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Antigen specificities of HIV-infected cells: A role in infection and persistence?

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ABSTRACT

Antigen-experienced memory CD4$^+$ T cells are the major target of HIV infection and support both productive and latent infections, thus playing a key role in HIV dissemination and persistence, respectively. Here, we reviewed studies that have shown direct association between HIV infection and antigen specificity. During untreated infection, some HIV-specific cells host productive infection, while other pathogen-specific cells such as cytomegalovirus (CMV) and *Mycobacterium tuberculosis* also contribute to viral persistence on antiretroviral therapy (ART). These patterns could be explained by phenotypic features differing between these pathogen-specific cells. Mechanisms involved in these preferential infection and selection processes include HIV entry and restriction, cell exhaustion, survival, self-renewal and immune escape. For instance, MIP-1β expressing cells such as CMV-specific memory cells were shown to resist infection by HIV CCR5 coreceptor downregulation/inhibition. Conversely, HIV-infected CMV-specific cells undergo clonal expansion during ART. We have identified several research areas that need further focus such as the role of other pathogens, viral genome intactness, inducibility and phenotypic features. However, given the sheer diversity of both the CD4$^+$ T cell repertoire and antigenic history of each individual, studying HIV-infected, antigen-experienced cells still imposes numerous challenges.

1. Introduction

HIV-infected CD4$^+$ T cells have been characterized over the past decades, unraveling their heterogeneity in terms of phenotype,[1–6] proviral landscape[7–12] and capacity to produce HIV.[6,13–16] Similarly, several studies have been conducted to define the antigenic specificity of cells that carry HIV genomes.[12,17–32] Only a small fraction of the highly diverse repertoire of CD4$^+$ T cells harboring unique T-cell receptors (TCR), which allows broad pathogen recognition, is infected by HIV. Antigen specificity of infected cells can play a central role in HIV biology for both infection establishment[33] and persistence during antiretroviral therapy (ART).[31] Systemic infection is established within days of infection during the viremic phase leading to the formation of two pools of cells: productively and latently infected cells.[33–37] Productively infected cells support the active production of infective viral particles, whereas latently infected cells do not produce viral particles but could do so in certain circumstances. Although the half-life of productively infected cells is short (approximately two days[38,39]), the viral reservoir (mostly composed of latently infected cells[40,41]) has an estimated half-life of 4 years.[13] This pool of cells can persist for decades during ART,[42–46] which is considered as the major obstacle to HIV eradication.

Some studies have shown preferential infection and/or persistence of cells with given antigenspecificities that could be explained by distinct functional and phenotypic characteristics of these cells.[21,22,24] Also, HIV-infected cells have been shown to persist mainly through clonal expansion on suppressive ART,[8,10,11,16,47] undergoing expansion and contraction processes in response to antigenic stimulations.[30,48] Altogether antigenic recognition by HIV-infected cells plays a key role at two specific times of HIV infection: initial infection and selection of life-long persistent cells. Here, we review the current knowledge in terms of preferential infection of pathogen-specific cells, underlying mechanisms and implications for future cure strategies.

2. Direct evidence of HIV infection of pathogen-specific cells

To this day, the antigen specificity of HIV-infected CD4$^+$ T cells has been studied for some pathogens among which the most common ones are reviewed here. In these studies, cells were obtained from viremic or ART-treated individuals and stimulated with peptides/virus lysates from different pathogens. The potential activation of T cells in response to this

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Table 1
Studies linking antigenspecificity and HIV infection.

Author	Year	Ref.	Pathogen	Antigen	Specificity detection assay	HIV detection assay	Participants (n)	Cohort details	Status	Conclusions
Simonetti et al	2021	[31]	CMV HIV	Lysate (Virusys) *gag* (JPT)	AIM assay (CD69, CD40L)	Dilutions + HIV sequencing or qVOA	10	USA, men/women, age 39-65y	ART	Larger infected clones in CMV-specific cells compared with HIV, without difference in intactness or inducibility, infection early or late during expansion
Cole et al	2021	[12]	CMV *M. tuberculosis*	NA NA	Single-cell TCR sequencing + specificity prediction	*p24* (KC57/28B7) + integration sites + HIV sequencing	8	Belgium, men, age 37-66y	ART	Link between integration in cancer-related genes (STAT5B) and antigen-specificty (flu)
Cattin et al	2021	[32]	*S. aureus* CMV	Lysate *pp65* (Miltenyi)	Monocyte-derived dendritic cells + proliferation (CFSE)	Integrated HIV DNA + *p24* supernatant	8	Canada, men, age 21-62y	ART	Higher levels of HIV DNA in *S. aureus*-specific cells compared to CMV
Gantner et al	2020	[30]	CMV Influenza	NA NA	Single -cell TCR sequnecing + specificty prediction	HIV-Flow	8	USA, men, age 32-67y	ART	Trend toward infected-cells being more CMV-specific, high variability between individuals, clone wax/wane over time
Mendoza et al	2020	[29]	CMV HIV Influenza	*pp65* (JPT) *gag* (JPT) CEFT (JPT)	AIM assay (CD69, 4-1BB, PD-L1)	Q4PCR + HIV sequencing	8	USA, men, age 30-48y	ART	Levels of infection highly variable between individuals, clones defective or intact in all specificities
Hey-Nguyen et al	2019	[28]	HIV CMV	*gag* (HRP) Cultured CMV on MRC5	AIM assay (CD25, OX40)	Total HIV DNA (*pol*) + cloning *gag/pol*	5	Australia, men, age 34-59y	ART	Similar HIV DNA levels between memory CD4, CMV and HIV-specific cells, distinct variants between specificities
Kristoff et al	2019	[27]	HIV CMV Influenza	*gag* (HRP, Sigma) *pp65* (Miltenyi) M1 antigen (eEnzyme, Miltenyi)	Monocyte-derived dendritic cells	*p24* expression (KC57 only) + HIV RNA supernatant	5	USA, age 47-77y	ART	CMV and HIV but not influenza drive latency reversal
Henrich et al	2017	[26]	CMV EBV	Lysate (Virusys) Lysate (Virusys)	ICS (IL-2 and/or IFNγ)	Total HIV DNA	5	USA, men	ART + cancer	Post-chemotherapy: HIV DNA was increased in CMV/EBV responsive CD4
Yek et al	2016	[25]	*S. pneumoniae*/HBV Influenza/HBV VZV/HBV VZV/HAV MMR DT	Vaccine Vaccine Vaccine Vaccine Vaccine Vaccine	Blood draw after vaccination	DNA ddPCR (*gag, 2-LTR*) + RNA ddPCR (*gag, polyA, tat-rev*)	13	Spain, men, age 29-52y	ART	Increase of unspliced RNA (*gag*) after influenza/HBV vaccination Increase of unspliced and *polyA* RNA after *S. pneumoniae*/HBV vaccination
Hu et al	2013	[24]	CMV *C. albicans*	CMV lysates (Advanced Biotechnologies) + *pp65* (JPT) C. albicans sonicate (Greer laboratories)	Proliferation (CFSE)	*In vitro* HIV infection + *p24* expression (KC57 only) + Total HIV DNA (*LTR*)	6	/	HIV-	HIV infection in CMV-specific CD4 T cells remain low (post-entry HIV restriction) compared to *C. albicans* Probable link with Th17 inflammatory response in *C. albicans*-specific cells
Jones et al	2012	[23]	Influenza HIV	Fluviral influenza vaccine *gag p55* (Austral)	Cytokine secretion assay	Proviral DNA	11	Canada, men, age 39-63y	ART	Higher levels of HIV DNA in influenza and CMV-specific

(*continued on next page*)

Table 1 (*continued*)

