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Review

What do mathematical models tell us about killing rates during HIV-1 infection?

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ABSTRACT

Over the past few decades the extent to which cytotoxic T lymphocytes (CTLs) control human immunodeficiency virus (HIV) replication has been studied extensively, yet their role and mode of action remain controversial. In some studies, CTLs were found to kill a large fraction of the productively infected cells relative to the viral cytopathicity, whereas in others CTLs were suggested to kill only a small fraction of infected cells. In this review, we compile published estimates of CTL-mediated death rates, and examine whether these studies permit determining the rate at which CTLs kill HIV-1 infected cells. We highlight potential misinterpretations of the CTL-killing rates from the escape rates of mutants, and from perturbations of the steady state viral load during chronic infection. Our major conclusion is that CTL-mediated killing rates remain unknown. But contrary to current consensus, we argue that killing rates higher than one per day are perfectly consistent with the experimental data, which would imply that the majority of the productively infected cells could still die from CTL-mediated killing rather than from viral cytopathicity.

1. Introduction

CD8$^+$ cytotoxic T lymphocytes (CTLs) are an indispensable arm of the adaptive immune system for protection against tumors, and against viral and bacterial infections. CTLs protect by killing infected cells and by various non-lytic mechanisms, including the secretion of interferon-γ, macrophage inflammatory proteins (MIP)-1α and MIP-1β [1,2]. The relative contribution of these two mechanisms in controlling infections is poorly understood, and an important parameter determining the extent of CTL protection is the rate at which they kill infected cells. Although CD4$^+$ T cells that have become productively infected with the human immunodeficiency virus type 1 (HIV-1) are known to die rapidly [3,4], recent studies into the role of CTLs in controlling HIV-1 infection have suggested that their mode of action is largely non-lytic [5–9]. This would imply that the majority of the productively infected cells are not killed, but die rapidly from infection (viral cytopathicity). Here we review these studies to see if they indeed support the minor contribution of the lytic effects of CTLs, and whether they are truly incompatible with rapid CTL killing rates. There are multiple ways to report CTL-mediated killing rates (see [10–12] for excellent overviews and how to interconvert these rates). In this review, we report published estimates of killing rates as the death rate of productively infected cells induced by all CTLs together.

Several kinetic parameters of HIV-1 and SIV infections are similar. During acute infection in their respective hosts, the two viruses initially replicate at comparable rates of 1.5–2.0/day [13–16], and during anti-retroviral therapy (ART) they exhibit similar viral load decay rates of 1–2/day [6,7,17,18]. One notable difference between HIV-1 and SIV is that HIV-1 immune escape rates were consistently lower than SIV escape rates [19]. Here, we primarily focus on CTL-mediated killing rates during HIV-1-infection and discuss a few published SIV estimates from studies that can only be performed in monkeys. As a side note, there is an ongoing debate on whether the CD4$^+$ T cell depletion and loss of HIV-specific immunity in longterm HIV patients is primarily due to CTL responses or to HIV-induced inflammation [20,21]. Our conclusions are independent of the mechanism(s) underlying CD4$^+$ T cell depletion, because the depletion of CD4$^+$ T cells during chronic infection occurs slowly (typically on a time scale of years), whereas the mathematical models discussed in this review only consider the faster dynamics occurring on a time scale of days.

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2. Estimating killing rates from escape rates

HIV and SIV mutate when they infect new target cells. If these mutations occur within CTL epitopes, they can reduce the ability of CTLs to recognize and control infected cells, thereby allowing the virus to escape CTL surveillance. Such escape mutants have a selective advantage over wild type viruses, and hence can outcompete them. The rate at which escape mutant replaces the wild type—defined as the escape rate—provides an indication of the efficiency of the CTL response that was evaded. However, escape mutations are often accompanied by fitness costs of the mutations, which slow down the escape rate [11,5]. The fitness costs can be estimated *in vitro* by competition assays [22–24] and *in vivo* from the rates at which escape mutants revert back to wild type in HLA-mismatched patients [5,25,26]. By accounting for fitness costs, researchers have tried to estimate the *in vivo* CTL-mediated killing rate from the escape rates of mutants (reviewed in [11]).

