# Cytotoxic T lymphocytes in HIV-1 infection: a killing paradox?

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*A decade after the first publications on HIV-1-specific cytotoxic T lymphocytes (CTLs) their role in the pathogenesis of AIDS is still equivocal. There is an ongoing debate as to whether HIV-1-specific CTLs control the viremia or mediate CD4$^+$ T-cell depletion and progression to AIDS. However, experimental data in favour of a beneficial role of HIV-1-specific CTLs are mounting. Here, Michèl Klein and colleagues try to depict and reconcile the current paradoxes of HIV-1-specific CTL responses.*

Cytotoxic T lymphocytes (CTLs) are part of the major host defence against trespassing intracellular pathogens. It has been shown *in vitro* that human immunodeficiency virus type 1 (HIV-1)-specific CTLs can eliminate virus-infected cells via MHC class I-restricted killing[1,2] and can interfere with HIV-1 replication via secretion of various antiviral cytokines (reviewed in Ref. 3), thus it is widely held that antiviral CTL responses to HIV-1 are 'salutary' to the infected host. However, after a decade of intense research on HIV-1-specific CTLs (Refs 1, 2) their precise role during the clinical course of HIV-1 infection is still not fully resolved. The majority of studies to date seem to support the concept that HIV-1-specific CTLs contribute to controlling viral replication and thus to delaying the onset of disease. However, several observations have suggested that HIV-1-specific CTLs are 'pathogenic' to the patient. HIV-1-specific CTLs have been detected in bronchoalveolar lavage fluids of some HIV-1-infected patients suffering from lymphocytic alveolitis or interstitial pneumonitis[2], and in cerebrospinal fluid of some infected patients suffering from neurological disorders[4]. Zinkernagel has even suggested that severe depletion of CD4$^+$ T cells and progression to AIDS result from the killing of huge numbers of infected cells by HIV-1-specific CTLs (Refs 5, 6). If this were true, it could greatly affect the current rationale of developing AIDS vaccines and treatment of HIV-1-infected patients.

In order to start composing a coherent model for the role of HIV-1-specific CTLs in the pathogenesis of AIDS, we need to (1) fully characterize HIV-1-specific CTL responses during the different clinical stages of disease, with respect to dynamics and epitope specificity; (2) understand why the virus persists in the face of vigorous and ongoing HIV-1-specific CTL responses; and (3) reveal why antiviral CTLs fail to provide lifelong protection from progression to AIDS in the vast majority of patients.

## HIV-1-specific CTL responses during the natural history of AIDS

### *CTLs during primary HIV-1 infection*

Upon sexual or parenteral transmission of HIV-1 it usually takes ~2–4 weeks before clinical symptoms of acute infection ensue. In about 50–70% of infected individuals an acute retroviral syndrome develops with mild influenza-like manifestations from which most patients usually fully recover. To date, HIV-1-specific CTL responses have only been documented in a limited number of patients with an acute retroviral syndrome[7–12]. HIV-1-specific CTLs have been observed as early as a few days following the onset of acute symptoms and in general before (neutralizing) antibody responses could be detected (see Fig. 1). The appearance of HIV-1-specific CTLs usually parallels a striking diminution of the viremia in infected patients. Likewise, elegant studies in rhesus monkeys have shown that upon deliberate infection with simian immunodeficiency virus (SIV), CTLs appear as early as 4–7 days post virus inoculation, and coincide with viral clearance from blood and lymph nodes[13,14]. Collectively these studies indicate that CTLs are recruited very early during the encounter with HIV-1, and that in part they may be responsible for the initial control of HIV-1 replication. Interestingly, some individuals were observed who remained HIV-1 seronegative despite frequent exposure to the virus, and seem to harbour HIV-1-specific CTL responses[15]. These CTL responses may be coincidental, demonstrating that some degree of viral replication has occurred, but could also suggest that the virus has been 'repelled' because of efficient HIV-1-specific CTL responses. Furthermore, in a group of women from Gambia who seemed to have escaped HIV-1 infection despite several years of high-risk sexual behaviour, three out of six patients had CTLs that recognized HIV-1 and HIV-2 crossreactive epitopes. This suggests that cellular immunity to HIV-2 protects against infection with HIV-1 (Ref. 16). Similarly, recent animal studies demonstrated that a live attenuated HIV-2 vaccine could protect cynomolgus macaques against development of AIDS for more than five years after infection with a pathogenic strain of SIV (Ref. 17). These results are quite encouraging, but the exact features for control and clearance of HIV-1 infection remain to be elucidated further. A critical note has been put forward by Phillips[18] who produced a mathematical model of termination of the primary viremia in the absence of HIV-1-specific immune responses. His theory seems to be supported by the lack of detectable HIV-1-specific CTL responses reported in some individuals. Clearly more systematic studies of patients with acute HIV-1 infection are needed with respect to other effector

