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Understanding the mechanisms and limitations of immune control of HIV

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Summary: A large number of experimental studies have been performed over the past decade in an attempt to develop a vaccine for human immunodeficiency virus (HIV). These studies have used a variety of approaches aimed at stimulating both antibody-mediated and cell-mediated immunity. Many of these experiments have been performed in macaque models of HIV. Analysis and modeling of the results of these studies provide the opportunity to investigate the mechanisms and limitations of viral control by humoral and cell-mediated immunity. These studies suggest that $CD8^+$ T cells do 'too little too late' to prevent the establishment of viral infection and latency. By contrast, passively administered antibody acts extremely early to reduce the initial inoculum and slow viral growth. In both cases, reduction in peak viral load appears crucial to the maintenance of $CD4^+$ T cells in acute infection and for effective long-term viral control. The insights gained from studies of simian human immunodeficiency virus infection have important implications for HIV vaccination. However, important questions remain as to whether differences in pathogenesis in HIV will lead to different 'rules of engagement' for immune control of virus.

Keywords: *HIV, vaccine, SHIV, cytotoxic T lymphocyte, antibody, mathematical modeling*

Introduction

An estimated 40 million people are currently infected with human immunodeficiency virus (HIV), with 5 million new infections and 3.1 million deaths in 2005. The vast majority of new infections occur in the developing world, where access to anti-retroviral drugs is limited. A vaccine for HIV presents the best possibility of halting the spread of the virus. Presently, work is progressing on trials of HIV vaccines in both humans and primates. HIV infection can be considered as having three key components: (i) the virus, (ii) the host target ($CD4^+$ T) cells, and (iii) the host immune response to the virus. To understand the dynamics of the host–pathogen interaction in HIV-infected individuals, it is necessary to develop a quantitative understanding of the various components and their interrelationship. For example, how effectively and by what

mechanism do $CD8^+$ T cells control virus? How much antibody is required to control virus, and how does it act? Quantitative analysis of these components will lead to a broader understanding of the host–pathogen interaction in HIV.

The importance of modeling in understanding HIV is illustrated by previous work analyzing viral dynamics in HIV infection. Although a general overview of viral load changes in HIV had been known for many years, the first mathematical models of viral kinetics in infected individuals revolutionized our knowledge of HIV infection (1, 2). The high rate of viral turnover and the existence of distinct compartments of infected cells, for example, has important implications for understanding the effects and limitations of drug therapy (1, 3–5). Similarly, quantitative analysis of the rate of turnover of $CD4^+$ T cells and the rates of replacement of these cells following anti-retroviral therapy provides insights into the dynamics of virus–target cell interactions and the course of immune reconstitution following therapy (6–9).

Quantitative models of the first two components of the host–viral interaction – virus and $CD4^+$ T cells – have elucidated key aspects of HIV infection important for the design of therapy protocols. Only limited quantitative studies of the effects of the specific immune control of the virus have been possible largely because of technological limitations. Early *in vitro* methods of measuring T-cell–mediated immune responses such as cytotoxicity (chromium release) assays and proliferation assays were not able to provide a direct quantitative measure of the immune response. Limiting dilution assays for assessing the numbers of responding cells were difficult and unreliable (10, 11). However, recent developments have provided the ability to directly measure the number of responding cells. Major histocompatibility complex (MHC) class I tetramers provide the ability to sort and count antigen-specific $CD8^+$ T cells (12). Similarly, intracellular cytokine staining and enzyme-linked immunospot assays allow measurement of the number of responding cells based on their functional status (10, 11). Thus, it is possible to follow changes in the number of responding T cells and in their function.

The advent of these techniques led to a better understanding of T-cell–mediated immune responses in HIV infection, and virus-specific $CD8^+$ T cells are thought to play an important role in the control of viral growth in both acute and chronic HIV infection. The importance of virus-specific $CD8^+$ T cell is suggested by several lines of evidence: (i) the decline in virus in acute infection coincides with the peak in virus-specific $CD8^+$ T-cell numbers (13, 14), (ii) depletion of $CD8^+$ T cells leads to increases in virus (15, 16), (iii) infusion of virus-specific $CD8^+$ T cell leads to a decrease in viral load (17), (iv) viral 'escape' of virus-specific $CD8^+$ T-cell recognition leads to increasing viral loads (18–20), and (v) human studies have suggested an association between virus-specific $CD8^+$ T-cell numbers and disease outcome in HIV infection (21).

Advances in techniques for measuring $CD8^+$ T-cell immune responses have also coincided with the evolution of a new generation of vaccines that permit induction of high levels of antigen-specific $CD8^+$ T cells. Antigen delivery systems involving DNA, live viral vectors, or chemical adjuvants have been used to generate antigen-specific $CD8^+$ T-cell responses directed toward HIV proteins (reviewed in 22). Virus-specific memory $CD8^+$ T cells induced by vaccination are thought to be useful in controlling infection both because of their increased numbers and because of their more rapid activation in response to antigen (23). Several studies in primates suggest that the presence of such vaccine-induced $CD8^+$ T-cell responses prior to infection leads to significantly reduced viral loads and acquired immunodeficiency syndrome mortality in infected animals (24–27), consistent with human studies, which have shown that lower HIV viral loads are associated with increased survival (28). However, these vaccines do not appear to be able to prevent persistent infection. It is unclear whether this inability to prevent chronic infection is a quantitative limitation (not enough specific $CD8^+$ T cells, not enough epitopes targeted) or a qualitative limitation (this type of immune response is inherently unable to mediate immunity). Moreover, there seem to be significant differences between the efficacies of vaccines in different animal models of HIV. Quantitative analysis of the dynamics of the virus–T-cell interaction provides an opportunity to address these issues and to advance our understanding of the possibilities and limitations of this new generation of vaccines.

A kinetic analysis of immune control

Viral control by $CD8^+$ T cells: too little too late

A number of studies of DNA or viral vectors in macaques have shown high levels of virus-specific $CD8^+$ T cells following vaccination. Infectious challenge of these macaques has shown reduced peak viral load and chronic viral load, although it appears that this does not mediate 'sterilizing immunity' or lead to viral elimination (24, 25, 27). Why memory T cells are unable to control early viral infection but control much higher viral loads later in infection poses an important question. Kinetic analysis of viral growth in early HIV infection provides insights into this phenomenon. This analysis shows that viral growth in the first 10 days following infection is not significantly affected by vaccination and the presence of high

numbers of virus-specific CD8$^+$ T cells (29, 30) (*Fig. 1*). That is, viral load is not significantly different between control and vaccinated animals on day 10 postinfection, nor is there any correlation between virus-specific CD8$^+$ T-cell numbers immediately prior to infection and viral loads 10 days after infection (31). Thus, during the first 10 days of infection, the presence of vaccine-induced virus-specific CD8$^+$ T cells (at levels as high as 25% of total CD8$^+$ T cells) has little impact on viral kinetics. However, after day 10, viral growth is significantly reduced in vaccinated animals, and vaccination leads to lower peak viral loads and in many cases long-term viral control (29, 31).

Analysis of the number of virus-specific CD8$^+$ T cells (detected by MHC class I tetramer) present prior to infection and their subsequent kinetics suggests a simple explanation for this delay in viral control: virus-specific CD8$^+$ T-cell numbers are not significantly increased above their preinfection levels until 10 days after infection, whether the animal was vaccinated or not (29, 32). A more precise timing of this initial increase can be estimated by taking the intersection of the original number of tetramer-positive cells with the growth curve of tetramer-positive CD8$^+$ T cells after day 10 (29). Such an analysis provides no evidence that memory T-cell responses are activated earlier than naive T-cell responses; growth in both populations commences around day 10. Further analysis suggests that this delay before the initial growth in T-cell numbers is also seen in mucosal tissues (30, 32) and is not overcome by higher T-cell numbers (31). On the contrary, higher preinfection T-cell numbers led to a greater delay (31), suggesting that competition for a limiting resource, such as antigen-presenting cells, may be playing a role. In fact, spatial models in which T cells move randomly at rates measured *in vivo* by two-photon microscopy die at some rate, proliferate only after encountering an antigen-presenting dendritic cell expressing the appropriate cognate peptide–MHC class I complex on its surface, also exhibit substantial delays before net T-cell expansion is observed (Jin B, Raychaudhuri S, Perelson AS, Chakraborty AK, manuscript submitted). Thus, the physical processes involved in antigen capture, processing, and presentation, and the need for T cells to interact with the appropriate antigen-presenting cell may limit the speed at which CD8$^+$ T-cell responses occur, particularly at the early stages of infection when antigen levels are low.

