig. 1B). Three days postinfection, 2.3% and 3.7% of total $CD4^+$ cells were infected under IL-2 and T_h17-polarizing conditions, respectively. By 5 days after infection, however, the percentage of cells expressing p24 was 4-fold higher in the presence of T_h17-polarizing cytokines, compared with IL-2-treated cells (Fig. 1B). Based on our kinetics experiments, we chose day 5 after infection as a time point at which the effects of T_h17-polarizing cytokines were most representative, compared with IL-2 treatment; the percentage of infected cells nearly tripled from 3.18 to 8.75 ($P < 0.0002$, $n = 10$) (Fig. 1C).

To determine whether our findings were specific to the CCR5-tropic HIV_{BaL} lab isolate, we repeated our experiments using two other isolates: HIV_{IIIB}, a CXCR4-tropic lab isolate, and HIV_{AD17}, a CCR5-tropic clone of a clinical transmitted-founder virus (47). Our results show that treatment with T_h17-polarizing cytokines increased p24 expression regardless of the HIV isolate used to infect the PHA-activated T cells (Fig. 1D).

We then determined the relative contribution of each of the polarizing cytokines to our observed changes in IL-17 and p24 expression. Accordingly, we infected PHA-activated cells from three donors with HIV_{BaL} and then added either IL-2 or one of the 15 possible combinations of T_h17-polarizing cytokines. Our data corroborated published effects of individual cytokines on IL-17 expression and HIV infection. For example, the addition of IL-1β and IL-6 or IL-23 tended to increase the number of IL-17-producing cells, but the addition of 2 ng/ml TGF-β consistently suppressed IL-17 expression (Fig. 1E). The combination of IL-1β and IL-23 generally produced the greatest increase in the percentage of HIV-infected cells, especially in the presence of TGF-β (Fig. 1E).

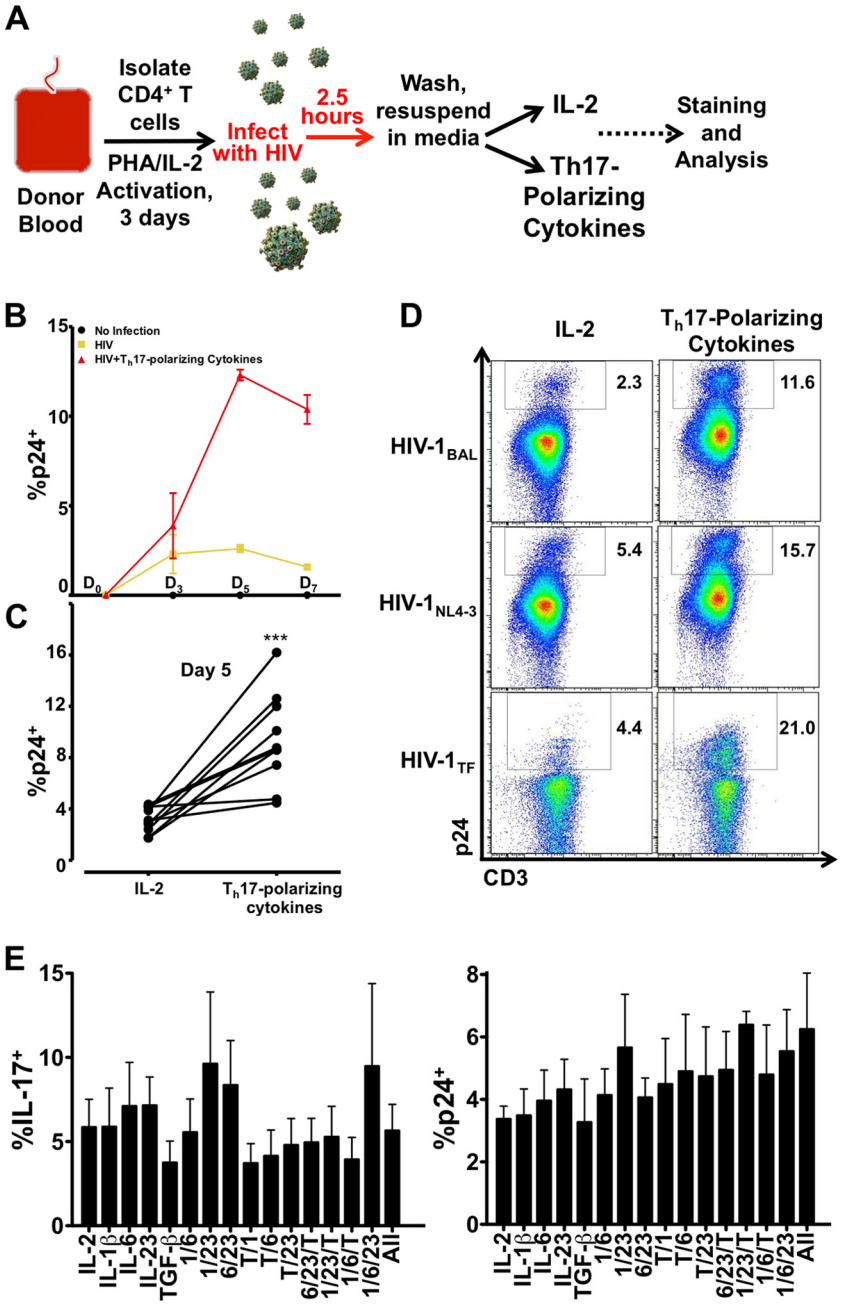

FIG 1 Effects of T_h17-polarizing cytokines on the percentage of HIV-infected, CD4 T cells. (A) Human $CD4^+$ T cells were isolated from peripheral blood by negative selection and then mitogenically activated with phytohemagglutinin (PHA; 5 μg/ml) and interleukin-2 (IL-2; 10 ng/ml) for 3 days prior to infection. Cells were then washed, infected with the indicated isolates of HIV for 2.5 h, washed again, and then resuspended in medium containing IL-2 or the T_h17-polarizing cytokines IL-1β, IL-6, and IL-23 (10 ng/ml) and TGF-β (2 ng/ml). (B and C) Time course (B) and day 5 comparison (C) of the total percentages of $p24^+$ cells from IL-2 and from T_h17-polarizing conditions. D_0, D_3, D_5, and D_7, days 0, 3, 5, and 7, respectively. (D) Representative plots of cells infected for 5 days with CCR5-tropic HIV_{BaL}, CXCR4-tropic HIV_{NL4-3}, or CCR5-tropic, transmitted-founder isolate HIV_{AD17}. (E) The indicated cytokines were added after infection with HIV_{BaL}. The IL-17 expression shown was in uninfected samples. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

T_h17 cells are preferential targets for productive HIV infection. To determine which $CD4^+$ T cell subsets were most likely to become productively infected by HIV, we analyzed the coexpression of cytokines, T cell markers, and p24 in human T cells activated with PHA and infected with HIV_{BaL} in the presence of IL-2 or T_h17-polarizing cytokines (Fig. 2). IL-17-expressing cells were enriched among infected cells, and the coenrichment of IL-17- and p24-expressing cells was significantly higher than that observed in IFN-γ^+ cells. When infected in IL-2-containing medium, IL-17^+ cells constituted only 3.1% of PHA-activated T cells but accounted for 18.4% of total HIV-1_{BaL}-infected cells ($P < 0.0001$). Notably, 19.8% of IL-17^+ cells became productively infected, compared with only 3.2% of total T cells or 9.4% of IFN-γ^+ IL-17^- cells ($P < 0.0001$ and $P < 0.003$, respectively) (Fig. 2A).

