Impact of antigen expression kinetics on the effectiveness of HIV-specific cytotoxic T lymphocytes

Carel A. van Baalen[1], Christophe Guillon[1], Minus van Baalen[2], Esther J. Verschuren[1], Patrick H. M. Boers[1], Albert D. M. E. Osterhaus[1] and Rob A. Gruters[1,3]

[1] Institute of Virology, Erasmus MC Rotterdam, Rotterdam, The Netherlands
[2] Institute of Biodiversity and Ecosystem Dynamics, Population Biology Section, University of Amsterdam, Amsterdam, The Netherlands
[3] UMR 2142 CNRS/bioMérieux, ENS de Lyon, Lyon, France

Recent studies indicate that the time required for virus-infected cells to become vulnerable for the activity of CTL is of significance for the capacity of CTL to control ongoing viral reproduction. To investigate whether this applies to the effectiveness of HIV-1-specific CTL, we measured virus production in cultures containing $CD4^+$ T cells inoculated with HIV at low multiplicity of infection, and CTL directed against an early protein, Rev, or a late protein, RT. The Rev-specific CTL prevented at least 2 $\log_{10}$ more HIV-1 production, in 10 days, than similar numbers of RT-specific CTL. To study how CTL effectiveness depends on variations in the potency of effector functions and kinetics of HIV protein expression, we developed a mathematical model describing CTL-target cell interactions during successive infection cycles. The results show that substantially higher CTL-mediated target cell elimination rates are required to achieve control as there is less time for CTL to act before infected cells release progeny virions. Furthermore, *in vitro* experiments with HIV recombinant viruses showed that the RT-specific CTL were at least as effective as the Rev-specific CTL, but only if the RT epitope was expressed as part of the early protein Nef. Together these results indicate that CTL control ongoing HIV reproduction more effectively if they are able to recognize infected cells earlier during individual viral replication cycles. This provides rationale for immunization strategies that aim at inducing, boosting or skewing CTL responses to early regulatory proteins in AIDS vaccine development.

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1 Introduction

HIV-specific cytotoxic T lymphocytes (CTL) can inhibit virus replication by killing infected cells and by secreting non-lytic antiviral factors [1]. The *in vivo* dynamics of CTL responses, viral loads and emergence of viral escape variants indicate that CTL exert considerable pressure on HIV replication during the primary and chronic stages of infection (for a recent review see [2]). In most individuals, however, CTL are not capable of controlling ongoing viral reproduction after primary viremia, and the underlying mechanisms may differ among individuals [2–4].

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Abbreviations: Rev: Early protein **RT:** Late protein R_0**:** Virus' reproduction ratio **MOI:** Multiplicity of infection

Previously we observed that prognosis for HIV-1-infected individuals [5] and resolution of primary viremia in SIV-infected macaques [6] were better if CTL responses were not only directed against structural viral proteins, but also against the regulatory proteins Rev and Tat. These findings support the hypothesis that the latter are more suitable to control HIV by virtue of their specificity for early viral proteins [5, 7]. Their presence also adds to the breadth of the CTL response, which has been reported to be beneficial for control of HIV infection [8–10].

Here we further investigate the hypothesis that the ability of CTL to recognize infected cells earlier during a viral replication cycle contributes to the effectiveness of CTL in controlling ongoing HIV reproduction, as has been reported for CTL in other viral infections [11, 12]. The HIV proteins Tat, Rev and Nef have been detected at 6 h following acute infection in T cells [13]. These early proteins

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are translated from the first, multiple spliced, transcripts [14, 15] that can be detected between 6 to 8 h after infection of T cells with HIV-1 [16]. Unspliced transcripts encoding stuctural polyproteins, *e.g.* Gag and Pol, are not detected until later and increasing levels after approximately 24 h coincide with onset of virion release [14].

Most HIV-specific CTL that have been analyzed for their antiviral capacity were directed against epitopes derived from intermediate and late proteins [17–20]. Here, $CD4^+$ T cells are inoculated with HIV at low multiplicity of infection (MOI) and cocultured with CTL directed against Rev (early) or RT (late). The results from these experiments are used to calibrate a matemathical model that simulates CTL-target cell interaction during multiple infection cycles. This allows us to analyze how variations at the effector and target cell level can influence CTL effectiveness. Predictions of the model are tested *in vitro* with HIV recombinants constructed to express CTL epitopes with different kinetics.