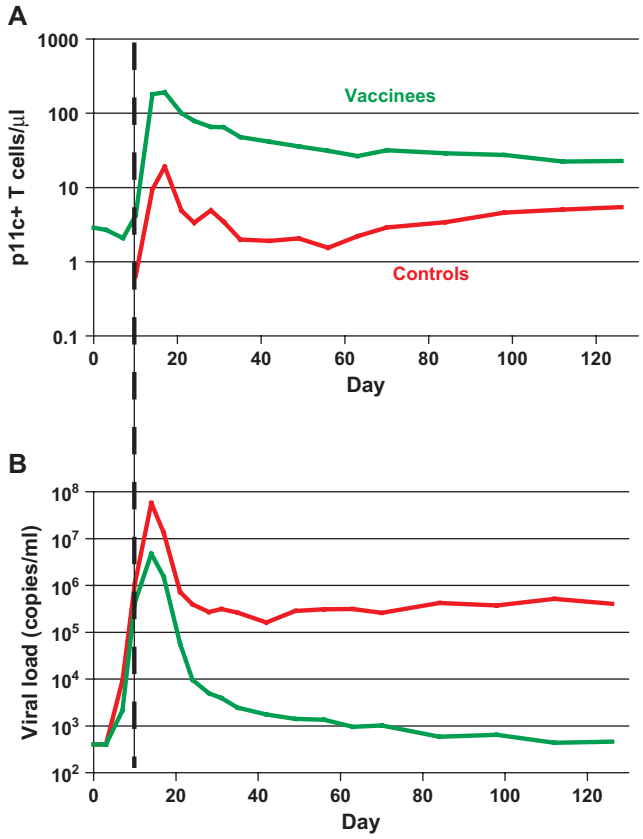

***Fig. 1.* Delayed CD8$^+$ T-cell expansion and viral control.** (A) The number of virus-specific CD8$^+$ T cells (p11c$^+$ T cells, specific to the p11c epitope in gag) in vaccinated animals is not significantly increased from preinfection levels until day 10 postinfection. (B) Similarly, there is no significant difference in viral loads between control and vaccinated macaques up to day 10 postinfection. Data plotted are geometric mean p11c$^+$ T-cell number and viral load from Barouch *et al.* (24).

The timing of the initial virus-specific CD8$^+$ T-cell growth coincides with the early viral control in macaques. Thus, for example, if virus-specific CD8$^+$ T-cell expansion is observed to start at day 10, then there is little functional effect of virus-specific CD8$^+$ T cells on virus growth prior to day 10, suggesting that a late 'activation' of virus-specific CD8$^+$ T cells is responsible for both the delay in virus-specific CD8$^+$ T-cell growth and effector function.

The delay in CD8$^+$ T-cell activation following infection might in some circumstances seem entirely trivial: viral control is still initiated at low levels of virus, and peak viral loads are significantly reduced. However, the ability of HIV-infected cells to convert to latency can lead to persistent infection, even if virus is controlled relatively early. The number of infected cells present at the time of initial virus-specific CD8$^+$ T-cell control ($\sim$10 days) can be estimated from an understanding of viral production and clearance rates. Such analysis suggests that a minimum of $\sim$200,000 cells are productively infected on day 10, when virus-specific CD8$^+$ T cells become active (29). Thus, even if only 1/1000 productively infected cells converts to latency, persistent infection will already be well established prior to the time of virus-specific CD8$^+$ T-cell activation. Thus, the delay in growth of virus-specific CD8$^+$ T cells and control of

virus provides a window of opportunity for the uncontrolled viral growth in early infection and the establishment of a pool of latently infected $CD4^+$ T cells.

Other studies of viral and virus-specific $CD8^+$ T-cell kinetics provide important insights into the limitations of immune control of simian human immunodeficiency virus (SHIV). A delay in the control of viral growth is not restricted to SHIV as it has also been observed in influenza (33, 34) and lymphocytic choriomeningitis virus (LCMV) (35) infection in mice. In these infections, the early viral load (up to approximately day 3) does not differ between controls and vaccinees. However, vaccinated animals bring the infection under control more rapidly after this time. In the case of these acute infections, the absence of a latent stage means that virus can be cleared by the virus-specific $CD8^+$ T-cell response, despite the uncontrolled period of early viral growth. The ability of SHIV-infected cells to convert to latency prior to the onset of virus-specific $CD8^+$ T-cell–mediated immunity suggests that virus-specific $CD8^+$ T cells cannot prevent viral persistence.

The inability of vaccination to prevent the establishment of chronic infection in simian immunodeficiency virus (SIV)/SHIV models appears to be a question of the kinetics of virus-specific $CD8^+$ T-cell growth. This finding suggests that current virus-specific $CD8^+$ T-cell–inducing vaccines may be inherently restricted in their ability to recognize low levels of infection and control early viral growth and thus may allow viruses to 'sneak through' to establish persistent infection (36).

A threshold level of antigen to stimulate $CD8^+$ T-cell proliferation

The mechanisms of delay in the virus-specific $CD8^+$ T-cell control of SHIV infection could either be intrinsic to the T cells (i.e. it always takes 10 days for $CD8^+$ T cells to become activated effectors) or be extrinsic to the T cells themselves (i.e. T cells receive a delayed external signal to proliferate). It seems very unlikely that a 10-day delay in virus-specific $CD8^+$ T-cell activation is intrinsic to macaque T cells because significant T-cell proliferation can be observed as early as 1 week post-vaccination (37). However, it seems quite likely that differences in the delivery of external signals (i.e. antigenic stimulation) to the virus-specific $CD8^+$ T cell may explain the difference between SHIV infection and vaccination: vaccination involves the delivery of a large bolus of antigen, which would be expected to stimulate a response soon after administration. By contrast, infection involves the administration of small doses of antigen, which replicate and increase in number over time. It is unsurprising that virus-specific $CD8^+$ T cells are unable to recognize extremely small doses of antigen present at the time of initial infection and that some 'threshold' level of antigen may be required to trigger virus-specific $CD8^+$ T-cell activation. In the case of SHIV, this threshold would appear not to be reached until around day 10 postinfection. This finding suggests that achieving earlier virus-specific $CD8^+$ T-cell activation may require a higher sensitivity of T cells to low doses of antigen. Thus, higher avidity T-cell responses may be the key to earlier virus control. In this context, it is interesting to consider that an inverse correlation has been observed between antigen dose and T-cell avidity (38–40). Thus, there may be a trade-off between the magnitude and timing of T-cell control of virus. By contrast, repeated small doses of vaccine have been suggested to increase the avidity of the response (41). However, it remains to be seen whether a virus-specific $CD8^+$ T cell of sufficiently high avidity to prevent the establishment of latency can be generated.

The establishment of memory

In acute infections of mice, such as LCMV, an early peak of virus-specific $CD8^+$ T cells is observed around days 7–10, followed by a rapid decay of approximately 90% of cells and a persistence of a stable memory pool (42). In SHIV, the peak in virus-specific $CD8^+$ T-cell number occurs later at around days 14–17 following infection, and there is a subsequent decay until they reach approximately 5–40% of their peak levels (29) (*Fig.* 2). The profile of decay of the cells is consistent with two populations of virus-specific $CD8^+$ T cells with different half-lives: a short-lived putative 'effector' population with a half-life of around 3 days and a long-lived putative 'memory' population with a half-life of approximately 150 days (29) (*Fig.* 2*A*). In vaccinated animals challenged with SHIV, the early phase of decay of virus-specific $CD8^+$ T cells is substantially slower, and a larger proportion of cells appear to enter the memory pool than in naive animals (29) (*Fig.* 2*B*). This enhanced memory formation may result from a number of factors: (i) increased $CD4^+$ T-cell help (because of preservation of $CD4^+$ T-cell numbers in vaccinated animals) may enhance memory function (43, 44), (ii) memory cells (produced by vaccination) may have a slower decay rate than naive cells (in a primary response) (45) perhaps because they have all already acquired the appropriate phenotype for survival [such as interleukin-7 receptor expression (46)], and (iii) the persistence of antigen in control animals may lead to 'exhaustion' of virus-specific $CD8^+$ T cells (although contraction of $CD8^+$ T cells has in other circumstances been found to be independent of antigen persistence) (45).