Author	Year	Ref.	Pathogen	Antigen	Specificity detection assay	HIV detection assay	Participants (n)	Cohort details	Status	Conclusions
			CMV	CMV Ag (Virion)						cells compared to HIV, no significant effect of vaccination
Geldmacher et al	2010	[22]	*M. tuberculosis*	PPD + ESAT6+CFP10	ICS (TNFα ± IL-2/ IFNγ)	Total HIV DNA (*gag*)	10	Tanzania/ Ghana, men/ women	VIR + active TB	Higher levels of HIV DNA in M. tuberculosis-specific cells compared to other CD4 (effect of IL-2 and MIP-1β)
Casazza et al	2009	[21]	CMV	*pp65* (JPT)	ICS (IFN)	Total HIV DNA (*gag*)	8	USA, men, age 21-62y	VIR	Lower levels of HIV DNA in CMV-specific cells compared to other CD4 cells (effect of MIP-1β)
Renoux et al	2005	[20]	TT/DT *M. tuberculosis*	Vaccine PPD	Blood draw + delayed-type hypersensitivity skin patches	SIV *env* sequencing	2 (SIV)	/	VIR	Specific viral quasispecies for each antigen stimulation
Douek et al	2002	[18]	HIV CMV	Peptides (whole) peptides (whole)	ICS (CD69, IFNγ)	Total HIV DNA (*gag*)	16	USA	VIR/ ART	Higher levels of HIV DNA in HIV-specific cells when compared to other CD4 and CMV-specific cells
Demoustier et al	2002	[19]	HIV CMV *M. tuberculosis*	*p24* (Protein Sciences) *nef* (Intracell) CMV (Biowhittaker) PPD (Pasteur-Merieux)	Limiting dilution-based culture assay	HIV RNA in supernatants	5	France	ART	Infected $CD4^+$ T cells include HIV-specific cells (5-100-fold higher than those specific for CMV or *M. tuberculosis*)
Cheynier et al	1998	[17]	*M. tuberculosis*	BCG	Blood TCRBV PCR and skin tests	SIV *env* sequencing	1 (SIV)	France	VIR	Specific viral variant emerging after stimulation

CFSE: Carboxyfluorescein succinimidyl ester, AIM: Antigen-induced marker, ICS: Intracellular cytokine staining, TCR: T-cell receptor, SIV: Simian immunodeficiency virus, qVOA: quantitative viral outgrowth assay, Q4PCR: quadruplex PCR assay, VIR: Viremic, ART: Antiretroviral therapy, PPD: purified-protein derivative, TB: tuberculosis, MMR: measles-mumps-rubella, DT: Diphteria toxin, TT: Tetanus toxoid,CMV: cytomegalovirus, HBV: hepatitis B.

antigenic stimulation was then determined by measuring either activation markers (e.g. CD69, CD40L), cytokine production (e.g. IL-2, IFNγ) or cell proliferation (i.e. CFSE). By combining single-cell sorting of infected cells (*p24*+) to TCRβ DNA sequencing, other investigators have identified T cell antigen specificity by predicting it from the TCR sequence, which is limited by the inference of TCRβ chains only, and by the limited number of public data sets of CD4-derived TCRs. In all these studies, HIV was detected by measuring HIV DNA, viral RNA/protein expression or viral particle release (Table 1).

3. Productive infection in untreated HIV-infected individuals

3.1. HIV

HIV-specific $CD4^+$ T cells have been the first population studied for hosting productive infection as they are activated by HIV antigens and also exposed to infection by antigen presenting cells, making them at high risk of HIV infection. The first studies assessing the specificity of infected cells were thus done on samples collected in a population of mostly viremic individuals (a mix of different clinical settings including acute infection and post-ART interruption) with a high $CD4^+$ T cell count (median 600 cells/mm^3). Douek et *al.* demonstrated that HIV-specific cells are enriched in HIV DNA compared to CMV-specific or total $CD4^+$ T ones.[18] Besides, they have shown that during post-ART interruption, HIV-infected HIV-specific cells re-expand preferentially. This indicates that the contribution to the pool of infected cells from a certain antigenic specificity may vary according to the stage of HIV infection. However, one cannot exclude that productively infected cells do not respond properly to antigenic stimulation due to CD4 downregulation by HIV proteins (*nef, env* and *vpu)* and that these HIV-specific HIV-infected cells in the study above mentioned were in fact latently infected.[49]

3.2. Cytomegalovirus (CMV)

Most people living with HIV are co-infected with CMV,[50,51] which after an acute phase causes a chronic infection with episodic reactivations. When measuring HIV DNA in untreated individuals, total DNA levels were lower in CMV-specific $CD4^+$ T cells than in other $CD4^+$ T cells.[21] This relative low permissiveness to HIV infection was explained by the autocrine secretion of MIP-1β blocking/downregulation of CCR5 on CMV-specific cells. Interestingly enough, it has been observed that CMV-specific $CD4^+$ T cells have a lower rate of *in vitro* HIV infection, even when MIP-1β was neutralized, thus suggesting post-entry HIV restriction, compared with *Candida albicans*-specific cells.[24] These differences might be explained by polarization differences as CMV-specific cells are mostly Th1 and regulatory T cells (Tregs) and *C. albicans*-specific cells mostly T helper 17 cells (Th17).

3.3. Mycobacterium tuberculosis

Both *M. tuberculosis* infection and vaccination are frequent in people living with HIV worldwide.[52] Firstly, in a non-human primate model, simian immunodeficency virus (SIV) sequencing after recall vaccination has shown that different viral quasispecies emerged following recall with *M. tuberculosis*.[20] Similarly, the emergence of a distinct SIV variant was noticed after vaccination with BCG.[17] In untreated humans, total HIV DNA quantification revealed an approximately 2.5-fold enrichment

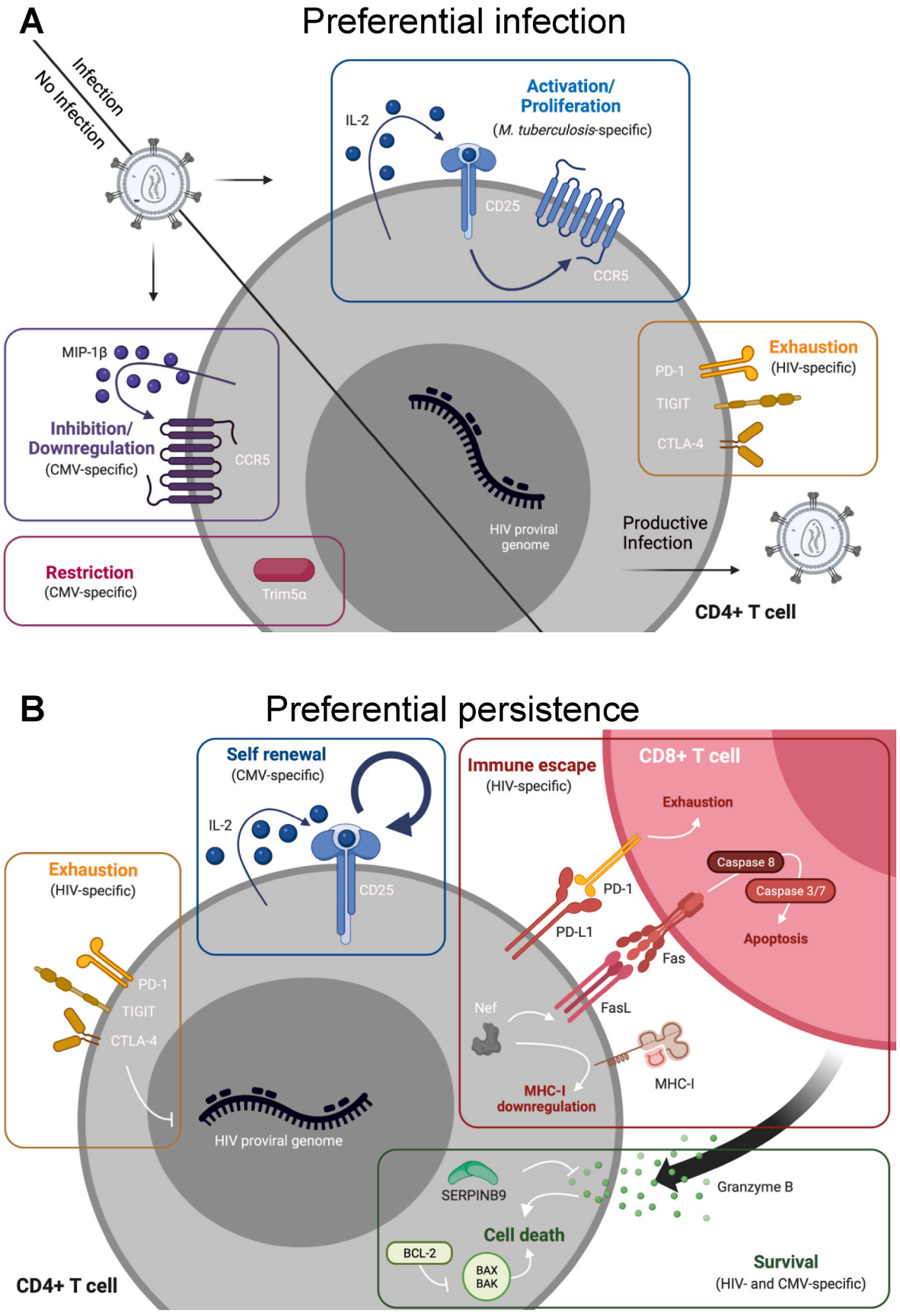

Fig. 1. Host cell factors associated with HIV infection and persistence.

in HIV infection in *M. tuberculosis*-specific CD4$^+$ T cells, in comparison with other CD4$^+$ T cells.[22] The authors, also showed that *M. tuberculosis*-specific CD4$^+$ T cells expressing IL-2 (showing a capacity to proliferate[2]) but not MIP-1β (allowing CCR5 recognition[21]) were more susceptible to HIV-infection.

