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Memory CD8$^+$ T cells in HIV infection

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THE ROYAL SOCIETY

Memory CD8+ T cells in HIV infection

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Cytotoxic T lymphocytes (CTLs) play a central role in the control of persistent HIV infection in humans. The kinetics and general features of the CTL response are similar to those found during other persisting virus infections in humans. During chronic infection there are commonly between 0.1 and 1.0% of all $CD8^+$ T cells in the blood that are specific for immunodominant virus epitopes, as measured by HLA class I peptide tetramers. These figures are greatly in excess of the numbers found by limiting dilution assays; the discrepancy may arise because in the latter assay, CTLs have to divide many times to be detected and many of the HIV-specific $CD8^+$ T cells circulating in infected persons may be incapable of further division. Many tetramer-positive T cells make interferon-γ, β-chemokines and perforin, so are probably functional. It is not known how fast these T cells turn over, but in the absence of antigen they decay in number. Impairment of CTL replacement, because $CD4^+$ T helper cells are depleted by HIV infection, may play a major role in the development of AIDS.

Keywords: AIDS; HIV; cellular immunity; cytotoxic T lymphocytes; memory; HLA

1. HIV INFECTION

Human immunodeficiency virus-1 (HIV-1) is a lentivirus that causes a persistent infection in humans. It infects $CD4^+$ T cells and monocytes and ultimately reduces the number of the former and impairs their function. There is good evidence that the $CD8^+$ T cells control the infection, for a time at least, though ultimately this immune response fails (Rowland-Jones *et al*. 1997). Recently, it has been shown that, in early HIV infection, antigen-specific $CD4^+$ T cells are specifically depleted and are lacking thereafter, although some patients with very slow progression of the infection retain antigen-specific $CD4^+$ T-cell activity (Rosenberg *et al*. 1997). In progressing patients, it is likely that the impairment of $CD4^+$ T-cell help contributes to the loss of $CD8^+$ T-cell function and ultimate failure to control the infection.

A central feature of HIV infection, which has prognostic implications, is the progressive loss of $CD4^+$ T cells. This cannot be attributed entirely to destruction of cells infected with HIV as these represent under 0.1% of peripheral $CD4^+$ T cells at most stages of infection (Chun *et al*. 1997). There are several other possible explanations. Many $CD4^+$ T cells may be lost very early in the infectious cycle when they express virus proteins and become targets for cytotoxic T lymphocytes (CTLs), but before they generate new virus particles (Wain Hobson 1995). There is impairment of $CD4^+$ T-cell generation from the thymus and therefore a defect on the supply side (Fauci *et al*. 1996; Douek *et al*. 1998). In addition, apoptosis of uninfected $CD4^+$ and $CD8^+$ T cells occurs in HIV infected persons (Ameisen & Capron 1991): there is often a non-specific activation of lymphocytes that leads to fas expression, making them susceptible to lysis by fas ligand-bearing cells. Fas-ligand expression on T cells can be induced by tat and by nef in HIV infection (Xu *et al*. 1997). Destruction of uninfected $CD4^+$ T cells may also result from partial activation by free gp120 (Banda *et al*. 1992). It is probable that all of these mechanisms contribute to the depletion of $CD4^+$ T cells that is central to the pathogenesis of the acquired immunodeficiency syndrome (AIDS).

Despite impaired $CD4^+$ T-cell function, the $CD8^+$ T-cell response to HIV infection is very vigorous and resembles the CTL response to other persisting viruses, Epstein–Barr virus (EBV) and cytomegalovirus (CMV), neither of which causes serious immunosuppression. The evidence that CTLs are important in the control of HIV comes from several sources and is now very strong. First, there is a temporal inverse relationship between viraemia and the CTL response: the initial high virus level is brought down as the CTL response appears and late virus escape is associated with low levels of CTLs (Borrow *et al*. 1994; Koup *et al*. 1994; Rowland-Jones *et al*. 1997). In the chronic phase of infection, there is an inverse relationship between virus load and the number of CTLs specific for the immunodominant epitopes (Ogg *et al*. 1998). This relationship is consistent with the recent demonstration in simian immunodeficiency virus (SIV) infection that treatment of infected macaques with anti-CD8 caused a rapid rise in virus levels, both in acute and chronic infection (Jin *et al*. 1999; Schmitz *et al*. 1999). However, the relationship between the virus load and specific $CD8^+$ T-cell number is not simple. Although high levels of CTLs induced in the acute phase should suppress the virus, this would remove the antigenic stimulus necessary to stimulate the CTLs. Initially, a high number of