In the later phase of T-cell memory formation, differences are again observed between naive and vaccinated animals. In

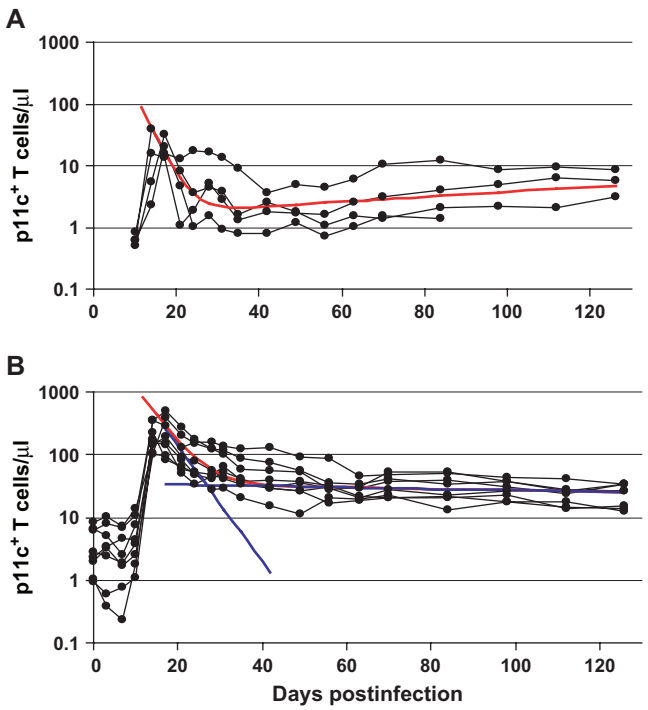

***Fig. 2.* Slow decay of memory CD8$^+$ T cells in vaccines and memory inflation in controls.** (A) The rapid decay of virus-specific CD8$^+$ T cells from its peak in control animals is followed by a slow increase over time (in parallel with increasing viral loads in these animals). This increase has been termed 'memory inflation' (48). (B) In contrast, viral control in vaccinated animals is associated with a slow decay of virus-specific CD8$^+$ T cells with a half-life of around 150 days. Data plotted are p11c$^+$ T-cell numbers from individual control (A) or vaccinated (B) animals from Barouch *et al.* (24). The red solid lines represent model fitting of the data using a two-phase decay model [as described in Davenport *et al.* (29)]. In the lower panel, the decay of the rapidly decaying ('effector' population) and the slowly decaying ('memory' population) are shown separately in blue.

vaccinated animals, as observed above, there is a slow decay of virus-specific CD8$^+$ T cells with a half-life of approximately 150 days. By contrast, in many naive animals, there is an increase rather than a decrease in virus-specific CD8$^+$ T-cell numbers over time (*Fig.* 2*A*). This increase in numbers parallels an increase in viral load in these animals (*Fig.* 1); thus, it appears that virus-specific CD8$^+$ T-cell proliferation may be driven by virus rather than controlling it (47). In some cases, the virus-specific CD8$^+$ T-cell numbers in naive animals in late disease approach those seen in acute infection. However, unlike in acute infection, this late increase in virus-specific CD8$^+$ T cells does not appear to control viral load. This increase in virus-specific CD8$^+$ T-cell numbers with advancing infection has also been noted in both murine and human cytomegalovirus infection and has been termed 'memory inflation' (48, 49). The association between increased viral load and increased virus-specific CD8$^+$ T-cell numbers in advancing infection is consistent with a model of virus driving T-cell proliferation but is inconsistent with the role of T cells in controlling virus, suggesting that virus-specific CD8$^+$ T cells are a 'passenger' rather than a 'driver' of viral load (50). One explanation for this inconsistency is that CD8$^+$ T cells become increasingly less effective in controlling virus (e.g. because of lack of CD4$^+$ T-cell help) (43, 44). Another explanation is that CD8$^+$ T cells become exhausted, possibly because of upregulation of programmed death-1 (51), or progressive senescence of high-affinity responses leads to 'clonal succession' toward poorly functional T cells (52, 53). Reduced CD8$^+$ T-cell function in advanced disease has been observed in HIV (53, 54). However, other more general mechanisms such as replicative senescence may reduce the long-term efficacy of CD8$^+$ T cells because of chronic antigenic stimulation (52, 55–58).

Mechanisms of viral control

Viral load in acute SHIV infection peaks around days 14–17, and this peak is followed by the decay of virus to a set point viral level. The reason for viral decay in SHIV and HIV is usually attributed to immune control of virus. An alternative to the immune-mediated viral control of virus is a 'target cell–limited' model, in which viral growth is determined largely by the availability of target CD4$^+$ T cells for infection (59). In this model, an early peak and drop in viral load is observed in the absence of immune control simply because the virus 'runs out' of target cells to infect. A number of attempts have been made to differentiate target cell–limited versus immune control models of HIV infection (60–62) as this understanding has important implications for vaccination. The almost complete depletion of CD4$^+$ T cells in acute SHIV infection of unvaccinated macaques (24, 27) supports the notion that viral growth may be target cell limited in this infection and that massive infection and death of CD4$^+$ T cells may indeed be the factor limiting early viral growth (*Fig.* 3). However, in vaccinated animals, viral growth is constrained prior to the severe depletion of CD4$^+$ T cells. Thus, viral growth is controlled before the animal runs out of CD4$^+$ T cells to infect, suggesting a role for immune control of virus following vaccination.

Experimental work in macaques shows that high virus-specific CD8$^+$ T-cell numbers are associated with a low peak and chronic levels of viral load (24, 25, 27), although the cellular and molecular mechanisms by which this viral control is achieved are unclear. There has been substantial debate on whether the kinetics of virus during acute infection can be explained either by cytolytic or non-cytolytic mechanisms of immune control (59, 62, 63), and several different approaches have been taken to investigate the effects of the CD8$^+$ T-cell