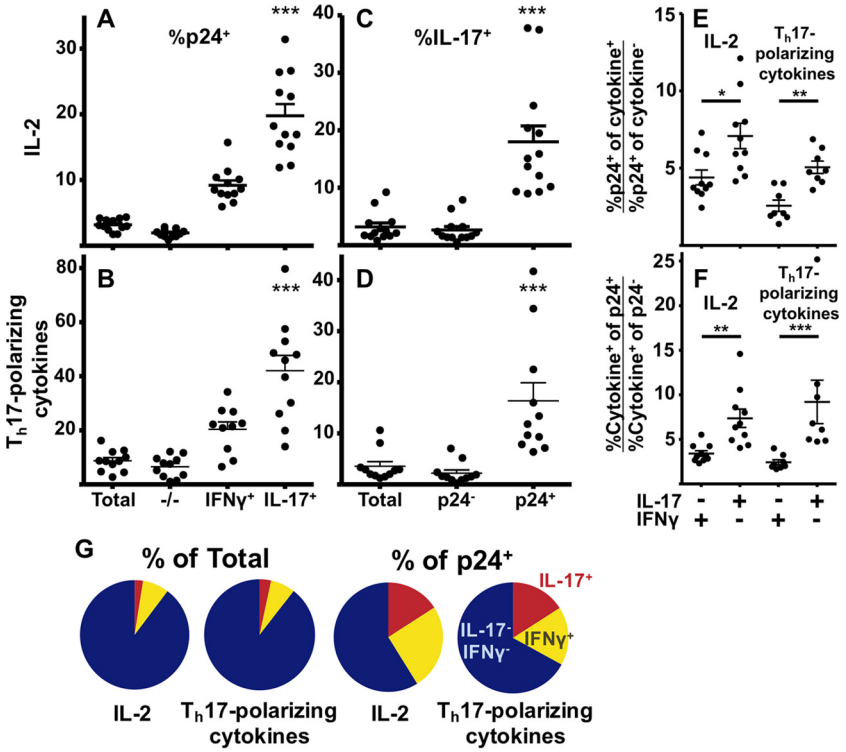

FIG 2 Comparison of total and infected IL-17- or IFN-γ-producing cells. PHA-activated $CD4^+$ T cells were infected with HIV_{BaL} for 5 days in IL-2 or the T_h17-polarizing cytokines IL-1β, IL-6, and IL-23 (10 ng/ml) and TGF-β (2 ng/ml). (A and B) Comparison of p24 expression among total, $IL\text{-}17^-$ $IFN\text{-}\gamma^-$, $IL\text{-}17^+$, and $IL\text{-}17^-$ $IFN\text{-}\gamma^+$ T cells. (C and D) Comparison of IL-17 expression among total, $p24^-$, or $p24^+$ T cells. (E) Fold enrichment of $p24^+$ cells among cells that either express or lack IFN-γ or IL-17. (F) Fold enrichment of IFN-γ- or IL-17-single-positive cells among $p24^+$ cells, relative to $p24^-$ cells. (G) Percentages of IL-17- and IFN-γ-expressing cells were measured by flow cytometry. Blue depicts the proportion of $IL\text{-}17^-$ $IFN\text{-}\gamma^-$ cells, red shows $IL\text{-}17^+$ cells, and yellow shows $IFN\text{-}\gamma^+$ $IL\text{-}17^-$ cells. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$.

The coenrichment of IL-17- and p24-expressing cells was even more pronounced under T_h17-polarizing conditions. Whereas 19.8% of $IL\text{-}17^+$ cells became infected under T_h0 conditions, 42.1% became infected in the presence of T_h17-polarizing cytokines ($P < 0.001$, $n = 11$). Whether infected in the presence of IL-2 or of T_h17-polarizing cytokines, $IL\text{-}17^+$ cells accounted for about one-fifth of total $p24^+$ cells. T_h17-polarizing cytokines also tended to decrease the proportion of infected cells that express IFN-γ (Fig. 2C, D, and G).

$IFN\text{-}\gamma^+$ and $IL\text{-}17^+$ cells were both enriched among infected cells from T_h0 conditions. Nevertheless, when accounting for the proportion of total cells, $IL\text{-}17^+$ cells were significantly more likely to become productively infected by HIV than were $IFN\text{-}\gamma^+$ cells (Fig. 2A and B). In PHA-activated T cells, $IL\text{-}17^+$ cells were infected at significantly higher rates than $IFN\text{-}\gamma^+$ cells (Fig. 2E and F). $IL\text{-}17^+$ $IFN\text{-}\gamma^-$ cells and $IL\text{-}17^+$ $IFN\text{-}\gamma^+$ cells had similar rates of infection (19.4% and 20.7%, respectively).

We found that T_h17-polarizing cytokines rendered T cells significantly more permissive to productive HIV infection, even when washed away prior to infection. Based on this observation, we hypothesized that T_h17 signaling pathways promote HIV replication intracellularly, through postentry mechanisms.

T_h17 polarization renders T cells more likely to become productively infected by HIV. To achieve greater T_h17 polarization and discern between pre- and postinfection effects of our polarizing cytokines, we polarized cells before infection as shown in Fig. 3A. T_h17 cells express the chemokine receptor CCR6. Accordingly, primary human $CD4^+$ T cells were sorted according to their CCR6 expression; activated with anti-CD3 and anti-CD28 in the presence of IL-2 or T_h17-polarizing cytokines; and then washed, infected, and resuspended in medium containing only IL-2 and IL-23 (Fig. 3A).

Very few (mean = 0.43%, $n = 6$) of T cell receptor (TCR)-activated, $CCR6^-$ cells expressed IL-17, even when activated under T_h17-polarizing conditions (0.74%). Roughly a third (30.9%) of $CCR6^+$ cells expressed IL-17 upon activation in the presence of T_h17-polarizing cytokines. A 20.5% proportion of $CCR6^+$ cells produced IL-17 when activated in the presence of IL-2 (Fig. 3C). We observed high donor variability in the expression of IL-17 among $CCR6^+$ cells under T_h0 conditions, with some producing very few $IL\text{-}17^+$ cells and others expressing nearly as many as in donor-matched $CCR6^+$ cells from T_h17-polarizing conditions. The percentage of IL-17-expressing cells peaked on days 3 to 5, immediately after activation, gradually decreasing to less than half of the initial peak level by day 9 (data not shown).

Representative plots in Fig. 3B that show low percentages of $IL\text{-}17^+$ cells coexpressing p24 need to be analyzed in context. For example, in the HIV_{BaL}-infected, $CCR6^-$, T_h0-polarized cells, the representative plot shows that only 0.02% of $IL\text{-}17^+$ cells are $p24^+$. However, in this example T_h17 cells made up only 0.1% of total cells and yet accounted for 11% of total infected cells [0.02%/(0.02% + 0.16%)]. Furthermore, while only 0.18% of all cells were $p24^+$, 20% of IL-17-expressing cells were infected.