4. Latent infection on antiretroviral therapy

4.1. HIV

Conflicting results obtained in individuals on ART showed both enrichment and depletion of HIV-infected cells in the pool of HIV-specific cells. Demoustier et *al.* have shown that, infected CD4$^+$ T cells are highly enriched in HIV-specific cells, much more than CMV or *M. tuberculosis*-specific ones (5–100 fold enrichment).[19] When quantifying HIV DNA, Hey-Nguyen et *al.* have shown that total DNA levels are similar in HIV and CMV-specific memory CD4$^+$ T cells.[28] However, Jones and *al.* have focused on proviral DNA and described lower levels in HIV-specific CD4$^+$ T cells than in CMV and influenza-specific cells.[23] These differences could be explained by technical variations in the ways of measuring specificity, HIV infection and using other pathogens as comparators. Ultimately, it has been observed that monocyte-derived dendritic cells (MDC1) presentation of HIV and CMV peptides to their specific CD4$^+$ T cells facilitates HIV latency reversal, much more than with influenza peptides, suggesting that HIV-specific and CMV-specific cells can be infected and produce viral particles upon stimulation.[27] These experiments were done in the absence of ART, making it impossible to precisely quantify the frequency of reservoir cells. These results might thus be explained by a higher activation in response to stimulation, allowing for more efficient virus replication *ex vivo*.

4.2. CMV

Total HIV DNA levels were increased in CMV-specific CD4$^+$ T cells in individuals on ART post-chemotherapy.[26] Also, using specificities predictions based on TCRβ sequences and clonotypes analysis, some expanded clonotypes were shown to be reactive to CMV and influenza, with high variability between individuals on ART.[30] Combining T cell stimulation and HIV sequencing, Mendoza et *al.* showed a clonal expansion of T cell clones carrying defective or intact latent proviruses in CMV-specific cells.[29] Using a similar approach, Simonetti and al. have described that the antigen stimulation of T cells is followed by a clonal expansion with larger clones in CMV-specific CD4$^+$ T cells, in comparison with HIV-infected CD4$^+$ T cells and that infection could occur early or late during the expansion process.[31]

By combining TCR and integration sites sequencing in single cells, Cole et *al.* have revealed that HIV-infected CD4$^+$ T cells predicted CMV-, influenza-, and *M. tuberculosis*-specific harboring provirus integrated in cancer-associated genes (e.g. STAT5B) in individuals on ART. Thus, we can hypothesize that mechanisms driving HIV persistence (integration-induced and antigen-induced proliferation) could be intermingled.[12] Altogether, a positive selection via both antigen and HIV integration driven expansion has also been suggested by others,[31] given the high frequency of proviruses in genes linked to HIV persistence.[53]

4.3. Influenza

Flu incidence in individuals living with HIV is similar than in HIV-negative individuals[54] and vaccination has been recommended for decades for individuals living with HIV,[55] which makes influenza-specific cells potential targets for HIV. Viral reactivation in individuals on ART after vaccination has shown increased levels of HIV transcription after influenza vaccination (unspliced *gag* RNA). However, there were no measurable changes in HIV DNA or plasma HIV RNA.[25] Also, clonal expansion of an emerging influenza-specific clone has also been identified during ART.[30]

4.4. Miscellaneous

When measuring HIV DNA in individuals on ART after stimulation with *S. aureus* and CMV antigens, it has been observed that HIV reservoir levels were higher in *S. aureus*-specific compared with CMV-specific CD4$^+$ T cells (around 5 fold higher). Cattin et *al.* have also described a role of dendritic cells driving the proliferation of these specific cells.[32] *S. pneumoniae*/hepatitis B (HBV) vaccination led to viral reactivation in individuals on ART as shown by increased levels of unspliced *gag* and *polyA* HIV RNA.[25]

Altogether, the antigen specificity of HIV-infected cells seem to vary according to the clinical and therapeutic history of people living with HIV. Also, the individual infection/vaccination history plays a role in the availability of memory cells from a given antigen specificity at the time of infection and can thus create a high inter-individual variability.

5. Host cell factors influencing susceptibility to HIV infection

5.1. HIV entry

Cells expressing CCR5, the major HIV coreceptor, are highly infected with HIV and support both productive and latent infections.[6,33,56] Conversely, low CCR5 expression was shown to protect HIV-specific CD4$^+$ T cells from infection in elite controllers.[57] CCR5 expression varies according to T cell lineage, memory differentiation and localization.[58] Peripheral blood Th1 express high levels of CCR5.[59,60] Peripheral blood Th17 (CCR4+CCR6+ and CXCR3+CCR6+) and cervical Th17 were shown to be highly permissive to HIV-1 infection due to increased CCR5 expression.[61,62] T follicular helper (Tfh) cells from lymph nodes and spleens express CCR5[63] and have been shown to be highly HIV-infected.[64,65] Interestingly enough, CCR5 is increasingly expressed as cells become more differentiated (e.g. central memory to effector memory)[66] and central memory cells have been shown to harbor most of the HIV DNA[67] and effector memory cells inducible HIV.[6] *M. tuberculosis* specific CD4$^+$ T cells show increased expression of CCR5[58] and thus higher HIV infection rates (2.5 fold enrichment).[22] CMV-specific CD4$^+$ cells that produce MIP-1β either block and/or display lower expressions of CCR5 and are thus less frequently infected by HIV (Fig. 1A).[21] Other memory CD4$^+$ T cells targeting other pathogens, such as rhinoviruses,[68] have been shown to express high levels of CCR5, but their role as HIV reservoirs has not been investigated.

5.2. Restriction

Factors targeting multiple steps of the viral replication cycle such as CCR5-mediated entry, the uncoating, reverse transcription, release and *env* processing (recently reviewed in[69]) have been described. These restriction factors are able to block HIV replication and include the following proteins: apolipoprotein B mRNA-editing enzyme catalytic polypeptide-like 3G (APOBEC3G), tetherin/bone marrow stromal cell antigen 2 (BST2)/CD317, sterile α motif and histidine-aspartate domain-containing protein1 (SAMHD1) and tripartite-motif-containing 5α (TRIM5α, essentially in non-human primates[70]). The APOBEC3G levels have been shown to be increased in Th1 cells[71] but not in Th17 cells.[72] HIV-specific cells from elite controllers maintain a highly differentiated Th1 phenotype through infection,[73] and could thus resist infection in part through APOBEC3G restriction. Functional cell subsets express various levels of SAMHD1.[74] The SAMHD1^{low} CD4$^+$ T cells are highly enriched in both productively and latently infected cells.[75] Also, CMV-specific cells express higher levels of TRIM factors.[24] Altogether, some restriction factors are differentially expressed during infection and according to compartments[76] and T cells subsets,[74,77,78] likely modulated by cytokines and interferons,[79–82] which could be linked to specificity.