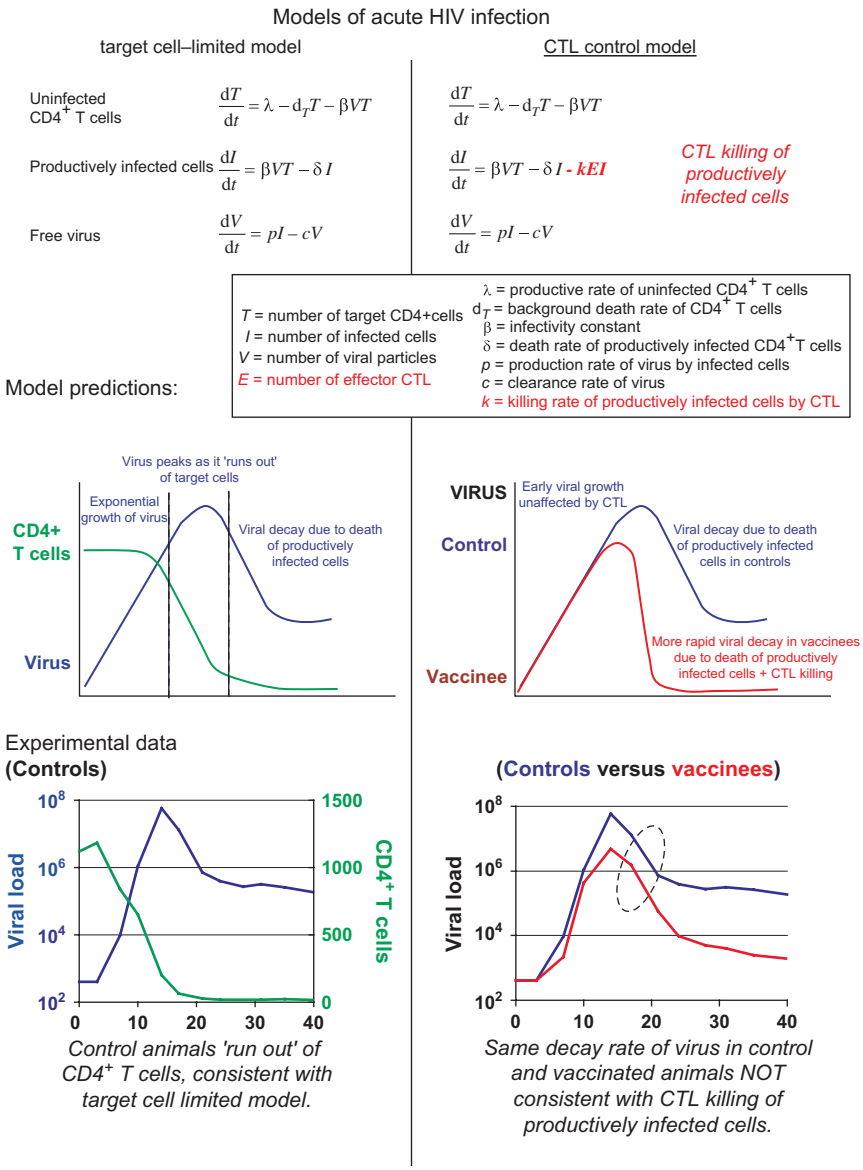

Fig. 3. Target cell–limited versus cytotoxic T-lymphocyte (CTL) control of HIV. The basic equations of simple target cell–limited (left) and CTL-mediated (right) models of HIV control are shown above (and explained in box below). In the target cell–limited model, productively infected cells decay at a constant rate (δ) because of viral cytopathic effect/activation-induced cell death. In the CTL control model, infected cells are expected to die at a higher rate in vaccinees because of killing by CTLs (at rate *kEI*). The viral load and $CD4^+$ T-cell curves in control monkeys (left) conform to the predictions of the target cell–limited model. However, whereas the model of CTL-mediated control of virus growth predicts more rapid decay of virus in the presence of CTLs, this result is not observed in the experimental data (circled). Data plotted are geometric mean viral load and mean $CD4^+$ T-cell number from individual control or vaccinated animals from Barouch *et al.* (24).

response on viral dynamics: depletion of $CD8^+$ T cells (15, 16), blocking of T-cell activation (62), and boosting of virus-specific $CD8^+$ T-cell responses through vaccination (29, 31).

HIV-infected cells may die because of a number of mechanisms: direct viral cytopathic effect, activation-induced death (because the virus preferentially infects activated cells, which may have a short intrinsic survival time), and virus-specific $CD8^+$ T-cell–mediated killing of infected cells. If the latter is the dominant effect mediating viral control, then we should expect a slower decay of virus in the absence of $CD8^+$ T cells and a more rapid decay in the presence of higher virus-specific $CD8^+$ T-cell numbers. Thus, we analyzed the mechanisms of $CD8^+$ T-cell control of HIV following induction of stronger virus-specific $CD8^+$ T-cell responses through vaccination. In these circumstances, despite a strong effect of virus-specific $CD8^+$ T-cell responses on total viral loads (controlling virus to >2 logs lower than unvaccinated animals in some cases), there is no significant change in the decay rate of infected cells (29, 31) during acute infection (*Fig.* 3). Thus, virus-specific $CD8^+$ T-cell responses are controlling virus without affecting the net decay rate of productively infected cells. Vaccinated animals achieve lower viral loads because viral decay continues for longer, not because it occurs more rapidly (*Fig. 1B*).

A number of mechanisms could explain this apparent paradox. First, in unvaccinated animals, virtually all $CD4^+$ T cells are infected at the peak of infection; thus, during the decay phase of virus, there are few uninfected cells to infect. The experimentally observed decay rate of virus is related to the net rate of decay of infected cells (which reflects the balance

between death rate of infected cells and infection of new cells). In control animals where $CD4^+$ T-cell depletion is almost 100%, the 'new' infection rate of $CD4^+$ T cells is almost zero and thus the net decay rate of virus is very close to the actual death rate of infected cells. By contrast, in vaccinated animals, $CD4^+$ T cells are not as severely depleted, and death of productively infected $CD4^+$ T cells may be balanced by reinfection of new cells. Thus, the 'real' death rate of infected cells could be higher in vaccinated animals, but this death is balanced by infection of new $CD4^+$ T cells during the period immediately after the peak of viral load, so that the net decay rate appears unchanged. However, this scenario seems unlikely as it would require an exact balance between the increased death rate of infected $CD4^+$ T cells and the infection rate of uninfected $CD4^+$ T cells to observe the same net decay rate. An alternative explanation for the unchanged decay rate of virus in vaccinated animals is that virus-specific $CD8^+$ T cells control virus through non-cytolytic mechanisms such as inhibition of viral production or viral infectivity by, for example, impairment of viral entry into cells by chemokines or inhibition of intracellular viral production by cytokines. However, it seems somewhat counterintuitive that cytotoxic T lymphocytes may play a predominantly non-cytolytic role in HIV. A final explanation, which incorporates traditional cytolytic mechanisms, is that virus-specific $CD8^+$ T cells are only able to kill a cell during a short 'window' period soon after viral protein production and antigen presentation begins (*Fig.* 4). However, as viral products build up and the cell prepares for viral budding, viral proteins such as nef may downregulate MHC class I expression (64), leading to a loss of virus-specific $CD8^+$ T-cell recognition. In this scenario, although virus-specific $CD8^+$ T cells mediate viral control through cytolysis, they do not directly kill productively infected cells, instead eliminating cells early in their life cycle. Thus, they do not affect the observed decay rate of productively infected cells. All these mechanisms are consistent with the data, and further experimentation and modeling are required to differentiate these possibilities.

Antibody acts early

Most vaccination strategies induce both virus-specific T-cell and antibody responses to the virus, and it is thus hard to tease apart the differing effects of these immune mechanisms on viral load. Passive administration of antibody provides an opportunity to investigate the effects of antibody alone and has been shown to be capable of producing 'sterilizing immunity' to SHIV in some animals. Analysis of the viral kinetics of those animals that became infected following vaccination shows, in contrast to $CD8^+$ T-cell–mediated immunity, that antibody acts early (*Fig.* 5). An approximately 700-fold difference in viral load is observed as early as day 7 postchallenge (65). However somewhat surprisingly, antibody appears to have a relatively weak effect on reducing the viral growth rate, reducing it by only 25%. This effect would be expected to reduce the viral load at day 7 by only approximately eightfold (65). Thus, the effects of antibody on viral growth cannot account for the 700-fold reduction in viral load at day 7, and there must also have been a significant (nearly 100-fold) reduction in the initial inoculum of virus that was able to reproduce after entering the host. Thus, it seems the dominant effect of antibody is to neutralize the initial inoculum at the time of viral entry, but if this neutralization is not achieved, antibody is unable to prevent subsequent viral growth.

The passively administered antibody has a short half-life (approximately 1–2 weeks); thus, its protective effect is rapidly

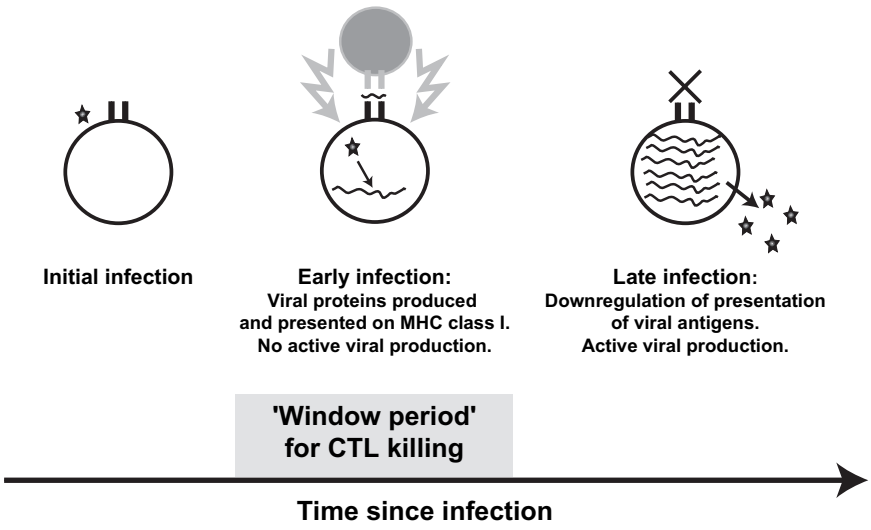

***Fig.* 4. The 'window period' hypothesis for $CD8^+$ T-cell killing in SHIV infection.** The experimental data are not consistent with increased killing of productively infected cells. Therefore, $CD8^+$ T-cell control of virus must either be mediated by non-cytolytic mechanisms or killing of infected cells must occur in a 'window period' of infection prior to when cells become productively infected.