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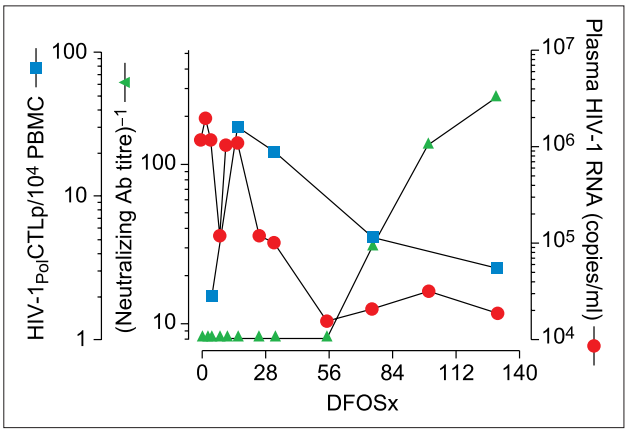


**Fig. 1.** *Temporal associations of immune responses with initial control of the viremia in acute HIV-1 infection. Data depicted in this graph were drawn from studies reported by Koup, Safrit and co-workers[7,8,84-87]. The patient was a homosexual male who seroconverted for HIV-1 eight days following onset of symptoms (DFOSx) of the acute retroviral syndrome. The viremia (red) markedly diminished at 28 DFOSx and the virologic setpoint was reached by 56 DFOSx. HIV-1-specific CTL responses were demonstrated as early as 4 DFOSx, with CTLs directed against Pol (blue) being the most pronounced. Minor responses to Gag and Env were also observed[7,8,84,85]. Neutralizing antibody responses (green) against early autologous HIV-1 isolates were first detected in the plasma at 77 DFOSx (Ref. 86). The early virus isolates were macrophage-tropic[87]. Sequence analysis of the gp120-V3 region showed a marked homogeneous virus population, at least until one year after the original presentation[8], and without any evidence for viral escape from the HLA-B7-restricted gp120-V3 CTL epitope RPNNNTRKSI (Ref. 85). After the acute phase, the CD4$^+$ T-cell counts returned to relatively normal values and the patient remained asymptomatic during the follow up of their studies. Abbreviations: Ab, antibody; CTL, cytotoxic T lymphocyte; HIV-1, human immunodeficiency virus type 1; PBMC, peripheral blood mononuclear cell.*

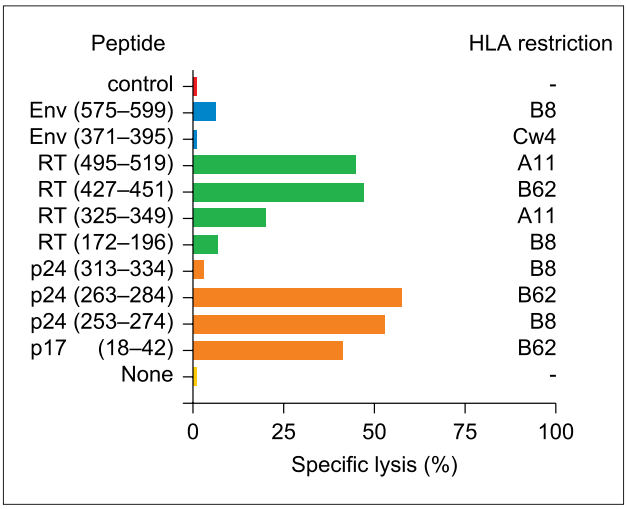


**Fig. 2.** *Simultaneous recognition of multiple HIV-1-derived CTL epitopes in cultures of unstimulated PBMCs. Data depicted in this graph were drawn from studies reported by Walker, Johnson and co-workers[1,23,24,88–90]. The graph represents experiments with freshly isolated and unstimulated PBMCs of an HIV-1 seropositive individual. PBMCs were directly tested for cytotoxicity in a standard 6 h $^{51}$Cr-release assay using Epstein–Barr virus (EBV)-transformed autologous B-lymphoblastoid cell lines that had been pulsed with the indicated synthetic peptides previously demonstrated to contain CTL epitopes (100 μg ml$^{-1}$)[91]. Numbers in parentheses indicate the position of the first and last amino acid of the peptide. For each peptide the HLA-restriction element is indicated on the right. By analyzing the epitope specificity of HIV-1-specific clones from this individual it was found that up to 14 distinct HIV-1 CTL epitopes were recognized[91]. No extensive clinical follow up data were reported on this individual: at the time the experiments were carried out he was asymptomatic, and although the single published determination of his CD4$^+$ T-cell counts (145 cells/μl) and HIV-1 RNA levels (94 800 copies/ml) indicate a significant increased risk for progression to AIDS, as of recently he is still alive and doing well. At this point he is on extensive anti-retroviral therapy (B.D. Walker, pers. commun.). Abbreviations: see Fig. 1 legend.*

functions of the immune system to refute or to support a causal role of HIV-1-specific CTLs.

