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The dynamics of the cellular immune response to HIV infection: implications for vaccination

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Recent advances in measuring T-cell responses to viruses have led to new insights into how these T cells respond. In the acute infection there are massive $CD8^+$ T-cell responses to both Epstein–Barr virus (EBV) and to human immunodeficiency virus (HIV). Many of these T cells are effector cells and only a minority appear to be capable of maintaining immunological memory. In persistent virus infections, high levels of antigen-specific effector cells persist. If virus does not persist, the effectors fade in number but memory is maintained and is primed to react rapidly to a new challenge. A vaccine that stimulates only T-cell responses may protect when these memory cells respond rapidly enough to generate high numbers of effectors before the infecting virus becomes established.

Keywords: HIV; Epstein–Barr virus; $CD8^+$ T cells; memory; vaccination

1. INTRODUCTION

The study of the cellular immune response to infectious agents has been transformed by the recent introduction of major histocompatibility complex (MHC) tetramers (Altman *et al*. 1996; McMichael & O'Callaghan 1998). These are complexes of four MHC class I molecules containing the same epitope peptide linked by a biotin molecule to streptavidin. Such reagents enable direct visualization of antigen-specific T cells for the first time. In parallel, methods for intracellular cytokine staining of antigen-specific T cells have also been introduced (Butz & Bevan 1998; Pitcher *et al*. 1999). Use of these two technical advances has altered our view of how T cells respond to an invading pathogen. The biggest surprise was the magnitude of the $CD8^+$ T-cell response to virus infections, ten to 100 times larger than many expected (Altman *et al*. 1996; Butz & Bevan 1998; Murali-Krishna *et al*. 1998; Tan *et al*. 1999). Nearly all the activated T cells seen in an acute infection are virus specific, very few are activated as bystanders. These findings permit a revision of how T cells respond, of their dynamics and what they do.

2. ACUTE HUMAN IMMUNODEFIENCY VIRUS AND SIMIAN IMMUNODEFICIENCY VIRUS INFECTION

In acute human immunodeficiency virus (HIV) and simian immunodeficiency virus (SIV) infection, the virus replicates in an uncontrolled manner, reaching a peak of over 10^6 particles per millilitre of blood in about three weeks. The $CD8^+$ T-cell response peaks a few days later and at this time there is a fall in the viraemia. It has long been argued that these antigen-specific T cells are responsible for the reduction in virus load (Koup *et al*. 1994). Strong support for this view comes from studies in macaques where $CD8^+$ T cells were removed by antibody treatment *in vivo*; when challenged with SIV the viraemia was not controlled until the $CD8^+$ T cells recovered from their suppression (Schmitz *et al*. 1999). The peak of the $CD8^+$ T-cell response reaches 1–10% of all $CD8^+$ T cells in the blood, both for HIV (Wilson *et al*. 2000) and SIV (Kuroda *et al*. 1999). It is not possible to measure the number of HIV-specific T cells in uninfected and unexposed people because the assays are not sensitive and accurate enough, but it is safe to estimate that the number is less than one in a million. Therefore it is likely that the T cells responding at the peak of the infection have divided over 15 times (figure 1). Essential to the use of the tetramer assay is knowledge of the dominant epitope peptide and it does appear that for many viruses the acute phase immune response is focused on very few epitopes out of the many that are theoretically possible (Chen *et al*. 2000). It looks as if the immunodominance is decided early on, for reasons that are still not clear (Chen *et al*. 2000).

HIV and SIV infect dendritic cells and $CD4^+$ helper T cells, both of which are critical cells of the immune system. Their infection could affect the $CD8^+$ T-cell response. Although the primary $CD8^+$ T-cell response to HIV looks large, it appears to be smaller than the acute $CD8^+$ T-cell response to Epstein–Barr virus (EBV) (Callan *et al*. 1998). Here $CD8^+$ T cells specific for EBV have been found at levels as high as 44% of $CD8^+$ T cells in the blood of patients with infectious mononucleosis; the range is between 5% and 45%. In acute infection with lymphocytic choriomeningitis virus in mice, similar high numbers were observed (Murali-Krishna *et al*. 1998).

