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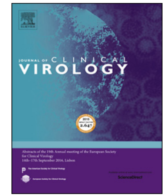

Review

Cytotoxic CD4$^+$ T-cells during HIV infection: Targets or weapons?

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ABSTRACT

Classically, CD4$^+$ T-cells have been referred as cytokine-producing cells and important players in immune responses by providing soluble factors that potentiate several effector immune functions. However, it is now evident that CD4$^+$ T-cells can also elaborate cytotoxic responses, inducing apoptosis of target cells. Cytotoxic CD4$^+$ T cells (CD4$^+$ CTLs), exhibit cytolytic functions that resemble those of CD8$^+$ T-cells; in fact, there is evidence suggesting that they may have a role in the control of viral infections. In this article, we discuss the role of CD4$^+$ CTLs during HIV infection, where CD4$^+$ CTLs have been associated with viral control and slow disease progression. In addition, we address the implication of CD4$^+$ CTLs in the context of antiretroviral therapy and the partial reconstitution of CD8$^+$ T-cells effector function.

1. Introduction

CD4$^+$ T-cells are critical components of immunity, and by producing various cytokines and expressing co-stimulatory ligands, they contribute to innate immune responses, potentiate CD8$^+$ T-cell effector functions, and promote B-cell antibody production. CD4$^+$ T-cells also limit exacerbated immune responses through various immunoregulatory mechanisms [1]. Nonetheless, it has been observed that CD4$^+$ T-cells may have direct antiviral effector functions through the lysis of infected cells [2]. Indeed, it has been proposed that cytotoxic CD4$^+$ T-cells (CD4$^+$ CTLs) constitute a unique CD4$^+$ T-cell profile, which is generated *via* the interaction of human leukocyte antigen class II (HLA-II)/peptide with T-cell receptor (TCR) in the presence of specific polarizing factors [3]. Importantly, a protective role of CD4$^+$ CTLs has also been suggested in infections caused by several viruses such as Epstein–Barr virus [4], dengue virus [5], and influenza virus [6]. However, they may also have a pathogenic role in viral hepatitis [7]. In addition, the antitumor activities of CD4$^+$ CTLs have been demonstrated [8,9]. Therefore, CD4$^+$ CTLs responses may be relevant in cases of dysfunction or immune evasion of other cytotoxic cells, such as CD8$^+$ T-cells and natural killer (NK) cells, or in tissues with regulated cell traffic, such as lymphoid follicles, which exhibit low levels of cytotoxic cells because of low expression of the follicle homing receptor CXCR5 [10].

Antigen-specific CD4$^+$ CTLs have been detected in patients with HIV infection [11–15]. Moreover, despite the high degree to which CD4$^+$ T-cells are compromised in HIV infection [16], this cell population may have antiviral activities in some scenarios. For a detailed discussion on the origin, phenotypes, and effector functions of CD4$^+$ CTLs and their roles in antiviral immunity, readers are referred to few published papers [2,3,17–19]. In the present review, we discuss the mechanisms underlying CD4$^+$ CTL induction during HIV infection and the phenotypes, effector mechanisms, and roles in disease progression. We focus on patients treated for HIV infection, in whom CD4$^+$ T-cells can be reconstituted following viral suppression induced by antiretroviral therapy (ART).

2. Origins and phenotypes of CD4$^+$ CTLs in HIV infection

To develop into effector subsets, naive CD4$^+$ T-cells receive activating signals from professional antigen-presenting cells (APCs), such as dendritic cells, macrophages, and B-cells. These signals are delivered *via* HLA-II/peptide/TCR interactions and are accompanied by secondary co-stimulatory signals, such as CD80/CD86 binding to CD28. The third signal is mediated by local cytokines and is primarily responsible for polarization to a specific CD4$^+$ T-cell effector profile [20]. This effector specificity requires a differentiation program that directs CD4$^+$ T-cells toward a particular cytotoxic profile. Similar to other CD4$^+$ T-cell populations, such as Th1, Th2, and Th17 cells, CD4$^+$ CTLs receive specific yet undefined, cytokine signals to express transcriptional and effector profiles that sustain lytic activity [2]. Type-I interferons (IFN) and interleukin (IL)-2 may provide the third signal