5.3. Exhaustion and infection

Immune check point molecules are co-inhibitory receptors which downmodulate immune responses to prevent hyperimmune activation, minimize collateral damage, and maintain peripheral self-tolerance. The expression of several of these molecules by HIV-infected cells, including the programmed cell death protein 1 (PD-1), cytotoxic T-lymphocyte-associated protein 4 (CTLA-4), T -cell immunoreceptor with immunoglobulin and ITIM domains (TIGIT), T-cell immunoglobulin and mucin containing protein-3 (Tim3) and lymphocyte-activation gene 3 (LAG3), has been reported in numerous studies.[3,4,6,33,83–86] Productively infected cells were shown to be highly enriched PD-1+ CD4 T cells in blood[6,33] and lymph nodes.[3,64] However, targeting PD-1 *in vitro* did not show an effect on the frequency of productively infected cells.[86] Cells from different antigenic specificities (e.g. HIV, CMV, EBV, *M. tuberculosis*) displayed various levels of immune check point molecules, with sometimes some conflicting results that may be explained by some clinical differences in the cohorts studied (e.g. PD-1, TIGIT, CTLA-4).[84,87–89] As an example, PD-1, TIGIT and CD200 expression on HIV-specific $CD4^+$ T-cells depends on their polarization and cytokine production profile.[84] Also, CMV-specific effector memory $CD4^+$ T cells did not show an increase in PD-1 expression when compared to other effector memory cells.[90] These differential expression levels could explain preferential infection of pathogen-specific T cell populations.

5.4. Miscellaneous

Infected cells are also enriched in cell subsets expressing other markers. For instance, $CD2^{high}$,[91] $CD30^+$,[92] $CD57^{-1}$ $CD20^+$,[93] $CD161^+$[94] were all enriched in latently infected cells in individuals on suppressive ART. Recently, controversial data showed CD32a expression could be a marker of HIV replication-competent reservoir.[95–99] Besides, both cell subsets harboring integrins α4β7 and α4β1, mediating migration to the gut or inflamed tissues, respectively, have been shown to be highly susceptible to HIV infection.[6,100,101] Although, this could play a role in the selective infection of pathogen-specific cells, the frequency of these cell subsets in antigen-specific cell populations, has not yet been evaluated.

Mechanisms involved in these preferential infection and selection processes include HIV entry, restriction, exhaustion, survival, self-renewal and immune escape.

Bcl2: B-cell lymphoma 2, FasL: Fas ligand, MHC-I: major histocompatibility complex class I, MIP-1β: macrophage inflammatory protein 1β, PD-L1: Programmed cell-death ligand 1, PD-1: Programmed cell-death protein 1, TRIM5α: tripartite-motif-containing 5α.

6. Host cell factors influencing persistence

6.1. Self-renewal

Effector cytokine IL-2 is an early cytokine expressed by less differentiated memory $CD4^+$ T cells, such as central memory cells that is involved in both proliferation and differentiation of antigen-specific cells. The capacity of central memory cells to produce IL-2 upon antigen stimulation[102] maintains the pool of latently infected central memory cells by self-renewal, whereas the effector memory reservoir is maintained by homeostatic proliferation.[2] Furthermore, cells expressing the IL-2 receptor α chain (CD25) were shown to predominantly support productive[103] and latent infection.[104] Similarly, Tregs, expressing high levels of CD25, were highly enriched in HIV DNA and could produce infectious virions during ART.[105,106] Some CMV-specific $CD4^+$ T cells were shown to be Tregs[107] that express high levels of CD25, which could explain the preferential persistence of HIV-infected CMV-specific during ART through clonal expansion (Fig. 1B).[31] However, CMV-specific do not express high levels of CD127 (IL-7 receptor), which might be due to their Treg polarization as well, and thus may not be sensitive to IL-7-induced proliferation.[90]

6.2. Survival

Overexpression of pro-survival factor B-cell lymphoma 2 (Bcl2) and serinen proteinase inhibitor B9 (serpin B9) have been recently described among latently (but not productively) infected cells,[108,109] causing a possible resistance to $CD8^+$ T cell-mediated killing. Besides, Collora et *al.* have also noted a trend toward higher levels of expression of serpin B9 in HIV- and CMV-specific cells compared to memory cells. Thus, persistence of HIV-infected CMV-specific cells on ART could be attributed to preferential survival.

6.3. Immune escape

HIV has evolved mechanisms to evade the host's immune responses. For instance, HIV proteins such as *nef*, *vpu* and *env* mediate CD4 and/or MHC class I downregulation, allowing infected cells to avoid cytotoxic $CD8^+$ T lymphocyte killing.[110–113] Recently, it was shown that *nef* activity is higher in cells with a more differentiated memory subset.[114]

Other molecules expressed by HIV-infected cells could induce apoptosis or senescence of cytotoxic $CD8^+$ T lymphocytes and thus be involved in HIV immune escape mechanisms. Fas ligand (FasL) expression has been shown to be higher on infected cells in SIV and Friend virus models.[115–117] FasL may protect infected cells from cytotoxic $CD8^+$ T cell killing, thus providing a route for escaping immune responses.

Similarly, both virions and infected cells were recently shown to display high levels of PD-L1, an immune check point ligand that could induce exhaustion of $CD8^+$ T cells.[118] PD-L1 expression differs at the specificity level[119] and could thus be a mechanism of preferential persistence.

6.4. Exhaustion and persistence

Immune check point molecules may play a role in HIV persistence as infected cells expressing multiple immune check points simultaneously including PD-1, TIGIT, and the lymphocyte-activation gene 3 (LAG3) were found to be highly enriched during ART.[4,6] In a recent large immune profiling analysis, it was shown that reservoir cells were progressively selected, starting at ART initiation (including cells that undergo clonal expansion), according to their phenotypic features, including immune check point markers.[120] Altogether, this suggests that these inhibitory receptors not only suppress T-cell activation but consequently also HIV transcription and therefore favor HIV latency.[86,120] Thus, targeting PD-1 *in vitro* could induce latency reactivation.[83,121] PD-1 and CTLA-4 are expressed on HIV-specific cells at higher levels then on CMV-specific or their memory counterparts[122,123] and could thus help selecting persistent HIV-infected HIV-specific cells during ART.

Altogether, some key phenotypic features have been highlighted from previous studies, but these features have not been characterized in all types of antigen-specific cells and some data remains controversial. Also, these pathways may not solely explain preferential infection/persistence and researching other pathways is needed.

7. Conclusions

HIV-infected cells consist mostly of antigen-experienced memory $CD4^+$ T cells.[67] Given the diversity of both their repertoire and the antigenic history of each individual living with HIV, studying HIV-infected, antigen-experienced cells is challenging. Some $CD4^+$ T cells were shown to support productive infection such as HIV- and *M. tuberculosis*-specific cells during untreated infection. The work of Koup and colleagues has identified host cell factors that could shape the landscape of HIV-infected cells, showing that MIP-1β expression on CMV-specific cells renders them less permissive to infection. However,

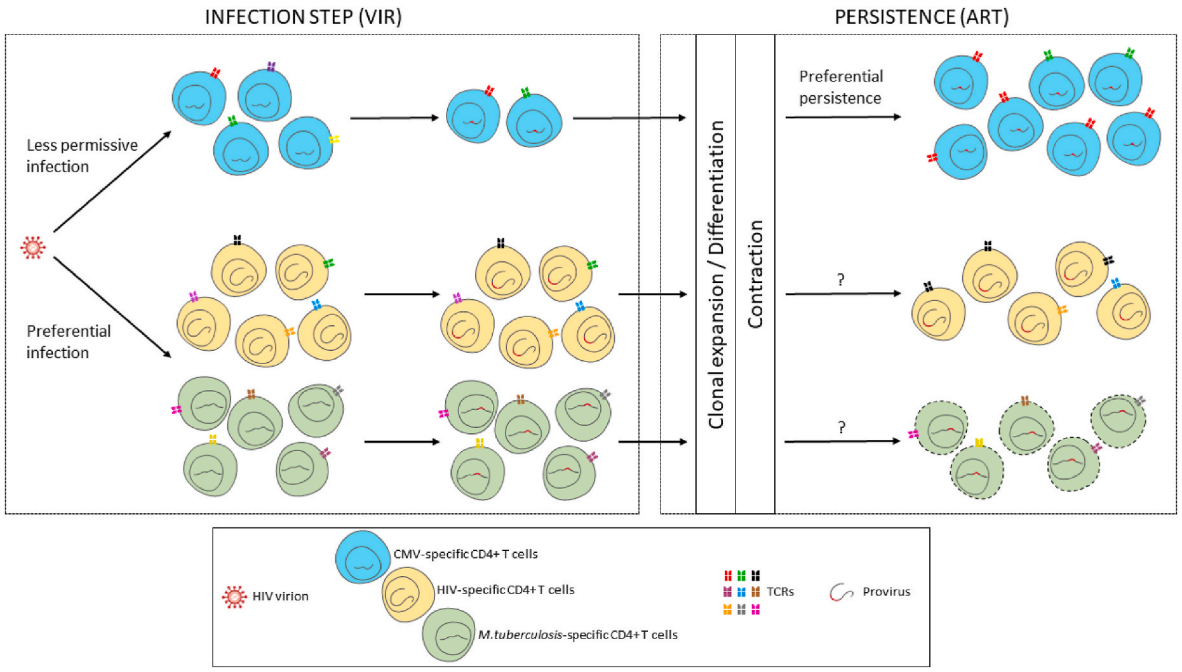

Fig. 2. Antigen specificities of HIV-infected cells during untreated HIV infection and on antiretroviral therapy.

specific cellular factors favoring permissiveness and persistence of other antigen-specific cells are still to be identified. On the other hand, latent HIV infection during ART has been described in HIV-, CMV-, influenza-, *M. tuberculosis*- and *S. aureus*-specific cells, with various degrees of enrichment. Interestingly enough, although HIV-infected CMV-specific cells are less permissive to HIV infection, they undergo a clonal expansion process called memory inflation which does not contract in the long term.[124] These cells become an important pool of latently infected cells during ART, likely due to repeated antigenic exposures associated with frequent CMV reactivations (Fig. 2).