2.1. Early escape rates

Asquith et al. [5] examined longitudinal measurements of the frequencies of immune escape mutants and found very slow escape and reversion rates in 12 HIV-1-infected individuals at different stages of infection. Combining the estimated escape and reversion rates, and considering a model where CTLs control by killing infected cells, they estimated a median killing rate by the escaped CTL response of 0.04/day during the acute phase of the infection. Since the total death rate of productively infected $CD4^+$ T cells is about $\delta = 1$/day [17] (see below), they concluded that a CTL response targeting a single epitope is responsible for the death of only a minor fraction of virus-producing cells, i.e., at most 4%. In patients mounting CTL responses to, say, five epitopes, the contribution of CTL killing to the death of productively infected $CD4^+$ T cells would still be just 20%, suggesting that most infected cells die by viral cytopathic effects. Although later studies using the same approach frequently find at least 10-fold faster escapes [27–29] and more rapid reversions [26], other recent studies confirm that both immune escapes and reversions can be slow [30]. One possible explanation for these slow immune escape rates is that CTLs largely control by non-lytic mechanisms [9]. Immune escapes from non-lytic CTLs impose lower selection pressures than escapes from lytic responses because cells infected with escaped virus remain susceptible to non-specific factors secreted by the CTL in their neighborhood [9].

At least two other explanations for slow immune escape rates have been suggested, both implying that the published escape rates underestimate the actual CTL killing rates. First, there are some general issues associated with estimating killing rates from escape rates, i.e., by sequencing the HIV-1 quasi species in a patient at various time points and fitting simple population genetic models to the time course describing the fraction of mutated sequences within each epitope [31]. One major problem with such models is that the escapes are treated independently, whereas in reality the immune escapes appear more or less sequentially, i.e., subsequent escapes can only evolve after previous immune escapes have become established. This competition is known as “clonal interference” in population genetics [32,33], and several authors have recently shown that clonal interference can markedly decrease the escape rate inferred independently for each epitope [31,34–37]. For example, Kessinger et al. [35] developed a novel mathematical model combining the escape data from all epitopes, and showed that their estimation procedure markedly increased the most likely set of escape rates. Another major problem with this kind of data is that the time courses are underpowered due to sampling that is fairly infrequent [38] and that is not deep enough to detect the exact arrival time of the escape mutants. By testing clonal interference models on simulated data, it was indeed found that the estimated escape rates are very sensitive to the sampling frequency and to the sequencing depth [35,37], casting additional doubt on the estimated escape rates [31,35]. Next generation sequencing (NGS) methods have been used to sequence much deeper [39], but due to their prohibitive costs, the sampling frequency remains limited. An interesting approach is to reconstruct whole genome haplotypes from the NGS data [40], which allows one to observe the clonal interference directly and to estimate the escape rate of all the major haplotypes.

A second line of explanations for slow immune escape that does not involve non-lytic mechanisms is that large fitness costs can explain at least some of the slow escapes observed. Several immune escapes confer severe fitness defects, which can be observed *in vivo* by poor replication of the virus in subsequent HLA-mismatched patients [26] or *in vitro* by viral competition assays [24,22,23]. Additionally, the reversion rates estimated *in vivo* also suffer from clonal interference, and could hence be markedly underestimated. Finally, several immune escape mutations ultimately become repaired by compensatory mutations increasing the replicative fitness of the virus. Following transmission to HLA-mismatched patients such a repaired immune escape may have a high fitness, and hence reverts very slowly.