2 Results

2.1 Inhibition of HIV reproduction by CTL

Non-immortalized $CD4^+$ TCL2H7 cells were inoculated with primary HIV-1 at low MOI. Production of extracellular p24 could be monitored during 10 days without restimulation or addition of fresh cells. The virus used, HIV-$1_{ACH320.2A.2.1}$ (HIV-$1_{2.1WT}$) was detectable from day 4 onwards and increased exponentially over a 3 $\log_{10}$ range, until a plateau was reached by day 9 (Fig. 1a) [21] cf. [22].

CTL were added at different ratios and the CD8/CD4 cell ratio was followed by flow cytometry. In cultures containing RT-CTL and CD4 cells at a 1:10 ratio on day 2, HIV production was delayed, resulting in a 2 $\log_{10}$ reduction of p24 levels by day 10 (Fig. 1a). A tenfold lower initial CD8/CD4 cell ratio resulted in exponentially increasing p24 levels between day 6 and 10, reaching tenfold higher p24 levels (Fig. 1b). Sequence analyses of extracellular virus revealed no mutations in the epitope or flanking regions (data not shown), despite the continued presence of RT-CTL (Fig. 1a, b).

In cultures with Rev-CTL at similar CD8/CD4 cell ratios as the RT-CTL in Fig. 1b, virus remained undetectable (Fig. 1c). Only in cultures initiated with tenfold less Rev-CTL, low p24 levels ($<$0.2 ng/ml) were detected at the end, when $CD8^+$ cell numbers had decreased to the detection limit (Fig. 1d). No mutations were found in the epitope or flanking regions (data not shown).

2.2 Cytolytic capacity of the CTL

We tested whether the Rev-CTL and the RT-CTL differed in their capacity to lyse vulnerable target cells. TCL2H7 cells were labeled with the minimal peptide-epitopes and incubated with different numbers of effector cells in chromium-release assays. The lowest effector/target cell (E/T) ratio required to lyse all vulnerable cells was 4:1 for both CTL populations, and both achieved half-maximal lysis at E/T =0.7:1 (Fig. 2). Low MOI was mimicked by addition of tenfold excess of unlabeled TCL2H7 cells. This did not significantly reduce the percentage of cells lysed by the CTL at various E/T ratios (data not shown).

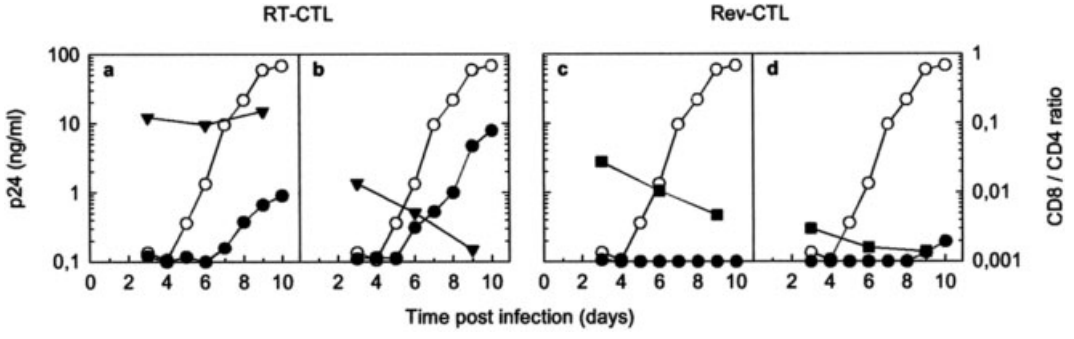

Fig. 1. Inhibition of HIV reproduction by RT- and Rev-CTL. (a–d) Kinetics of virus production by TCL2H7 cells infected with HIV-$1_{2.1WT}$ and cultured without (open circles) or with (closed circles) CTL at different ratios. Virus production levels were quantified by p24 ELISA [43]. Cocultures were initiated with 2×10^5 $CD4^+$ T cells and two quantities of RT-CTL (a and b; triangles), or Rev-CTL (c and d; squares). The CD8/CD4 cell ratio was assessed by flow cytometry. Similar results were obtained in four independent experiments.