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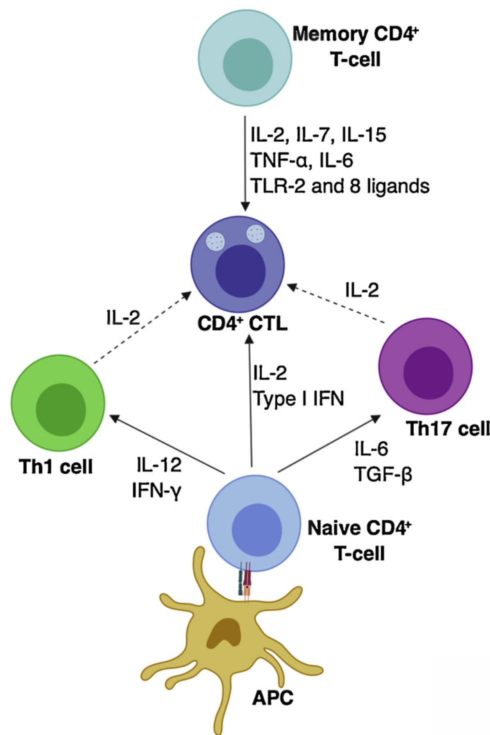

Fig. 1. Model of differentiation of CD4$^+$ CTLs. Priming of naïve CD4$^+$ T-cells by an APC in the presence of IL-2 and type I IFN promote the polarization of CD4$^+$ CTLs. Moreover, CD4$^+$ CTLs can originate from Th1 and Th17 subsets, most likely in response to IL-2. Finally, in inflammatory conditions, such as HIV infection, memory CD4$^+$ T-cells express cytotoxic molecules through the action of common γ-chain cytokines IL-2, IL-7 and IL-15, TNF-α and IL-6, or TLR-2 and 8 ligands.

required for CD4$^+$ CTL polarization (Fig. 1). In mouse CD4$^+$ T cells, these cytokines induce cooperative action of the transcription factors B lymphocyte-induced maturation protein-1 (Blimp-1) and T-bet *via* the signal transducer and activator of transcription (STAT) 2 for upregulating the cytotoxic molecules granzyme B and perforin [21]. The transcription factor eomesodermin (Eomes) is also an important regulator of CD4$^+$ CTL effector functions [22,23]. CD4$^+$ T-cells expressing class I–restricted T-cell–associated molecule (CRTAM) preferentially acquire cytotoxic profiles following activation, because this surface receptor promotes the expression of Eomes, granzyme B, and perforin [24]. Moreover, considering the plasticity of CD4$^+$ T-cell responses [25], memory Th1, Th17, or other cell subsets could eventually reprogram their transcriptional profiles in favor of cytotoxic potential (Fig. 1). Accordingly, co-stimulation of antigen-specific Th1 cells with OX40 and 4-1BB by APCs in the presence of IL-2 promotes the acquisition of T-bet, Eomes, and cytotoxic activities [22]. In the presence of IL-2, Th1 and Th17 cells also reportedly co-express granzyme B with IFN-γ and IL-17, respectively [26]. In addition, common γ-chain (IL-2, IL-7, IL-15) and pro-inflammatory (TNF-α and IL-6) cytokines, along with Toll-like receptor (TLR)-2 and TLR-8 ligands, can promote granzyme B release and viral replication in HIV-infected memory CD4$^+$ T-cells [26] (Fig. 1). These observations indicate that under the inflammatory conditions of HIV infection, activated memory CD4$^+$ T-cells exhibit cytotoxic potential, despite active HIV replication.