### *CTLs during the asymptomatic period of HIV-1 infection*

After seroconversion, the plasma levels of HIV-1 RNA usually stabilize within several months around the so-called 'virologic setpoint'. A variable asymptomatic period follows which appears to correlate with the level of this residual virus replication[19]. The median time from HIV-1 seroconversion to clinical AIDS in adults has been estimated to be ~8–10 years. Vigorous HIV-1-specific CTL responses have been observed in most asymptomatic individuals studied to date. The precursor frequencies of HIV-1-specific CTLs as determined in limiting dilution assays typically range from $10^{-3}$–$10^{-5}$, and represent mainly memory CTLs (Refs 20–22). High levels of circulating CTL effectors can often be demonstrated directly *ex vivo* without the need for restimulation and expansion *in vitro*[1,23,24]. In addition, measurements of epitope-specific CTLs by quantitation of T-cell receptor β (TCRβ) mRNA transcripts and flow cytometric analysis of CTLs with tetrameric peptide–HLA complexes have indicated extraordinary levels of circulating HIV-1-specific effector CTLs ranging up to several percent of peripheral blood mononuclear cells (PBMCs)[11,25,26].

To date, the exact relationship between the effector and memory components of the HIV-1-specific CTL responses is unresolved[27]. It could be hypothesized that the ratio of HIV-1-specific effector CTLs to memory CTLs reflects how successfully virus replication is contained. For example, if virus replication is well controlled, the number of circulating effector CTLs might be low while memory CTL frequencies are either high or normal. In the case of a high ratio with increased numbers of effector CTLs for a prolonged period of time, this would probably predict more-rapid disease progression. In addition, one could envisage in the case of anti-retroviral treatment of HIV-1-infected patients that phenotypic analysis of HIV-1-specific CTLs could give complementary information when viral load becomes undetectable.

Another feature of HIV-1-specific CTL responses is that they typically involve recognition of a large array of epitopes from multiple HIV-1 proteins (see Fig. 2). The list of CTL epitopes has