2.2. Late escape rates

In the study by Asquith et al. [5] the immune escape rates tended to be even slower in chronically infected patients, i.e., about 0.008/day than in acute stage. They were also found to decrease over time in a study following three HIV-1-infected individuals over one year [28]. Moreover, a recent study following about 120 HIV-1-infected individuals starting at a median of 11 weeks post seroconversion found very few escapes over the subsequent 2–3 years of chronic infection [30]. The apparent decrease in the immune escape rate has been attributed to a lower killing efficiency during chronic infection due to exhaustion of CTLs [5,12,31], and due to the decrease in CTL numbers after the acute phase [11].

However, other explanations for the escape rate decrease over time are possible. First, a trivial explanation could again be the small number of time points in these studies, because the sampling frequency typically decreases over time [37]. Second, similar observations have been made in mathematical models in which the data is “sampled” very frequently [38]. Van Deutekom et al. [41] and Ganusov et al. [28] demonstrated that escape rates are expected to decrease during chronic infection due to an increase in the number of CTL responses. This is because multiple CTL responses can collectively induce rapid death, while the contribution of each individual CTL response remains small. As a result, the selective advantage for the virus to escape just one CTL response decreases [41], leading to slow escape. Consistent with this, a recent study showed that broad Gag responses are associated with few escapes and low viral load in HIV-1-infected individuals [42]. Thus, slow escape rates during chronic infections are to be expected for broad immune responses, and are not indicative of non-lytic or poor control by CTLs.

Summarizing, the current data on the immune escape and reversion rates in HIV-1 infection have been described with oversimplified mathematical models, and are not rich enough for reliably estimating the contribution of CTLs during the acute and chronic phases of HIV-1 infection, let alone the relative contribution of lytic and non-lytic mechanisms. Fortunately, with the current rise of NGS approaches [39,40], and the novel models taking clonal interference into account [31,34–37], this is expected to improve once we can afford to sample the viral quasi species very frequently. This is important because immune escape and reversions provide natural *in vivo* experiments providing information on the role of CTLs in HIV-1 infection.

3. Estimating killing rates from ART data

During chronic HIV/SIV infection, the plasma viral load remains constant over many years due to the balance between viral replication and clearance mechanisms, i.e., a steady state. In several studies the steady state was perturbed, and the ensuing viral dynamics were analyzed to estimate the kinetic parameters of viral replication and CTL-mediated killing rates. The approaches used to perturb steady state can be classified into three broad categories: treatment with anti-retroviral therapy (ART), depletion of $CD8^+$ cells, or adoptive transfer of $CD8^+$ T cells. In the remainder of this review, we focus on estimates of killing rates during chronic HIV/SIV infection obtained using these three methods.

Ho et al. [3] and Wei et al. [4] examined the dynamics of HIV-1 following ART with one or two protease inhibitors, and found that the plasma viral load declines very rapidly after treatment. In later studies, the viral load was found to decline in multiple phases [43,44], and using an even more potent combination of drugs [17], it was found that the initial downslope of the viral load is about $\delta = 1$/day. These downslopes were estimated by fitting a basic model (Fig. 1A, [46]) to the viral load, in which this initial downslope, δ, reflects the death rate of productively infected $CD4^+$ T cells [3,4,43,44,17].

A meta-analysis of various clinical data sets showed that δ hardly depends on the $CD4^+$ T cell count or on the pre-treatment viral load [18]. This was surprising because the death rate of productively infected $CD4^+$ T cells represents both "normal death" and CTL killing, i.e., $\delta = d_P + K$, where d_P is the normal death rate (including viral cytopathic effects), and K is the rate at which infected cells are killed. Since δ was so invariant in the meta-analysis, and patients with different viral loads probably have different CTL responses, it was proposed that CTLs hardly affect the death rate of productively infected $CD4^+$ T cells [18]. This is reminiscent of the minor contribution of killing estimated from the escape rates [5] and hence again suggests that CTLs largely control by non-lytic mechanisms.