HLA-II expression is restricted to professional APCs under steady-state conditions, and compromise of these cells by disease may further limit the production of CD4$^+$ CTLs. Yet, at least three sources of antigen presentation have been associated with HIV infection. First, HIV-infected dendritic cells or macrophages, or APCs that have phagocytosed other infected cells, can process and present antigens to naive CD4$^+$ T-cells or antigen-specific CD4$^+$ CTLs in a HLA-II-dependent manner [27,28]. Second, non-APCs can upregulate HLA-II molecules following pathogen challenge or cytokine stimulation. For instance, in the presence of inflammatory conditions or after stimulation with IFN-γ, intestinal epithelial cells upregulate HLA-II molecules [29–31] and may be targets of CD4$^+$ CTLs. Third, activated CD4$^+$ T cells can bind HIV gp120 *via* surface receptors, and after processing can present identifying peptides to other antigen-specific CD4$^+$ T-cells [32]. Other examples of T–T interactions have been reported [33–35], and these may also play important roles in the induction of CD4$^+$ CTL responses to HIV infection of CD4$^+$ T cells. Finally, it should be noted that CD4$^+$ CTLs are non-specifically cytotoxic following recognition of envelope protein-expressing target cells [11]. These observations are relevant to scenarios of active viral replication and high antigen burden, and partly explain the CD4$^+$ CTL responses elicited by a HIV envelope gp160 vaccine in immunized volunteers [36–38].

HLA-II-restricted HIV-specific CD4$^+$ CTLs were first detected 30 years ago in cerebrospinal fluids from patients with acquired immunodeficiency syndrome [39]. In later studies, vaccination of seronegative volunteers with an HIV Env protein vaccine elicited CD4$^+$ T-cell responses with HLA-II regulated lytic activity [36–38]. Other reports also show recognition of Gag, Env and/or Pol peptides by CD4$^+$ T-cells and *in vitro* lysis of peptide-pulsed or HIV-infected target cells [11,40,41]. The first *ex vivo* study of CD4$^+$ CTLs in patients with HIV infection was performed by Appay et al, who showed the presence of an important proportion of perforin-expressing CD4$^+$ T cells. These had a CD27$^-$ CD28$^-$ CCR7$^-$ CD45RA$^-$ CD45RO$^+$ CD11a$^+$ CD11b$^+$ phenotype [12], that is related to late stages of differentiation [42]. Interestingly, perforin$^+$ CD4$^+$ T-cell numbers were increased in HIV seroconverted patients compared with seronegative controls, and these cells exhibited an activated phenotype (CD38 and Ki-67 expression); the number of these cells were further augmented during chronic infection [12]. Consistent with the fully differentiated stage, perforin-expressing CD28$^-$ CD4$^+$ T-cells had reduced proliferative capacity, and this was related to clonal expansion during the early stages of HIV infection [12]. Additionally, an expanded subset of p24-specific CD45RO$^+$ CCR7$^-$ CCR5$^+$ CD4$^+$ T-cells with lytic capacity was found in peripheral blood from a long-term non-progressive patient [14]. This cell subset also expressed NK cell markers such as CD161 and CD244, but the absence of the TCR Vα24, characteristic of NKT-cells [14]. CCR5 expression, and the activated state indicated by high expression of CD38 was associated with high susceptibility of the CD4$^+$ CTL subset to HIV infection [26,43], thus limiting antiviral potential. Recently, Johnson et al. evaluated transcription and phenotype profiles of CD4$^+$ CTLs in patients with HIV infection [15], and these data were consistent with previous reports showing a fully mature profile and expression of NK cell markers [12,14]. Specifically, the CD4$^+$ CTLs in these studies were characterized by a CD57$^+$ KLRG1$^+$ CD161$^+$ IL-7R$^-$ phenotype and expression of T-bet and Eomes, similar to that observed in CD8$^+$ T-cells [15]. Studies in Simian Immunodeficiency virus (SIV)-infected macaques have also demonstrated a CD45RA$^-$ CD28$^+$ CD95$^+$ CCR7$^-$ phenotype in CD4$^+$ CTLs [44]. Phenotypes of CD4$^+$ CTLs in patients with HIV infection are listed in Table 1. Collectively, these data indicate that memory CD4$^+$ CTLs are generated early during HIV infection after APC priming and differentiation from other CD4$^+$ T-cell effector subsets (Fig. 1). Due to activation and expression of the HIV co-receptor CCR5, CD4$^+$ CTLs are susceptible to HIV infection and are likely depleted rapidly. Yet a fraction of CD4$^+$ CTLs is clonally expanded and acquires a fully differentiated quiescent profile with low proliferative capacity. This profile is maintained throughout chronic infection.