An analysis combining data from all conditions from six do-

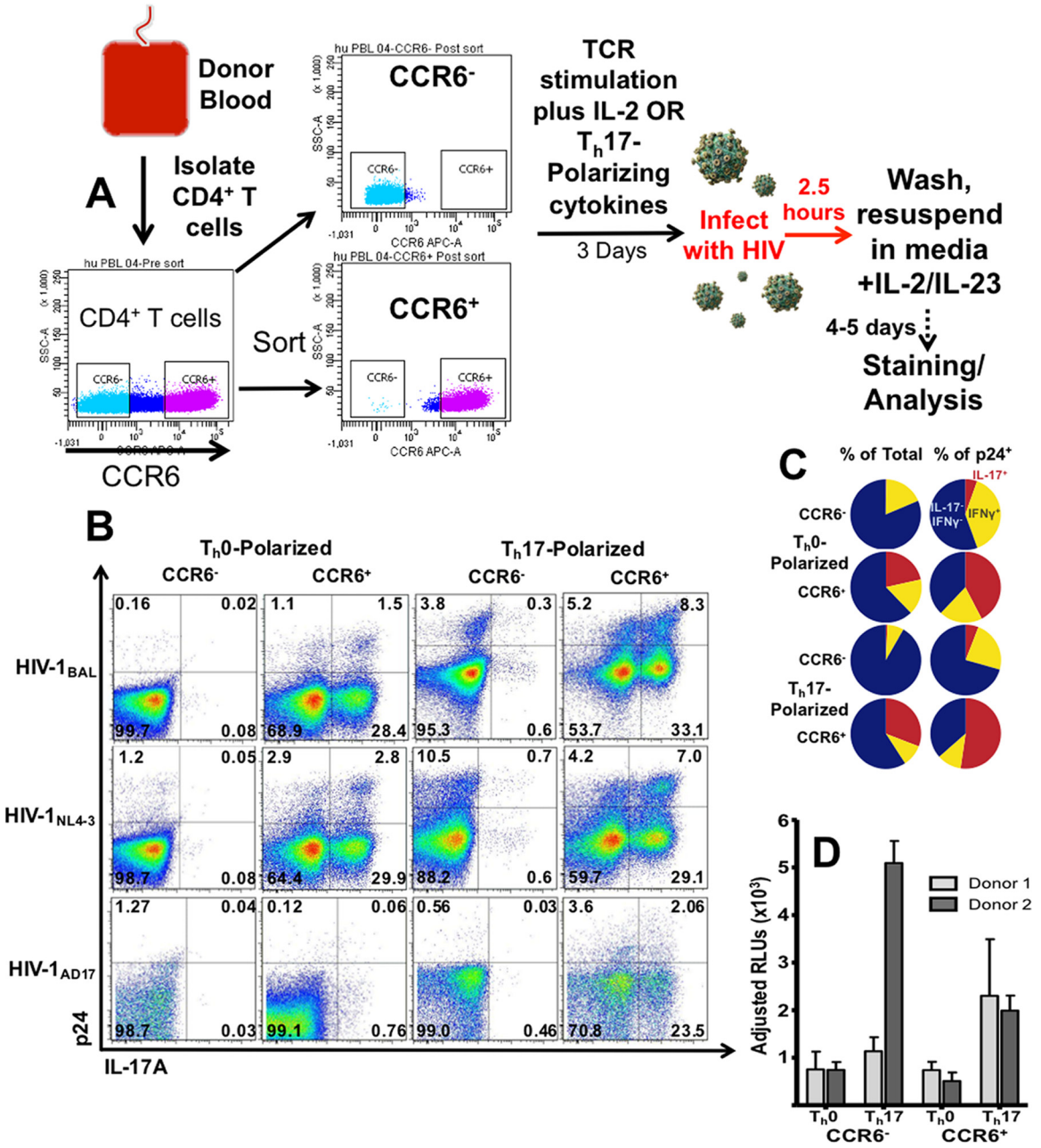

FIG 3 HIV infections of CCR6-sorted, prepolarized cells. (A) Cells were sorted according to their expression of CCR6 and then TCR stimulated in the presence of T_h0- or T_h17-polarizing cytokines (IL-2 and IL-1β, IL-6, IL-23, and TGF-β, respectively) for 3 days. Cells were then infected with the indicated isolates of HIV, washed, resuspended in medium with IL-2/IL-23, and analyzed by flow cytometry 5 days postinfection. *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$. (B) Representative flow cytometry plots depicting p24 and IL-17 expression in T_h0- or T_h17-polarized cells infected with the indicated isolates of HIV. (C) Percentages of IL-17- and IFN-γ-expressing cells were measured by flow cytometry. Blue depicts the proportion of IL-17$^-$ IFN-γ$^-$ cells, red shows IL-17$^+$ cells, and yellow shows IFN-γ$^+$ IL-17$^-$ cells. (D) Five nanograms of p24/ml from the supernatant of HIV_{BaL}-infected T cells under the indicated conditions (or the equivalent volume from condition-matched, uninfected cells) was added to preseeded TZM-BL cells in triplicate. Luciferase activity was measured 48 h later, and relative light unit (RLU) measurements from infected supernatants were adjusted by subtracting the RLU from their corresponding uninfected supernatants.

nors reveals that the disproportionately higher rates of IL-17 and p24 coexpression were more common than that of IFN-γ and p24. Overall, IL-17$^+$ cells had greater than a 2-fold-higher percentage of p24$^+$ cells than total cells in 21 out of 24 samples (range, 2.1- to 14-fold). However, IFN-γ$^+$ IL-17$^-$ cells from only 8/24 samples had greater than 2-fold increases in their percentage of p24$^+$ cells (range, 2.2- to 6.3-fold), and 5/24 samples had fewer IFN-γ$^+$ IL-17$^-$ cells among p24$^+$ cells, relative to total cells.

IL-17$^+$ cells made up only 6.2% of total T cells when all conditions were averaged but accounted for over 18% of all infected cells ($P < 0.002$).