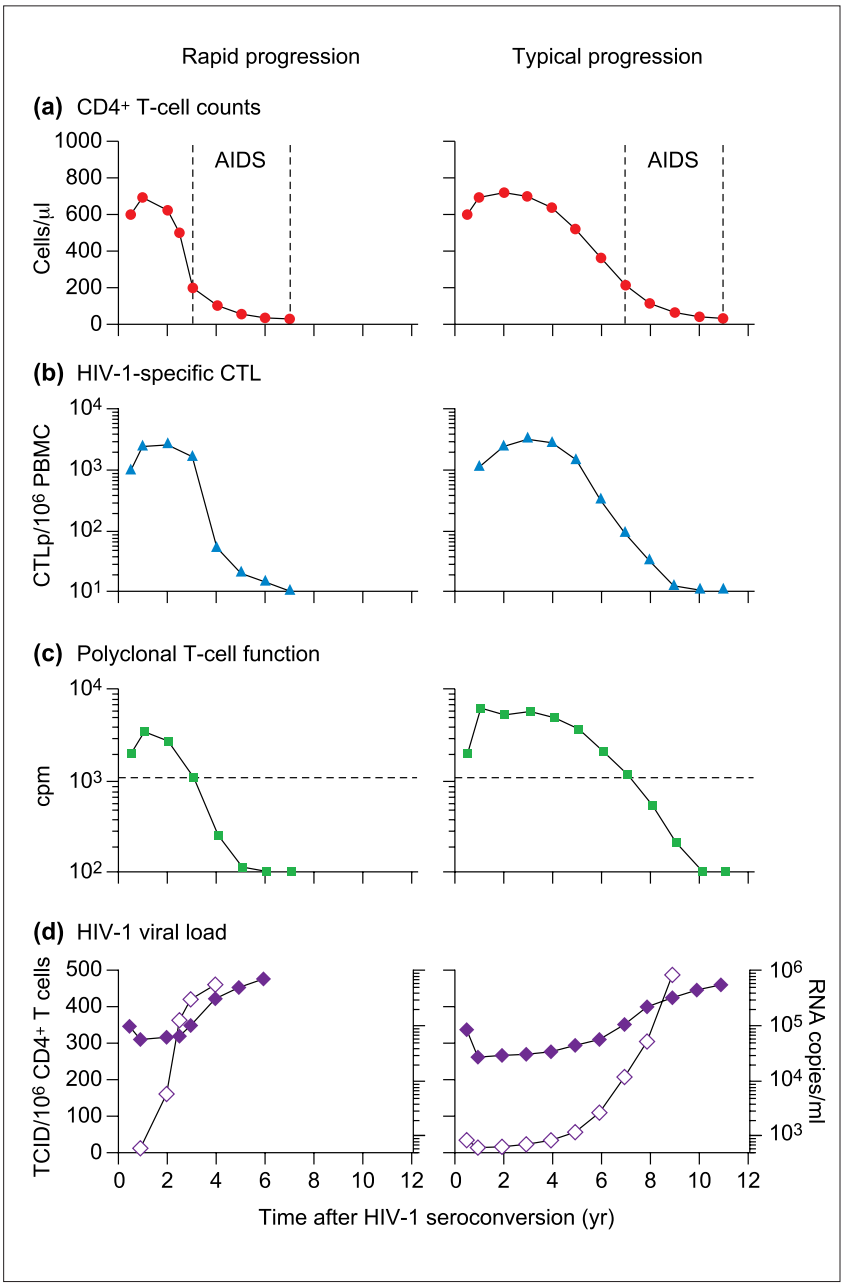


**Fig. 3.** *Natural history of HIV-1 infection. This composite graph represents a free impression of longitudinal and cross-sectional data currently available in the literature*[21–23,29–31]*. Panels on the left represent rapid progressors to AIDS within three to seven years from HIV-1 seroconversion. Panels on the right represent typical progressors who develop AIDS within 7 to 11 years. (a) Longitudinal CD4⁺ T-cell counts (red) during the years following HIV-1 seroconversion; (b) HIV-1-specific CTLp frequencies (blue) determined with limiting dilution analysis; (c) polyclonal T-cell function to CD3 monoclonal antibody in whole blood lymphocyte cultures (green); and (d) HIV-1 viral load representing the number of circulating CD4⁺ T cells productively infected with HIV-1 (TCID) (open purple), and the number of HIV-1 RNA copies/ml (filled purple). Note (1) the relatively high virologic setpoint after the acute phase of infection; (2) the increase of HIV-1 infected CD4⁺ T cells in the face of vigorous HIV-1-specific CTL responses for rapid progressors, in violent contrast to typical progressors where viral load significantly increased only after substantial loss of HIV-1-specific CTL responses; and (3) the loss of antiviral CTL responses that parallels deterioration of the polyclonal T-cell function. Abbreviations: see Fig. 1 legend.*

frequently been updated (see, for example, Ref. 28). Characterization of CTL epitopes recognized by dominant HIV-1-specific CTL responses during the natural history of HIV-1 infection is clearly relevant for future vaccine development. Information on HIV-1-derived CTL epitopes is now compiled in the Los Alamos HIV Molecular Immunology database, and has been made widely accessible through the internet (Korber, B.T.M., Brander, C., Walker, B.D. *et al.*, eds; http://hiv-web.lanl.gov/immuno/).

### *CTL during progression to AIDS*

Many studies have shown that HIV-1-specific CTL responses deteriorate during disease progression[21–23,29–31]. From longitudinal studies on HIV-1-specific CTLs and viral load it follows that there are at least two scenarios for disease progression. In rapid progressors the viral load generally seems to increase in the face of strong HIV-1-specific CTL responses suggesting that HIV-1 has escaped antiviral CTL responses. Another pattern is observed in more-typical progressors, in whom the viral load seems to increase only after a substantial loss of HIV-1-specific CTLs (see Fig. 3). The reasons for the apparent failure of CTLs to contain HIV-1 replication may be diverse.

As mentioned above, it has been proposed by some investigators that HIV-1-specific CTL responses are deleterious to the infected host[5,6]. In general however, the observations to date do not seem to support this concept. The most obvious counter argument would be the fact that CD4⁺ T-cell numbers do not recover when HIV-1-specific CTL responses deteriorate during progression to AIDS (Refs 21–23, 29–31), whereas it has been shown that most late-stage patients can still regain considerable numbers of CD4⁺ T cells during potent anti-retroviral therapy[32,33]. Likewise, infusions of large amounts of non-specifically expanded autologous CD8⁺ T cells, although the frequency of HIV-1-specific CTLs is largely unknown, have been well tolerated and without any deleterious effect on CD4⁺ T-cell counts[34]. It seems more acceptable that HIV-1-specific CTLs are associated with some inflammatory side-effects characteristic of late-stage HIV-1