3. Effector mechanisms of CD4$^+$ CTLs during HIV infection

Similar to CD8$^+$ T-cells, CD4$^+$ CTLs mediate cytolytic activities through granule-dependent and independent mechanisms [2] (Table 1 and Fig. 2). An early study of the lytic capacity of CD4$^+$ T-cells from patients with HIV infection showed induction of target cell death

Table 1
CD4$^+$ CTLs features during HIV infection.

Phenotype	Effector mechanism	Recognized HIV proteins	Role in disease progression	References
N/A	Non-granule-dependent cytotoxicity	Env, Gag	N/A	[11,47]
N/A	Perforin-mediated cytotoxicity; IFN-γ, TNF-α and β-chemokines secretion.	Gag (p24)	N/A	[41,47,52]
CD45RO$^+$ CD11a$^+$ CCR7$^-$	Perforin-mediated cytotoxicity, IFN-γ and TNF-α secretion.	N/A	N/A	[12]
CD45RO$^+$ CCR7$^-$ CCR5$^+$ CD161$^+$ CD244$^+$	Granzyme B/Perforin-mediated cytotoxicity; IFN-γ secretion.	Gag	N/A	[14,26,43]
N/A	Degranulation (CD107a expression); IL-2, and IFN-γ secretion.	Gag	No differences between progressor and non-progressors in CD107a$^+$ CD4$^+$ T-cells	[50]
CD57$^+$ KLRG1$^+$ IL-7R$^-$	Degranulation (CD107a expression); Granzyme B/Perforin-mediated cytotoxicity; IFN-γ secretion.	Gag	Higher HIV-specific CD4$^+$ CTLs in elite controllers and in patients with lower viral set point.	[15,48]

N/A: Not assessed; KLRG1: killer-cell lectin like receptor G1; IFN: Interferon.

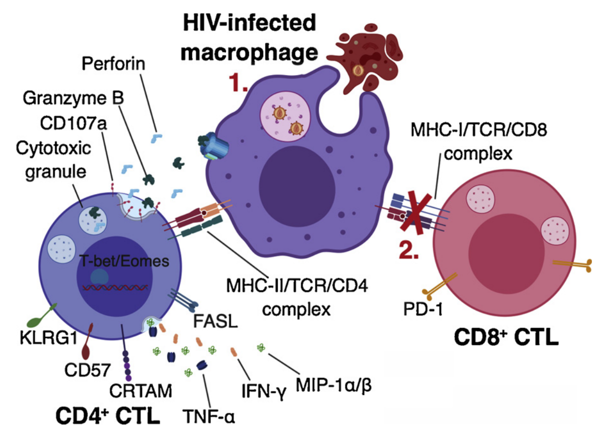

Fig. 2. Two scenarios of CD4$^+$ CTLs anti-HIV control. CD4$^+$ CTLs are characterized by the expression of CD57, KLRG1, CRTAM, T-bet and Eomes. They also exhibit granule-dependent (release of cytotoxic molecules) and independent (FASL) cytotoxic mechanisms and can secrete IFN-γ, TNF-α and β-chemokines. The first scenario of an antiviral role of CD4$^+$ CTLs during HIV infection is in the case of HIV-infected macrophages or dendritic cells (1), which can present endocytic or cytosolic antigens in an HLA-II-restricted manner. A second scenario is in the setting of a poor CD8$^+$ T-cells response (2), such as in the presence of HIV escape mutations, immune evasion, and exhaustion, evidenced by the expression of the inhibitory receptor programmed death (PD)-1.