Recently, Klatt et al. [6] and Wong et al. [7] independently studied the contribution of CTL-mediated killing, K, during SIV-infection to the death rate of productively infected cells, δ. The viral dynamics were studied following ART in both $CD8^+$ cell-depleted and control rhesus macaques infected chronically with SIV. Note that targeting the CD8 molecule for CTL depletion also depletes other CD8-expressing cells (such as NK cells) [6]. Nevertheless, the rate at which the plasma viral load declined was similar between CD8-depleted and control macaques. By fitting the basic model (see Fig. 1A) to the viral load, the authors estimated similar death rates, δ, of virus-producing cells in CD8-depleted (where $\delta = d_P$) and control hosts (where $\delta = d_P + K$). Because this implies that K is very small, this again suggests a negligible role of CTLs in the death of virus-producing cells.

3.1. Interpreting ART and CD8-depletion data with two-stage mathematical models

The above studies estimated lifespans of the productively infected cells using the basic model of virus dynamics (Fig. 1A), which ignores several aspects of viral replication that are relevant to HIV-1 infection. For instance, it does not account for the eclipse phase, i.e., the time period between viral entry and production of viruses [45]. In other words, the basic model assumes that the infected cells start producing virus particles immediately after infection.

Klenerman et al. [47] developed an HIV/SIV-specific mathematical model that includes two infected cell stages and a viral production stage for the infected cells (i.e., three-stage viral replication; similar to the two-stage model shown in Fig. 1B). They assumed that antigen expression and ensuing recognition by CTLs only occurred in the viral production stage (late-stage killing model). Furthermore, they argued that the rate, γ, at which HIV-infected cells transit to antigen-expressing stage is the slowest timescale of HIV replication. In that case, the observed downslope of the viral load during ART, δ, reflects this slow transition rate. Because the average duration of the eclipse phase, $1/\gamma$, is about one day [45], this is in excellent agreement with a decay rate of $\delta = 1$/day, and with the independence of the viral load and the $CD4^+$ T cell count [17,18].

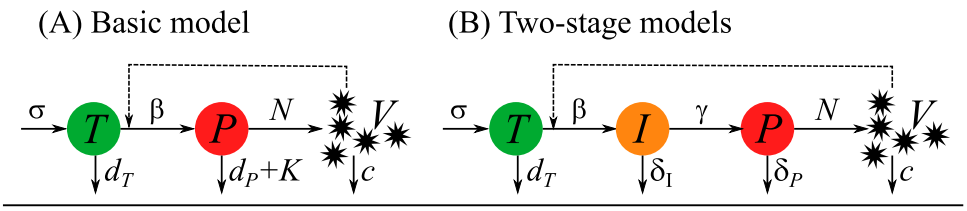
Further, when Wick et al. [49] performed stochastic simulations similar to the late-stage killing model with a rapid CTL-mediated killing rate of $K \approx 7$/day, they found approximately equal viral decay rates between CD8-depleted and control hosts when γ was the rate limiting step. Although the Wick et al. [49] simulation model differs from the late-stage killing model in several details, analysis of a similar deterministic model (Fig. 1B) confirms its consistency with the CD8-depletion experiments (i.e., viral decay rate reflects $\gamma + d_I$; Fig. 2A). Therefore, in both the two- and three-stage variants of the late-stage killing model, CTL-mediated killing can be rapid, if it acts only in late stages of infection [47,49] and thus separates the timescales of viral replication and CTL lysis. However, Klatt et al. [6] argue that the time scale of infected cells in the eclipse phase is not necessarily slow, because some HIV epitopes are expressed on infected cells as early as an hour after infection [50]. Thus, infected cells could be susceptible to CTL-mediated killing immediately after infection.

It is unclear whether the timescale of the eclipse phase is indeed the slowest process in the short life of productively infected cells. Therefore, Althaus et al. [48] used the mathematical model of Fig. 1B to show that early antigen expression followed by rapid CTL-mediated killing of infected cells at an early stage within the eclipse phase (early-stage killing model) is also consistent with the similar decay rates of viral loads observed in CD8-depleted and control hosts (Fig. 2B). Because CTLs in this model only kill infected cells in the eclipse phase, the viral decay rates are independent of CTL activity. These decay rates now represent the death rate of virus-producing cells, which can be large due to viral cytopathicity alone (i.e., $\delta = d_P$; Fig. 1).