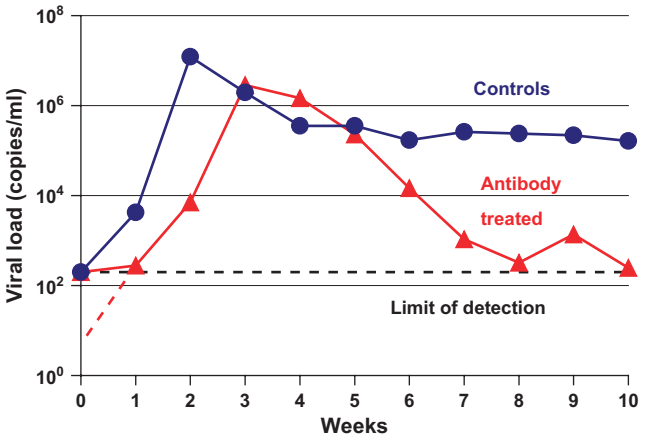

Fig. 5. **Antibody acts early after infection.** Viral loads are reduced approximately 700-fold in antibody-treated animals (red) compared with controls (blue) as early as day 7 after infection, the viral growth rate is only reduced approximately 25%. The reduced viral growth rate can only account for around eightfold of the difference in viral load at day 7, suggesting that antibody treatment reduced the initial viral inoculum by approximately 100-fold (dashed red line). Data plotted are geometric mean viral load following intravaginal infection for control animals and those treated with ≥2 monoclonal antibodies from Mascola *et al.* (85, 65).

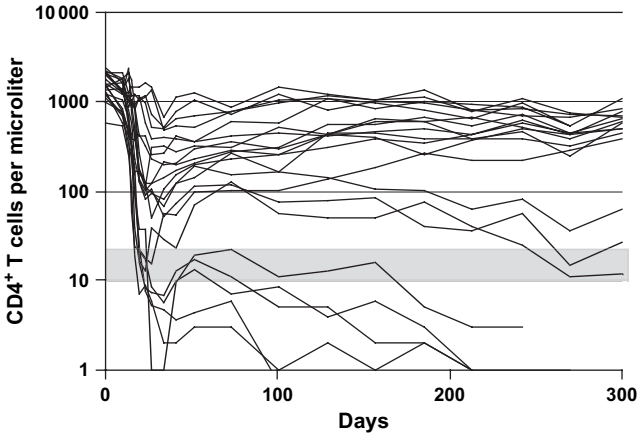

Fig. 6. **Early CD4$^+$ T-cell depletion determines long-term outcome in SHIV infection.** SHIV infection results in early and profound depletion of CD4$^+$ T cells. The extent of this early depletion is a major determinant of long-term outcome. A drop in CD4$^+$ T cells to below 10–20 cells/μl (shaded area) is followed by a long-term decline in CD4$^+$ T cells. By contrast, animals that maintain CD4$^+$ T cells above this level experience a long-term recovery in CD4$^+$ T cells. Data plotted are CD4$^+$ T-cell counts from individual control and vaccinated animals from group A of Shiver *et al.* (27).

lost. However, antibody-treated animals had significantly reduced viral loads during late infection, when antibody had been largely cleared (66). This outcome suggests that despite the transient nature of passive antibody, its presence early in infection is able to 'program' the long-term outcome of infection, most likely through the preservation of CD4$^+$ T-cell numbers and the subsequent increased 'help' for both CD8$^+$ T-cell responses and endogenous antibody responses.

Early programming of the outcome of infection

In HIV, the target cells for infection, CD4$^+$ T cells, are also central to the development of a potent immune response. Thus, early depletion of CD4$^+$ T cells can effectively abrogate the later development of protective immunity. In contrast, even a relatively minor increase in the number of CD4$^+$ T cells that 'survive' initial infection can lead to a significant improvement in outcome (67). There may be a delicate balance between immune control and immunodeficiency soon after infection (68). In SHIV-89.6P infection, for example, it appears that if a critical level of CD4$^+$ T cells can be maintained in acute infection (approximately 20 cells/μl), then the animals go on to do well (*Fig.* 6). Animals that experience a loss of CD4$^+$ T cells, having less than approximately 20 cells/μl, experience a continued decline in CD4$^+$ T-cell numbers and an increase in viral load, whereas those that have CD4$^+$ T-cell responses above this level experience a long-term recovery in CD4$^+$ T cells. This observation is made even in control animals, where a small proportion of animals maintain CD4$^+$ T-cell numbers above this threshold in acute infection and go on to maintain long-term CD4$^+$ T-cell counts. Vaccination acts to decrease average peak viral load and CD4$^+$ T-cell depletion, hence increasing the proportion of animals that maintain CD4$^+$ T cells above this threshold. Passive antibody therapy (65) or anti-retroviral drug therapy (69) have similar effects.

The importance of the level of CD4$^+$ T-cell depletion in acute infection to long-term outcome led us to study the kinetics CD4$^+$ T-cell depletion in SHIV infection (70). We found a very close correlation between the peak viral load observed in acute infection and the level of CD4$^+$ T-cell depletion over the subsequent days (70). Modeling of the relationship between viral load and CD4$^+$ T-cell infection and death allowed us to estimate the 'viral infectivity' (*Fig.* 7). Knowledge of viral infectivity allows prediction of the level of CD4$^+$ T-cell depletion that will be seen for a given peak viral load and conversely allows the prediction of how effective a reduction in viral load will be at preserving CD4$^+$ T-cell numbers (*Fig.* 8). Preservation of CD4$^+$ T cells in acute infection has important downstream consequences for immunity to SHIV: CD4$^+$ T-cell help may facilitate the development of both CD8$^+$ T-cell memory responses as well as antibody responses to the virus, which may in turn lead to better viral control, reduced chronic CD4$^+$ T-cell depletion, and effective recovery (*Fig.* 9). Reduced viral loads may also lead to a decreased risk of developing immune escape variants of SHIV because there is a smaller viral

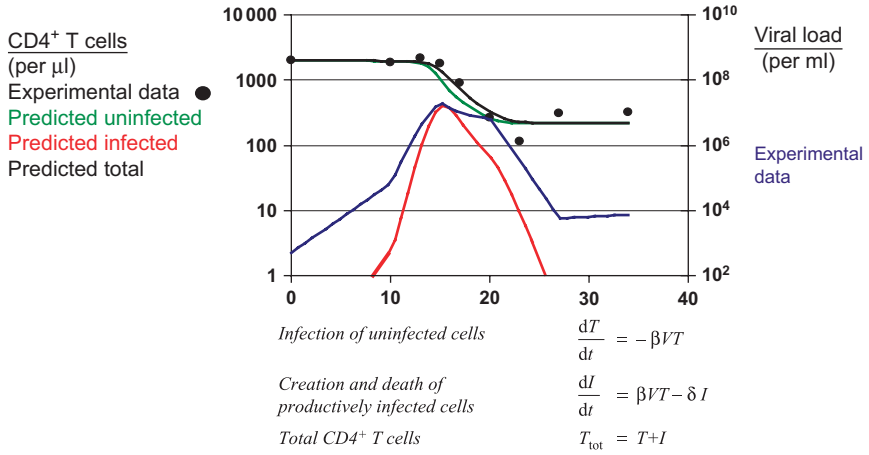

Fig. 7. Kinetics of CD4$^+$ T-cell depletion in SHIV. SHIV viral load determines the rate of CD4$^+$ T-cell depletion in acute SHIV infection (70). The equations describing the infection and death of CD4$^+$ T cells in acute SHIV infection are shown below (see *Fig.* 3 for description of parameters). The experimentally observed viral load (blue, shown on right-hand axis) determines the rate and extent of CD4$^+$ T-cell infection in acute infection. The predicted total CD4$^+$ T-cell count [black line, the sum of infected (red) and uninfected (green) cells] is fitted to the experimentally observed CD4$^+$ T-cell count (black dots) by non-linear least-squares regression where the infectivity parameter (β) is allowed to vary. Data shown are fit to one animal from Shiver *et al.* (27).

population in which mutations can develop. Thus, viral control over a short period during acute infection can tip the balance in favor of immune control. The profound long-term impact of lowering viral loads in acute SHIV infection provides room for optimism that a similar phenomenon may exist in the case of HIV infection.

