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THE ROYAL SOCIETY

Immunological memory and acquired immunodeficiency syndrome pathogenesis

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Infection with the human immunodeficiency virus results in profound perturbations in immunological memory, ultimately resulting in increased susceptibility to opportunistic infections and acquired immunodeficiency syndrome (AIDS). We have used rhesus macaques infected with the simian immunodeficiency virus (SIV) as a model to understand better the effects of AIDS virus infection on immunological memory. Acute infection with SIV resulted in significant deficits in $CD4^+$ helper responses to cytomegalovirus (CMV) as well as CMV-specific cytotoxic T-lymphocyte and neutralizing antibody responses. Reactivation of CMV was associated with high levels of SIV replication and suppression of both T-helper and cytotoxic responses to CMV. We have also studied the effects of SIV infection on T-cell turnover in non-human primates. T-cell turnover was evaluated using the nucleoside analogue bromodeoxyuridine (BrdU) in combination with five-colour flow cytometric analysis. T cells in normal animals turned over at relatively rapid rates, with memory cells turning over more quickly than naive cells. In SIV-infected animals, the labelling and elimination rates of both $CD4^+$ and $CD8^+$ BrdU-labelled cells were increased by two- to threefold compared with normal controls. Further analysis of immunological memory in non-human primates should offer the opportunity to extend immunological insights from murine models to the pathogenesis and prevention of AIDS.

Keywords: human immunodeficiency virus; simian immunodeficiency virus; T-cell turnover; cytomegalovirus; bromodeoxyuridine

1. INTRODUCTION

Just as the topic of memory is central to the field of immunology, so is it of primary importance to our efforts to understand the pathogenesis of human immunodeficiency virus (HIV) infection. There are a number of questions related to immunological memory in HIV infection, ranging from those that impact on our understanding of the hallmark feature of acquired immunodeficiency syndrome (AIDS) and the increased susceptibility to opportunistic infections, to those that impact on the development of an effective AIDS vaccine (table 1).

Addressing these questions in HIV-infected people has often proven quite difficult. Experimental limitations inherent in human studies significantly constrain the ability to define precisely the mechanisms that lead to the loss of recall responses against microbial pathogens or to determine which specific immune responses are likely to protect against HIV infection. To a large extent, the difficulties of answering these questions are also encountered in animal models used for the study of HIV infection. One of the most widely used animal models for the study of AIDS is infection of macaques with simian immunodeficiency virus (SIV), a primate lentivirus closely related to HIV (Desrosiers 1990). However, despite the significant usefulness of this model for vaccine trials and pathogenesis research, it has been difficult to rigorously elucidate mechanisms of immunological memory in SIV-infected macaques. Techniques commonly used in murine models, such as adoptive transfer experiments or the creation of knockout or transgenic mice, are not currently feasible in non-human primates.

This overview will highlight several selected topics on immunological memory that are of central importance to AIDS pathogenesis. Relevant insights derived from murine models will be briefly discussed, along with recent data from our laboratory and others that address these issues.

2. WHAT IS IMMUNOLOGICAL MEMORY?

Ongoing research on the nature of immunological memory has been marked by debate regarding the precise definition of memory. One school of thought argues that memory should be broadly defined by the ability of the host to resist reinfection (Zinkernagel 1996). Another proposes that memory should be defined as an enhanced immune response that occurs as a result of antigen stimulation. These definitions become even more problematic when immune response to infectious agents, such as herpesviruses, that persist in the host are considered. In

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Table 1. *Immunological memory and AIDS*

What mechanisms are responsible for loss of memory responses to microbial pathogens, leading to opportunistic infections?
What are rates of T-cell turnover in normal and HIV-infected subjects?
What role do $CD4^+$ T cells play in the maintenance of cytotoxic T lymphocytes specific for HIV and other pathogens?
What memory responses protect against HIV infection?
Is persistence of antigen (or continued antigen presentation) required for protective immunity against HIV?

these instances, virus-specific immune responses in a chronically infected animal will comprise both activated effector cells and memory cells without effector-cell function. Loss of the ability to resist reactivation of a chronic infection reflects both a failure of activated effector cells and of memory cells to give rise to effector cells. On a practical basis, it is difficult to distinguish these two components of the immune response (activated effector cells versus memory cells without effector-cell function) using standard assays of cell-mediated immune responses, most of which involve *in vitro* restimulation with antigen. Given the difficulties of differentiating these distinct populations of lymphocytes in primates (humans or monkeys), for the purposes of this discussion we shall refer to a memory response as the ability to detect an immune response to a specific antigen, including classic recall antigens (such as tetanus toxoid) as well as persistent agents like cytomegalovirus (CMV).