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CTLs should reduce virus load and at equilibrium one might expect a positive correlation between the two. (This has been found for human T-cell lymphotropic virus (HTLV)-1 in humans; C. Bangham, D. Wodarz and G. Ogg, personal communication). The negative association may result from the virus suppressing the CTL response indirectly by impairing $CD4^+$ T-cell help. Thus there would be a quasi-equilibrium that would reflect the balance between antigenic stimulation by virus, removal of antigen by CTLs and the suppressive effect. As virus load increases, the CTL response becomes less effective; nevertheless the CTLs control virus load until late in the infection. This controlling effect of CTLs is demonstrated by the selection of escape mutants during HIV and SIV infection by CTLs (Borrow *et al*. 1997; Couillin *et al*. 1995; Goulder *et al*. 1997*a*,*b*; McMichael & Phillips 1997; Price *et al*. 1997). Whether CTLs suppress virus on their own is more uncertain. Certainly the macaque anti-CD8 experiments imply that CTLs are largely responsible for control of the virus (Schmitz *et al*. 1999). As indicated above, the specific $CD4^+$ T-cell response appears to be greatly impaired. The antibody response is generally regarded as being of little help, although the selection of mutant viruses with alterations in the virus envelope implies escape from antibody neutralization (Nara *et al*. 1990).

Why does the CTL response not control HIV infection? Failure to eliminate the virus is not unique, for example EBV and CMV are not removed but both are permanently controlled. Both have evolved mechanisms to evade the CTL response (Ploegh 1998), which is vigorous, and so a balance must be struck between antigenic stimulation, specific impairment of the CTLs and virus persistence. HIV has also evolved mechanisms for escaping the CTL response (reviewed by McMichael 1998). The most obvious is that the virus can infect cells as provirus and remain silent, not expressing virus proteins and therefore invisible to antibody and T cells, at least until the cell is activated. Careful quantitative experiments show that 10^{-4}–10^{-5} T cells may carry virus in this form (Chun *et al*. 1997). Just as such virus is hard to eliminate with drug therapy, CTLs would not be expected to be able to eliminate such virus. However, the majority of infected cells are in productive cycle and express virus proteins. In this cycle, there is a period before virus production where they express virus proteins and are therefore targets for CTLs; if the latter are very active they could kill many infected cells before they release new virus particles. However the virus escape strategies may thwart this. One, shared by many other persisting viruses, is downregulation of major histocompatibility complex (MHC) class I molecules (McMichael 1998; Ploegh 1998). It has been clearly shown that the virus nef protein mediates this effect (Collins *et al*. 1998), but it is not apparent until about 48 h after the initiation of the infection. Another nef function is to up-regulate fas ligand, making infected cells capable of killing fas-bearing activated T cells including specific CTL (Xu *et al*. 1997). Evasion also occurs by mutation of the epitopes recognized by CTL. HIV replicates in very high numbers, typically over 10^9 virions per day with a mutation rate between 10^{-4} and 10^{-5} (Perelson *et al*. 1996; Ploegh 1998). The virus genome is around 10^4 bases and so each new virus particle carries at least one new mutation and each possible mutation, and most pairs, must be made every day. Given that CTLs reduce virus load, probably by killing, there must be intense selective pressure for the virus escape mutants (McMichael 1998; McMichael & Phillips 1997). This is borne out by demonstrations of the evolution of escape mutants in longitudinal studies and by highly suggestive data in cross-sectional studies (Borrow *et al*. 1997; Couillin *et al*. 1995; Goulder *et al*. 1997*a*,*b*; Price *et al*. 1997).