In SHIV infection, profound CD4$^+$ T-cell depletion is observed in the peripheral blood of infected animals during the first few weeks of infection. The CXCR4 tropism of SHIV-89.6P allows it to target essentially all CD4$^+$ T cells (71). However, in early HIV and in many SIV infections, the virus is CCR5 tropic and thus its target cell range is restricted to CCR5$^+$ (predominantly memory and activated) CD4$^+$ T cells (72). During acute infection with CCR5-tropic viruses, relatively little depletion of total CD4$^+$ T-cell numbers is observed in peripheral blood (73, 74), suggesting that the dynamics of CD4$^+$ T-cell depletion may not be as straightforward as those observed in CXCR4-tropic SHIV infection. Studies of the depletion of memory cells in peripheral blood suggest that depletion of these cells is also relatively mild (75). However, a profound depletion of CD4$^+$ T cells is observed in the gut during CCR5-tropic SIV (76, 77) and HIV infection (78, 79). The gut is rich in CCR5$^+$CD4$^+$ memory T cells, which are the main target cells for infection. It therefore seems likely that a similar dynamic of massive target cell infection and death in acute infection may exist in HIV and SIV infection, but in these cases, it is limited to memory cells in the gut. Determining whether the same relationship between peak viral load and

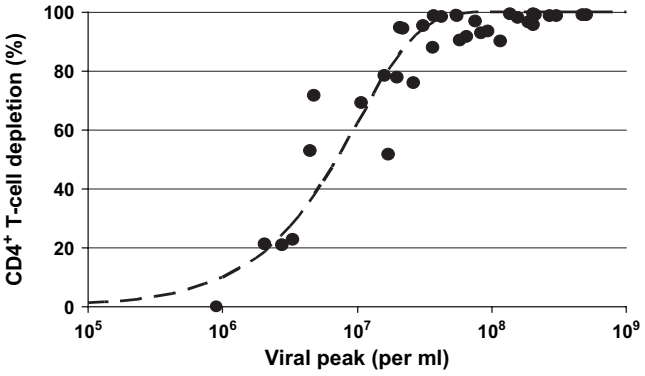

Fig. 8. Peak viral load predicts CD4$^+$ T-cell depletion in SHIV infection. The peak viral load and extent of CD4$^+$ T-cell depletion 1 week after this peak are plotted for individual control and vaccinated animals [data from Shiver *et al.* (27)]. The best-fit curve for this relationship is also plotted [detailed in Davenport *et al.* (70)]. This relationship allows prediction of the extent of control of peak viral load that is required to preserve CD4$^+$ T-cell immunity.

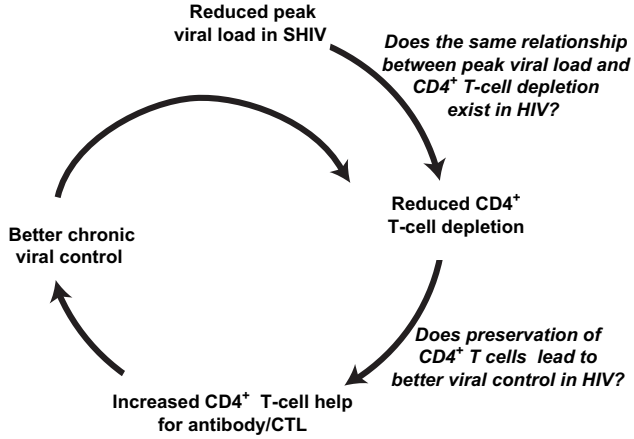

Fig. 9. Reduction in peak viral load leads to a cycle of viral control in SHIV infection. The ability of vaccines to reduce peak viral load has a profound effect on outcome in SHIV infection. Reduced peak viral load leads to reduced CD4$^+$ T-cell depletion, which is thought to lead to better T-cell help for both antibody and CD8$^+$ T-cell responses to virus. Whether the relationship between early viral load and CD4$^+$ T-cell depletion is as strong in HIV as in SHIV is not known. Similarly, it is not clear whether early CD4$^+$ T-cell preservation can maintain immune control of HIV during chronic infection.

$CD4^+$ T-cell preservation exists in HIV as in SHIV will be important to understanding the likely impacts of reducing peak viral loads in HIV. Thus, further studies of $CD4^+$ T-cell dynamics in CCR5-tropic SIV infection and HIV infection are clearly required. In addition, a major difference between SHIV infection and HIV infection is the potential for generation of neutralizing antibodies to the former. In the case of SHIV, the maintenance of $CD4^+$ T-cell help may facilitate neutralization of virus. However, it is unclear whether neutralizing antibodies can be readily generated in HIV, whether $CD4^+$ T-cell help is present or not (80).

Implications of a kinetic understanding of immune control

An understanding of the dynamics of immune control of HIV has important implications for rational vaccine design. In the case of cell-mediated immunity, although $CD8^+$ T-cell responses appear unable to prevent chronic infection, their ability to reduce peak viral loads and preserve $CD4^+$ T-cell help in acute infection may play a major role in improving the long-term outcome of infection. Immune escape is a major factor limiting the efficacy of virus-specific $CD8^+$ T-cell–mediated viral control, and again control of peak viral loads may reduce the viral population in which mutation can occur and be selected. Moreover, by focusing on epitopes where immune escape inflicts significant 'fitness costs' to the virus, it may be possible to select for weakened viral variants that have a reduced ability to infect and kill $CD4^+$ T cells. In the case of humoral immunity, the ability of antibody to neutralize the initial inoculum is crucial. However, given that the initial inoculum is likely to be small following sexual transmission, neutralization may be achieved with relatively low antibody titers. Finding a conserved target for such antibodies remains an ongoing area of research. However, if neutralization of the initial inoculum is not possible, early control of viral load may still provide long-term benefits by improving $CD4^+$ T-cell help for the evolving immune responses.

The benefits for the individual of a vaccine that reduces viral load and preserves $CD4^+$ T cells are amplified when the whole population is considered. Several epidemiological models have been developed to investigate the potential of 'disease-modifying' vaccines (which do not prevent infection but slow disease progression) to control the HIV epidemic (81–84). These analyses suggest that such vaccines will have a profound effect in slowing the epidemic, despite their inability to prevent initial infection. However, reductions in viral load of the order of 10-fold may be required to see significant effects (81).