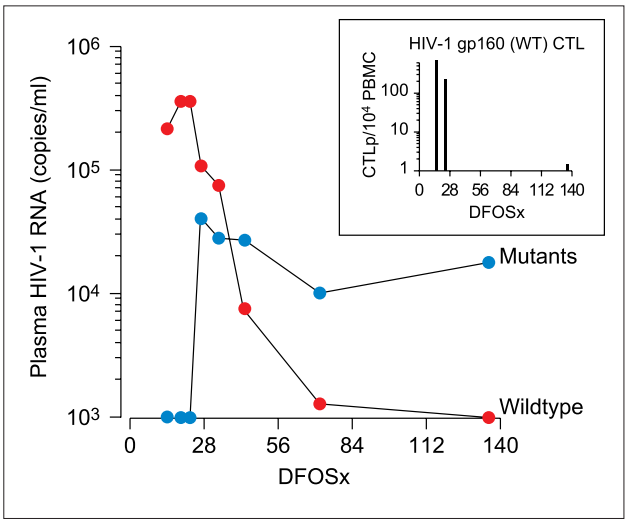


**Fig. 4.** *Emergence of HIV-1 escape variants from a dominant CTL response. Data depicted in this graph were drawn from studies by Borrow, Shaw and co-workers[9–11,92–94]. The patient was a homosexual male who presented with symptomatic primary HIV-1 infection 20 days after a single encounter with a patient with AIDS (Ref. 92). He seroconverted for HIV-1 23 days following onset of symptoms (DFOSx) of the acute retroviral syndrome. The viremia reached peak levels at 20 DFOSx and decreased significantly thereafter[93,94]. The first detectable HIV-1-specific CTL responses were observed at 16 DFOSx (Refs 9–11) and were exclusively directed against the HLA-B44 restricted wild-type (WT) gp160 CTL epitope AENLWVTVY (Ref. 11). This response was exceptional since it comprised at least 5% of the circulating PBMCs, although later it declined significantly (see insert)[9–11]. From 30 DFOSx on, CTLs with other specificities were also observed[9,11]. The early WT isolates (red) were T-cell tropic and syncytium-inducing[92]. Sequence analysis of the WT-immunodominant gp160 CTL epitope AENLWVTVY revealed that escape mutants (blue) appeared from 30 DFOSx, which were no longer recognized by the initial CTL response to this epitope. After an initial rebound, CD4⁺ T-cell counts quickly dropped below 200 cells/μl at 212 DFOSx and the patient died at 1601 DFOSx (Ref. 11). Abbreviations: see Fig. 1 legend.*

infection[2,4], but these events are probably of less significance to the initiation of the disease process.

### *CTLs during long-term survival of HIV-1 infection*

In a small proportion of HIV-1-infected individuals, which represents the right-hand extreme of disease progression, an extraordinarily benign disease course beyond the median time to AIDS is observed. This group of so-called 'long-term survivors' appears heterogeneous and seems to contain mostly very slow progressors and maybe some true non-progressors. It is expected that HIV-1-specific CTLs from long-term survivors have distinct features that contribute to prolonged maintenance of the asymptomatic phase. Several studies of long-term survivors have shown robust and persistent HIV-1-specific CTL responses[22,35–38] involving simultaneous recognition of multiple CTL epitopes[38–42]. These CTL responses coincide with relatively low numbers of HIV-1-infected cells and intuitively point towards a situation of efficient viral control. Furthermore, it has been suggested that qualitative differences in the initial immune response to HIV-1 are responsible for distinct clinical outcomes[43,44]. Pantaleo *et al.* have reported that simultaneous expansions of many TCR Vβ families during primary HIV-1 infection, probably resembling a broadly directed HIV-1-specific CTL response, at least protects against rapid progression to AIDS (Ref. 43). In addition, Van Baalen *et al.* have shown that early CTL responses from long-term survivors were more-frequently targeted at epitopes in Tat and Rev compared with those of rapid progressors[44]. This suggests that CTLs directed against early viral proteins are more effective in controlling viral load, because they are thought to kill HIV-1-infected cells before a major release of virions takes place.

Central to the controversy on HIV-1-specific CTLs are the findings that some long-term survivors seem to have rather low levels of HIV-1-specific CTLs (Ref. 37). However, this condition may be a consequence of infection with attenuated viruses[45] or may be caused by genetic defects in HIV-1 co-receptor expression[46], both of which could result in a lower level of viral replication. Although extensive phenotypic analysis of HIV-1-specific CTLs has yet to be performed[27], it could be expected that in these patients there is a much lower magnitude of effector CTL responses. Even more of a paradox are the findings that patients who progress to AIDS usually mount strong HIV-1-specific CTL responses during their asymptomatic period[22], sometimes even involving epitopes recognized by CTLs from long-term survivors[23,39,40,47]. However, consistent associations between HLA alleles and time to AIDS (for example, HLA-B8 and B35 and rapid disease progression[48]; and HLA-B27 and B57 and long-term survival[39,49]) indicate that there may be qualitative differences between HIV-1-specific CTL responses. Either structural or functional peculiarities of these HLA molecules, affecting the number or the kind of CTL epitopes presented[50], or the level of crosstalk with other cell types involved in the immune response to HIV-1 (such as interactions with inhibitory receptors on natural killer cells[51]) could be responsible for the observed associations.