However, several limitations to these studies can be highlighted. Most of those providing direct evidence of infection of antigen specific cells used several assays aimed at determining both antigenspecificity (e.g. differences between assays, peptides, markers) and HIV infection, that prevent straight forward comparisons of their results. Although Th1 cells were described to harbor more frequently intact HIV proviruses,[10] intactness and inducibility information in latently infected antigen specific cells is scarce. Untreated/on ART comparisons are rarely possible: the role of influenza-specific cells to support productive infection during untreated infection and the contribution of *M. tuberculosis*-specific cells as viral reservoir during ART have not been studied as yet and are to be further studied. Some antigens remain unexplored; as an example, it was shown that Th17 cells are particular targets of HIV, thus, the role of intestinal and vaginal microbiota-specific cells should be evaluated.[125,126] Indeed, most studies have included mainly men (sometimes only them). There may be some variation in antigen specificities and phenotypic features of infected cells in women.

Overall, we know that latent proviruses are archived in memory cells during untreated infection before ART is initiated.[34,35] Thus, latent infection is established in antigen-experienced memory cells that reflect previous immunizations with common and opportunistic pathogens (e.g. CMV, *M. tuberculosis*) or vaccines (e.g. influenza). Depending on the history of immunizations, the pool of latently infected cells is likely to be both diverse and variable between individuals. Further persistence of these cells during ART could then depend on both their phenotypic characteristics (e.g. pro-survival signaling) and the persistence of their antigen (e.g. chronic versus acute resolutive infections). HIV-infected cells specific to chronic infections with reactivations like CMV infection are thus at higher risk of expanding and persisting than in other cells such as influenza-specific cells. Targeting cells reactive to a given pathogen highly susceptible to harbor HIV might be of interest in an HIV cure perspective. Removing antigen exposures could favor the contraction of some HIV-infected clones, but it is unknown is this would help decreasing the size of the viral reservoir. Another issue is antigen-specificities of infected cells variability, that should be defined in a personal antigenic-profiling of infected cells before any intervention. It could be of interest to retrospectively analyze reservoir modifications in HIV/CMV vaccine trials, to see if some specific clones were expanded after vaccination. Conversely, letermovir (a recently approved anti-CMV drug) could dampen CMV reactivation and thus decrease antigen exposure and thus stop inflationary memory and maybe help the contraction of CMV-specific HIV-infected clones.

Preferential infection drives productive infection during off ART, whereas preferential persistence of latently infected cells occurs during ART.

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Declaration of competing interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

Data availability

No data was used for the research described in the article.

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References

1 Brenchley JM, et al. T-cell subsets that harbor human immunodeficiency virus (HIV) in vivo: implications for HIV pathogenesis. *J Virol*. 2004;78:1160–1168. https://doi.org/10.1128/jvi.78.3.1160-1168.2004.

2 Chomont N, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009;15:893–900. https://doi.org/10.1038/nm.1972.

3 Banga R, et al. PD-1(+) and follicular helper T cells are responsible for persistent HIV-1 transcription in treated aviremic individuals. *Nat Med*. 2016;22:754–761. https://doi.org/10.1038/nm.4113.

4 Fromentin R, et al. CD4+ T cells expressing PD-1, TIGIT and LAG-3 contribute to HIV persistence during ART. *PLoS Pathog*. 2016;12, e1005761. https://doi.org/10.1371/journal.ppat.1005761.

5 Gosselin A, et al. HIV persists in CCR6+CD4+ T cells from colon and blood during antiretroviral therapy. *AIDS*. 2017;31:35–48. https://doi.org/10.1097/QAD.0000000000001309.

6 Pardons M, et al. Single-cell characterization and quantification of translation-competent viral reservoirs in treated and untreated HIV infection. *PLoS Pathog*. 2019;15, e1007619. https://doi.org/10.1371/journal.ppat.1007619.

7 Bruner KM, et al. Defective proviruses rapidly accumulate during acute HIV-1 infection. *Nat Med*. 2016;22:1043–1049. https://doi.org/10.1038/nm.4156.

8 Hiener B, et al. Identification of genetically intact HIV-1 proviruses in specific CD4(+) T cells from effectively treated participants. *Cell Rep*. 2017;21:813–822. https://doi.org/10.1016/j.celrep.2017.09.081.

9 Wagner TA, et al. HIV latency. Proliferation of cells with HIV integrated into cancer genes contributes to persistent infection. *Science*. 2014;345:570–573. https://doi.org/10.1126/science.1256304.

10 Lee GQ, et al. Clonal expansion of genome-intact HIV-1 in functionally polarized Th1 CD4+ T cells. *J Clin Invest*. 2017;127:2689–2696. https://doi.org/10.1172/JCI93289.

11 Einkauf KB, et al. Intact HIV-1 proviruses accumulate at distinct chromosomal positions during prolonged antiretroviral therapy. *J Clin Invest*. 2019;129:988–998. https://doi.org/10.1172/JCI124291.

12 Cole B, et al. In-depth single-cell analysis of translation-competent HIV-1 reservoirs identifies cellular sources of plasma viremia. *Nat Commun*. 2021;12:3727. https://doi.org/10.1038/s41467-021-24080-1.

13 Siliciano JD, et al. Long-term follow-up studies confirm the stability of the latent reservoir for HIV-1 in resting CD4+ T cells. *Nat Med*. 2003;9:727–728. https://doi.org/10.1038/nm880.

14 Baxter AE, et al. Single-cell characterization of viral translation-competent reservoirs in HIV-infected individuals. *Cell Host Microbe*. 2016;20:368–380. https://doi.org/10.1016/j.chom.2016.07.015.

15 Yukl SA, et al. HIV latency in isolated patient CD4(+) T cells may be due to blocks in HIV transcriptional elongation, completion, and splicing. *Sci Transl Med*. 2018;10. https://doi.org/10.1126/scitranslmed.aap9927.

16 Sannier G, et al. Combined single-cell transcriptional, translational, and genomic profiling reveals HIV-1 reservoir diversity. *Cell Rep*. 2021;36, 109643. https://doi.org/10.1016/j.celrep.2021.109643.

17 Cheynier R, et al. Antigenic stimulation by BCG vaccine as an in vivo driving force for SIV replication and dissemination. *Nat Med*. 1998;4:421–427. https://doi.org/10.1038/nm0498-421.

18 Douek DC, et al. HIV preferentially infects HIV-specific CD4+ T cells. *Nature*. 2002;417:95–98. https://doi.org/10.1038/417095a.

19 Demoustier A, et al. In patients on prolonged HAART, a significant pool of HIV infected CD4 T cells are HIV-specific. *AIDS*. 2002;16:1749–1754. https://doi.org/10.1097/00002030-200209060-00006.

20 Renoux C, Wain-Hobson S, Hurtrel B, Cheynier R. Antigenic stimulation specifically reactivates the replication of archived simian immunodeficiency virus genomes in chronically infected macaques. *J Virol*. 2005;79:11231–11238. https://doi.org/10.1128/JVI.79.17.11231-11238.2005.

21 Casazza JP, et al. Autocrine production of beta-chemokines protects CMV-Specific CD4 T cells from HIV infection. *PLoS Pathog*. 2009;5, e1000646. https://doi.org/10.1371/journal.ppat.1000646.