IFN-γ expression was highest among cells from T_h0 condi-

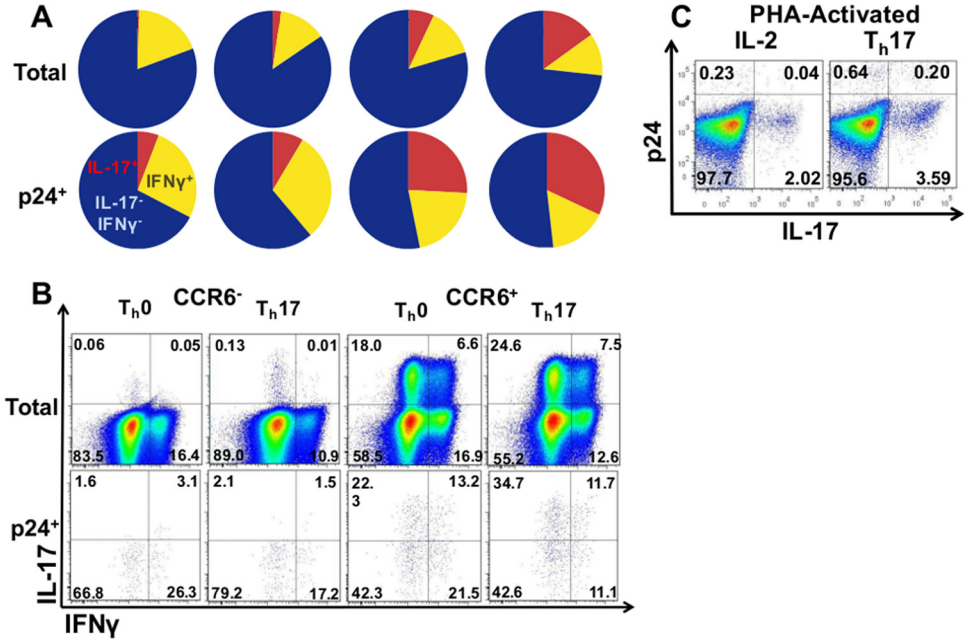

FIG 4 IL-17 and IFN-γ expression among CCR6-sorted, prepolarized cells infected with a pseudotype HIV vector, HIV_{AMLV}. (A) Pie charts of average percentages of IL-17- and IFN-γ-expressing cells among CCR6-sorted cells that were T_h0 or T_h17 polarized and then infected with an *Env*-deficient HIV vector in an amphotropic murine leukemia virus (AMLV) envelope for 4 days. Total and $p24^+$-gated cells from 5 donors are shown. (B) Flow cytometry plots from the donor whose cells became most T_h17 polarized. (C) Representative plots of IL-17 and p24 expression in phytohemagglutinin (PHA)-activated CD4 T cells infected for 4 days with HIV_{AMLV} in the presence of IL-2 or T_h17-polarizing cytokines.

tions, and levels were similar between $CCR6^+$ and $CCR6^-$ cells (16.3% and 18.2%, respectively). Activation under T_h17-polarizing conditions yielded fewer IFN-γ^+ cells (7.5% for $CCR6^-$ and 10.0% for $CCR6^+$ cells) (Fig. 3C).

Upon establishing our culture system and analyzing T cells from the sorted, polarized conditions, we wanted to compare HIV_{BaL}, HIV_{IIIB}, and HIV_{AD17} infections among various subpopulations. We chose 4 to 5 days postinfection to maximize the percentage of live, polarized cells present during infection and to ensure the detectability of $p24^+$ cells by flow cytometry.

Remarkably, IL-17^+ cells from HIV_{BaL} $CCR6^+$, T_h17-polarizing conditions made up more than half of all infected cells (Fig. 3B and C) Further, the effects of T_h17-polarizing cytokines on HIV infection occurred among both $CCR6^+$ and $CCR6^-$ cells and with all HIV isolates used (Fig. 3B). Among HIV_{BaL}-infected cells, T_h17 polarization increased the percentage of $p24^+$ cells by 8.9- and 5.8-fold in $CCR6^-$ and $CCR6^+$ cells, respectively ($P < 0.02$). $CCR6^+$ cells had 4.0-fold ($P < 0.07$)- and 2.6-fold ($P < 0.04$)-higher percentages of total infected cells in T_h0- and T_h17-polarized cells, respectively (Fig. 3B and C). Sorted, polarized cells from four donors were infected with HIV_{IIIB}, and another three were infected with transmitted-founder HIV_{AD17} and analyzed. T_h17 polarization increased the percentage of HIV-infected cells among all three HIV isolates (Fig. 3B).

Similar to PHA-activated cells, T_h17 cells from T cells that were sorted based on their expression of CCR6 and activated with TCR stimulation were preferential targets for productive HIV infection. Both IL-17^+ and $CCR6^+$ cells exhibited a higher percentage of HIV-infected cells relative to total cells, regardless of the viral isolate used. The increasing percentage of IL-17^+ cells among $p24^+$ cells resulting from T_h17 polarization was proportional to that seen in total T cells. However, T_h17 polarization resulted in decreased percentages of IFN-γ^+ cells among infected cells (Fig. 3B and C).

To address the possibility that viruses produced under different conditions may differ in infectivity, we added a standardized p24 concentration (5 ng/ml) from supernatants of sorted, polarized, HIV_{BaL}-infected cells to TZM-BL cells. TZM-BL cells express luciferase, dependent on HIV long terminal repeat (LTR) promoter-driven transcriptional activity (Fig. 3D). HIV-dependent luciferase activity was measured in triplicate samples of supernatants. To correct for luciferase activity that could be due to cellular effects resulting from differences in the volume or polarizing treatments of supernatant added, we subtracted the luciferase activity of uninfected control supernatants from that of their corresponding infected samples. Two of the donors showed increased luciferase activity (mean, 4.7-fold-higher relative light units) in samples treated with supernatants from T_h17-polarized cells, relative to T_h0-polarized cells (Fig. 3D).

T_h17 cells are highly permissive to productive HIV infection by a pseudotyped HIV vector, HIV_{AMLV}. To control for the contribution of entry-level (susceptibility) differences to the HIV infection of T_h17 cells, we infected PHA-activated or T_h0/T_h17-polarized cells with an amphotropic murine leukemia virus (AMLV)-pseudotyped HIV. HIV_{AMLV} lacks the *Env* gene, thus preventing completion of the viral replication cycle. However, HIV_{AMLV} is complemented with an AMLV envelope, which utilizes the widely expressed PiT2 receptor for cell entry, and can therefore infect many mammalian cell types. Despite using an HIV vector that enters cells independently of HIV receptors, the nonspreading infection still produced a disproportionately high percentage of productively infected IL-17^+ cells (Fig. 4).

IL-17$^+$ cells were consistently more likely to become productively infected by HIV_{AMLV} than IL-17$^-$ or IFN-γ^+ cells, regardless of polarizing conditions. On average, T_h17 cells were overrepresented among p24$^+$ cells by 2.9-fold ($n = 5$, Student's t test, $P < 0.001$; median, 2.7-fold, and range, 1.5- to 22.1-fold increases in frequency of IL-17$^+$ cells among p24$^+$ cells, relative to total T cells). IFN-γ^+ IL-17$^-$ cells were also more likely to become productively infected by HIV_{AMLV} but to a lesser degree (Fig. 4A and B) (1.6-fold enrichment in p24$^+$ cells, $P < 0.03$; median, 1.4-fold; range, −0.3- to 5.5-fold). When added postinfection to PHA-activated T cells, T_h17-polarizing cytokines increased the percentage of productively infected cells by 262% (Fig. 4C, $n = 2$).

HIV replicates more efficiently in IL-17-expressing cells. Our hypothesis that T_h17 cells are more permissive to HIV replication predicts that IL-17-expressing cells will produce more virus than IL-17$^-$ cells. In our flow cytometry experiments, we noticed that infected T_h17 cells tended to have a higher fluorescence intensity of p24. Therefore, we compared the geometric mean intensities of p24 from HIV_{BaL}-infected T_h17 cells and IL-17$^-$ cells.