In acute infectious mononucleosis, the expanded $CD8^+$ T-cell population is capable of function (Callan *et al*. 2000). They can kill antigen-expressing target cells at about the level expected. They release cytokines (γ-interferon

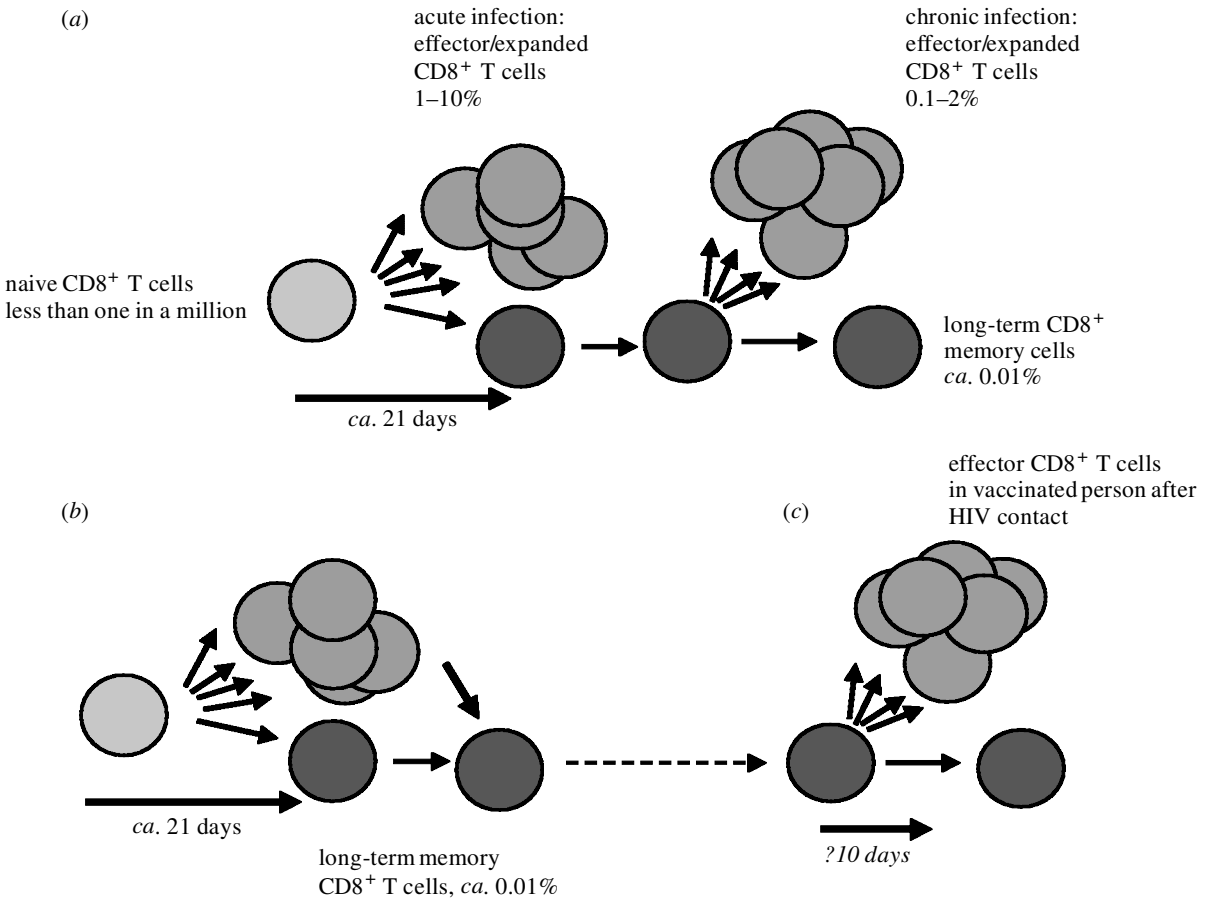

Figure 1. Possible dynamics of $CD8^+$ T-cell response to (*a*) HIV, (*b*) a vaccine and (*c*) HIV after vaccination. In (*a*), the calculated numbers of naive HIV-specific T cells (estimate), expanded effectors (detected with tetramers) and long-term memory cells (detected by limiting dilution assay) are shown. In (*b*), the similar response to a non-persisting $CD8^+$ T-cell stimulating vaccine is shown. (*c*) The response to HIV in a vaccinated person several months after vaccination. Figures are based on Hanke *et al.* (1999), Wilson *et al.* (2000) and Evans *et al.* (1999).