22 Geldmacher C, et al. Preferential infection and depletion of Mycobacterium tuberculosis-specific CD4 T cells after HIV-1 infection. *J Exp Med*. 2010;207:2869–2881. https://doi.org/10.1084/jem.20100090.

23 Jones RB, Kovacs C, Chun TW, Ostrowski MA. Short communication: HIV type 1 accumulates in influenza-specific T cells in subjects receiving seasonal vaccination in the context of effective antiretroviral therapy. *AIDS Res Hum Retrovir*. 2012;28:1687–1692. https://doi.org/10.1089/AID.2012.0115.

24 Hu H, et al. Distinct gene-expression profiles associated with the susceptibility of pathogen-specific CD4 T cells to HIV-1 infection. *Blood*. 2013;121:1136–1144. https://doi.org/10.1182/blood-2012-07-446278.

25 Yek C, et al. Standard vaccines increase HIV-1 transcription during antiretroviral therapy. *AIDS*. 2016;30:2289–2298. https://doi.org/10.1097/QAD.0000000000001201.

26 Henrich TJ, et al. Human immunodeficiency virus type 1 persistence following systemic chemotherapy for malignancy. *J Infect Dis*. 2017;216:254–262. https://doi.org/10.1093/infdis/jix265.

27 Kristoff J, et al. Type 1-programmed dendritic cells drive antigen-specific latency reversal and immune elimination of persistent HIV-1. *EBioMedicine*. 2019;43:295–306. https://doi.org/10.1016/j.ebiom.2019.03.077.

28 Hey-Nguyen WJ, et al. HIV-1 DNA is maintained in antigen-specific CD4+ T cell subsets in patients on long-term antiretroviral therapy regardless of recurrent antigen exposure. *AIDS Res Hum Retrovir*. 2019;35:112–120. https://doi.org/10.1089/AID.2018.0235.

29 Mendoza P, et al. Antigen-responsive CD4+ T cell clones contribute to the HIV-1 latent reservoir. *J Exp Med*. 2020;217. https://doi.org/10.1084/jem.20200051.

30 Gantner P, et al. Single-cell TCR sequencing reveals phenotypically diverse clonally expanded cells harboring inducible HIV proviruses during ART. *Nat Commun*. 2020;11:4089. https://doi.org/10.1038/s41467-020-17898-8.

31 Simonetti FR, et al. Antigen-driven clonal selection shapes the persistence of HIV-1-infected CD4+ T cells in vivo. *J Clin Invest*. 2021;131. https://doi.org/10.1172/JCI145254.

32 Cattin A, et al. RALDH activity induced by bacterial/fungal pathogens in CD16(+) monocyte-derived dendritic cells boosts HIV infection and outgrowth in CD4(+) T cells. *J Immunol*. 2021;206:2638–2651. https://doi.org/10.4049/jimmunol.2001436.

33 Gantner P, et al. HIV rapidly targets a diverse pool of CD4+ T cells to establish productive and latent infections. *Immunity*. 2023;56(3):653–668.e5. https://doi.org/10.1016/j.immuni.2023.01.030.

34 Abrahams MR, et al. The replication-competent HIV-1 latent reservoir is primarily established near the time of therapy initiation. *Sci Transl Med*. 2019;11. https://doi.org/10.1126/scitranslmed.aaw5589.

35 Brooks K, et al. HIV-1 variants are archived throughout infection and persist in the reservoir. *PLoS Pathog*. 2020;16, e1008378. https://doi.org/10.1371/journal.ppat.1008378.

36 White JA, et al. Complex decay dynamics of HIV virions, intact and defective proviruses, and 2LTR circles following initiation of antiretroviral therapy. *Proc Natl Acad Sci U S A*. 2022;119. https://doi.org/10.1073/pnas.2120326119.

37 Fray EJ, et al. Antiretroviral therapy reveals triphasic decay of intact SIV genomes and persistence of ancestral variants. *Cell Host Microbe*. 2023;31:356–372 e355. https://doi.org/10.1016/j.chom.2023.01.016.

38 Wei X, et al. Viral dynamics in human immunodeficiency virus type 1 infection. *Nature*. 1995;373:117–122. https://doi.org/10.1038/373117a0.

39 Perelson AS, Neumann AU, Markowitz M, Leonard JM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell life-span, and viral generation time. *Science*. 1996;271:1582–1586. https://doi.org/10.1126/science.271.5255.1582.

40 Chun TW, et al. In vivo fate of HIV-1-infected T cells: quantitative analysis of the transition to stable latency. *Nat Med*. 1995;1:1284–1290. https://doi.org/10.1038/nm1295-1284.

41 Chun TW, et al. Quantification of latent tissue reservoirs and total body viral load in HIV-1 infection. *Nature*. 1997;387:183–188. https://doi.org/10.1038/387183a0.

42 Chun TW, et al. Presence of an inducible HIV-1 latent reservoir during highly active antiretroviral therapy. *Proc Natl Acad Sci U S A*. 1997;94:13193–13197. https://doi.org/10.1073/pnas.94.24.13193.

43 Finzi D, et al. Identification of a reservoir for HIV-1 in patients on highly active antiretroviral therapy. *Science*. 1997;278:1295–1300. https://doi.org/10.1126/science.278.5341.1295.

44 Wong JK, et al. Recovery of replication-competent HIV despite prolonged suppression of plasma viremia. *Science*. 1997;278:1291–1295. https://doi.org/10.1126/science.278.5341.1291.

45 Finzi D, et al. Latent infection of CD4+ T cells provides a mechanism for lifelong persistence of HIV-1, even in patients on effective combination therapy. *Nat Med*. 1999;5:512–517. https://doi.org/10.1038/8394.

46 Leyre L, et al. Abundant HIV-infected cells in blood and tissues are rapidly cleared upon ART initiation during acute HIV infection. *Sci Transl Med*. 2020;12. https://doi.org/10.1126/scitranslmed.aav3491.

47 Cohn LB, et al. HIV-1 integration landscape during latent and active infection. *Cell*. 2015;160:420–432. https://doi.org/10.1016/j.cell.2015.01.020.

48 Wang Z, et al. Expanded cellular clones carrying replication-competent HIV-1 persist, wax, and wane. *Proc Natl Acad Sci U S A*. 2018;115:E2575–E2584. https://doi.org/10.1073/pnas.1720665115.

49 Gantner P, et al. HIV rapidly targets a diverse pool of CD4(+) T cells to establish productive and latent infections. *Immunity*. 2023. https://doi.org/10.1016/j.immuni.2023.01.030.

50 Berry NJ, et al. Seroepidemiologic studies on the acquisition of antibodies to cytomegalovirus, herpes simplex virus, and human immunodeficiency virus among general hospital patients and those attending a clinic for sexually transmitted diseases. *J Med Virol*. 1988;24:385–393. https://doi.org/10.1002/jmv.1890240405.

51 Robain M, Carre N, Dussaix E, Salmon-Ceron D, Meyer L. Incidence and sexual risk factors of cytomegalovirus seroconversion in HIV-infected subjects. The SEROCO Study Group. *Sex Transm Dis*. 1998;25:476–480. https://doi.org/10.1097/00007435-199810000-00006.

52 Bell LCK, Noursadeghi M. Pathogenesis of HIV-1 and Mycobacterium tuberculosis co-infection. *Nat Rev Microbiol*. 2018;16:80–90. https://doi.org/10.1038/nrmicro.2017.128.

53 Coffin JM, et al. Integration in oncogenes plays only a minor role in determining the in vivo distribution of HIV integration sites before or during suppressive antiretroviral therapy. *PLoS Pathog*. 2021;17, e1009141. https://doi.org/10.1371/journal.ppat.1009141.

54 Balachandran N, et al. Incidence of laboratory-confirmed influenza among HIV-infected versus HIV-uninfected individuals in two districts of Ghana, 2014 to 2016. *Am J Trop Med Hyg*. 2021;105:783–787. https://doi.org/10.4269/ajtmh.20-1437.

55 Gallagher KM, et al. Predictors of influenza vaccination in HIV-infected patients in the United States, 1990-2002. *J Infect Dis*. 2007;196:339–346. https://doi.org/10.1086/519165.

56 Dragic T, et al. HIV-1 entry into CD4+ cells is mediated by the chemokine receptor CC-CKR-5. *Nature*. 1996;381:667–673. https://doi.org/10.1038/381667a0.