3. ABNORMALITIES IN MEMORY PHENOTYPE AND FUNCTION IN HIV AND SIV INFECTION

The vast majority (>98%) of HIV replication *in vivo* occurs in activated $CD4^+$ T lymphocytes (Perelson *et al.* 1996; Schnittman *et al.* 1989). Efficient replication in T cells requires activation in order to ensure efficient reverse transcription of viral RNA, translocation into the nucleus and integration into the host genome (Bukrinsky *et al.* 1992; Stevenson *et al.* 1990; Zack *et al.* 1990). Since memory T cells (or more precisely, memory-phenotype T cells) have increased rates of cell division and activation (Michie *et al.* 1992; Rosenzweig *et al.* 1998; Tough & Sprent 1994) (see § 5), these are likely to be preferentially infected and killed by HIV. HIV infection of antigen-specific T cells may also occur following presentation of the cognate antigen (Margolick *et al.* 1987), especially when the antigen-presenting cell is infected with HIV (Mann *et al.* 1990). Even after T-cell activation, memory-phenotype ($CD45RO^+$) $CD4^+$ T cells support higher levels of HIV replication and are more susceptible to the HIV-induced cytopathic effect (Chun *et al.* 1997; Schnittman *et al.* 1990; Spina *et al.* 1997; Woods *et al.* 1997). The mechanism for increased replication of HIV in memory cells has not been precisely defined but appears to reflect events in the HIV replicative cycle after virus entry (Spina *et al.* 1997; Woods *et al.* 1997).

Preferential replication of HIV in memory-phenotype cells may also be explained by the selective distribution of chemokine receptors, some of which serve as co-receptors for HIV entry. After binding to CD4, the HIV-1 envelope glycoprotein undergoes a conformational change that allows it to bind to a chemokine receptor, which serves as a necessary co-receptor for virus entry into the cell (Wu *et al.* 1996). The differential expression of chemokine receptors on different cell types and the selective binding of HIV-1 strains to chemokine receptors play a significant role in determining the selective tropism of HIV-1 strains for specific cell populations. Although a wide variety of chemokine receptors can potentially serve as co-receptors for HIV entry, the dominant receptors for HIV are CCR5 and CXCR4 (D'Souza & Harden 1996). CCR5, which is predominantly used by HIV strains in early stages of infection, is selectively expressed on memory-phenotype cells ($CD45RA^{low}$, $CD45RO^+$, $CD26^+$) *in vivo* (Bleul *et al.* 1997). Thus, HIV-1 strains that use CCR5 as a co-receptor will selectively target memory-phenotype cells.

It is therefore not surprising that HIV and SIV infection induce significant abnormalities in the function of memory T cells. Early studies documented that HIV infection resulted in a selective suppression of proliferative responses to antigens such as tetanus toxoid and influenza virus, even prior to the onset of $CD4^+$ T-cell depletion (Clerici *et al.* 1989; Lane *et al.* 1985). Similar findings have been observed in SIV-infected macaques (Mills *et al.* 1993). The mechanisms responsible for T-helper cell dysfunction include both functional abnormalities, which can be partially reversed *in vitro* (Clerici *et al.* 1993, 1994), and depletion of antigen-specific cells (Mann *et al.* 1990).

Both HIV and SIV infection also induce phenotypic perturbations in the normal distribution of memory and naive cells. Based on the selective replication of HIV in activated cells, one might anticipate a selective depletion of memory-phenotype cells during the course of HIV infection. However, most clinical studies have failed to observe a selective depletion of memory-phenotype $CD4^+$ T lymphocytes in HIV-infected subjects (Chou *et al.* 1994; Giorgi & Detels 1989; Reddy & Grieco 1991), although some studies have reported a selective depletion of $CD4^+CD29^+$ lymphocytes (de Martini *et al.* 1988; Van Noesel *et al.* 1990). These largely negative results may in part reflect the imprecise definition of true memory cells using phenotypic markers, especially when only single markers such as CD45RA are examined. Interestingly, when memory- and naive-phenotype cells are assessed using a combination of CD45RA and CD62L, HIV infection results in a depletion of naive-phenotype ($CD45RA^+CD62L^+$) $CD4^+$ and $CD8^+$ lymphocytes (Roederer *et al.* 1995). Several factors may contribute to the selective depletion of $CD45RA^+CD62L^+$ T lymphocytes, including increased conversion of naive to memory-phenotype cells, decreased production of naive-phenotype cells, or some combination of the above.

Infection of macaques with SIVmac251, a heterogeneous pathogenic SIV stock, results in a selective depletion of memory-phenotype cells, with a relative preservation of naive cells. In chronically infected animals with moderately advanced disease, naive-phenotype cells expressing both CD45RA and CD62L may constitute

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over 80% of the remaining $CD4^+$ T cells (M. Rosenzweig and R. P. Johnson, unpublished data). Similar findings have been reported by Willerford *et al.* (1990), who noted a selective depletion of $CD44^+CD4^+$ T lymphocytes in SIV-infected macaques. There may therefore be significant differences between SIV-infected macaques and HIV-infected humans on the effects of lentivirus infection on naive- and memory-phenotype lymphocytes.