Indeed, upon analysis of the p24 fluorescence intensity among IL-17$^+$ cells under PHA-activating conditions, we found a significant increase in cell-associated p24 compared with IL-17$^-$ cells (Fig. 5A) ($P < 0.04$) or IFN-γ^+ cells (data not shown; $P < 0.03$).

Prepolarized cells exhibited the same trend, regardless of CCR6 expression or polarizing conditions (Fig. 5B). Infections with HIV_{IIIB} and transmitted-founder HIV_{AD17} produced similar results (Fig. 3B and data not shown).

Moreover, HIV_{AMLV}-infected T_h17 cells had higher p24 mean fluorescence intensities (MFIs) (Fig. 5D). IL-17$^+$ CD4 T cells, independently of their CCR6 expression or polarizing conditions, tended to have a higher p24 MFI than p24$^+$ IL-17$^-$ cells. This was especially true for CCR6$^+$-sorted cells, which had 2.9-fold ($P < 0.01$)- and 3.1-fold ($P < 0.008$)-higher p24 MFIs than IL-17$^-$ cells. T_h17 polarization generally increased overall p24 MFI, but this trend did not reach statistical significance, perhaps due to a lower n ($P < 0.09$). PHA-activated T cells exhibited a similar trend. The addition of T_h17-polarizing cytokines during infection also resulted in heightened p24 MFI among IL-17$^+$ cells and tended to increase overall p24 fluorescence intensity, regardless of IL-17 expression (Fig. 4C and 5A [$P < 0.02$] and data not shown).

To determine whether T_h17-polarized cells also produced more secreted virus, we infected equal numbers of sorted, polarized cells and then subjected the supernatants to p24 ELISA and viral titer analyses. Consistent with our data suggesting that HIV replicates more efficiently in T_h17 cells, the supernatant p24 level was highest from T_h17-polarized cells. Among the five donors tested, we detected an average 6.9-fold increase ($P < 0.0008$) in supernatant p24 from infected, T_h17-polarized, CCR6$^+$ cells. This increase was relative to donor-matched, T_h0-polarized CCR6$^-$ cells, which were relatively devoid of T_h17 cells (mean percents IL-17$^+$ were 30.9 and 0.4, respectively). Supernatant p24 levels from CCR6$^+$ T_h17-polarized cells were also 2.7 ($P < 0.02$)- and 3.5 ($P < 0.007$)-fold higher than those from donor-matched T_h17-polarized, CCR6$^-$ cells and T_h0-polarized CCR6$^+$ cells, respectively (Fig. 5E).

Thus, T_h17-polarized cells inherently produce larger amounts of secreted, infectious virus than do T_h0-polarized cells.

T_h17-polarized cells have diminished expression of RNase A genes and proteins. Next, we attempted to identify changes in gene expression among T_h17-polarized cells that could potentially promote viral replication intracellularly. To confirm that our sorting and polarization conditions produced meaningful changes in gene expression, we performed a microarray analysis of RNA extracted from CCR6$^-$, T_h0-polarized cells and donor-matched CCR6$^+$, T_h17-polarized cells (Fig. 6A).

After correction for multiple testing, our differential gene expression analysis shows that genes associated with the T_h17 phenotype, such as IL-23 receptor, retinoic acid receptor (RAR)-related orphan receptor C (RORγc), and MIP-3α/CCL20, are significantly upregulated in the T_h17-polarized population. Gene set enrichment analyses (GSEAs) revealed an immunologic signature in the T_h17-polarized cells consistent with genes upregulated in memory CD4 T cells compared to naive cells (false-discovery rate [FDR] q value of <0.001 [48]) with a consistent pattern for downregulated genes. GSEA reactome analyses showed a signature comparable to those of reported reactomes for HIV infection (FDR = 0.023), of host interaction of HIV factors (FDR = 0.0031), and of *vif*-mediated degradation of APOBEC3G (FDR = 0.024), indicating that the T_h17 cells are enriched in factors that favor HIV replication. Analyses of signaling pathways showed that T_h17-polarized cells upregulated genes that are *myc* targets (FDR = 0.006), by tumor necrosis factor alpha (TNF-α) signaling via NF-κB (FDR = 0.02).

Among the genes that were most significantly downregulated in the T_h17-polarized cells, we noticed two members of the RNase A family known to have antiviral activity, i.e., RNase 2 (Fig. 6B, log fold change [logFC] = −4.928, adjusted P value = 0.003) and RNase 3 (logFC = −2.533, adjusted P value = 0.02). A third member of the same family which had not previously been tested against HIV, RNase 6, had a relatively weaker downregulation and false-discovery rate (Fig. 6B) (logFC = −1.081, adjusted P value = 0.12). Known restriction factors, such as APOBEC3G and SamHD1, were not differentially expressed in T_h17-polarized cells from the three donors tested (data not shown).

Based on these findings, we harvested protein from CCR6-sorted, polarized cells and compared their levels of expression of RNases 2, 3, and 6 by immunoblotting. While RNase 2 was not in detected in activated T cells with the antibody that we used, RNases 3 and 6 were expressed at higher levels in the T_h0-polarized cells than in donor-matched cells from T_h17-polarizing conditions. The steady-state RNase 3 and RNase 6 protein expression levels were 57% and 41%, respectively, lower in T_h17-polarized cells ($n = 5$, $P < 0.05$) (Fig. 6C). Thus, our microarray data and confirmatory immunoblotting assays revealed a sharp decrease in the expression of RNase 3 and RNase 6 upon T_h17 polarization.

We next activated CD4 T cells from 5 donors in the presence of IL-2 or T_h17-polarizing cytokines, infected cells with HIV_{IIIB}, washed them, resuspended them in medium, and then added recombinant human RNases (Fig. 6D). We measured p24 expression by flow cytometry, comparing RNase-treated cells with untreated controls.

Recombinant RNases 2 and 6 significantly inhibit HIV infection at 200 nM and 1 μM, respectively (Fig. 6D) ($P < 0.05$ at all concentrations). At the doses that we tested, RNase 3 did not significantly inhibit HIV infection in either T_h0- or T_h17-polarized cells. Since RNase 3 had been previously tested and found to be antiviral, we postulate that the dose of the protein that we used was not sufficient to inhibit HIV under our assay conditions. Nonetheless, the inhibition of HIV by RNases 2 and 6 was most pronounced in T_h17-polarized cells. When we tested RNase 2, the