## Persistence of HIV-1 in the face of vigorous antiviral CTL responses

Despite seemingly potent CTL responses, HIV-1 is almost never completely eradicated from the body but instead persists for many years at the virologic setpoint[19]. It seems likely that release of virions from HIV-1-infected cells, which are relatively resistant or not readily accessible to CTL-mediated killing, or other types of immune responses, will significantly contribute to this level of residual viral replication. The ability of the virus to escape from CTL recognition and the broad cell tropism of HIV-1 (i.e. specific target-cell preference) are both considered to confer viral persistence in the face of vigorous HIV-1-specific CTL responses.

### *Viral escape from CTL recognition*

Recent studies on viral dynamics have revealed unexpectedly rapid kinetics of HIV-1 replication[32]. The virion half-life has been estimated to be six hours and the total number of virions produced per

day at $10^{10}$ (Ref. 52). Assuming a minimum burst size of ~100 virions per cell, the total number of productively infected cells is ~$10^8$. Given the error rate of HIV-1 reverse transcriptase in the order of $10^{-4}$–$10^{-5}$ per base, and the size of the viral genome of $10^4$ base pairs, the number of mutant viruses that appear each day is tremendous[53]. This mutation rate results in a steady accumulation of random mutations in the viral genome. Only those mutations will persist in the viral quasispecies that do not significantly interfere with functional or structural constraints of viral proteins. At the same time, virus variants are continuously subjected to strong selective forces, which result in accumulation of mutations at specific sites of the viral genome. Mutant viruses may appear in the course of disease that carry mutations in CTL epitopes that affect CTL recognition; either by interfering with intracellular processing and transport of viral peptides, or with peptide–MHC–TCR interactions. Although initially some concern was raised, there is now an increasing body of experimental evidence showing that escape of HIV-1 from CTL recognition can result in viral persistence[11,12,54–58]. In cases where the HIV-1-specific CTL response is oligoclonal and involves recognition of only a single variable epitope, the virus can easily adapt and dramatic acceleration of progression to AIDS has been observed[11,56]. Adoptive transfer of an *ex vivo* expanded autologous Nef-specific CTL clone resulted in the emergence of viral escape mutants lacking the specific epitope sequence recognized, while the patient shows rapid progression to AIDS (Ref. 56). The same phenomenon has been observed by Borrow *et al.* who reported two patients suffering from acute HIV-1 infection[11]. In the face of a strong and oligoclonal expansion of $CD8^+$ T cells[9], which reflected the dominant HIV-1 gp160-specific CTL response to a single HLA-B44-restricted CTL epitope[10,11], there was a rapid accumulation of viruses that had escaped this dominant CTL response. The patients subsequently progressed to AIDS and died within three years. The level of reduction of wild-type virus, the kinetics with which mutant viruses appeared, and the genetic pathways by which the virus escaped CTL recognition bear significant similarities to the kinetics with which drug-resistant strains of HIV-1 emerge during antiretroviral therapy[59]. This indicates that HIV-1-specific CTLs can exert selective pressure on the viral quasispecies. As such, these observations are strong evidence that HIV-1-specific CTLs are relevant for controlling viral replication *in vivo* (see Fig. 4).

As mentioned above, most HIV-1 infected individuals mount polyclonal CTL responses to the virus involving simultaneous recognition of many epitopes. Not only will this seriously limit the opportunities for the virus to escape *in vivo*[60], it has also proved difficult to observe this phenomenon *in vitro*. However, it was recently shown for four asymptomatic HIV-1-infected individuals that particular variants dominated the viral quasispecies only for a short period of time as they were later eliminated by HIV-1-specific CTLs (Ref. 57). Depending on the dominance of the CTL response from which the virus escapes, the virus will experience a transient decrease of the cytolytic force of the immune system and subsequently a temporary selective advantage *in vivo*. Once CTL responses with new specificities are mounted, or CTLs from subdominant responses are boosted, the viral load is expected to return to more or less steady-state values. This transient escape mechanism may be operational during the asymptomatic period when HIV-1-specific CTL responses are not yet affected. During the clinical course of HIV-1 infection, when the immune competence of the infected host is gradually decaying (i.e. when the selection pressure by CTLs subsides), the need for the virus to adapt to antiviral immune responses will slowly disappear and the fittest virus strains will be selected. To further explore viral dynamics under the selective constraints of broad immune responses, mathematical models may need to be adjusted with more experimental data to allow for definitive statements on these temporary, and often non-linear virus–host interactions[60,61].