In conclusion, the viral load dynamics is determined by the timescale of the slowest process, and CTL lysis need not be slow. The two-stage model is a simple extension of the basic model, and underlines how the rate limiting step alters the interpretation of the initial viral decay. More sophisticated models either with multiple cellular compartments [52,43], or with n stages from infection to viral production [53,54] have been proposed for a complete description of all three phases of viral decay. Nevertheless, the two-stage model with CTL-mediated killing of infected cells either in the early or late stage can qualitatively explain the results from ART studies (phase I) with or without CD8-depletion. Importantly, this implies that the rate of CTL-mediated killing can be larger than 1/day.

3.2. Which model quantitatively describes the ART and CD8-depletion data best?

Recently, Elemans et al. [8] studied whether or not the above models (i.e., the basic, early-stage killing, and late-stage killing; Fig. 1) can provide a good quantitative description of the CD8 depletion/ART data of Klatt et al. [6]. Fitting these models to the data, they found that all three models poorly described the experimental data [8]. This is puzzling as both Klenerman et al. [47] and Althaus et al. [48] demonstrated that their respective models are qualitatively consistent with the experiments over a wide range of parameters. A potential explanation for these conflicting findings is that Elemans et al. [8] required the target cell levels in the model to describe the observed $CD4^+$ T cell counts, and considered the specific CTL



Model	Model-specific parameters	Predicted viral decay rate, δ	
		Control	With CD8-depletion
Basic [4,46]	-	$\delta = d_P + K$	$\delta = d_P$
Late-stage killing [19,47]	$\delta_I = d_I$, $\delta_P = d_P + K$	$\delta = \min(\gamma + d_I,\ d_P + K)$	$\delta = \min(\gamma + d_I,\ d_P)$
Early-stage killing [48]	$\delta_I = d_I + K$, $\delta_P = d_P$	$\delta = \min(\gamma + d_I + K,\ d_P)$	$\delta = \min(\gamma + d_I,\ d_P)$

Fig. 1. Summary of various models used to describe HIV dynamics. The scheme summarizes the three models discussed: (A) basic and (B) two-stage infection models in which CTL-mediated killing acts either during eclipse phase or during virus-production phase. HIV viral particles, V, infect healthy CD4$^+$ T cells, T, at a rate β. Following infection, infected cells, I, transit the eclipse phase at a rate γ to become virus-producing cells, P, which produce new virus particles. Healthy CD4$^+$ T cells are produced at a constant rate, σ, and die at a rate d_T, and free virus particles are cleared at a rate c. Infected cells in the eclipse phase, I, and productively infected cells, P, die normally at a rate d_I and d_P, respectively. Additionally, they can be killed by CTL at a rate K, which, depending on the model increases their total death rates to δ_I and δ_P, respectively. The predicted viral decay rates, δ, are summarized in the accompanying table, where $\min(x, y)$ represents the minimum of x and y. Note that the basic model can be obtaining from the two-stage models by making the eclipse phase very short (i.e., by letting $\gamma \to \infty$).

response to be proportional to the observed CD8$^+$ T cell counts. Contrary to these assumptions, the frequency of HIV-specific CTLs within the pool of CD8$^+$ T cells could change over time following CD8 depletion, and the total number of CD4$^+$ T cells is probably a poor indicator for the number of susceptible target cells. Thus, it remains possible that the two-stage models shown in Fig. 1B can quantitatively account for the viral load data.