4. IMMUNOLOGICAL MECHANISMS UNDERLYING THE DEVELOPMENT OF AIDS-RELATED OPPORTUNISTIC INFECTIONS

One of the characteristic features of AIDS is the loss of immunological responses to a wide variety of microbial agents. Ultimately, deficits in immune function lead to an increased susceptibility to routine pathogens such as herpes simplex virus as well as organisms like *Pneumocystis carinii* that normally infect only individuals with weakened immune systems. Although opportunistic infections are a hallmark of AIDS immunosuppression, the mechanisms involved in loss of immunity to microbial pathogens are not well understood. As noted above, defects in cellular immune responses to recall antigens in HIV-infected individuals have been well described. However, there is little information on the relative importance of HIV-induced deficits of $CD4^+$, $CD8^+$ T- and B-cell responses to reactivation of opportunistic infections or on the specific mechanisms involved in these deficits.

Since HIV productively infects $CD4^+$ T lymphocytes, loss of $CD4^+$ T-cell function in HIV-infected individuals is likely to be a consequence, at least in part, of direct infection. However, it is not clear how $CD8^+$ T- and B-lymphocyte functions are also affected. Is dysfunction of humoral immunity and cytotoxic T lymphocytes (CTL) related to loss of CD4 help or are they independent of CD4 dysfunction? CD40–CD40 ligand (CD40L)-mediated interactions between B and T cells are required for generation of thymus-dependent humoral immune responses (Grewal & Flavell 1998) and may in part account for B-cell functional abnormalities in AIDS. The role of $CD4^+$ T lymphocytes in induction and maintenance of virus-specific $CD8^+$ CTL is complex and has largely been studied in murine models using $CD4^+$ T-cell-depletion experiments or major histocompatibility complex (MHC) class-II-deficient mice. Studies with influenza virus, lymphocytic choriomeningitis virus (LCMV) and murine γ-herpesvirus (MHV-68), infections in which $CD8^+$ CTL are critical for control of viral replication, have shown that $CD4^+$ T lymphocytes are not essential for initial virus clearance or generation of a primary CTL response in acute infection (Battegay *et al.* 1994; Cardin *et al.* 1996; Matloubian *et al.* 1994; Tripp *et al.* 1995). In contrast, chronic LCMV infection in $CD4^+$ T-cell-depleted or T-cell-deficient mice results in ineffective $CD8^+$ CTL responses and establishment of a chronic carrier state (Battegay *et al.* 1994; Matloubian *et al.* 1994). The inability of $CD8^+$ CTL to maintain control of LCMV replication in the absence of $CD4^+$ T-cell help may be due to either deletion or anergy (Zajac *et al.* 1998). MHC class-II-knockout mice infected with MHV-68 also have lethal reactivation of disease (Cardin *et al.* 1996). Interestingly, MHV-specific $CD8^+$ CTL as assessed by frequency of tetramer positive cells, interferon-γ-secreting cells or CTL precursors in limiting dilution assays were preserved, suggesting an *in vivo* functional defect in CTL (rather than CTL exhaustion) in the absence of CD4 help (Stevenson *et al.* 1998). $CD4^+$ T-lymphocyte-derived cytokines may be essential for maintenance or function of CTL. Recently, Borrow *et al.* (1998) showed that the generation but not maintenance of $CD8^+$ CTL to LCMV was affected in CD40L-deficient mice and the mechanism appeared to be due to absence of CD40L on $CD4^+$ rather than $CD8^+$ T lymphocytes.

To define better the immunological events associated with increased susceptibility to opportunistic infections, we have analysed reactivation of CMV in rhesus macaques infected with SIV. CMV infection is one of the most common opportunistic viral infections in patients with AIDS and can lead to retinitis, encephalitis and pulmonary and gastrointestinal disease (Drew 1992). CMV infection is widely prevalent in captive rhesus macaques, and the natural history and clinical manifestations are in many respects similar to those in humans (Asher *et al.* 1974; Swack & Hsiung 1982). Like humans, immunocompetent animals have a persistent but asymptomatic infection. In the setting of immunosuppression, CMV reactivates, resulting in CMV disease. CMV is the most common viral opportunistic infection seen in SIV-infected macaques developing AIDS (Lackner *et al.* 1994; Simon *et al.* 1992). Immune responses to rhesus CMV are similar to that described in humans, including the presence of a $CD8^+$, MHC class-I-restricted CTL response (Kaur *et al.* 1996).

We therefore initiated a prospective study of the effects of SIV infection on CMV-specific cellular and humoral responses. Four CMV-seropositive, tetanus-toxoid-immunized adult rhesus macaques were inoculated intravenously with SIVmac251 and followed for one year or until death. A comprehensive array of immunological assays were undertaken, including measurements of CMV-specific neutralizing antibodies, anti-CMV and anti-tetanus toxoid immunoglobulin (IgG), CMV and tetanus-specific proliferative precursor frequencies, and CMV CTL activity. In addition, serial measurements of plasma CMV DNA by semiquantitative polymerase chain reaction (PCR) were performed to study the association between CMV reactivation and CMV-specific immune responses. To quantify more precisely T-helper responses, proliferative responses were analysed in limiting dilution precursor frequency assays.