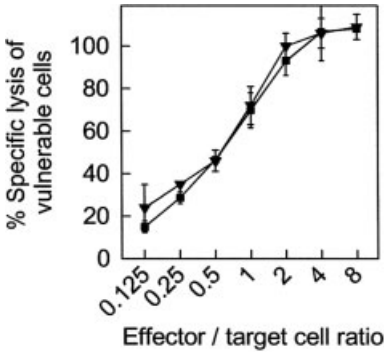

Fig. 2. Cytolytic capacity of the RT- and Rev-CTL. Peptide-labeled TCL2H7 cells were incubated with RT-CTL (triangles) or Rev-CTL (squares) at indicated effector/target cell ratios in a chromium-release assay (mean of triplicates). Maximal lysis was reached by 5 h at effector/target ratios >4; $RT_{244–252}$- and $Rev_{67–75}$-peptide labeled target cell populations contained 100% and 80% vulnerable cells, respectively. Results are expressed as percentage of maximal lysis of vulnerable cells, allowing estimation of the number of CTL required for half maximal lysis.

2.3 An in silico model for CTL effectiveness

To explore how effectiveness of HIV-specific CTL depends on variability in potency of effector mechanisms and kinetics of antigen expression, we developed a mathematical model, based on a previous model by Klenerman et al. [23]. Fig. 3 depicts the stages susceptible cells go through after infection and when they encounter specific CTL. In both models, elimination of infected cells is an exponential decay process at rate α, and elimination can begin when infected cells become vulnerable at time a_V after infection. The model presented here takes into account that progeny virions can start new infection cycles at a maximum rate of *m* per day.

To obtain estimates for *m* we fitted values in the model of p24 levels observed in the *in vitro* experiments shown in Fig. 1 and 5. The estimates ranged from 12–14 infections/day. Considering essentially the same parameter values for the onset of virus production (a_P = 1 day), and the lifetime of productively infected cells (a_D–a_P =2.5 days) as Klenerman et al., this implies that, in absence of CTL, one infected cell will cause 30–35 new infections. This number, the virus' reproduction ratio (R_0) is within the range of estimates obtained during primary viremia *in vivo* [24].

The virus' R_0 should be reduced to less than 1 to quench ongoing viral reproduction. By how much a specific CTL population will reduce R_0 depends on its action, which has two components, a_V and α. From the model framework, depicted in Fig. 3, the virus' R_0 can be derived and is equal to:

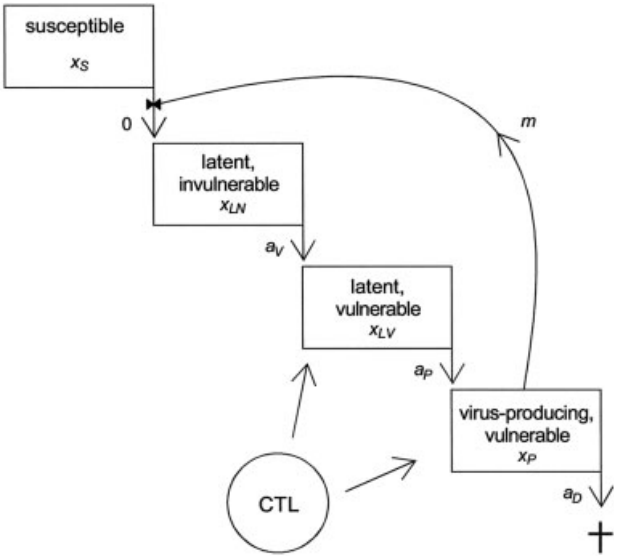

Fig. 3. Schematic representation of the model combining virus kinetics and infection cycles. Densities of susceptible cells, non-vulnerable latent cells, latent cells vulnerable to CTL attack and virus-producing cells (vulnerable) are given by x_S, x_{LN}, x_{LV} and x_P respectively. The 'age' of a cell measures the time since infection: cells become vulnerable to CTL attack at age a_V, start to produce virus at age a_P and die at age a_D. The rate at which infected cells are eliminated from the replication cycle by CTL is α. The reproduction ratio R_0 of the virus in absence of CTL is $R_0^- = m(a_D - a_P)$; in presence of CTL it is given by equation (1) in Sect. 2.3; the relative amount of virus reproduction prevented by CTL is therefore $F = R_0 / R_0^-$.

$$R_0 = m e^{\alpha a_V} \frac{e^{-\alpha a_P} - e^{-\alpha a_D}}{\alpha} \quad (1)$$

which is the product of the infection rate *m* and survival of infected cells during the producing stage.