References

1. Ho DD, Neumann AU, Perelson AS, Chen W, Leonard JM, Markowitz M. Rapid turnover of plasma virions and CD4 lymphocytes in HIV-1 infection. Nature 1995; **373**:123–126.
2. Wei X, et al. Viral dynamics in human immunodeficiency virus type 1 infection. Nature 1995; **373**:117–122.
3. Perelson AS, Neuman AU, Markowitz M, Leonard LM, Ho DD. HIV-1 dynamics in vivo: virion clearance rate, infected cell lifespan and viral generation time. Science 1996; **271**:1582–1586.
4. Perelson AS, et al. Decay characteristics of HIV-1-infected compartments during combination therapy. Nature 1997;**387**: 188–191.
5. Perelson AS. Modelling viral and immune system dynamics. Nat Rev Immunol 2002; **2**:28–36.
6. Mohri H, Bonhoeffer S, Monard S, Perelson AS, Ho DD. Rapid turnover of T lymphocytes in SIV-infected rhesus macaques. Science 1998;**279**:1223–1227.
7. Mohri H, et al. Increased turnover of T lymphocytes in HIV-1 infection and its reduction by antiretroviral therapy. J Exp Med 2001;**194**:1277–1287.
8. Sachsenberg N, et al. Turnover of CD4+ and CD8+ T lymphocytes in HIV-1 infection as measured by Ki-67 antigen. J Exp Med 1998;**187**:1295–1303.
9. Ribeiro RM, Mohri H, Ho DD, Perelson AS. In vivo dynamics of T cell activation, proliferation, and death in HIV-1 infection: why are CD4+ but not CD8+ T cells depleted? Proc Natl Acad Sci USA 2002;**99**: 15572–15577.
10. Murali-Krishna K, et al. Counting antigen-specific CD8 T cells: a reevaluation of bystander activation during viral infection. Immunity 1998;**8**:177–187.
11. Butz EA, Bevan MJ. Massive expansion of antigen-specific CD8+ T cells during an acute virus infection. Immunity 1998;**8**: 167–175.
12. Altman JD, et al. Phenotypic analysis of antigen-specific T lymphocytes. Science 1996;**274**:94–96.
13. Koup RA, et al. Temporal association of cellular immune responses with the initial control of viremia in primary human immunodeficiency virus type 1 syndrome. J Virol 1994;**68**: 4650–4655.
14. Borrow P, Lewicki H, Hahn BH, Shaw GM, Oldstone MB. Virus-specific CD8+ cytotoxic T-lymphocyte activity associated with control of viremia in primary human immunodeficiency virus type 1 infection. J Virol 1994;**68**:6103–6110.
15. Jin X, et al. Dramatic rise in plasma viremia after CD8(+) T cell depletion in simian immunodeficiency virus-infected macaques. J Exp Med 1999;**189**:991–998.
16. Schmitz JE, et al. Control of viremia in simian immunodeficiency virus infection by CD8+ lymphocytes. Science 1999;**283**:857–860.
17. Lieberman J, et al. Safety of autologous, ex vivo-expanded human immunodeficiency virus (HIV)-specific cytotoxic T-lymphocyte infusion in HIV-infected patients. Blood 1997;**90**:2196–2206.
18. Phillips RE, et al. Human immunodeficiency virus genetic variation that can escape cytotoxic T cell recognition. Nature 1991;**354**:453–459.
19. Koenig S, et al. Transfer of HIV-1-specific cytotoxic T lymphocytes to an AIDS patient leads to selection for mutant HIV variants and subsequent disease progression. Nat Med 1995;**1**:330–336.

20. Barouch DH, et al. Eventual AIDS vaccine failure in a rhesus monkey by viral escape from cytotoxic T lymphocytes. Nature 2002;**415**:335–339.
21. Musey L, Hughes J, Schacker T, Shea T, Corey L, McElrath MJ. Cytotoxic-T-cell responses, viral load, and disease progression in early human immunodeficiency virus type 1 infection. N Engl J Med 1997; **337**: 1267–1274.
22. Letvin NL, Barouch DH, Montefiori DC. Prospects for vaccine protection against HIV-1 infection and AIDS. Annu Rev Immunol 2002;**20**:73–99.
23. Ahmed R, Gray D. Immunological memory and protective immunity: understanding their relation. Science 1996;**272**:54–60.
24. Barouch DH, et al. Control of viremia and prevention of clinical AIDS in rhesus monkeys by cytokine-augmented DNA vaccination. Science 2000;**290**:486–492.
25. Amara RR, et al. Control of a mucosal challenge and prevention of AIDS by a multiprotein DNA/MVA vaccine. Science 2001;**292**:69–74.
26. Chen X, et al. Protection of rhesus macaques against disease progression from pathogenic SHIV-89.6PD by vaccination with phage-displayed HIV-1 epitopes. Nat Med 2001;**7**:1225–1231.
27. Shiver JW, et al. Replication-incompetent adenoviral vaccine vector elicits effective anti-immunodeficiency-virus immunity. Nature 2002;**415**:331–335.
28. Mellors JW, Rinaldo CR Jr, Gupta P, White RM, Todd JA, Kingsley LA. Prognosis in HIV-1 infection predicted by the quantity of virus in plasma. Science 1996;**272**: 1167–1170.
29. Davenport MP, Ribeiro RM, Perelson AS. Kinetics of virus-specific CD8+ T cells and the control of human immunodeficiency virus infection. J Virol 2004;**78**: 10096–10103.
30. Reynolds MR, et al. CD8+ T-lymphocyte response to major immunodominant epitopes after vaginal exposure to simian immunodeficiency virus: too late and too little. J Virol 2005;**79**:9228–9235.
31. Davenport MP, et al. High-potency human immunodeficiency virus vaccination leads to delayed and reduced CD8+ T-cell expansion but improved virus control. J Virol 2005;**79**:10059–10062.
32. Abdel-Motal UM, et al. Kinetics of expansion of SIV Gag-specific CD8+ T lymphocytes following challenge of vaccinated macaques. Virology 2005;**333**:226–238.
33. Doherty PC, Christensen JP. Accessing complexity: the dynamics of virus-specific T cell responses. Annu Rev Immunol 2000;**18**: 561–592.
34. Flynn KJ, Belz GT, Altman JD, Ahmed R, Woodland DL, Doherty PC. Virus-specific CD8+ T cells in primary and secondary influenza pneumonia. Immunity 1998;**8**:683–691.
35. Bachmann MF, Kundig TM, Hengartner H, Zinkernagel RM. Protection against immunopathological consequences of a viral infection by activated but not resting cytotoxic T cells: T cell memory without "memory T cells"? Proc Natl Acad Sci U S A 1997;**94**:640–645.
36. Stevenson PG, Belz GT, Castrucci MR, Altman JD, Doherty PC. A gamma-herpesvirus sneaks through a CD8(+) T cell response primed to a lytic-phase epitope. Proc Natl Acad Sci USA 1999;**96**:9281–9286.
37. Subbramanian RA, et al. Magnitude and diversity of cytotoxic-T-lymphocyte responses elicited by multiepitope DNA vaccination in rhesus monkeys. J Virol 2003;**77**:10113–10118.
38. Alexander-Miller MA, Leggatt GR, Berzofsky JA. Selective expansion of high- or low-avidity cytotoxic T lymphocytes and efficacy for adoptive immunotherapy. Proc Natl Acad Sci USA 1996;**93**:4102–4107.
39. Bullock TN, Mullins DW, Engelhard VH. Antigen density presented by dendritic cells in vivo differentially affects the number and avidity of primary, memory, and recall CD8(+) T cells. J Immunol 2003;**170**: 1822–1829.
40. Rees W, et al. An inverse relationship between T cell receptor affinity and antigen dose during CD4(+) T cell responses in vivo and in vitro. Proc Natl Acad Sci USA 1999;**96**:9781–9786.
41. Monsurro V, et al. Kinetics of TCR use in response to repeated epitope-specific immunization. J Immunol 2001;**166**:5817–5825.
42. De Boer RJ, Oprea M, Antia R, Murali-Krishna K, Ahmed R, Perelson AS. Recruitment times, proliferation, and apoptosis rates during the CD8(+) T-cell response to lymphocytic choriomeningitis virus. J Virol 2001;**75**: 10663–10669.
43. Matloubian M, Concepcion RJ, Ahmed R. CD4+ T cells are required to sustain CD8+ cytotoxic T-cell responses during chronic viral infection. J Virol 1994;**68**:8056–8063.
44. Belz GT, Wodarz D, Diaz G, Nowak MA, Doherty PC. Compromised influenza virus-specific CD8(+)-T-cell memory in CD4(+)-T-cell-deficient mice. J Virol 2002;**76**:12388–1293.
45. Badovinac VP, Porter BB, Harty JT. Programmed contraction of CD8(+) T cells after infection. Nat Immunol 2002;**3**:619–626.
46. Kaech SM, Tan JT, Wherry EJ, Konieczny BT, Surh CD, Ahmed R. Selective expression of the interleukin 7 receptor identifies effector CD8 T cells that give rise to long-lived memory cells. Nat Immunol 2003;**4**:1191–1198.
47. Mollet L, et al. Dynamics of HIV-specific CD8(+) T lymphocytes with changes in viral load. J Immunol 2000;**165**:1692–1704.
48. Karrer U, et al. Memory inflation: continuous accumulation of antiviral CD8+ T cells over time. J Immunol 2003;**170**:2022–2029.
49. Komatsu H, Sierro S, Cuero VA, Klenerman P. Population analysis of antiviral T cell responses using MHC class I-peptide tetramers. Clin Exp Immunol 2003;**134**:9–12.
50. Zafiropoulos A, Barnes E, Piggott C, Klenerman P. Analysis of 'driver' and 'passenger' CD8+ T-cell responses against variable viruses. Proc Biol Sci 2004; **271**(Suppl.):S53–S56.
51. Barber DL, et al. Restoring function in exhausted CD8 T cells during chronic viral infection. Nature 2006;**439**:682–687.
52. Davenport MP, Fazou C, McMichael AJ, Callan MFC. Clonal selection, clonal senescence, and clonal succession: the evolution of the T cell response to infection with a persistent virus. J Immunol 2002;**168**:3309–3317.
53. Papagno L, et al. Immune activation and CD8(+) T-cell differentiation towards senescence in HIV-1 infection. PLoS Biol 2004;**2**:E20.
54. Appay V, et al. HIV-specific CD8(+) T cells produce antiviral cytokines but are impaired in cytolytic function. J Exp Med 2000;**192**:63–75.
55. Effros RB, et al. Shortened telomeres in the expanded CD28-CD8+ cell subset in HIV disease implicate replicative senescence in HIV pathogenesis. AIDS 1996;**10**:F17–F22.
56. Effros RB, Pawelec G. Replicative senescence of T cells: does the Hayflick Limit lead to immune exhaustion? Immunol Today 1997;**18**:450–454.
57. Pawelec G, Akbar A, Caruso C, Effros R, Grubeck-Loebenstein B, Wikby A. Is immunosenescence infectious? Trends Immunol 2004;**25**:406–410.
58. Mittler JE, Levin BR, Antia. R T-cell homeostasis, competition, and drift: AIDS as HIV-accelerated senescence of the immune repertoire. J Acquir Immune Defic Syndr Hum Retrovirol 1996;**12**:233–248.
59. Phillips AN. Reduction of HIV concentration during acute infection: independence from a specific immune response. Science 1996;**271**:497–499.
60. De Boer RJ, Perelson AS. Target cell limited and immune control models of HIV infection: a comparison. J Theor Biol 1998;**190**:201–214.
61. Regoes RR, Wodarz D, Nowak MA. Virus dynamics: the effect of target cell limitation and immune responses on virus evolution. J Theor Biol 1998;**191**:451–462.
62. Regoes RR, Antia R, Garber DA, Silvestri G, Feinberg MB, Staprans SI. Roles of target cells and virus-specific cellular immunity in primary simian immunodeficiency virus infection. J Virol 2004;**78**:4866–4875.