During acute infection, coincident with peak SIV replication, we observed a transient suppression of CMV and tetanus-specific proliferative precursors at one to two weeks, prior to the onset of $CD4^+$ T lymphocytopenia (figure 1). By four to eight weeks, in most animals there was partial recovery of CMV-specific proliferative responses, but these remained at 25–50% of pre-infection values. In contrast, tetanus-specific proliferative precursor frequencies returned to baseline levels during this period. In one animal with rapidly progressive SIV disease (macaque 239.95) that was never able to control SIV replication, we did not observe a significant recovery of proliferative responses to either tetanus or CMV. Suppression of proliferative responses was partly reversed by *in vitro* anti-retroviral therapy at one week but not eight

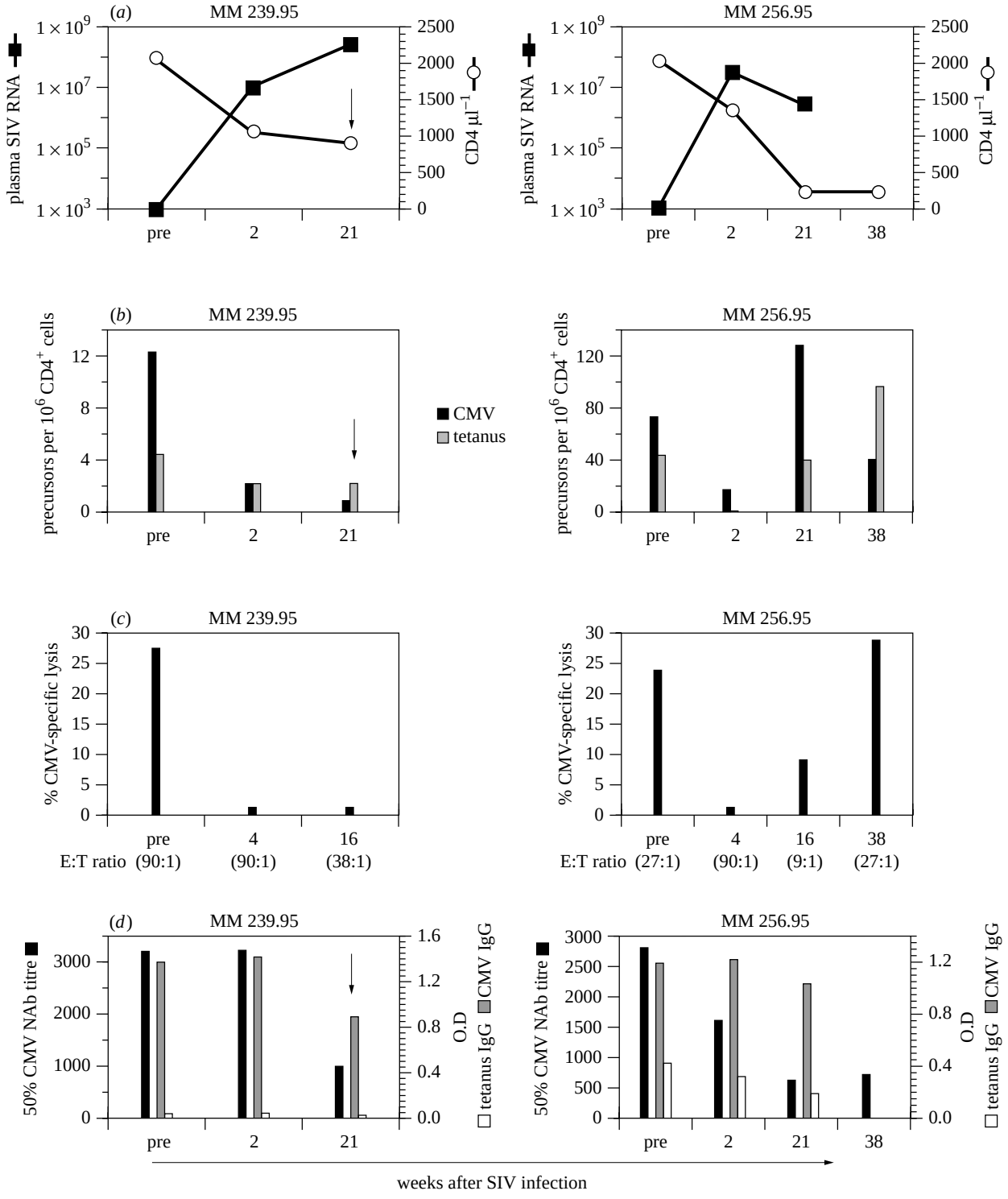

Figure 1. Longitudinal evaluation of CMV and tetanus-specific immune responses in two CMV-seropositive rhesus macaques inoculated intravenously with SIVmac251. Temporal association between plasma SIV RNA and peripheral CD4 counts (*a*), CD4 function (*b*), CTL activity (*c*) and humoral immunity (*d*) are shown. Mm 239.95 was a rapid progressor and died of AIDS at 21 weeks (depicted by arrow). Mm 256.95 is a normal progressor and was alive at 38 weeks after SIV inoculation. At necropsy, Mm 239.95 had histological evidence of disseminated CMV disease. Plasma SIV RNA was measured by a quantitative reverse-transcriptase PCR assay that has a lower limit of detection of 1000 RNA copies ml^{-1} plasma (Suryanarayana *et al.* 1998). CD4 function was assessed by [^{3}H]thymidine uptake in antigen-specific proliferative assays. Precursor frequency per 10^6 peripheral blood mononuclear cells (PBMC) was determined by limiting dilution analysis and frequency per 10^6 $CD4^+$ cells calculated from the percentage of peripheral $CD4^+$ T lymphocytes. CMV-specific CTL activity in PBMC was measured in standard chromium release assays after *in vitro* CMV-specific stimulation (Kaur *et al.* 1996). Results are depicted as the percentage of CMV-specific lysis and denote the difference in lysis between CMV-infected and uninfected target cells. CMV-specific neutralizing antibodies to the American Type Culture Collection strain of rhesus CMV were determined and the reciprocal of 50% neutralization titres shown. CMV and tetanus-specific IgG were measured by an enzyme-linked immunosorbent assay and the optical density values for sera tested at 1:50 dilution are shown.

weeks after infection (data not shown), which suggested the presence of a recoverable pool of antigen-specific $CD4^+$ T lymphocytes at least early in infection.

Acute SIV infection was also associated with suppression of CMV-specific CTL activity two to eight weeks after infection (figure 1). In three macaques, CTL activity had recovered to variable levels within four weeks of the onset of immunosuppression. The mechanisms responsible for loss of CMV-specific CTL activity remain unclear. Although suppression of $CD4^+$ T helper function could lead to loss of CTL activity, the temporal profile of suppression of CMV-specific proliferative function was not consistently concordant with loss of CTL activity. Further, in *in vitro* assays, CMV-specific CTL effectors could be expanded and detected even in the absence of $CD4^+$ T lymphocytes (data not shown).