2.4 Requirements for CTL to control virus spread

We varied individual parameters to test their influence on CTL effectiveness. Input values for the moment that cells become vulnerable for CTL (early: a_V =0.3 days; late: a_V =0.9 days) were the same as analyzed by Klenerman et al. [23]. In Fig. 4a the whole shaded area represents the amount of virus produced in absence of CTL. If RT-CTL can eliminate target cells from 0.9 days on, the fraction of cells that survive decays rapidly and virus production is reduced by 90%. Rev-CTL, that can start recognizing cells at 0.3 days, reduce virus production by 98% if they eliminate cells at the same rate as RT-CTL (α =3.0 in this

example). Although Rev-CTL have a slightly larger impact on virus production/infection cycle, these calculations do not resolve whether $R_0 < 1$ can be achieved. In Fig. 4b, the fraction of virus production that has to be prevented to meet this criterium (shaded area) has been calculated as function of the viral reproduction capacity (R_0 or m). At low viral reproduction ratio, a small reduction of the amount of virus produced will suffice. However, virus production should be reduced by more than 97% during each infection cycle at moderate infection rates, like for HIV-1 in T cells (m=12–14).

If vulnerability develops at other times than analyzed above, the α required to achieve $R_0 < 1$ changes (Fig. 4c). For CTL directed against early expressed epitopes, an α in the order of 2 to 3 day^{-1} suffices. But as there is less time to act before onset of virion release, CTL should eliminate infected cells at markedly higher rates. The beneficial effect of early recognition is more pronounced if the infection rate is larger (cf. m=6, 12, 24 in Fig. 4c). These results indicate that CTL have a higher per cell capacity to control HIV reproduction, if they have more time to eliminate infected cells before onset of virion release.

2.5 Impact of epitope expression kinetics on CTL effectiveness *in vitro*

To test this *in vitro*, we constructed recombinant viruses containing sequences encoding epitopes derived from late proteins inserted into the early expressed *nef* gene [21]. HIV-$1_{2.1RN}$ contains the RT-epitope recognized by the RT-CTL used in this study, and HIV $1_{2.1EN}$ contains a previously described Env-epitope [25]. Insertion of the epitopes did not perturb production of full-length Nef [21] or Nef-mediated down-modulation of HLA class I expression on the cell surface (data not shown). Cells expressing the recombinant Nef proteins were recognized by their cognate CTL [21], indicating that the epitopes were correctly processed and presented. Both HIV-$1_{2.1RN}$ and HIV-$1_{2.1EN}$ replicated with similar kinetics as HIV-$1_{2.1WT}$ (Fig. 5a–d, open symbols, cf. [21]).

TCL2H7 cells were infected with HIV-$1_{2.1EN}$ and HIV-$1_{2.1RN}$, and cocultured with different numbers of RT-CTL. The CD8/CD4 cell ratios were ~ 1:3 and 1:50 on day 2 and declined thereafter (Fig. 5a–d). The effect of the RT-CTL on HIV-$1_{2.1EN}$ was dose dependent, with minimal reduction of virus replication at low density, and a 3 to 4-day delay at higher CD8/CD4 cell ratios (Fig. 5a, b), comparable with the effect in cultures infected with HIV-$1_{2.1WT}$ (data not shown). This indicates that the effectiveness of RT-CTL was not affected by the Env-epitope in Nef. By contrast, HIV-$1_{2.1RN}$ was completely suppressed by the