57 Claireaux M, et al. Low CCR5 expression protects HIV-specific CD4+ T cells of elite controllers from viral entry. *Nat Commun*. 2022;13:521. https://doi.org/10.1038/s41467-022-28130-0.
58 Zaunders J, et al. Mapping the extent of heterogeneity of human CCR5+ CD4+ T cells in peripheral blood and lymph nodes. *AIDS*. 2020;34:833–848. https://doi.org/10.1097/QAD.0000000000002503.
59 Bonecchi R, et al. Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med*. 1998;187:129–134. https://doi.org/10.1084/jem.187.1.129.
60 Loetscher P, et al. CCR5 is characteristic of Th1 lymphocytes. *Nature*. 1998;391:344–345. https://doi.org/10.1038/34814.
61 Gosselin A, et al. Peripheral blood CCR4+CCR6+ and CXCR3+CCR6+CD4+ T cells are highly permissive to HIV-1 infection. *J Immunol*. 2010;184:1604–1616. https://doi.org/10.4049/jimmunol.0903058.
62 McKinnon LR, et al. Characterization of a human cervical CD4+ T cell subset coexpressing multiple markers of HIV susceptibility. *J Immunol*. 2011;187:6032–6042. https://doi.org/10.4049/jimmunol.1101836.
63 Miller SM, et al. Follicular regulatory T cells are highly permissive to R5-tropic HIV-1. *J Virol*. 2017;91. https://doi.org/10.1128/JVI.00430-17.
64 Perreau M, et al. Follicular helper T cells serve as the major CD4 T cell compartment for HIV-1 infection, replication, and production. *J Exp Med*. 2013;210:143–156. https://doi.org/10.1084/jem.20121932.
65 Colineau L, et al. HIV-infected spleens present altered follicular helper T cell (tfh) subsets and skewed B cell maturation. *PLoS One*. 2015;10, e0140978. https://doi.org/10.1371/journal.pone.0140978.
66 Sallusto F, Lenig D, Forster R, Lipp M, Lanzavecchia A. Two subsets of memory T lymphocytes with distinct homing potentials and effector functions. *Nature*. 1999;401:708–712. https://doi.org/10.1038/44385.
67 Chomont N, et al. HIV reservoir size and persistence are driven by T cell survival and homeostatic proliferation. *Nat Med*. 2009;15:893–900. https://doi.org/10.1038/nm.1972.
68 Muehling LM, et al. Single-cell tracking reveals a role for pre-existing CCR5+ memory Th1 cells in the control of rhinovirus-A39 after experimental challenge in humans. *J Infect Dis*. 2018;217:381–392. https://doi.org/10.1093/infdis/jix514.
69 Hadpech S, Moonmuang S, Chupradit K, Yasamut U, Tayapiwatana C. Updating on roles of HIV intrinsic factors: a review of their antiviral mechanisms and emerging functions. *Intervirology*. 2022;65:67–79. https://doi.org/10.1159/000519241.
70 Stremlau M, et al. The cytoplasmic body component TRIM5alpha restricts HIV-1 infection in Old World monkeys. *Nature*. 2004;427:848–853. https://doi.org/10.1038/nature02343.
71 Vetter ML, Johnson ME, Antons AK, Unutmaz D, D'Aquila RT. Differences in APOBEC3G expression in CD4+ T helper lymphocyte subtypes modulate HIV-1 infectivity. *PLoS Pathog*. 2009;5, e1000292. https://doi.org/10.1371/journal.ppat.1000292.
72 Christensen-Quick A, et al. Human Th17 cells lack HIV-inhibitory RNases and are highly permissive to productive HIV infection. *J Virol*. 2016;90:7833–7847. https://doi.org/10.1128/JVI.02869-15.
73 Vingert B, et al. HIV controllers maintain a population of highly efficient Th1 effector cells in contrast to patients treated in the long term. *J Virol*. 2012;86:10661–10674. https://doi.org/10.1128/JVI.00056-12.
74 Ruffin N, et al. Low SAMHD1 expression following T-cell activation and proliferation renders CD4+ T cells susceptible to HIV-1. *AIDS*. 2015;29:519–530. https://doi.org/10.1097/QAD.0000000000000594.
75 Hani L, et al. Proliferative memory SAMHD1low CD4+ T cells harbour high levels of HIV-1 with compartmentalized viral populations. *PLoS Pathog*. 2019;15, e1007868. https://doi.org/10.1371/journal.ppat.1007868.
76 Singh R, et al. TRIM5alpha and TRIM22 are differentially regulated according to HIV-1 infection phase and compartment. *J Virol*. 2014;88:4291–4303. https://doi.org/10.1128/JVI.03603-13.
77 Rahmberg AR, Rajakumar PA, Billingsley JM, Johnson RP. Dynamic modulation of expression of lentiviral restriction factors in primary CD4(+) T cells following simian immunodeficiency virus infection. *J Virol*. 2017;91. https://doi.org/10.1128/JVI.02189-16.
78 Abdel-Mohsen M, et al. Expression profile of host restriction factors in HIV-1 elite controllers. *Retrovirology*. 2013;10:106. https://doi.org/10.1186/1742-4690-10-106.
79 Peng G, Lei KJ, Jin W, Greenwell-Wild T, Wahl SM. Induction of APOBEC3 family proteins, a defensive maneuver underlying interferon-induced anti-HIV-1 activity. *J Exp Med*. 2006;203:41–46. https://doi.org/10.1084/jem.20051512.
80 Blasius AL, et al. Bone marrow stromal cell antigen 2 is a specific marker of type I IFN-producing cells in the naive mouse, but a promiscuous cell surface antigen following IFN stimulation. *J Immunol*. 2006;177:3260–3265. https://doi.org/10.4049/jimmunol.177.5.3260.
81 Asaoka K, et al. A retrovirus restriction factor TRIM5alpha is transcriptionally regulated by interferons. *Biochem Biophys Res Commun*. 2005;338:1950–1956. https://doi.org/10.1016/j.bbrc.2005.10.173.
82 Goujon C, Malim MH. Characterization of the alpha interferon-induced postentry block to HIV-1 infection in primary human macrophages and T cells. *J Virol*. 2010;84:9254–9266. https://doi.org/10.1128/JVI.00854-10.
83 Fromentin R, et al. PD-1 blockade potentiates HIV latency reversal ex vivo in CD4(+) T cells from ART-suppressed individuals. *Nat Commun*. 2019;10:814. https://doi.org/10.1038/s41467-019-08798-7.
84 Brunet-Ratnasingham E, et al. Immune checkpoint expression on HIV-specific CD4+ T cells and response to their blockade are dependent on lineage and function. *EBioMedicine*. 2022;84, 104254. https://doi.org/10.1016/j.ebiom.2022.104254.
85 Chew GM, et al. TIGIT marks exhausted T cells, correlates with disease progression, and serves as a target for immune restoration in HIV and SIV infection. *PLoS Pathog*. 2016;12, e1005349. https://doi.org/10.1371/journal.ppat.1005349.
86 Evans VA, et al. Programmed cell death-1 contributes to the establishment and maintenance of HIV-1 latency. *AIDS*. 2018;32:1491–1497. https://doi.org/10.1097/QAD.0000000000001849.
87 Parry HM, et al. PD-1 is imprinted on cytomegalovirus-specific CD4+ T cells and attenuates Th1 cytokine production whilst maintaining cytotoxicity. *PLoS Pathog*. 2021;17, e1009349. https://doi.org/10.1371/journal.ppat.1009349.
88 Pollock KM, et al. Differences in antigen-specific CD4+ responses to opportunistic infections in HIV infection. *Immun Inflamm Dis*. 2015;3:141–153. https://doi.org/10.1002/iid3.50.
89 Raziorrouh B, et al. Inhibitory molecules that regulate expansion and restoration of HCV-specific CD4+ T cells in patients with chronic infection. *Gastroenterology*. 2011;141:1422–1431. https://doi.org/10.1053/j.gastro.2011.07.004, 1431 e1421-1426.
90 Abana CO, et al. Cytomegalovirus (CMV) epitope-specific CD4(+) T cells are inflated in HIV(+) CMV(+) subjects. *J Immunol*. 2017;199:3187–3201. https://doi.org/10.4049/jimmunol.1700851.
91 Iglesias-Ussel M, Vandergeeten C, Marchionni L, Chomont N, Romerio F. High levels of CD2 expression identify HIV-1 latently infected resting memory CD4+ T cells in virally suppressed subjects. *J Virol*. 2013;87:9148–9158. https://doi.org/10.1128/JVI.01297-13.
92 Hogan LE, et al. Increased HIV-1 transcriptional activity and infectious burden in peripheral blood and gut-associated CD4+ T cells expressing CD30. *PLoS Pathog*. 2018;14, e1006856. https://doi.org/10.1371/journal.ppat.1006856.
93 Serra-Peinado C, et al. Expression of CD20 after viral reactivation renders HIV-reservoir cells susceptible to Rituximab. *Nat Commun*. 2019;10:3705. https://doi.org/10.1038/s41467-019-11556-4.
94 Li X, et al. CD161(+) CD4(+) T cells harbor clonally expanded replication-competent HIV-1 in antiretroviral therapy-suppressed individuals. *mBio*. 2019;10. https://doi.org/10.1128/mBio.02121-19.
95 Descours B, et al. CD32a is a marker of a CD4 T-cell HIV reservoir harbouring replication-competent proviruses. *Nature*. 2017;543:564–567. https://doi.org/10.1038/nature21710.
96 Abdel-Mohsen M, et al. CD32 is expressed on cells with transcriptionally active HIV but does not enrich for HIV DNA in resting T cells. *Sci Transl Med*. 2018;10. https://doi.org/10.1126/scitranslmed.aar6759.
97 Osuna CE, et al. Evidence that CD32a does not mark the HIV-1 latent reservoir. *Nature*. 2018;561:E20–E28. https://doi.org/10.1038/s41586-018-0495-2.
98 Bertagnolli LN, et al. The role of CD32 during HIV-1 infection. *Nature*. 2018;561:E17–E19. https://doi.org/10.1038/s41586-018-0494-3.
99 Perez L, et al. Conflicting evidence for HIV enrichment in CD32(+) CD4 T cells. *Nature*. 2018;561:E9–E16. https://doi.org/10.1038/s41586-018-0493-4.
100 Cicala C, et al. The integrin alpha4beta7 forms a complex with cell-surface CD4 and defines a T-cell subset that is highly susceptible to infection by HIV-1. *Proc Natl Acad Sci U S A*. 2009;106:20877–20882. https://doi.org/10.1073/pnas.0911796106.
101 Sivro A, et al. Integrin alpha4beta7 expression on peripheral blood CD4(+) T cells predicts HIV acquisition and disease progression outcomes. *Sci Transl Med*. 2018;10. https://doi.org/10.1126/scitranslmed.aam6354.
102 Maus MV, et al. Extensive replicative capacity of human central memory T cells. *J Immunol*. 2004;172:6675–6683. https://doi.org/10.4049/jimmunol.172.11.6675.
103 Borvak J, et al. Expression of CD25 defines peripheral blood mononuclear cells with productive versus latent HIV infection. *J Immunol*. 1995;155:3196–3204.
104 Rocco J, Mellors JW, Macatangay B. J. Regulatory T cells: the ultimate HIV reservoir? *J Virus Erad*. 2018;4:209–214.
105 Jiao YM, et al. CD4+CD25+CD127 regulatory cells play multiple roles in maintaining HIV-1 p24 production in patients on long-term treatment: HIV-1 p24-producing cells and suppression of anti-HIV immunity. *Int J Infect Dis*. 2015;37:42–49. https://doi.org/10.1016/j.ijid.2015.06.008.
106 Dunay GA, et al. Assessment of the HIV-1 reservoir in CD4+ regulatory T cells by a Droplet Digital PCR based approach. *Virus Res*. 2017;240:107–111. https://doi.org/10.1016/j.virusres.2017.07.008.
107 Lyu M, et al. Dissecting the landscape of activated CMV-stimulated CD4+ T cells in humans by linking single-cell RNA-seq with T-cell receptor sequencing. *Front Immunol*. 2021;12, 779961. https://doi.org/10.3389/fimmu.2021.779961.
108 Ren Y, et al. BCL-2 antagonism sensitizes cytotoxic t cell-resistant hiv reservoirs to elimination ex vivo. *J Clin Invest*. 2020. https://doi.org/10.1172/JCI132374.
109 Collora JA, et al. Single-cell multiomics reveals persistence of HIV-1 in expanded cytotoxic T cell clones. *Immunity*. 2022. https://doi.org/10.1016/j.immuni.2022.03.004.
110 Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. *Nature*. 1998;391:397–401. https://doi.org/10.1038/34929.
111 Lama J, Mangasarian A, Trono D. Cell-surface expression of CD4 reduces HIV-1 infectivity by blocking Env incorporation in a Nef- and Vpu-inhibitable manner. *Curr Biol*. 1999;9:622–631. https://doi.org/10.1016/s0960-9822(99)80284-x.
112 Cohen GB, et al. The selective downregulation of class I major histocompatibility complex proteins by HIV-1 protects HIV-infected cells from NK cells. *Immunity*. 1999;10:661–671. https://doi.org/10.1016/s1074-7613(00)80065-5.
113 Le Gall S, et al. Nef interacts with the mu subunit of clathrin adaptor complexes and reveals a cryptic sorting signal in MHC I molecules. *Immunity*. 1998;8:483–495. https://doi.org/10.1016/s1074-7613(00)80553-1.