Another possible escape mechanism is downregulation of major histocompatibilityy complex (MHC) class I molecules in HIV-1-infected cells. Partial downregulation has been ascribed to the viral proteins Tat and Nef (Refs 62, 63). Since it appears that only a few peptide–MHC class I complexes are needed per infected cell to activate the cytolytic machinery of HIV-1-specific CTLs, the level of down-regulation of MHC class I molecules in general may not be sufficient to completely resist CTL-mediated killing[64]. Another effect of Nef could be the upregulation of Fas ligand (FasL) expression in infected cells[65], which induces apoptosis of attacking CTLs. This situation may resemble the proposed FasL-mediated evasion of melanoma cells from tumour-specific cellular immune responses[66]. Virus-induced FasL expression could serve as a means to create local sanctuaries where HIV-1-infected cells are relatively protected from CTL-mediated killing. However, $CD4^+$ T cells and macrophages infected with HIV-1 are readily killed by CTLs *in vitro*[2,64], showing that Fas-mediated apoptosis of antiviral CTLs is probably not sufficient to abrogate HIV-1-specific CTL responses.

### *Viral persistence and broad cell tropism*

The recent identification of the co-receptors for HIV-1 has substantiated the long-standing observation that different HIV-1 strains vary in their ability to infect different cell types[67]. Containment of virus-infected cells by CTLs in various tissues may be complex. Some infected cell types may be efficiently eliminated by CTLs, whereas others may be relatively resistant to cell-mediated killing (such as cells from the macrophage lineage in the brain, bone marrow and epididymis). By analogy with hepatitis B virus infection (HBV), control of viral replication at sanctuary sites may be dependent on production of antiviral cytokines[68]. It has been shown that HIV-1-specific CTLs can secrete various soluble factors that interfere *in vitro* with HIV-1 replication [interferon γ, tumour necrosis factor α (TNF-α), $CD8^+$ T-cell antiviral factor (CAF), interleukin 16 (IL-16), and β-chemokines: RANTES, macrophage inflammatory protein 1α (MIP-1α) and MIP-1β] (reviewed in Ref. 3). Although these antiviral factors appear to extend the action-radius of CTLs, elimination of virus from cellular reservoirs resistant to CTL-mediated killing will be dependent on the natural half-life of the infected cells in which the virus reside and on viral cytopathic effects. As such, these infected cells are expected to contribute significantly to the virologic setpoint and to viral persistence.

Central to the debate on HIV-1-specific CTLs is our current lack of understanding of how the different measures of viral load relate to HIV-1-specific CTL responses. The exact relationships still need to be established between the level of residual virus replication as measured by the amount of HIV-1 RNA in the plasma (i.e. cell-free virions) and the numbers and different types of cells, in tissues or in the circulation, that give viable virus progeny. In addition, infected cells could contain a proviral DNA genome that is defective or just temporarily dormant. Latently infected cells, which express very few viral antigens, are virtually invisible to the immune system until they start to produce virions upon activation. Therefore, these cells may also constitute an important viral reservoir that contributes to persistence of HIV-1 infection.

Another important consequence of the cell tropism of HIV-1 is that cells that are susceptible to infection with the virus are crucial to a properly functioning immune system (see below). Since HIV-1 attacks the Achilles' heel of the cognate immune system (i.e. $CD4^+$ T cells and macrophages), it is likely that the virus eventually will destroy or at least, in part, will interfere with cells involved in the immune response to this virus.

## Loss of specific antiviral immune surveillance

Based on the current literature, at least two non-mutually exclusive scenarios could be proposed for declining HIV-1-specific CTL responses during progression to AIDS. The first scenario would be a physical exhaustion of HIV-1-specific CTL. The second would concern a functional depletion of antiviral CTLs due to lack of sufficient levels of $CD4^+$ T helper (Th) cells.

### *Exhaustion of HIV-1-specific CTLs*

For mice infected with lymphocytic choriomeningitis virus (LCMV) it has been shown that overwhelming virus infection results in exhaustion of the antiviral CTL response[69]. A similar situation may arise in HIV-1-infected patients. Long-standing observations of the $CD8^+$ T-cell compartment in HIV-1-infected patients show directly *ex vivo* detectable CTL effector activity which is uncommon to many other viral infections[1,23,24]. Furthermore, the number of $CD8^+$ T cells is usually increased with many cells carrying an activated phenotype, and with a high percentage of $CD8^+$ T cells primed for apoptosis[70,71]. In addition, telomeres of $CD8^+$ T cells from HIV-1-infected patients are considerably shorter than those of their $CD4^+$ T-cell counterparts, suggesting a prolonged history of peripheral expansion[72,73]. Taken together these results point towards continuous activation and expansion of specific and nonspecific (bystander) $CD8^+$ T cells[74], which could possibly lead to exhaustion of antiviral CTL responses.