Taken together therefore, there is strong evidence that CTLs play a major part in containing HIV infection, but a variety of mechanisms enable the virus to elude the attackers. The virus variability does present problems. The immune system is thought to be capable of recognizing almost any foreign protein, including variants, so why does the response not turn to alternative epitopes? There is evidence that it does and the resulting broadening of the immune response, in terms of the number of epitopes recognized (epitope spreading), is well described (Goulder *et al*. 1997*b*). However, there are constraints on the responses to closely related virus variants. 'Original antigenic sin', where the immune response appears locked into a response to the first version of the virus encountered, may apply in HIV infection (Klenerman & Zinkernagel 1998; McAdam *et al*. 1995). The mechanism is unknown but may relate to the high number of memory T cells compared to the very rare naive T cells specific for the new epitope. More memory than naive T cells may be triggered by a weakly cross-reacting new variant. The primary T-cell response, which is required to control the new variant, may also be hard to initiate when HIV has impaired the function of both antigen-presenting dendritic cells and $CD4^+$ T cells. A related phenomenon is antagonism, where T cells exposed to an altered epitope receive a partial signal that inhibits their ability to respond to the full agonist (Klenerman *et al*. 1994). A great excess of original virus epitopes could antagonize any T cell trying to respond to a new variant.

2. THE NATURE OF THE $CD8^+$ T-CELL RESPONSE TO HIV

In many respects the $CD8^+$ T-cell response to HIV resembles that to EBV and CMV, which is remarkable given the early failure of specific $CD4^+$ T-cell help (Rosenberg *et al*. 1997). A variety of techniques have been used to measure $CD8^+$ T cells that respond to HIV. These include lytic assays (Walker *et al*. 1987), Elispot/interferon-γ release assays (Lalvani *et al*. 1997), limiting dilution assays (LDA) (Carmichael *et al*. 1993), *ex vivo* T-cell receptor quantitation of T-cell clones of known specificity (Kalams *et al*. 1994; Moss *et al*. 1995) and tetramer binding assays (Altman *et al*. 1996). These assays measure function, proliferative capacity, clonality and antigen binding. Each method gives consistent but different results. HLA-peptide tetramer staining gives the highest numbers but, in its simplest form, does not reveal function. In most chronically HIV-infected persons 0.1–1.0% of $CD8^+$ T cells stain with tetramers formed with a single epitope peptide (Altman *et al*. 1996; Ogg *et al*. 1998). Elispot measures a function, antigen-induced interferon-γ or tumour necrosis factor (TNF) production,