Perturbations in the humoral arm of the CMV immune response were evident early after SIV infection in all four macaques (figure 1). Thus, CMV-specific neutralizing antibody titres had declined two- to sevenfold by 16 weeks of infection. However, decline in neutralizing antibody titres in itself was not sufficient for CMV reactivation and appeared to require loss of CMV-specific T helper and CTL function.

In summary, SIV infection resulted in early and widespread deficits in CMV-specific immune responses. As expected, SIV infection induced significant decreases in proliferative responses, although part of this effect appeared to be due to *in vitro* suppression of proliferative responses rather than *in vivo* depletion of the antigen-specific cells. We also observed significant suppression of CMV-specific CTL and neutralizing antibody responses after SIV infection. Further studies in this model should be helpful in defining the mechanisms by which SIV affects memory responses to herpesviruses and other microbial pathogens and in elucidating the relative roles of humoral and cellular immune responses in controlling reactivation of CMV.

5. T-CELL TURNOVER IN HIV AND SIV INFECTION

Although loss of $CD4^+$ T lymphocytes has long been recognized as a central feature of AIDS, until recently the dynamics of T-lymphocyte production and death have been poorly understood. Initial estimates based on the rebound of $CD4^+$ T cells after treatment of HIV-infected people with potent anti-retroviral agents suggested that $CD4^+$ cell turnover might be increased by as much as 78-fold as compared with normal individuals (Ho *et al.* 1995). However, subsequent work analysing the lengths of chromosomal telomeres, which are progressively shortened with cell division, suggested no increase in $CD4^+$ T-cell turnover (Wolthers *et al.* 1996). These widely discrepant results may in part be due to the fact that both of these studies relied on indirect techniques to assess $CD4^+$ T-cell turnover. In addition, interpretation of the data of Ho *et al.* (1995) was complicated by incomplete information regarding the rates of $CD4^+$ cell turnover in normal individuals.

Several previous studies have analysed T-cell turnover in rodents and humans. In mice, many investigators have employed the nucleoside analogue bromodeoxyuridine (BrdU) to track dividing cells *in vivo* (Rocha *et al.* 1990; Tough & Sprent 1994). BrdU is incorporated into the DNA of dividing cells, is not recycled after incorporation, and can be detected by flow cytometric analysis, thereby allowing simultaneous detection of the presence of BrdU in different subsets of lymphocytes defined on the basis of cell surface molecules. Analysis of the rates of BrdU incorporation in rodents has demonstrated a relatively rapid turnover of a significant population of peripheral T cells, with 30–50% of cells labelling in less than two weeks (Rocha *et al.* 1990; Tough & Sprent 1994). In the studies from Tough & Sprent (1994), memory-phenotype cells, as defined by expression of CD44, or relative lack of expression of CD45RB or CD62L, turned over at rates two- to threefold higher than those of naive-phenotype cells. In humans, data on T-cell turnover have been largely derived from analysis of radiation-induced chromosomal damage (McLean & Michie 1995; Michie *et al.* 1992). These studies also confirmed an increased turnover of memory-phenotype ($CD45RA^-$) cells, which were estimated to divide on average every 22 weeks, as compared with an average division time of 3.5 years for naive-phenotype cells (McLean & Michie 1995). The demonstration of shortened telomere lengths in $CD45RO^+$ $CD4^+$ T cells also supports an increased turnover of memory-phenotype lymphocytes in humans (Weng *et al.* 1995).