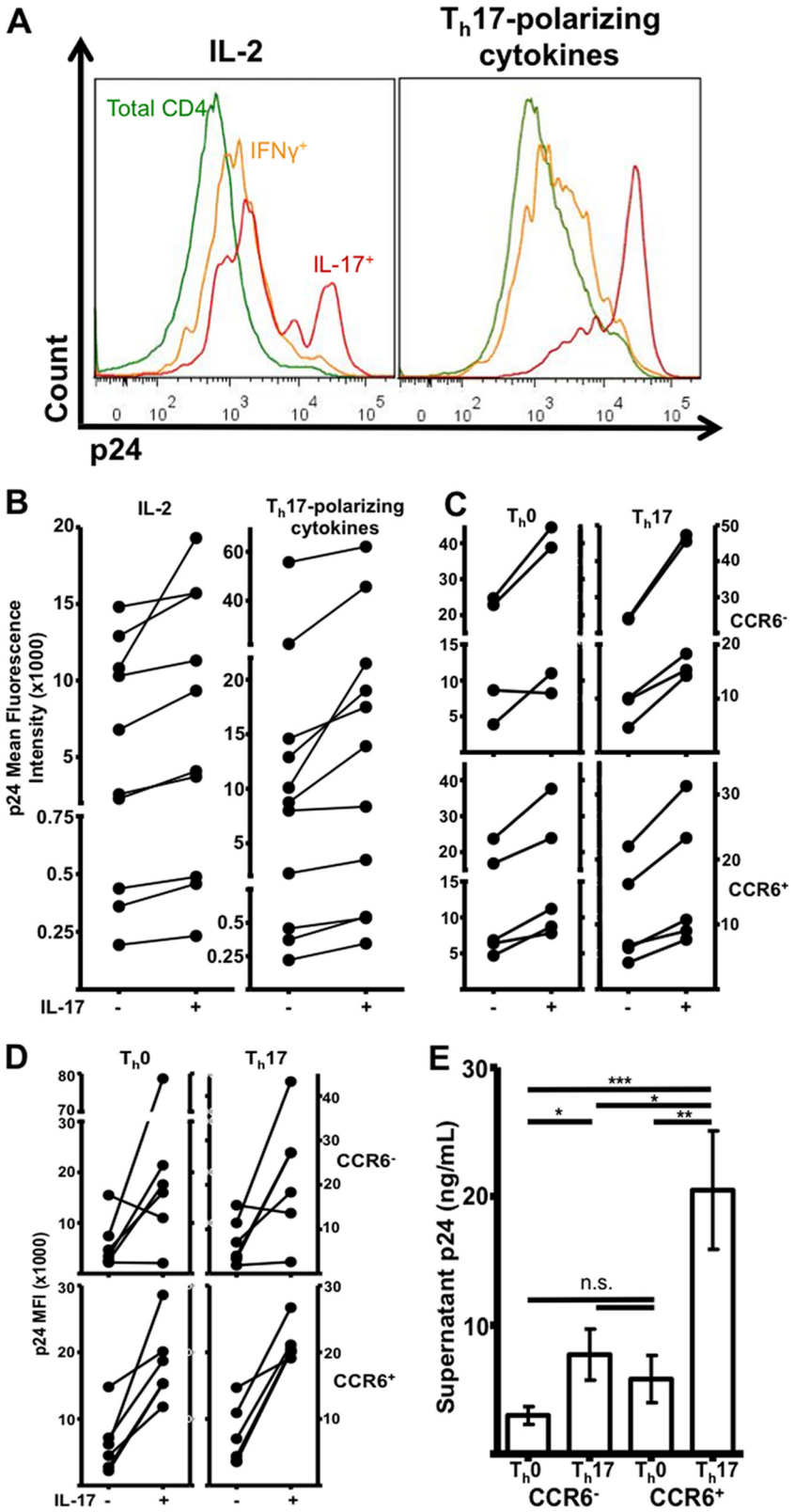

FIG 5 p24 mean fluorescence intensities (MFIs) among infected IL-17$^+$ and IL-17$^-$ cells and supernatant p24 from sorted, polarized cells infected with HIV_{BaL}. (A and B) Representative histograms (A) and donor-matched plots (B) of p24 geometric MFIs among IL-17$^+$ and IL-17$^-$, PHA-activated cells that were infected with HIV_{BaL} for 5 days in the presence of IL-2 or T_h17-polarizing cytokines. (C and D) p24 MFI plots from sorted, polarized cells that were infected with HIV_{BaL} (C) or HIV_{AMLV} (D). (E) Supernatant levels of p24 5 days postinfection, as measured by enzyme-linked immunosorbent assay (ELISA; $n = 5$). *, $0.01 < P < 0.05$; **, $0.001 < P < 0.01$; ***, $P < 0.001$. n.s., not significant.