114 Duette G, et al. The HIV-1 proviral landscape reveals that Nef contributes to HIV-1 persistence in effector memory CD4+ T cells. *J Clin Invest*. 2022;132. https://doi.org/10.1172/JCI154422.

115 Xu XN, et al. Evasion of cytotoxic T lymphocyte (CTL) responses by nef-dependent induction of Fas ligand (CD95L) expression on simian immunodeficiency virus-infected cells. *J Exp Med*. 1997;186:7–16. https://doi.org/10.1084/jem.186.1.7.

116 Xu XN, et al. Induction of Fas ligand expression by HIV involves the interaction of Nef with the T cell receptor zeta chain. *J Exp Med*. 1999;189:1489–1496. https://doi.org/10.1084/jem.189.9.1489.

117 Malyshkina A, et al. Fas Ligand-mediated cytotoxicity of CD4+ T cells during chronic retrovirus infection. *Sci Rep*. 2017;7:7785. https://doi.org/10.1038/s41598-017-08578-7.

118 Munoz O, et al. Active PD-L1 incorporation within HIV virions functionally impairs T follicular helper cells. *PLoS Pathog*. 2022;18, e1010673. https://doi.org/10.1371/journal.ppat.1010673.

119 Nikolova M, et al. Subset- and antigen-specific effects of Treg on CD8+ T cell responses in chronic HIV infection. *PLoS Pathog*. 2016;12, e1005995. https://doi.org/10.1371/journal.ppat.1005995.

120 Sun W, et al. Phenotypic signatures of immune selection in HIV-1 reservoir cells. *Nature*. 2023;614:309–317. https://doi.org/10.1038/s41586-022-05538-8.

121 Uldrick TS, et al. Pembrolizumab induces HIV latency reversal in people living with HIV and cancer on antiretroviral therapy. *Sci Transl Med*. 2022;14, eabl3836. https://doi.org/10.1126/scitranslmed.abl3836.

122 D'Souza M, et al. Programmed death 1 expression on HIV-specific CD4+ T cells is driven by viral replication and associated with T cell dysfunction. *J Immunol*. 2007;179:1979–1987. https://doi.org/10.4049/jimmunol.179.3.1979.

123 Kaufmann DE, et al. Upregulation of CTLA-4 by HIV-specific CD4+ T cells correlates with disease progression and defines a reversible immune dysfunction. *Nat Immunol*. 2007;8:1246–1254. https://doi.org/10.1038/ni1515.

124 Pera A, et al. CMV induces expansion of highly polyfunctional CD4+ T cell subset coexpressing CD57 and CD154. *J Leukoc Biol*. 2017;101:555–566. https://doi.org/10.1189/jlb.4A0316-112R.

125 Gosmann C, et al. Lactobacillus-deficient cervicovaginal bacterial communities are associated with increased HIV acquisition in young South African women. *Immunity*. 2017;46:29–37. https://doi.org/10.1016/j.immuni.2016.12.013.

126 Nganou-Makamdop K, et al. Translocated microbiome composition determines immunological outcome in treated HIV infection. *Cell*. 2021;184:3899–3914 e3816. https://doi.org/10.1016/j.cell.2021.05.023.