through calcium-independent mechanisms, and independently of the release of lytic granules [11]. FAS ligand or TNF-related apoptosis-inducing ligand (TRAIL) may underlie these effector mechanisms [45,46]. Other reports demonstrated granule-dependent cytolysis by HIV-specific CD4$^+$ CTLs, in which cell death was abrogated by the inhibitor of perforin-mediated killing concanamycin and/or by the chelating agent ethylene glycol tetraacetic acid (EGTA) [12,41]. Another report showed that HIV-specific CD4$^+$ CTL clones can differentially exert granule-dependent and independent cytotoxicity [47]. Resembling CD8$^+$ T-cells and NK cells, CD4$^+$ CTLs have been characterized as perforin- or granzyme-expressing cells [12,14,44,48], and the marker of cell degranulation CD107a [49] is also used to identify CD4$^+$ CTLs [15,50]. Moreover, transcriptomic analyses showed that, after antigen-specific stimulation, CD107a$^+$ CD4$^+$ CTLs express lytic granule-effector proteins such as granulysin, granzyme A, H, K and B, and perforin [15].

In addition to direct lytic mechanisms, CD4$^+$ CTLs secrete the antiviral cytokines IFN-γ and TNF-α [12,14,26,43], which promote antigen presentation and inflammatory responses [51]. The β-chemokines macrophage inflammatory protein (MIP)-1α and MIP-1β [41,47,52] also block HIV binding and entry into target cells [53,54]. Finally, virus-specific CD4$^+$ CTLs may upregulate IL-21 after antigen stimulation [15] and could promote humoral responses [44] or boost CD8$^+$ T-cells [55] during HIV/SIV infection. A summary of CD4$^+$ CTLs effector mechanisms in patients with HIV infection is shown in Table 1.

It is important to note that all of the studies described above were performed using blood-derived CD4$^+$ CTLs. Yet in contrast with blood cells, the cytolytic potential of CD4$^+$ T-cells was shown to be limited in lymphoid tissues [56], which are major sites of HIV/SIV replication [57]. These differing cytolytic profiles in blood and lymph nodes correspond with differential expression levels of T-bet and Eomes. In blood samples, granzyme B- and perforin-expressing CD4$^+$ CTLs preferentially expressed T-bet^{hi} and Eomes$^+$, whereas the frequency of this population was low–undetectable in lymph node tissues. This transcriptional profile is reflected by low expression of granzyme B and perforin in lymph node-confined CD4$^+$ T-cells, limited upregulation of cytotoxic molecules following HIV peptide stimulation, and delayed granule release. In concordant evaluations of transcriptional and functional signatures, CD107a$^+$ CD4$^+$ T-cells from blood and lymph nodes formed separate single-cell clusters, and effector molecules,