4. Estimating killing rates from the increase of the viral load upon CD8-depletion

In all studies where CD8$^+$ cells are depleted in chronically SIV-infected macaques it was consistently found that this results in a rapid transient increase of the viral load [6,7,55], suggesting that CD8-depletion results in an imbalance between viral production and clearance. Indeed, there was a negative correlation between the residual CD8$^+$ T cells surviving the depletion and the fold-increase in the viral load [7]. Because the killing rate at steady state was fast, both the early- and late-stage killing models readily account for such a rapid increase of the viral load increase upon CD8 depletion (Fig. 2), and both are consistent with the experimental observations. Since the killing rate in the basic model should not be faster than $\delta = 1$/day, the basic model can only account for these data if the major effect of CTLs is non-lytic [56].

A few studies directly estimated the CTL-mediated death rate by equating it to the rate at which the viral load increases upon CD8-depletion [6,7], and arrived at a killing rate between 0.25 and 0.8/day [7,8]. In the basic model of Fig. 1A one indeed expects the upslope of the viral load to reflect the killing rate K, but in the multi-stage models of Fig. 1B this is more complicated. Generally, the rate at which the viral load increases upon CD8-depletion reflects the effective replication rate of the virus in the chronic steady state just before the CD8$^+$ T cells were depleted. Compared to acute infection, the number of CD4$^+$ T cells is decreased during chronic stage.

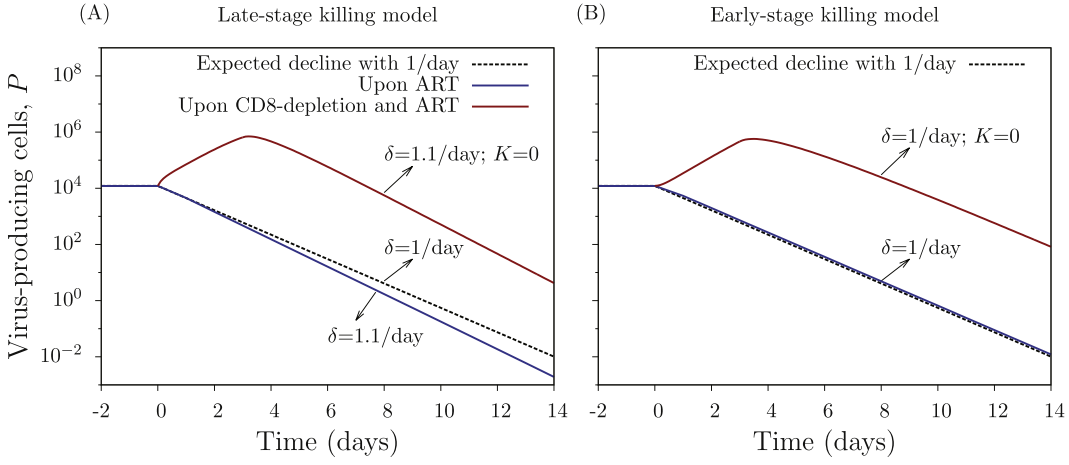


Fig. 2. Predictions of the decay of virus-producing cells following ART with or without CD8-depletion in the late-stage killing model (A) and in the early-stage killing model (B). Solid blue lines depict the dynamics of productively infected cells, P, upon ART starting at day 0. Solid red lines indicate their dynamics upon CD8-depletion at day 0 followed by ART starting at day 3. Dashed black lines depict the expected decline of virus-producing cells at a rate 1/day. During chronic infection, before CD8-depletion, the actual CTL killing rate in both models was about $K = 4.8$/day. The dynamics were computed with the differential equations corresponding to the scheme of Fig. 1B: $T' = \sigma - d_T T - \beta TV$, $I' = \beta TV - \delta_I I - \gamma I$, $P' = \gamma I - \delta_P P$ and $V = P$, with $\sigma = 10^6$ cells/day, $d_T = d_I = 0.1$/day, and $\gamma = 1$/day. The CTL dynamics and killing of infected cells obey the same functions and parameters as in [41], i.e., $E' = pEA/(h + A + E) - d_E E$ and $K = kE$, where the amount of antigen, A, is defined by P in the late-stage killing model, and by I in the early-stage killing model, and $p = 1.1$/day, $d_E = 0.1$/day and $k = 4 \times 10^{-5}$. In the early-stage killing model, a death rate of $d_P = 1$/day is used for productively infected cells such that decay rates are consistent with the experimental observations, i.e., $\delta = d_P$, whereas $d_P = 2$/day is used in the late-stage killing model (to guarantee that the eclipse phase remains the slowest time scale even after CD8-depletion). Infection rates, β, for both models are chosen following [51], such that the initial viral growth is 1.5/day (specifically $\beta = 9.1 \times 10^{-7}$ for the late-stage killing model, and 6.5×10^{-7} for the early-stage killing model). (For interpretation of the references to color in this figure legend, the reader is referred to the web version of the article.)