Currently, there is experimental evidence in support of the preferential loss of HIV-1-specific CTLs during disease progression[21,30,75,76]. It was shown both in a cross-sectional study[21] and in a longitudinal study[75], that HIV-1-specific CTLs are hardly detectable at low $CD4^+$ T-cell counts, whereas Epstein–Barr virus (EBV)-specific CTLs were seemingly unaffected at late-stage HIV-1 infection. Similarly, in some AIDS patients broad cytolytic CTL activity was preserved, whereas HIV-1-specific CTL activity was lost[30]. Furthermore, Pantaleo *et al.* have recently reported that in some patients with a primary HIV-1 infection a significant number of HIV-1-specific CTLs disappeared, which could not be attributed to escape from the virus epitopes recognized by these CTL clones[76]. By contrast, although it has been reported that HIV-1-specific CTL clones can persist in some patients for many years[77], it remains to be established in which situations clonal exhaustion of antiviral CTLs occurs and what the exact clinical effect of this could be.

### *Functional anergy of CTLs*

Alternatively, deterioration of HIV-1-specific CTL responses during disease progression can be explained by the fact that antiviral CTLs become functionally inactive. This phenomenon could result from continuous activation and persistent stimulation of HIV-1-specific CTLs, which may cause a state of hyporesponsiveness. In addition, lack of functional HIV-1-specific CTLs may be related to the progressive depletion of circulating $CD4^+$ T cells, the hallmark of HIV-1 infection. Of interest are elegant studies in mice experimentally infected with parasites[78], and studies in CD4-deficient mice[79–81], which have shown that in cases where T-cell function is altered or when absolute $CD4^+$ T-cell counts are severely reduced, the induction and maintenance of antiviral CTL responses can be affected.

Recent observations of patients treated with genetically modified HIV-1-specific CTL clones showed that HIV-1-infected patients with low $CD4^+$ T-cell counts and lacking delayed-type hypersensitivity (DTH) responses to several recall antigens could still generate primary MHC class I-restricted CTL responses to endogenously expressed foreign antigens[34]. Apparently, induction of MHC class I-restricted CTL responses is relatively preserved even when the immune system is already significantly damaged.

Still unexplained are early observations of polyclonal T-cell dysfunction when $CD4^+$ T-cell numbers are still relatively normal[82], and the conspicuous absence of HIV-1-specific $CD4^+$ Th cells in most infected patients[83], both of which could affect the maintenance of HIV-1-specific CTL responses.

## Concluding remarks

The composite picture from the current literature is in favour of a beneficial role for CTLs in HIV-1-infected individuals, and does not support the concept that HIV-1-specific CTL responses are deleterious to the infected host. It must be emphasized that HIV-1-specific CTLs *per se* do not mediate lifelong protection from progression to AIDS. HIV-1-specific CTL responses appear variable in their ability to control HIV-1 replication to the virologic setpoint. Therefore, HIV-1-specific CTLs may variably contribute to slowing down the rate of disease progression, but the exact determinants involved have not yet been revealed. The current data strongly point towards multiple mechanisms for viral persistence and for failure of HIV-1-specific CTL responses to control HIV-1 infection indefinitely. In the next decade, more-focused reseach will be required to

### Box 1. Future research strategies to further expand our understanding of HIV-1-specific CTLs

- Extensive characterization of timing, epitope specificity and phenotype of HIV-1 specific CTLs:
  (a) in patients during the acute phase of HIV-1 infection, because the virologic setpoint early in infection strongly correlates with the rate of disease evolution;
  (b) in long-term survivors, with the hope to find immune correlates of protection to AIDS;
  (c) in (typical) progressors, in order to further explore mechanisms which seem to frustrate antiviral CTL responses.
- Elucidation of temporal relationships between HIV-1-specific CTL responses and different measurements of HIV-1 viral load in situations where the quasi steady-state of virus replication and host immune responses is actively disturbed in order to gain more insight into 'cause and effect' of HIV-1-specific CTL responses.
- Continuous updating of the Los Alamos HIV Molecular Immunology database[a] with newly defined HIV-1-derived CTL epitopes, and more importantly, with relevant clinical and virological data on the patients studied. Serious efforts should be made to include data on the natural history of HIV-1 infection of previously reported cases, since a wealth of relevant unpublished data may still be at our disposal.

By addressing these research topics the controversies on HIV-1-specific CTLs will hopefully be resolved by the next decade. Ideally it would accelerate the development of effective vaccines and immunotherapeutic intervention strategies, especially for those unfortunate people who cannot bear the sometimes insufferable side-effects of antiviral therapies, and for all of those individuals in developing countries who do not readily have access to potent anti-retroviral drugs.

Abbreviations: CTL, cytotoxic T lymphocyte; HIV-1, human immunodeficiency virus type 1.
[a]Korber, B.T.M., Brander, C., Walker, B.D. *et al.*, eds; http://hiv-web.lanl.gov/immuno/

elucidate further the biological relevance of HIV-1-specific CTLs (see Box 1).

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