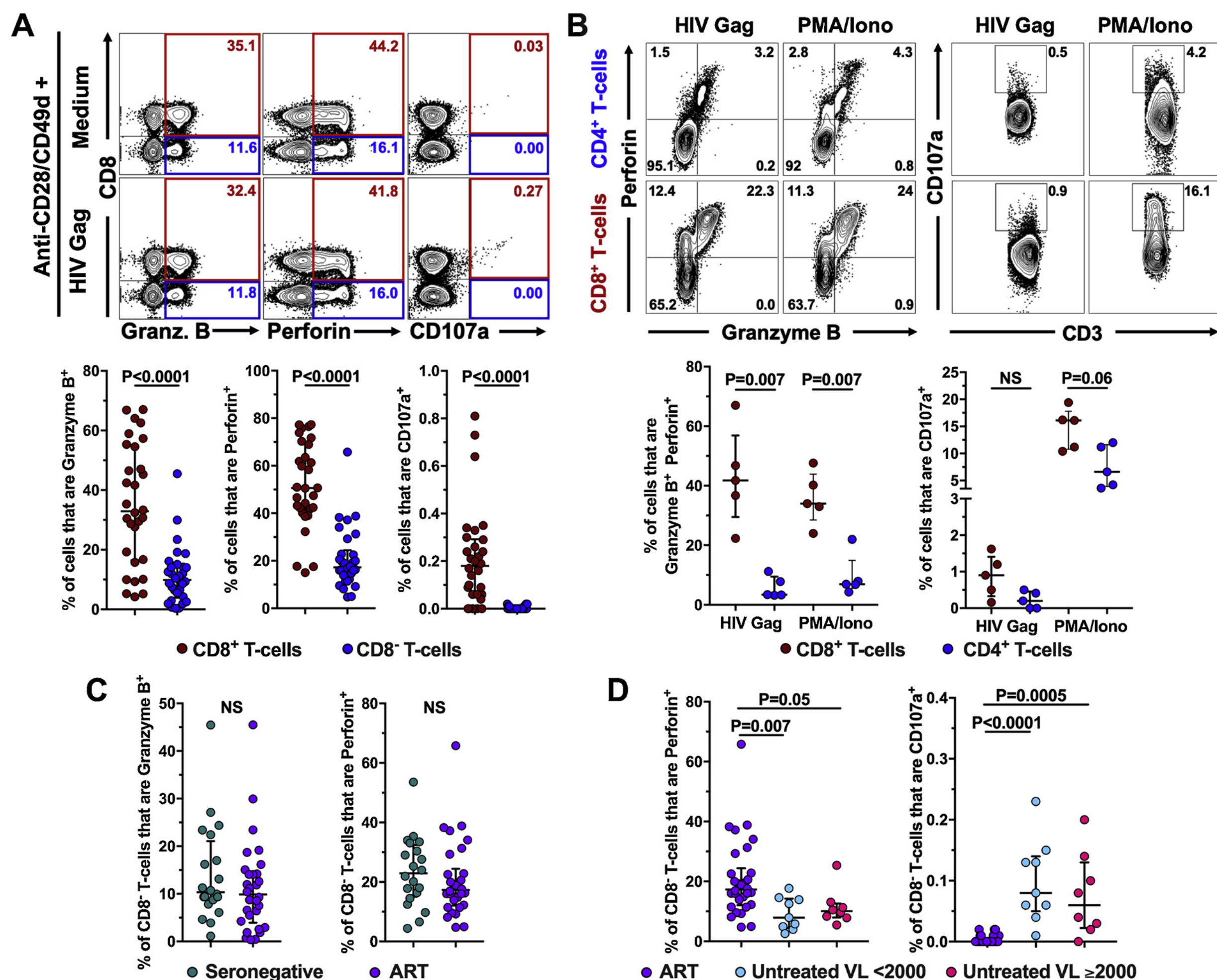

Fig. 3. Evaluation of the cytotoxic potential in circulating T-cells in HIV-infected patients under ART. A. Peripheral blood mononuclear cells from HIV-infected patients receiving ART for more than one year and with suppressed viral load and recovery of CD4$^+$ T-cell counts, where stimulated *ex vivo* with a pool of HIV Gag peptides, in the presence of co-stimulatory antibodies anti-CD28 and anti-CD49d. The expression of granzyme B, perforin and CD107a in T-cells (previous gate in singlet CD3$^+$ cells) was evaluated by flow cytometry after 12 h of culture. In the upper panels, representative contour plots from unstimulated and stimulated cells are shown. In red and blue, the proportions of CD8$^+$ and CD8$^-$ T-cells that are positive for each marker are shown, respectively. In the lower panels, the frequencies of granzyme B$^+$, perforin$^+$ and CD107a$^+$ in stimulated T-cells from all the patients included is shown. The background of CD107a in unstimulated cells was subtracted from peptide-stimulated cells. P value of the Wilcoxon test. **B.** Expression of granzyme B and perforin, as well as CD107a in CD4$^+$ and CD8$^+$ T-cells after stimulation under similar conditions to that described in **A**, in addition to polyclonal stimulation with PMA/Ionomycin. P value of the Wilcoxon test. NS: not statistically significant. **C.** Frequencies of granzyme B$^+$ and perforin$^+$ CD8$^-$ T-cells in seronegative volunteers and HIV-infected patients on ART. The P value of Mann-Whitney test was > 0.05, not statistically significant (NS). **D.** Frequencies of perforin$^+$ and CD107a$^+$ CD8$^-$ T-cells in HIV-infected patients on ART, and untreated patients, the latter classified according to their plasma viral load (< 2000 and ≥ 2000 RNA copies/mL). P value of the Kruskal-Wallis and Dunn's post-hoc tests (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

including IFN-γ, perforin, and β-chemokines, were poorly expressed in lymph node-confined CD107a$^+$ CD4$^+$ T-cells [56]. Taken together, these CD4$^+$ T-cell features confer limited cytolytic potential and immune surveillance capacity in lymph nodes. Similar characteristics have been reported for CD8$^+$ T cells [58], but despite the low cytotoxic potential of T-cells, HIV elite controller patients maintain low viral loads in the absence of therapy. Thus, non-cytolytic factors appear to be involved in the control of HIV in lymphoid tissues [56,58].