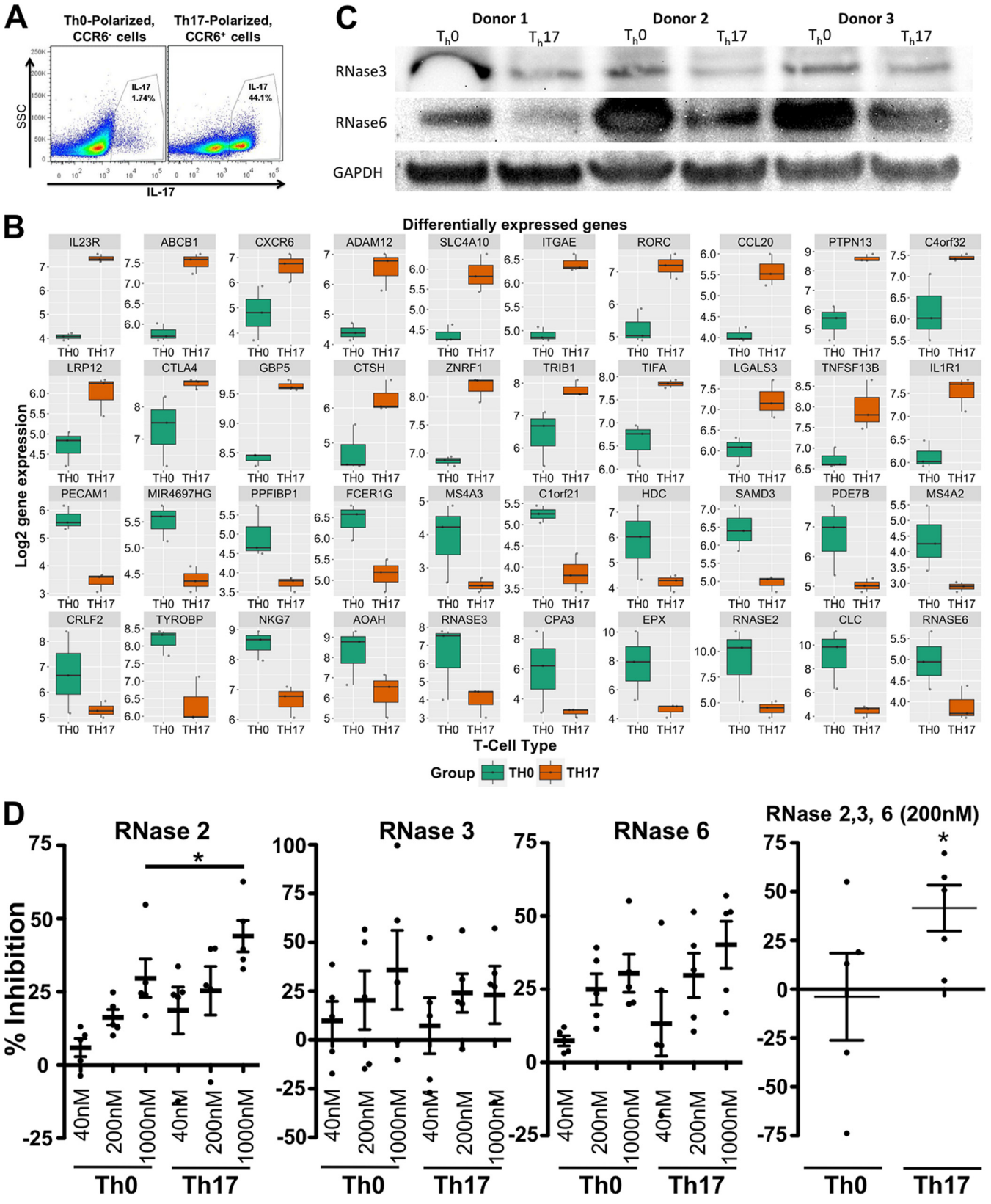

FIG 6 Comparison of RNase expression and HIV inhibition in T_h17-polarized cells with those in T_h0-polarized cells. (A) Representative flow cytometry plots depicting IL-17 expression among T_h0-polarized, $CCR6^-$ cells and T_h17-polarized, $CCR6^+$ cells. SSC, side scatter. (B) Box plot showing differential gene expression between T_h0- and T_h17-polarized cells. Total RNA from T_h0- or T_h17-polarized cells was analyzed by using microarrays. Relative levels of gene expression in T_h17-polarized cells and T_h0-polarized cells are shown. (C) Thirty micrograms of total protein from cell lysates of $CCR6^-$, T_h0-polarized and

heightened inhibition in T_h17-polarized cells reached statistical significance ($P < 0.05$) compared to T_h0-polarized cells. A similar trend was observed in RNase 6-treated cells. To our knowledge, these are the first data showing HIV inhibition by human RNase 6. When we tested all three RNases at 200 nM each, significant HIV inhibition was observed only in T_h17-polarized cells.

DISCUSSION

Here, we show that human peripheral blood $CD4^+$ T cells that express IL-17 are consistently more likely to become productively infected by HIV than IL-17^- or IFN-γ^+ cells regardless of activation method, polarizing conditions, or viral tropism (Fig. 1 to 4). Further, our data indicate that T_h17 cells not only are more susceptible to HIV entry but also mediate enhanced viral replication and production compared with IL-17^- $CD4^+$ T cells.

In their original work establishing the preferential loss of T_h17 cells in HIV-infected patients, Brenchley et al. noted that peripheral blood T_h17 cells were not preferentially infected compared to T_h1 cells, as measured by the copy number of integrated provirus in stimulated $CD27^+$ memory T cells (1). This seems to contradict the heightened susceptibility and permissiveness that we observed, but it is likely that cells with similar rates of successful viral integration could vary in their production of viral progeny (49). Consistent with this explanation, infected T_h17-polarized cells yielded higher levels of intracellular and supernatant p24 than did T_h0-polarized cells (Fig. 5D). Our use of peripheral rather than GALT $CD4^+$ T cells may account for differences in infectivity rates via changes in receptor expression and/or cellular activation. It is also possible that unintegrated viral DNA might contribute to p24 expression (50).

The design of our T_h17 enrichment strategy was based on another study (17), in which both IL-2 and IL-23 were added to the medium after polarization for all conditions, in order to maintain both T_h17 and non-T_h17 cells and ensure an appropriate comparison during the subsequent infection.

T_h17 depletion occurs early during acute HIV infection, and the mechanisms of the loss remain unclear, possibly including direct cytocidal effects, pyroptosis, and apoptosis (51–53). The loss of these cells compromises mucosal barriers and undermines the capacity of our immune system to defend against AIDS-associated ailments (4–7, 26, 54). The T_h17 cytokines IL-17 and IL-22 synergistically induce the expression of antimicrobial peptides (55). Our lab has shown that the antimicrobial peptide beta-defensin 2, which is robustly expressed in the oral mucosa of healthy individuals, is markedly decreased in HIV-infected subjects (56), possibly related to the loss of T_h17 cells. Notably, combined antiretroviral therapy initiated early during acute HIV infection prevents the loss of T_h17 cell numbers and polyfunctionality and reverses systemic and gut immune activation (3).

Our data suggest that T_h17 polarization could promote infection by two mechanisms. First, factors expressed in T_h17 cells (including transcription factors) might directly promote HIV replication. In accordance, a sequence analysis of several HIV-1 LTRs suggested the presence of putative RORγt consensus sequence binding sites (data not shown). If functional, these binding sites raise the possibility that the expression of proviral HIV may be directly modulated by RORγt activity. Additionally, STAT3 has been shown to promote HIV replication in human neonatal cord-blood mononuclear cells, and short hairpin RNA inhibition of STAT3 diminished HIV gene expression (57). Relevantly, the HIV-1 accessory protein Transactivator of Transcription (Tat) activates STAT3 in infected antigen-presenting cells, thereby driving the expression of T_h17-polarizing cytokines (58). HIV-pulsed dendritic cells also activate STAT3 in T cells *in vitro* (59). GP120 and Nef activate STAT3 as well. Thus, HIV directly activates the T_h17-related transcription factor STAT3 and supports the production of T_h17-polarizing cytokines.

Second, T_h17 polarization might inhibit the expression of necessary antiviral effectors (60). Our studies revealed that members of the RNase A superfamily were among the most strongly downregulated genes in T_h17-polarized cells; RNase 3 and RNase 6 protein expression was also diminished (Fig. 6C). Although the regulation of the expression of these RNases is poorly understood in T cells, members of the GATA family of transcription factors promote their expression in eosinophils (61). GATA3, a key transcription factor for the differentiation of T_h2 cells, is cross-inhibited by other differentiation pathways (62). T_h17 cells and other subsets may inhibit GATA transcription factors to stabilize non-T_h2 differentiation pathways, thereby inhibiting the expression of RNases.

The novel observation that RNase 6 inhibits HIV but is expressed at lower levels in T_h17-polarized cells than in T_h0-polarized cells provides new mechanistic insight into the unique vulnerability of T_h17 cells to HIV infection. Further studies are needed to characterize the HIV-inhibitory activity of RNase 6 and to measure function *in vivo*.

Our findings suggest that RNases may constitute an important defense against the HIV infection of CD4 T cells (as depicted in Fig. 7). Members of the RNase A superfamily directly inhibit a broad range of pathogens, can be secreted, and also modulate our immune system. Of the eight human RNase proteins that have been functionally characterized, four had already been shown to have HIV-inhibitory activity (63). The secretion of RNase 4 and RNase 5/angiogenin by T cells was recently identified as a mechanism of HIV inhibition (64). Recombinant human RNase 1, RNase 2, and RNase 5 each inhibit the HIV infection of primary human T cells *in vitro*, whether added before or after infection. The mechanisms of viral inhibition remain unclear, but antibodies to either RNase blocked HIV-inhibitory activity (65).