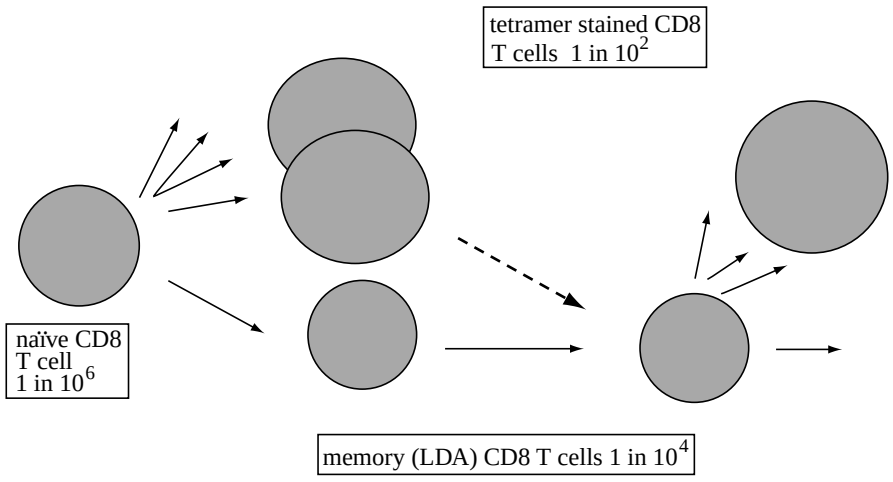

Figure 1. Model for stimulation of memory T-cell populations in HIV infection. Naive $CD8^+$ T cells with HIV-specific T-cell receptors are present at undetectable frequency, less that 1 in 10^6. In acute infection HIV-specific $CD8^+$ T cells are rapidly stimulated to divide and differentiate to generate specific T cells, detected with tetramers at frequencies of around 1 in 20–50. At this stage, memory T cells detected by LDA may be present at frequencies up to 1 in 10^3 but often lower. As the infection progresses, the tetramer-stained cells remain at frequencies of 1 in 1000 to 1 in 50. The LDA determined memory cells are commonly at 1 in 5000–10 000. Elispot assays indicate that at least 50% of tetramer-positive cells make interferon-γ and an unknown fraction can kill; all LDA-detected (or clonable) cells show lytic activity after antigen stimulation. The expanded T-cell population is short lived in the absence of persisting antigen. In persisting HIV infection, they may be long lived or, more likely, continually stimulated from the long-lived memory-cell pool.

and shows numbers that are about half those found by tetramer staining, a ratio that is similar to that found for other persistent viruses (Murali-Krishna *et al*. 1998). Quantitation of T-cell receptor (TCR) transcripts gives values for single clones that are close to those found with tetramers, although activated T cells may have more TCR mRNA than resting T cells. The LDA gives values 10–30-fold lower than tetramer staining but measures only T cells capable of dividing 10–14 times in response to antigen. When tetramer-positive cells from chronically HIV-infected persons are cloned, less than 10% of tetramer binding cells grow into antigen-specific clones *in vitro* (Dunbar *et al*. 1998; G. Ogg, unpublished results). This is consistent with the idea that only a subset of antigen-specific $CD8^+$ T cells are capable of multiple divisions (at least *in vitro*, but probably *in vivo* as well). Thus memory $CD8^+$ T cells, defined as T cells that have seen antigen, can be detected in different ways: (i) those that can proliferate and then function; (ii) those that can secrete interferon before they divide further; and (iii) those that can bind antigen, have unknown functional activity and may not be able to divide further. In HIV-1 and other virus-infected persons, the ratio of these is typically 1:5:10. These numbers are very similar in other persisting infections (L. Tan *et al*. 1999).

All of these assays detect T cells that can be regarded as memory cells in the sense that they have responded to antigen. It is clear that a given T-cell clone can appear in both the LDA population and the tetramer staining populations (Wilson *et al*. 1998), even though most of the latter die in culture. The population that can grow may represent truly long-lived cells that mediate memory in the original immunological sense (figure 1).

HIV is a persisting infection and, like EBV and CMV infection, T cells that stain with tetramer are maintained at high levels (0.1–3.0% of $CD8^+$ T cells) throughout the infection. As indicated above, most die in culture even with further antigen stimulation and cytokines. If this reflects what happens *in vivo*, there should be a continuous turnover of these T cells, the high level being maintained by continuous antigen stimulation of the memory pool. In this case, the role of the $CD4^+$ helper T cell may be critical in determining the equilibrium level $CD8^+$ T cells and therefore the virus load. Some evidence that tetramer-positive cells are short lived *in vivo* comes from CTL transfer experiments into HIV-infected patients, where they were found to apoptose rapidly, possibly on antigen contact (R. Tan *et al*. 1999). However, apart from this study, where the transferred cells were *in-vitro*-cultured clones, high turnover of antigen-specific T cells in HIV infection has not been formally shown, although a high turnover of the whole $CD8^+$ T-cell population has been shown (see Kaur *et al*., this issue). Therefore, it is still possible that the tetramer-binding population survives for long periods *in vivo*. In this case, $CD4^+$ T cells may help determine the longevity of the CTLs. Preliminary data from an experiment where a non-replicating, and therefore short-lived (vaccine), immunogen was used to stimulate SIV-specific CTLs, showed that, in the absence of persisting antigen, the tetramer-stained T cells disappeared rapidly from the blood, with a half-life of around six days (Hanke *et al*. 2000). This contrasts with the decay of tetramer-staining cells in patients treated with potent antiretroviral drugs where the half-life was 45 days (Ogg *et al*. 1999). This probably indicates that antigen persists and that the CTL response is re-equilibrating. It is likely therefore that antigen is necessary to maintain the high level of CTL seen in the persisting virus infections.