4. Roles of CD4$^+$ CTLs during untreated HIV infection

Antigen-specific CD4$^+$ T-cell responses have been associated with HIV control and slow disease progression [59–62]. However, HIV-specific CD4$^+$ T-cells are preferentially infected by HIV [63], and high viremia hampers their responses [64]. Thus, because antigen-specific CD4$^+$ T cells are the main targets of HIV, their antiviral roles remain controversial. Nonetheless, the roles of CD4$^+$ CTLs during HIV infection are supported by considerable evidence. Specifically, i) HIV elite controller patients exhibit higher frequencies of circulating HIV-specific CD107a$^+$ CD4$^+$ CTLs [15], ii) patients with high frequencies of circulating CD57$^+$ or granzyme A$^+$ CD4$^+$ CTLs develop lower viral set points [15], iii) CD8$^+$ T-cell depletion in SIV or SIV/HIV-infected macaques does not completely eliminate the lysis of peptide-pulsed target cells *in vivo*, suggesting a CD4-mediated cytotoxicity [65]. Although vestigial CD8$^+$ T-cells and NK cells cannot be excluded from these observations, these results are also supported by the emergence of virus-specific CD4$^+$ CTLs coinciding with viral control post-CD8 depletion [44]. Further confirming the roles of CD4$^+$ CTLs during HIV infection, iv) HIV or SIV-specific CD4$^+$ CTLs suppressed viral infection of CD4$^+$ T-cells [66,67] and macrophages [27,67], and v) both SIV and HIV Gag-specific CD4$^+$ CTLs exerted *in vivo* selective pressure, inducing escape mutations during their control [68,69]. In addition to these lines of evidence, the induction of CD4$^+$ CTLs by HIV vaccine candidates suggests that this population provides beneficial lytic activities [37,38]. Moreover, vaccine-induced CD107a$^+$ CD4$^+$ T-cells, but not cytokine-producing CD4$^+$ T-cells, are resistant to depletion following SIV

infection in macaques, resulting in lower viral replication [70]. These data suggest that this cell population controls viral loads through lytic mechanisms and by intrinsic virus resistance and should be enhanced by vaccine candidates.

Although antigen-specific $CD4^+$ CTLs are a target of HIV, particularly during acute infection, preserved $CD4^+$ CTL responses could play antiviral roles in three scenarios. First, $CD4^+$ CTLs are likely important for the lysis of infected macrophages and dendritic cells [27,67], because these cells could present a wider diversity of both endocytic and cytosolic antigens in a HLA-II-dependent manner [71]. Targeting of these cell types is hence pertinent because they act as cellular HIV reservoirs [72]. Secondly, $CD4^+$ CTLs may cooperate with $CD8^+$ T-cells to achieve lysis of infected cells [15], particularly in the context of a poor $CD8^+$ T-cell responses, such as those associated with CD8-escape mutations [73], viral evasion, downregulation of HLA-I molecules [74], and/or $CD8^+$ T-cell exhaustion [75] (Fig. 2). A third scenario follows ART-induced viral suppression and is discussed below. Thus, although HIV-specific $CD4^+$ CTLs are a target of HIV and are depleted early after infection, the remaining cells may contribute to viral control, particularly by targeting macrophages, dendritic cells, and non-APCs that are not readily eliminated by $CD8^+$ T cells. Hence, preserved $CD4^+$ CTL responses may comprise antiviral mechanisms in HIV non-progressor patients and could be enhanced by vaccine candidates and immunotherapeutic approaches.