63. Wick D, Self SG, Corey L. Do scarce targets or T killers control primary HIV infection? J Theor Biol 2002;**214**:209–214.
64. Collins KL, Chen BK, Kalams SA, Walker BD, Baltimore D. HIV-1 Nef protein protects infected primary cells against killing by cytotoxic T lymphocytes. Nature 1998;**391**:397–401.
65. Zhang L, et al. Effects of antibody on viral kinetics in simian/human immunodeficiency virus infection: implications for vaccination. J Virol 2004;**78**:5520–5522.
66. Mascola JR, et al. Protection of Macaques against pathogenic simian/human immunodeficiency virus 89.6PD by passive transfer of neutralizing antibodies. J Virol 1999;**73**:4009–4018.
67. Letvin NL, et al. Preserved CD4+ central memory T cells and survival in vaccinated SIV-challenged monkeys. Science 2006;**312**:1530–1533.
68. Korthals Altes H, Ribeiro RM, de Boer RJ. The race between initial T-helper expansion and virus growth upon HIV infection influences polyclonality of the response and viral set-point. Proc R Soc Lond, B, Biol Sci 2003;**270**:1349–1358.
69. Igarashi T, et al. Early control of highly pathogenic simian immunodeficiency virus/human immunodeficiency virus chimeric virus infections in rhesus monkeys usually results in long-lasting asymptomatic clinical outcomes. J Virol 2003;**77**:10829–10840.
70. Davenport MP, Zhang L, Shiver JW, Casmiro DR, Ribeiro RM, Perelson AS. Influence of peak viral load on the extent of CD4+ T-cell depletion in simian HIV infection. J Acquir Immune Defic Syndr 2006;**41**:259–265.
71. Nishimura Y, et al. Resting naive CD4+ T cells are massively infected and eliminated by X4-tropic simian-human immunodeficiency viruses in macaques. Proc Natl Acad Sci USA 2005;**102**:8000–8005.
72. Nishimura Y, et al. Highly pathogenic SHIVs and SIVs target different CD4+ T cell subsets in rhesus monkeys, explaining their divergent clinical courses. Proc Natl Acad Sci USA 2004;**101**:12324–12329.
73. Allen TM, et al. Tat-vaccinated macaques do not control simian immunodeficiency virus SIVmac239 replication. J Virol 2002;**76**:4108–4112.
74. Mattapallil JJ, Letvin NL, Roederer M. T-cell dynamics during acute SIV infection. AIDS 2004;**18**:13–23.
75. Picker LJ, et al. Insufficient production and tissue delivery of CD4+ memory T cells in rapidly progressive simian immunodeficiency virus infection. J Exp Med 2004;**200**:1299–1314.
76. Smit-McBride Z, Mattapallil JJ, McChesney M, Ferrick D, Dandekar S. Gastrointestinal T lymphocytes retain high potential for cytokine responses but have severe CD4(+) T-cell depletion at all stages of simian immunodeficiency virus infection compared to peripheral lymphocytes. J Virol 1998;**72**:6646–6656.
77. Mattapallil JJ, Douek DC, Hill B, Nishimura Y, Martin M, Roederer M. Massive infection and loss of memory CD4+ T cells in multiple tissues during acute SIV infection. Nature 2005;**434**:1093–1097.
78. Guadalupe M, et al. Severe CD4+ T-cell depletion in gut lymphoid tissue during primary human immunodeficiency virus type 1 infection and substantial delay in restoration following highly active antiretroviral therapy. J Virol 2003;**77**:11708–11717.
79. Brenchley JM, et al. CD4+ T cell depletion during all stages of HIV disease occurs predominantly in the gastrointestinal tract. J Exp Med 2004;**200**:749–759.
80. Jansen CA, et al. Analysis of the effect of highly active antiretroviral therapy during acute HIV-1 infection on HIV-specific CD4 T cell functions. AIDS 2005;**19**:1145–1154.
81. Davenport MP, Ribeiro RM, Chao DL, Perelson AS. Predicting the impact of a nonsterilizing vaccine against human immunodeficiency virus. J Virol 2004;**78**:11340–11351.
82. Smith RJ, Blower SM. Could disease-modifying HIV vaccines cause population-level perversity? Lancet Infect Dis 2004;**4**:636–639.
83. Anderson R, Hanson M. Potential public health impact of imperfect HIV type 1 vaccines. J Infect Dis 2005;**191**(Suppl.):S85–S96.
84. van Ballegooijen M, Bogaards JA, Weverling JG, Boerlijst MC, Goudsmit J. AIDS vaccines that allow HIV-1 to infect and escape immunologic control: a mathematic analysis of mass vaccination. J Acquir Immune Defic Syndr 2003;**34**:214–220.
85. Mascola JR, et al. Protection of macaques against vaginal transmission of a pathogenic HIV-1/SIV chimeric virus by passive infusion of neutralizing antibodies. Nat Med 2000;**6**:207–210.