(IFN-γ), tumour necrosis factor-α (TNF-α) and macrophage inflammatory protein-1β (MIP-1β)) rapidly on contact with antigen-presenting cells, although not all T cells can do this. However, on removal from the body, they die rapidly by apoptosis (Callan *et al.* 2000). Similarly, when stimulated by a non-persisting immunogen (DNA and recombinant modified vaccinia virus Ankara) the high $CD8^+$ T-cell peak decays rapidly with a half-life in the blood of about seven days (Hanke *et al.* 1999). The expanded T cells appear therefore to be end-stage T cells, functioning but not capable of dividing further. Memory is maintained by a minor subset of T cells within the same clonal population that are able to divide further (Tan *et al.* 1999). The functional status of T cells in acute HIV infection is not yet known; preliminary experiments suggest some impairment of cytokine release and of perforin expression (V. Appay, unpublished data).

In acute HIV infection there is an early $CD4^+$ T-cell response, but this has been hard to demonstrate, possibly because the acutely reacting T cells are infected and damaged by the HIV. However, Pitcher *et al.* (1999) have been able to demonstrate HIV-specific $CD4^+$ T cells by using intracellular cytokine staining (after a few hours of exposure to antigen). The number found was substantial in patients with stable infection but less than the number responding to cytomegalovirus, another persisting virus. Despite the clear presence of HIV-specific $CD4^+$ T cells, it has been almost impossible to demonstate them by the conventional antigen-stimulated proliferation assay; only a few long-term survivors show a response. However, Rosenberg *et al.* (1997) showed that it was possible to rescue this response by treating acutely infected persons very early with potent antiretroviral drugs. Thus there appears to be an early impairment of the $CD4^+$ T-cell response that might affect the initiation of the $CD8^+$ T-cell response. Nevertheless, the $CD8^+$ T-cell response is substantial, mostly functional and important in reducing virus load. The suspicion remains, however, that it is not as effective as it might be and that this could result in higher virus loads at the set-point, once the acute phase stabilizes, than might otherwise be found.

3. CHRONIC HIV INFECTION

In chronic HIV infection, during the asymptomatic phase, there is a strong ongoing anti-HIV $CD8^+$ T-cell response (Ogg *et al.* 1998). Often antigen-specific $CD8^+$ T cells constitute over 1.0% of all $CD8^+$ T cells when there is an identifiable, immunodominant T-cell response. Ogg *et al.* (1998) have shown that there is an

inverse correlation between the number of T cells specific for immunodominant gag and pol epitopes presented by HLA-A2 and the plasma virus RNA load. This is in a pseudo-equilibrium state and has been the subject of considerable discussion (see McMichael *et al*. 2000). In HTLV-1 infection, C. Bangham (personal communication) has shown that there is a positive correlation between virus load and $CD8^+$ T-cell number, suggesting that the negative correlation reported by Ogg *et al*. (1998) might reflect suppression of the $CD8^+$ T-cell response by the virus, presumably through the effects on HIV-specific $CD4^+$ T cells. Mathematical modelling arguments support this model (D. Wodarz and M. Nowak, personal communication), as does the obvious finding that no virus means no detectable HIV-specific $CD8^+$ T cells. We have suggested therefore that the relationship between virus load and cytotoxic T-lymphocyte (CTL) number is biphasic with a positive correlation at low virus load, but that above a certain virus level, HIV effectively suppresses the CTL response—most of the patients seen are in this second phase (D. Wodarz and M. Nowak, personal communication). This does not mean that the CTLs fail to suppress the virus—macaque experiments where the $CD8^+$ T cells are removed *in vivo* in ongoing SIV infection, resulting in higher virus loads, support this view (Jin *et al*. 1999; Schmitz *et al*. 1999). However it does argue that the $CD8^+$ T-cell response is not as effective as it might be in HIV infection.

This view is reinforced by the findings of Appay *et al*. (2000), who examined HIV-specific $CD8^+$ T-cell function in samples from chronically infected persons. Whereas these T cells responded well to antigen stimulation to make IFN-γ, TNF-α and MIP-1β, levels of perforin were very low. This was particularly striking when the HIV-specific T-cell responses were compared to cytomegalovirus-specific T cells in the same patients. The low perforin levels were found regardless of the virus load, even when undetectable as the result of aggressive antiretroviral drug therapy. Therefore it seems that perforin expression is impaired, perhaps a failure of $CD8^+$ T-cell maturation due to poor $CD4^+$ T-cell help (Zajac *et al*. 1998).