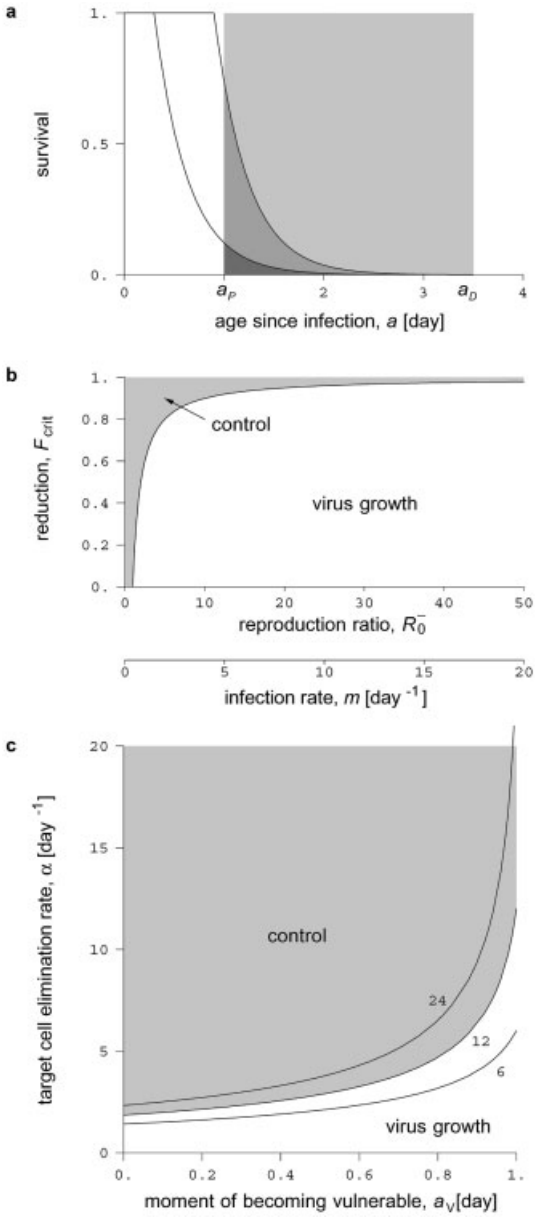

Fig. 4. Reduction of virus production by CTL. (a) Expected survival of infected cells as a function of age since infection, in the presence of CTL that either attack from age a_V =0.3 onward (left curve), or from age a_V =0.9 onward (right curve); both eliminate infected cells at rate α =3.0/day. The virus' R_0 is proportional to amount of virus produced (shaded areas: light in absence of CTL, intermediate for late-acting CTL, dark for early-acting CTL). (b) Critical reduction [difference between the shaded areas in panel (a)] of R_0 that is necessary for control of the infection. Also indicated is how the virus' R_0 depends on the rate of infection m. (c) Combinations of the moment of becoming vulnerable (a_V) and the target cell elimination rate (α) that result in control. In the shaded area $R_0 < 1$ for the case where the infection rate m =12; the curves indicate how the area changes with infection rate (m =6, 12 or 24).

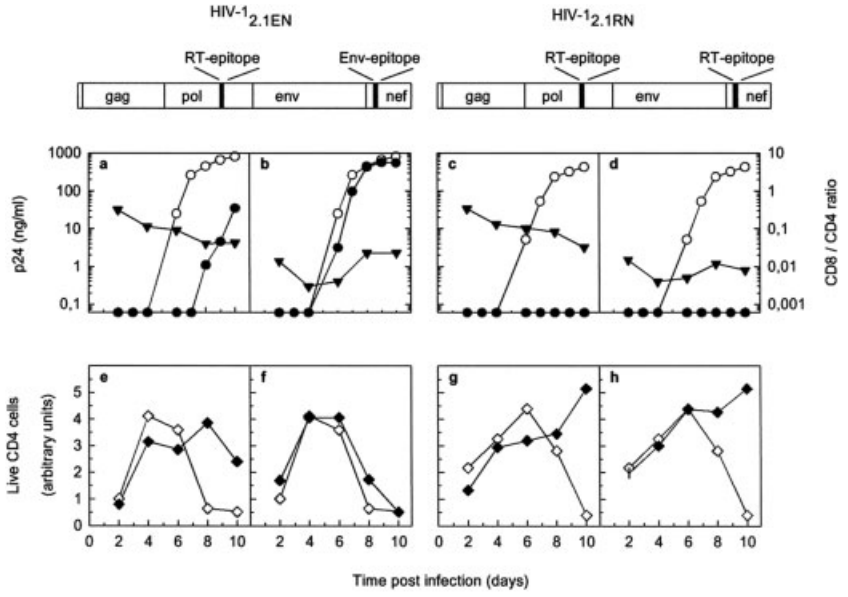

Fig. 5. Kinetics of antigen expression and the effectiveness of RT-CTL. Kinetics of virus production by $CD4^+$ TCL2H7 cells infected with recombinant HIV-$1_{2.1EN}$ (a, b), or HIV-$1_{2.1RN}$ (c, d). Extracellular p24 levels were quantified in cultures initiated with 3×10^5 $CD4^+$ T cells, containing a number of infected cells proportional to approximately 17 ID_{50}, together with no (open circles), 3×10^5 (a and c; closed circles) or 3×10^4 (b and d; closed circles) RT-CTL. The CD8/CD4 cell ratio in cultures containing RT-CTL (triangles) was assessed by flow cytometry. (e-h) Dynamics of life, $7AAD^-$, $CD4^+$ T cells during the culture period in absence (open diamonds) or presence (closed diamonds) of RT-CTL; (e-h correspond to the same cultures as a-d). Data are presented as event count ($\times10^{-3}$) acquired in 90 s from 200-μl samples. Similar results were obtained in three independent experiments.