5. Proposed role of $CD4^+$ CTLs during HIV infection in the presence of ART-induced viral suppression

Typically, HIV replication is suppressed by ART along with increasing $CD4^+$ T-cell counts, depending on nadir $CD4^+$ T-cell numbers or naive subpopulations at the time of treatment initiation [76–80]. Moreover, patients with HIV infection who recover $CD4^+$ T-cell counts are unlikely to experience counts below the threshold for opportunistic infection risk thereafter [81]. Thus, under conditions of ART-induced viral suppression, recovery of $CD4^+$ T-cells may facilitate cytolytic functions. Certainly, the lytic activity of $CD4^+$ CTLs in this setting would be significant, because some alterations in the $CD8^+$ T-cell cytotoxic program, in addition to persistent activation and exhaustion, are evident in patients with HIV infection and are not fully restored by ART-induced viral suppression [82–85], hence limiting antiviral activities.

Circulating $CD4^+$ CTLs have been detected in patients with HIV infection receiving ART [12,50]. However, suppression of viral load and antigenemia may promote migration of antigen-specific $CD4^+$ CTLs from the blood to lymphoid tissues, as shown with HIV-specific IFN^+ $CD4^+$ T cells [60] and HIV-specific $CD8^+$ T-cells [86]. However, our evaluations of cytotoxic phenotypes in different cohorts of HIV-infected individuals suggest that $CD107a^+$ $CD8^-$ T-cells, which frequencies highly coincide with $CD107a^+$ $CD4^+$ T-cells, are low but detectable after *ex vivo* HIV Gag stimulation of cells from patients receiving ART (Fig. 3A and B). $CD8^-$ or $CD4^+$ T-cells also contain granzyme B and perforin in their lytic granules, but at consistently lower concentrations than in $CD8^+$ T-cells (Fig. 3A and B). In addition, expression levels of granzyme B and perforin are similar between patients with HIV infection receiving ART and seronegative individuals, suggesting that the production of cytotoxic molecules by $CD4^+$ T cells is intact in these patients (Fig. 3C). Indeed, $CD8^-$ T-cells from patients receiving ART exhibit higher expression levels of perforin than cells from untreated patients, both with viral load < 2000 or ≥ 2000 RNA copies/mL (Fig. 3D). Finally, consistent with the migration of antigen-specific $CD4^+$ CTLs from blood to lymphoid tissues following viral suppression, patients receiving ART exhibit lower frequencies of Gag-specific $CD107a^+$ $CD8^-$ T-cells than untreated patients (Fig. 3D). Thus, although more studies are required to explore the roles of $CD4^+$ CTLs in patients with HIV infection who receive ART, these data support the cytotoxic potential of $CD4^+$ T cells.

6. Conclusions

Beyond help, $CD4^+$ T-cells can exert direct cytolytic functions and cooperate with other cytotoxic cell populations to control HIV infection. Yet, considering differences in $CD4^+$ T-cell profiles between blood and lymphoid tissues, the phenotypic, transcriptional, and functional signatures of $CD4^+$ CTLs could be better defined to elucidate their roles in the control of HIV infections. Moreover, considering that most patients with HIV infection receive ART, future studies should focus on this patient group. Such investigations will likely contribute to the understanding of protective T-cell cytolytic functions. Given that lymphoid tissues are sites of augmented HIV replication and act as viral reservoirs, and that lymphoid tissue T-cells are characterized by a lower granule-dependent cytotoxic potential, as indicated by their transcriptional profiles, these studies may distinguish between granule-independent or non-cytolytic mechanisms in terms of the control of viral replication, transcriptional and epigenetic regulation, and their relative efficacy as targets for immunotherapeutic strategies.

Ethical approval

Ethical Approval was provided by IRB of Sede de Investigacion Universitaria, Universidad de Antioquia (certificates 15-08-634 and 11-08–352).

CRediT authorship contribution statement

Alexandra Sanchez-Martinez: Data curation, Writing - original draft, Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing. **Federico Perdomo-Celis:** Data curation, Writing - original draft, Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing. **Liliana Acevedo-Saenz:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing. **Maria T. Rugeles:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing, Funding acquisition, Project administration. **Paula A. Velilla:** Conceptualization, Formal analysis, Investigation, Methodology, Visualization, Writing - review & editing, Funding acquisition, Project administration.

Declaration of Competing Interest

None declared.

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