The high number of tetramer-staining cells in late HIV infection implies that they are surviving well or are actively replaced, from long-term memory T, after apoptosis. We favour the latter, though data to distinguish these possibilities are lacking. The number of $CD8^+$ T cells specific for HIV remains high throughout the infection but the infection nevertheless progresses. Contributing factors to this are possible progressively greater impairment of $CD8^+$ T-cell function and the selection of escape mutants. Appearance and selection of escape mutants has now been shown in several patients and also in macaques (Evans *et al*. 1999; Goulder *et al*. 1997; Phillips *et al*. 1991). The consequences are uncertain, but at the very least new $CD8^+$ T-cell responses to alternative epitopes, i.e. new primary T-cell responses, must be required. Again, impaired $CD4^+$ T-cell help must weaken these new responses and could contribute significantly to the gradual and harmful increase in virus load that occurs as the infection progresses.

4. IMPLICATIONS FOR VACCINES

Given the difficulty of current strategies to stimulate effective HIV-neutralizing antibodies in animals and humans (Connor *et al*. 1998), much attention has now been directed towards stimulating $CD8^+$ T-cell responses. There are good data in animal models of other virus and parasite infections that CTL can protect against new infection (Brehm *et al*. 1997; Fu *et al*. 1999; Schneider *et al*. 1998). In HIV infection, extensive data from a small subset of African sex workers who are highly exposed to HIV but not infected, implies that their CTL response protects them from infection, as long as the CTL levels are maintained (Rowland-Jones *et al*. 1999). In macaque SIV infection models, data suggest that vaccine-induced CTL can protect or at least lower virus loads when infection occurs (Kent *et al*. 1998; Robinson *et al*. 1999).

How do CTLs protect against HIV infection? CTLs kill virus-infected cells and prevent them from producing new virus particles, but they cannot prevent infection, as they have no activity against naked virus. Furthermore, the kinetics of the antivirus CTL response shows that large numbers of activated effector T cells can be readily generated, but if antigen does not persist, they disappear rapidly (Hanke *et al*. 1999; figure 1*b*). Current vaccine constructs are designed not to persist, for safety reasons demanded by the regulatory authorities. Therefore it is likely that any protection will have to come from the long-term memory population.

Long-term memory T cells are probably best detected by limiting dilution assays, which demand multiple (about 12) divisions *in vitro* and maturation to effectors. The Elispot assay probably detects the same population in a situation where antigenic challenge occurred many months ago and antigen is no longer present, e.g. influenza infection, and detects very similar cell numbers to the limiting dilution assay (Lalvani *et al*. 1997). This indicates that long-term memory T cells can be activated to function (interferon secretion) within 6 h and before the cell divides (Lalvani *et al*. 1997). Thus in a person who was vaccinated several months ago by a CTL-inducing vaccine, there may not be instant killing of any HIV-infected cells that appear, but there is a response that differs substantially from that of the primary CTL response (figure 1*c*). The first advantage is in antigen-specific T-cell numbers: 10^6 for an unprimed person and 10^4 for a person with memory. This would give an advantage of seven divisions (2^7), about seven days. Second, it has been shown that memory T cells respond much more rapidly than memory T cells and, as indicated above, can release cytokines almost immediately (Tanchot *et al*. 1998); this could give an advantage of a few more days. Thus, the vaccine-primed person should be able to respond much more rapidly to the new infection, although the magnitude of the response might be less if the virus load was well controlled. In addition, the ability of a vaccine to stimulate a good T-helper-cell response may give the primed person a further crucial advantage, ensuring that all the virus-specific T cells are functional and again speeding up the response. This has to be balanced for HIV infection with the possibility that these activated T cells will be good host cells for the virus to infect. Whether these advantages add up

enough to clear an incoming HIV infection remains to be seen, but there is a chance and at the least, the level of HIV at the set point should be lower, with a better prognosis.

5. CONCLUSIONS

The studies reviewed here give a kinetic view of HIV infection that is of crucial importance in understanding the natural response and how effective it is. This understanding is crucial for design of vaccines that can prime the T-cell response to be able to control infection and even clear it when the infecting challenge is low dose.

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