In light of the relative paucity of data regarding T-cell turnover in AIDS, we established techniques for examining T-cell turnover in normal and SIV-infected macaques with the nucleoside analogue BrdU. In order to define precisely memory- and naive-phenotype T cells, we used a five-colour flow cytometric technique, which allowed simultaneous analysis of CD45RA and CD62L. Simultaneous analysis of the expression of these markers has been proposed to offer a more precise definition of naive-phenotype T cells in humans (Roederer *et al.* 1995). BrdU was administered by intraperitoneal injection for 10–14 days and the percentage of BrdU-labelled cells was determined twice weekly in different naive- and memory-phenotype populations.

As predicted from previous studies in mice (Tough & Sprent 1994) and humans (Michie *et al.* 1992), in uninfected rhesus macaques, memory-phenotype cells turned over at increased rates as compared with naive-phenotype cells (figure 2). However, the combined analysis of CD45RA and CD62L expression led to some findings not previously appreciated based on analysis of single memory markers. First, in $CD4^+$ T cells, expression of CD62L alone appeared to be sufficient for separating cells with high and low rates of turnover (figure 2). Regardless of the expression of CD45RA, $CD62^-CD4^+$ T cells had BrdU incorporation rates approximately twofold greater that those of $CD62L^+$ cells. In contrast, for $CD8^+$ T lymphocytes in normal animals, the combined use of CD45RA and CD62L resulted in the definition of populations of cells that differed by sevenfold in their rates of turnover. Naive-phenotype $CD8^+$ cells, which expressed both CD45RA and CD62L, had the lowest rate of BrdU uptake; memory-phenotype cells expressing one but not both of these markers had intermediate levels of turnover, and memory-phenotype cells lacking expression of both CD45RA and CD62L had the highest rate of BrdU incorporation (figure 2).

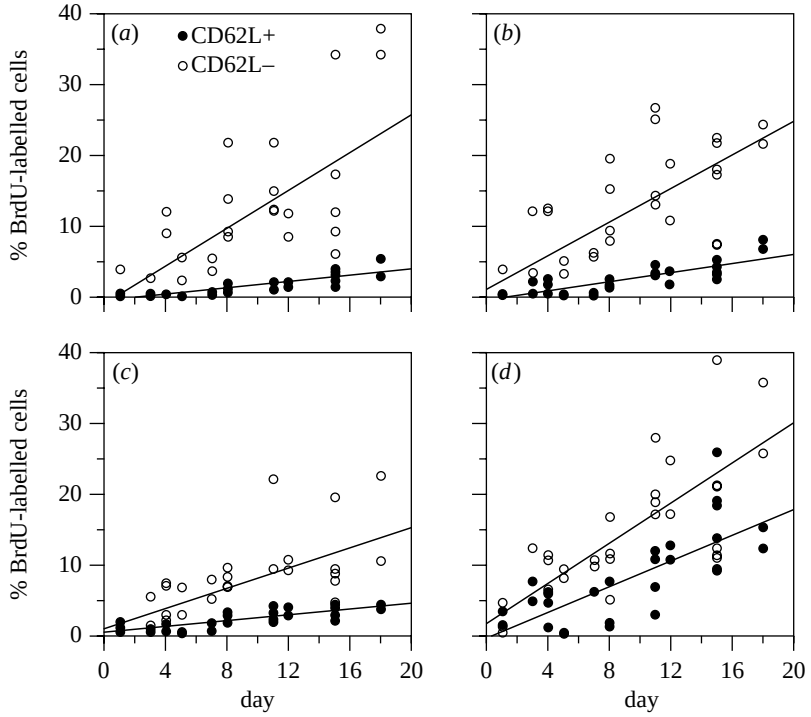

Figure 2. Analysis of rates of BrdU-labelling in memory and naive-phenotype lymphocytes in normal rhesus macaques. Uninfected rhesus macaques were labelled with BrdU for approximately two weeks and the percentage of BrdU-labelled cells was determined in peripheral blood lymphocytes twice a week for the duration of the labelling phase using five-colour flow cytometric analysis (Rosenzweig *et al*. 1998). Linear regressions of the percentage of BrdU-labelled cells are shown for the indicated cell population. (*a*) $CD45RA^+$, $CD4^+$; (*b*) $CD45RA^-$, $CD4^+$; (*c*) $CD45RA^+$, $CD8^+$; (*d*) $CD45RA^-$; $CD8^+$.