RT-CTL, even at low density (Fig. 5c, d). No changes were observed in the primary sequence of the recombinant *nef* genes of the virus recovered at the end of the experiment (data not shown).

2.6 Effect of CTL on $CD4^+$ T cells in the presence of HIV

In the same experiment we followed the number of viable $CD4^+$ cells. In absence of CTL, the number of $CD4^+$ cells declined concurrently with the increasing virus levels, indicating that cell death was related to virus production (Fig. 5e–h). In presence of RT-CTL, $CD4^+$ cell death occurred later and paralleled the delay in the HIV-$1_{2.1EN}$ production (Fig. 5e, f). The enhanced control of HIV-$1_{2.1RN}$ reproduction by the RT-CTL was associated with continued proliferation of the $CD4^+$ cells (Fig. 5g, h), similarly to uninfected $CD4^+$ cell cultures (data not shown). Thus, expression of the RT-epitope as part of the *nef* gene not only rendered the RT-CTL more effective in inhibiting virus reproduction, it also enabled them to protect the $CD4^+$ cell population from virus-related cell death.

3 Discussion

3.1 Different effectiveness of CTL directed against early or late HIV proteins

This study provides evidence for the contribution of early target cell recognition to the capacity of HIV-1-specific CTL to control HIV reproduction in $CD4^+$ T cell populations. Previously reported data indicated that CTL directed against the early Rev protein prevented slightly more virus production during a single infection cycle than CTL directed against the late RT protein; >97% and ~92% reduction by 48 h after infection, respectively [26]. Here, residual progeny virus could start successive infection cycles and the small difference was shown to increase markedly over time. After 10 days, the Rev-specific CTL had prevented at least 2 $\log_{10}$ more virus production than a similar number of RT-specific CTL, which was not compensated for by tenfold more effector cells. These results are in line with the notion that if CTL are to be antivirally active, they have to lyse infected cells within a given time window [27], and that small differences in their capacity to reduce virus production can have dramatic effects on overall virus control due to the capacity of virus populations to expand exponentially [11, 28].

3.2 Differences in potency of CTL effector mechanisms or target cell vulnerability

Several alternative mechanisms could explain these results. Our analyses showed that neither escape by mutation of virus nor differences in direct cytolytic capacity of the effector cells are likely explanations for the lower effectiveness of the RT-specific CTL. Moreover, the latter required less peptide for half maximal lysis than the Rev-specific CTL [26, 29], and at saturating peptide levels, both effector cell populations lysed half of the target cells at similar E:T ratio (this study). Possible differences in the number of vulnerable cells that one effector cell can eliminate during its lifetime, the rate of serial killing, or the efficiency of non-cytolytic effector mechanisms require further analyses.

The abundance of RT-epitopes on the surface of infected cells has been shown to be a limiting factor for CTL-mediated lysis of chronically infected immortalized T cells [30]. However, in our experiments, which utilize activated T cells, primary virus isolates, and allow for multiple infection cycles, similar fractions of infected cells were lysed by the RT- and Rev-specific CTL [26]. Furthermore, high-level antigen expression does not suffice for complete CTL-mediated control if the antigen, *e.g.* Gag, is expressed late [19].

The significance of antigen expression kinetics was further investigated with a recombinant virus that encoded a minimal RT-epitope in the early expressed *nef* gene. The epitope is expected to be generated earlier and at higher levels, which both would result in earlier vulnerability of the target cells. The recombinant virus remained undetectable in the presence of the RT-specific CTL for 10 days, demonstrating that the CTL were not functionally impaired. That the parental virus continued to spread, without escape by mutation, despite the RT-CTL, is therefore more likely due to their inability to attack enough infected cells before progeny virions initiated new infection cycles.

Our data on MHC class I expression (not shown) indicated that it is unlikely that the epitope insert in the *nef* gene merely affected the ability of Nef to reduce MHC class I expression [31]. This could have caused a larger fraction of infected cells to become vulnerable before the onset of virion release. Furthermore, early epitopes are more likely than late epitopes to be presented before Nef can interfere with their presentation. Although further studies are required to measure the average and range of the time that infected cells are vulnerable before producing progeny virus, our results indicate that timing is a critical factor in CTL-mediated control of HIV reproduction.