Therefore, the availability of suitable target cells should also be low at the onset of the CD8-depletion, and the rate at which the viral load increases is expected to be lower than during acute infection, i.e., approximately 1.5/day [13–15,57]. Indeed, the observed viral load increases of 0.25–0.8/day [7,8] are consistent with this reasoning. Similar rates of viral load increase, about 0.3–1.2/day, occur in other CD8-depletion studies [55,56,58,59]. In the model simulations of CD8-depletion (Fig. 2) the productively infected cells, and hence the viral load, increased at a realistic rate of about 1.3/day, whereas the CTL-mediated killing rate at steady state was almost 5/day. Therefore, the rate at which the viral load increases upon CD8-depletion need not always reflect the killing rate, and rapid killing rates can be consistent with the moderate viral load increase rates observed in the data.

5. Killing rates from adoptive transfer studies

More than a decade ago, Brodie et al. [60] transferred *ex-vivo* activated HIV-specific CTLs into chronically HIV-1-infected individuals, and followed the resulting dynamics of productively infected cells (defined by the expression of HIV-mRNA) and CTLs in the blood. To the best of our knowledge, this is the only study involving adoptive transfer of CTLs into HIV-1-infected humans, and provides an opportunity to directly estimate the killing rates. Fitting a mathematical model to these measurements, Wick et al. [61] estimated the killing rate due to the transferred CTLs as ranging from 1.6/day to 9.8/day [61,12]. This range thus represents the highest killing rate estimate so far.

However, the model by Wick et al. [61] described the data rather poorly, particularly around 4 days post CTL transfer, at which time the CTLs in the blood reached nadir. This could be because the simulations considered only CTLs and infected cells in the blood, ignoring their interactions in lymphoid tissues. Indeed, at day 4 Brodie et al. [60] found 10-fold more CTLs in lymph nodes (LNs) than in the blood. Moreover, LN-residing CTLs preferentially co-localized with HIV-infected cells, suggesting that CTLs in the LN continue to kill infected cells at this time point. Because the frequency of CTLs in the LNs was measured at only a single time point, it remains unknown whether they preferentially accumulated in the LN, and what fraction of killing occurs in the LN. Therefore, the estimated death rates are likely inaccurate.

6. Conclusions

Over the past decades, researchers have used various experimental and modeling approaches to determine the importance of CTLs in mediating lytic control of HIV-1 infection. In this review, we have examined several estimates for CTL-mediated death rates of HIV-1 infected cells. We conclude that there is currently not a single robust estimate, and have highlighted some of the difficulties associated with inferring killing rates from escape rates. Unfortunately, models where productively infected cells are killed largely at an early stage [48], at a late stage [47], or where CTL action occurs largely through non-lytic effects [8] can all explain the data, and it thus remains unknown which of them is correct. This calls for novel experiments that can distinguish between these mechanisms. In conclusion, published estimates of CTL-mediated killing rates remain uncertain, and we have only added to this uncertainty by arguing that rapid killing rates are also consistent with the data. Thus, CTL killing could after all be the major cause of death of productively infected $CD4^+$ T cells.

Conflict of interest

The authors declared that they have no competing interests.

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