In influenza-specific $CD8^+$ T-cell responses, the virus antigen disappears and the CTL response decays so that after several months or years the response is close to, or

below, the level of detection by tetramer (1 in 5000 $CD8^+$ cells) (Dunbar *et al*. 1998; Lalvani *et al*. 1997). In humans who have not been infected with influenza virus for at least a year, Elispot-detected T cells were at levels close to those measured by the LDA. This suggests a decay of activated T cells, leaving only the long-term memory T cells. It is interesting that the antigen-specific Elispot assay detected memory T cells that could be activated by epitope peptides to secrete interferon-γ in as little as 6 h (Lalvani *et al*. 1997). These cells can be cloned efficiently after tetramer staining and fluorescence-activated cell sorter sorting (Dunbar *et al*. 1998). This suggests that the three assays (tetramer staining, Elispot and LDA) converge when antigen has disappeared, leaving only the long-term memory.

The bulk of the tetramer-positive T cells in the persisting human virus infections stain with anti-perforin and anti-β-chemokine antibodies and therefore contain lytic granules. Elispot assays show that around half can be induced to secrete interferon. However, in a mouse model, where lymphocytic choriomeningitis virus replicates at a high rate or where CD4 T-cell function is grossly impaired, tetramer-positive $CD8^+$ T cells that do not function can be demonstrated (Gallimore *et al*. 1998; Zajac *et al*. 1998). This form of silencing may not be a major feature of the anti-HIV $CD8^+$ T-cell response because of the ample evidence that $CD8^+$ T cells are effective against HIV. However, Walker and colleagues have described rare, rapidly progressing patients whose $CD8^+$ T cells lyse virus-infected cells, but cannot inhibit virus replication *in vitro* (B. D. Walker, unpublished results). What determines such responses needs further investigation.

In conclusion, persisting virus infections stimulate strong $CD8^+$ T-cell responses that are maintained by the continuous presence of antigen. In one sense, these are not really memory responses as there is constant stimulation. The $CD8^+$ T cells can mediate several functions, lysis, interferon and TNF release, and chemokine release (V. Appay and S. Rowland-Jones, unpublished data). It is possible, but not certain, that there may be subsets that mediate different functions. There could also be functionally silent T cells, either overstimulated or lacking CD4 T-cell help (Gallimore *et al*. 1998; Zajac *et al*. 1998), but this has not yet been shown for HIV. It is clear that not all of the expanded T cells in HIV infection or other persistent virus-specific T cells can divide further. There is therefore likely to be an end-stage $CD8^+$ T cell that is short lived in the absence of antigen and may be continuously replaced in HIV-infected persons. Ultimate breakdown of this replacement, probably because of its dependence on $CD4^+$ T-cell help, may well contribute to the pathogenesis of AIDS. Finally, the importance of the activated $CD8^+$ T-cell response in the control of persisting virus infections is evident from the elaborate means that these viruses have evolved to evade this response. CMV has at least six proteins that downregulate HLA class I expression (Ploegh 1998). EBV limits virus protein expression in latently infected cells and Epstein–Barr nuclear antigen, the major remaining protein, has its own way of inhibiting its degradation by the proteasome (Levitskaya *et al*. 1995). HIV nef downregulates HLA class I expression (Collins *et al*. 1998) and the up-regulation of fas ligand is another possible evasion strategy (Xu *et al*. 1997). Escape mechanisms are probably essential for the persistence of the virus and are likely to be present in all viruses that persist.

These viruses teach us about T-cell memory, but leave several questions unanswered. In particular, when viruses such as influenza are eliminated, it is unclear what state the memory $CD8^+$ T cells are in. The expanded populations decay rapidly, but a population is left that can reactivate with accelerated kinetics. How long this population persists in the absence of antigen in humans is unresolved although the indications are that they turnover continuously, possibly stimulated by type 1 interferon and IL-15 (Sprent *et al*., this issue; Tanchot *et al*., this issue). In this way they can persist for at least two years—the life span of the mouse. Whether such populations could last 70 years in humans is still to be resolved, but earlier experiments on the human influenza virus-specific CTL response indicated a slow decay in the absence of reinfection (McMichael *et al*. 1983). Knowledge of the different states of $CD8^+$ T cells, and their life spans and antigen and cytokine dependence, will lead to a full understanding of T-cell memory with implications for the better design of vaccines.

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