3.3 Mathematical model for CTL-target cell interaction and viral dynamics

Direct measurement of intervals between onset of target cell vulnerability and virion release is problematic due to the many factors that govern triggering of CTL by infected cells, including MHC-peptide abundance and TCR density, CTL differentiation, and the intrinsic potency of effector mechanisms. Moreover, whether a certain interval is sufficient for CTL to control virus spreading depends on kinetics of virus reproduction and rates of serial target cell elimination by CTL, as well.

Integration of experimentally determined values in mathematical models can help to assess the relative contribution of some parameter to the final outcome. Complete control of ongoing viral reproduction by CTL requires that they reduce the virus' reproduction ratio R_0 to less than 1. We analyzed two components of CTL pressure on R_0: the time at which target cells become vulnerable to CTL (a_v), and the CTL-mediated target cell elimination rate (α). In the present model, target cell elimination refers to loss of the infected cell in terms of their ability to contribute to new infection cycles. Biologically, this could be achieved both by lysis of infected cells and by suppression of viral protein production via non-cytolytic mechanisms.

The results show that if CTL eliminate infected cells from the reproduction cycle with an exponential decay rate of approximately 2 to 3/day, they can control the infection, provided that infected cells become vulnerable within approximately 16 h after infection. After that time, the target cell elimination rate, *e.g.* the effector cell number, has to increase considerably as there is less time before release of progeny virus begins. These results reveal how antigen expression kinetics in infected cells can influence the per cell capacity of CTL to control ongoing viral reproduction. They provide a plausible explanation for variations in effectiveness of Rev- and RT-specific CTL.

3.4 Implications for pathogenesis of HIV infection and vaccine design

The present data indicate that the association of CTL responses directed against Rev and Tat with slower rates of disease progression [5] can be explained by the ability of CTL directed against early proteins to start eliminating infected cell earlier during the eclipse phase. Yet, Nef-specific CTL have not been associated with better control of HIV infection [5, 32]. This could be explained by the fact that the *nef* gene, unlike *tat* and *rev*, does not overlap with other open reading frames, and variation resulting in escape can be frequently tolerated [33].

Evasion of HIV from immune pressure during the different stages of infection has been attributed to escape by mutation [2], persistence of virus in latently infected cells, immune-privileged sites [34] or FDC-networks [35], and impairment of immune responses [2–4]. Our data suggest that the failure of CTL to control ongoing viral reproduction after primary viremia *in vivo* may also result, in part, from the fact that the dominant CTL response is generally directed against late proteins, which is associated with lower pressure exerted by these CTL on the reproduction of HIV [36].

Several studies have shown that vaccination with regulatory proteins induces effective immune responses. Vaccination with Tat, alone or in combination with Rev, gave mild transient viremia and a beneficial follow up after challenge in macaques [37–39]. More recently we observed that pre-existing CTL responses in Rev- and Tat-vaccinated cynomolgus macaques correlated with better control of primary SIV viremia than in Gag-and Pol-vaccinated macaques [40]. Considering the number of proteins, their size and levels of expression, it is unlikely that the higher protection level induced by the Rev/Tat vaccine was due to more broadly directed immune responses. Collectively, our data provide rationale for further evaluation of immunization strategies aimed at induction, boosting or skewing CTL responses to early regulatory proteins in AIDS vaccine development.

4 Materials and methods

4.1 Cells

Generation of the $CD8^+$ $Rev_{67–75}$SAEPVPLQL-specific, HLA-B14-restricted, TCC108 CTL clone, the $CD8^+$ $RT_{244–252}$ IVLPEKDSW-specific, HLA-B57-restricted, TCL1C11 CTL line and the polyclonal $CD4^+$ TCL2H7 cell line (HLA-B14,-B57) has been described [26, 29]. Cells were stimulated with phytohemagglutinin-L (PHA, 1 μg/ml; Boehringer Mannheim) and gamma irradiated feeder cells every 10–14 days as described [26]. Cell concentrations were kept between 0.3×10^6/ml and 1.0×10^6/ml.