One caveat of our study was its dependence on peripheral blood as the source of T cells. The GALT and vaginal mucosae represent unique anatomical compartments in which local antigens, cytokines, chemokines, and localization factors such as integrins differ from those present in blood. Whether or not GALT T_h17 cells also demonstrate such proneness to productive HIV infection relative to other subsets remains unclear. However, our findings are consistent with an *in vivo* SIV transmission study showing that vaginal mucosal T_h17 cells, which made up less than

$CCR6^+$, T_h17-polarized CD4 T cells from three donors was denatured and separated according to size by SDS-PAGE, transferred to a PVDF membrane, and then probed for the indicated RNase expression with anti-RNase antibodies. The loading control was glyceraldehyde 3-phosphate dehydrogenase (GAPDH). (D) T_h0- and T_h17-polarized CD4 T cells were infected with HIV_{IIIB}, washed, and then resuspended in medium containing the indicated RNases. Percent inhibition was calculated from the percentage of $p24^+$ cells, as measured by flow cytometry.

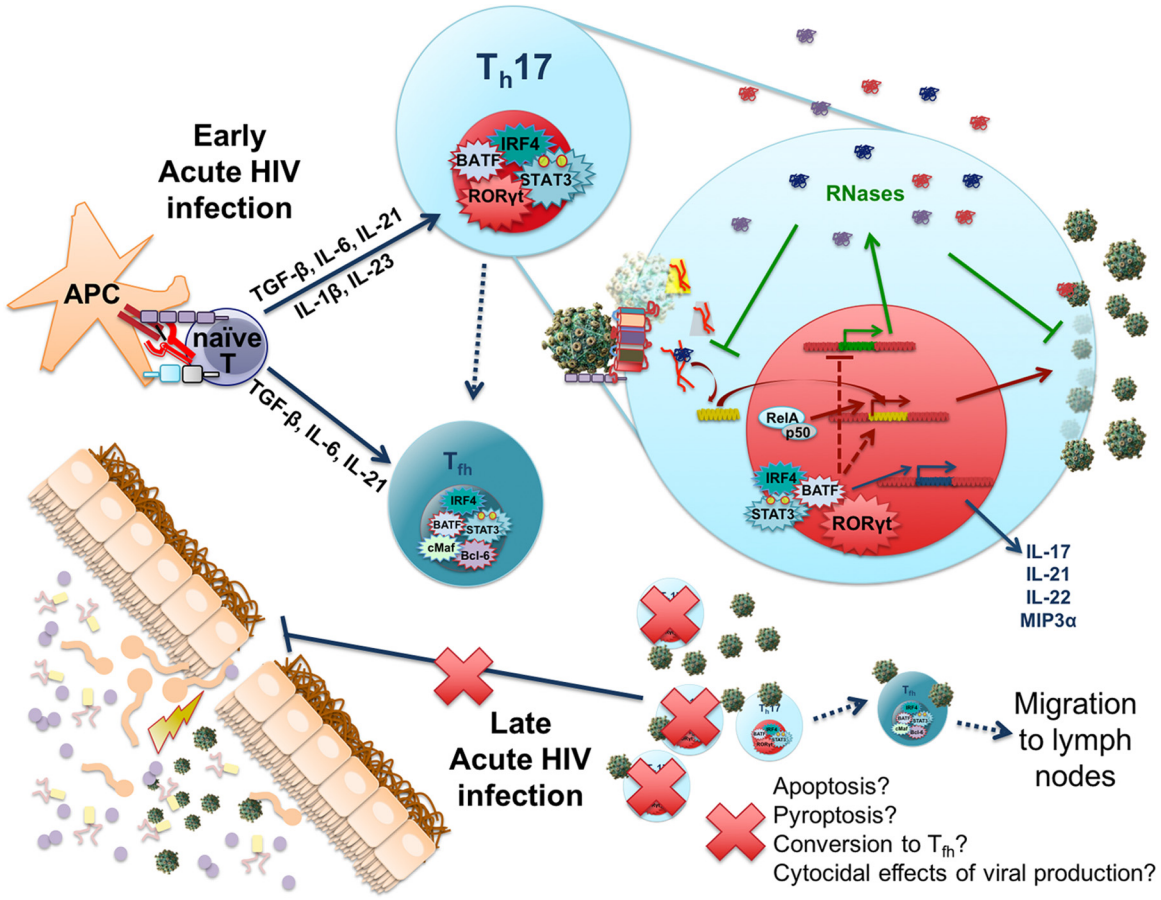

FIG 7 Potential mechanisms and effects of T_h17 permissiveness during acute HIV infection. Heightened permissiveness to HIV in response to T_h17- or T_{fh}-polarizing cytokines may promote viral replication by direct transcriptional modulation of proviral and/or antiviral response genes. The subsequent loss of T_h17 cells could be attributable to programmed cell death, conversion to T_{fh} cells, or the cytopathic effects of high virus production. The loss of T_h17 cells then promotes the disruption of mucosal immunity. APC, antigen-presenting cell.

one-fifth of total $CD4^+$ T cells, accounted for over four-fifths of productively infected cells (66).

A second caveat is that our CCR6-sorted cells were cultured with both IL-2 and IL-23 after TCR stimulation and polarization. We found that IL-23 was necessary to preserve the T_h17 subset during infection, but IL-23 may selectively activate T_h17 cells relative to other subsets in our culture system. Nonetheless, T_h17 cells from our PHA/IL-2-activated cells (which were never treated with IL-23) showed disproportionately higher rates of infection than IFN-γ^+ cells and also heightened p24 MFI (Fig. 1, 2, 4, and 5). Furthermore, IL-2 is an inhibitor of T_h17 function, and we are comparing the infection of IL-17^+ cells with that of IFN-γ^+ cells, which are also highly activated.

If IL-23 contributes to increased p24 production within T_h17 cells, the IL-23R-mediated activation of STAT3, which is activated in both T_h17 and T_{fh} cells, should be further investigated for its potential role in promoting productive HIV infection (67). Such a finding would strengthen our argument that T_h17 and T_{fh} differentiation (and unique activation pathways) are specifically linked to increased HIV permissiveness.

Our results provide deeper insight into the mechanisms underlying the preferential targeting of T_h17 cells during HIV infection, compared with other $CD4^+$ T cell subsets. In our model, given their relative abundance at sites of HIV sexual transmission, T_h17 cells constitute a major source of viral production during acute infection (Fig. 7). Our data suggest that the transcriptional and translational environment of T_h17 cells may directly promote the expression of HIV proteins. It is also possible that infected T_h17 cells directly become T_{fh} cells (32, 33), which would then be capable of migration to secondary lymphoid tissues (Fig. 7), where they are capable of replication at high levels, despite their low expression of CCR5 (68, 69).

Our findings may aid in therapeutic intervention strategies aimed at preventing viral production during acute HIV infection or reducing the size of the viral reservoir in people living with HIV.

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A.C.-Q. performed the majority of experiments and wrote the manuscript. M.L. and L.S. performed experiments and provided helpful suggestions and manuscript edits. L.M. analyzed microarray data, provided expert advice for its interpretation, and edited the manuscript. A.D. provided HIV_{AD17} stocks, designed research, and edited the manuscript. A.G.-D. designed the research, analyzed data, and edited the manuscript.

The authors have no conflicts of interest to declare.

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