In SIV-infected animals, rates of turnover of $CD4^+$ T cells were increased by two- to threefold, as reflected by increased rates of BrdU labelling and more rapid decay of BrdU-labelled $CD4^+$ T cells (figure 3). Unexpectedly, there was also an increased turnover of $CD8^+$ T cells of comparable magnitude in SIV-infected animals. The observation that turnover rates of both $CD4^+$ and $CD8^+$ T cells in SIV-infected animals were increased by a similar degree suggested the possibility that the turnover of these cell populations might be linked. Indeed, in both normal and SIV-infected animals, the rates of BrdU incorporation for $CD4^+$ and $CD8^+$ cells were closely correlated ($r = 0.74$, $p < 0.003$, linear regression analysis) (Rosenzweig *et al*. 1998). A similar relationship was observed for rates of decay of BrdU-labelled cells.

An increased turnover of $CD4^+$ and $CD8^+$ T cells in SIV-infected animals was observed in both naive- and memory-phenotype subpopulations (figure 4). The increased decay of BrdU-labelled cells in SIV-infected animals was particularly notable for naive-phenotype ($CD45RA^+CD62L^+$) cells, for which the half-lives of BrdU-labelled $CD4^+$ and $CD8^+$ T cells were decreased by seven- to ninefold.

Although these data provide conclusive results regarding relative rates of T-cell turnover, several caveats should be kept in mind with regard to efforts to calculate absolute rates of cell division or cell death using BrdU. Depending in part on the means of BrdU administration and the cell population examined, the efficiency of BrdU incorporation into dividing cells may be significantly less than 100%. Variation in the efficiency of BrdU labelling of cells in different lymphoid compartments has been reported by some investigators (Rocha *et al*. 1990) but not others (Schittek *et al*. 1991). In our study, since BrdU is rapidly degraded *in vivo* (Kriss *et al*. 1963; Kriss & Revesz 1962), the once daily administration of BrdU was likely to label lymphocytes for only a portion of the day. Similar problems occur with the interpretation of the decay rate of BrdU-labelled cells. The disappearance of BrdU-labelled cells from peripheral blood will reflect the combination of the effects of cell death, cell division (leading to a progressive dilution of the BrdU signal to a point where it cannot be detected) and redistribution to extravascular compartments. Based on *in vitro* experiments, we estimate that a BrdU-labelled cell will revert to a BrdU-negative cell after approximately four to seven cell divisions (M. Rosenzweig and R. P. Johnson, unpublished data). In addition, analysis of the decay of BrdU-labelled cells within different naive- and memory-phenotype lymphocyte populations will be affected by interconversion among the subsets, especially for transformation of naive cells into memory cells. Although these considerations suggest some caution in calculating absolute rates of cell proliferation and death based on BrdU administration, mathematical models for determining these rates have recently been derived (Mohri *et al*. 1998) and should prove useful in interpreting cell turnover data generated using BrdU.

Our data have several general implications for T-cell turnover, independent of their implications for AIDS pathogenesis. First, our results confirm the increased turnover of memory-phenotype cells previously observed in other studies in mice and humans (Michie *et al*. 1992; Tough & Sprent 1994). However, the simultaneous analysis