4.2 Viruses

HIV-$1_{ACH320.2A.2.1}$, referred to as HIV-$1_{2.1WT}$, is a molecular clone of a primary, non-syncytium-inducing virus isolate from patient 320 of the Amsterdam Cohort Studies on HIV-1 infection and AIDS [41, 42]. Recombinant HIV-$1_{2.1RN}$ and HIV-$1_{2.1EN}$ were generated by replacing the *nef* gene of HIV-$1_{2.1WT}$ with the recombinant *nef* genes containing the sequences encoding IVLPEKDSW (2.1rn) or ERYLKDQQL (2.1en) [21]. Virus stocks, generated by transfection of 293T cells, were used to infect TCL2H7 cells. Virus production was monitored with a p24-ELISA as described [43]. For estimation of the fraction of infected cells at the start of the experiment, six fivefold dilutions of the inoculated cells were cultured in quadruplicate with uninfected TCL2H7 cells for 14 days. The fraction p24-positive wells for each of the dilutions was used to calculate the ID_{50} (Kärber estimate).

4.3 Flow cytometry

At indicated times, 110-μl samples of the cultures were centrifuged and cells were incubated with CD4-fluorescein isothiocyanate, CD8-phycoerytrine (Dako), and 7 amino-actinomycine-D (7AAD) (Sigma) for 20 min, washed, resuspended in 200 μl PBS containing 2% paraformaldehyde (Merck), and analyzed on a FACScan (Becton Dickinson). This allows discrimination between CTL ($CD4^-CD8^+$) and target cells ($CD4^+CD8^-$), and live ($7AAD^-$) and dead ($7AAD^+$) cells [44].

4.4 Chromium-release assays

Lysis of peptide-labeled target cells was assessed as described previously [26]. Mean values of triplicate incubations were calculated as follows: % specific lysis =100×[(experimental release–spontaneous release)/(maximal release–spontaneous release)]. Synthetic peptides corresponding to HIV-1 $RT_{244–252}$ IVLPEKDSW and $Rev_{67–75}$SAEPVPLQL were manufactured by EVL (Woerden, The Netherlands).

4.5 Mathematical model

Our dynamic model extends the static model analyzed by Klenerman et al. [23], and describes changes in densities of virus-susceptible cells, infected but invulnerable cells, cells vulnerable to CTL attack, and virus-producing cells (Fig. 3). The dynamics are given by a set of four delay-differential equations:

Susceptible cells (x_S) become infected at rate r (see equation A5)

$$dx_S(t)/dt = -r(t) \quad \text{(A1)}$$

Infected cells that are latent, *i.e.* do not yet produce virus, and not vulnerable to CTL attack (x_{LN}), become vulnerable at $t = a_V$

$$dx_{LN}(t)/dt = r(t) - r(t-a_V) \quad \text{(A2)}$$

Latent but vulnerable cells (x_{LV}) are eliminated by CTL at rate α and start to produce virus at $t = a_P$

$$dx_{LV}(t)/dt = r(t-a_V) - \alpha - r(t-a_P)e^{-a(a_P-a_V)} \quad \text{(A3)}$$

Virus producing cells (x_P) are eliminated by CTL at rate α or die at $t = a_D$ due to the virus

$$dx_P(t)/dt = r(t-a_P)e^{-a(a_P-a_V)}\,\alpha - r(t-a_D)e^{-a(a_D-a_V)} \quad \text{(A4)}$$

Recruitment of latent cells (infection) at time t is given by

$$r(t) = mx_S(t)\,x_P\,(t) \quad \text{(A5)}$$

Initial conditions are $x_S\,(0) = 1$, $x_{LN}\,(0) = x_{LV}\,(0) = x_P\,(0) = 0$; the model is started with a short pulse of infection (for details on analyses of delay-differential equations, see Gurney et al. [45]). As in the static model, we assume that infected cells start to produce virus at $t = a_P = 1$ day after infection. An insignificant difference is that Klenerman et al. [23] assume distributed cell deaths after the onset of production of virus (cells die with a rate of $c = 0.4$/day) whereas we assume that all virus-producing cells die at a fixed moment (at $t = a_D = 1/c = 2.5$ days after the onset of virus production). We did the analysis assuming distributed cell deaths, and obtained virtually the same results. The most significant change to the model is that multiple infection cycles occur. That is, a virus-producing cell generates newly infected cells with a maximum rate of m/day (m is the rate of infection in the beginning of the experiment, the effective rate will of course decline in the course of the experiment due to depletion of susceptible cells).

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Correspondence: Albert D. M. E. Osterhaus, Institute of Virology, Erasmus MC Rotterdam, PO BOX 1738, 3000 DR, Rotterdam, The Netherlands
Fax: +31-10-408-9485
e-mail: Osterhaus@viro.fgg.eur.nl

M. van Baalen's present address: Institut d'Ecologie, CNRS UMR 7625, Université Pierre et Marie Curie, Paris, France