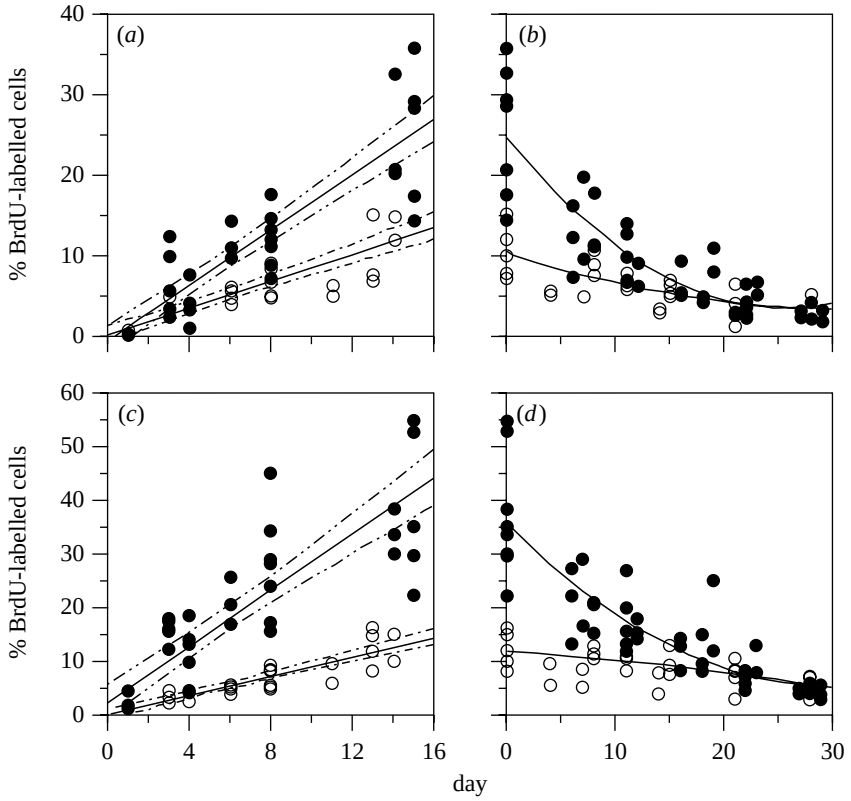

Figure 3. Serial analysis of BrdU-labelled cells during BrdU labelling (*a*, *c*) and decay (*b*, *d*) in normal and SIV-infected macaques. (*a*, *b*) $CD4^+$, (*c*, *d*) $CD8^+$. Macaques were labelled with BrdU for approximately two weeks and then observed during a washout phase of four weeks. The percentage of BrdU-labelled cells was determined in peripheral blood lymphocytes twice a week for the duration of the labelling and washout phases. Each symbol represents an individual time-point for a given animal; open circles are normal animals, filled circles are SIV-infected animals. For the labelling phase, a linear regression of the percentage of BrdU-labelled cells with 95% confidence intervals is shown for normal and SIV-infected animals. For the decay phase, a nonlinear regression of the percentage of BrdU-labelled cells is shown for normal and SIV-infected animals. Reproduced, with permission, from Rosenzweig *et al.* (1998).

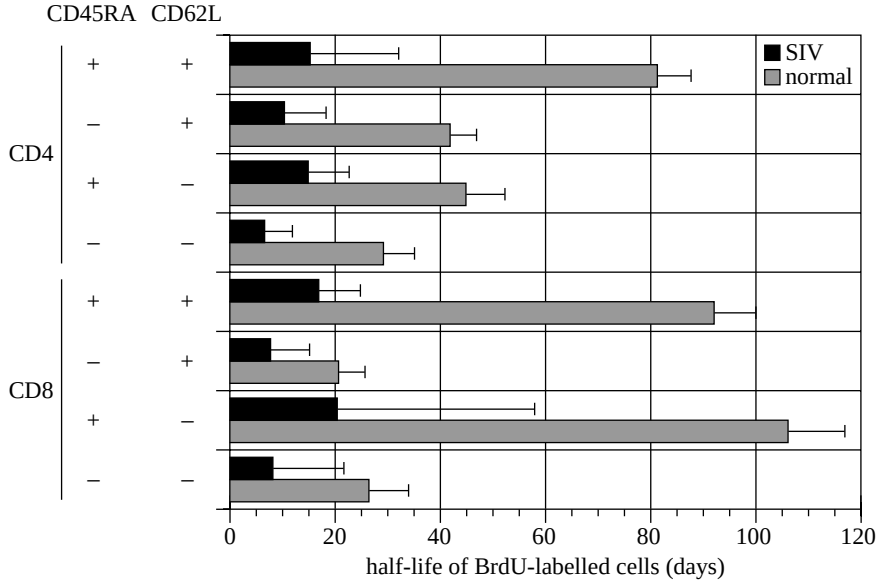

Figure 4. Decay of BrdU-labelled naive- and memory-phenotype T cells in normal and SIV-infected macaques. BrdU was administered to normal and SIV-infected macaques as described in the legend to figure 3. Using five-colour flow cytometric analysis, the decay of BrdU-labelled cells was determined in naive and memory T lymphocytes based on simultaneous analysis of expression of both CD45RA and CD62L. The half-life ($T_{1/2}$) of BrdU-labelled cells was calculated from the decay constant calculated using a nonlinear regression from the formula $T_{1/2} = \ln 2/\text{exponential decay rate}$. This value may not represent the true $T_{1/2}$ of the indicated cell population, as the disappearance of BrdU-labelled cells will reflect the combined contributions of both cell division and cell death, as well as conversion of a BrdU-labelled cell to other phenotype cells (see § 5 for expanded discussion).

of both CD45RA and CD62L expression allowed further breakdown of populations of $CD8^+$ T cells that varied widely in their rates of turnover. Second, our results suggest the overall rates of T-cell turnover in primates are faster than previously appreciated. Conversion of our calculated elimination rates to estimates of the average life spans of BrdU-labelled cells yields approximate values of 16 weeks for naive ($CD45RA^+$) T cells and seven weeks for memory ($CD45RA^-$) T cells. Although these estimated life spans of BrdU-labelled cells reflect the combined contributions of cell death and cell division, regardless of the exact contribution of these two processes, these values represent a significant increase over previous estimates of T-cell turnover rates in humans based on analysis of radiation-induced chromosomal damage (McLean & Michie 1995). Finally, the finding that rates of $CD4^+$ and $CD8^+$ T cells are significantly correlated with each other, both in normal and SIV-infected animals, suggests the existence of homeostatic mechanisms that coordinate turnover of both lineages. Similar findings have recently been obtained in normal and HIV-infected humans using glucose labelled with a non-radioactive isotope to assess T-cell turnover (Hellerstein *et al.* 1999). These results contrast with the paradigm of T-cell homeostasis, which proposes that the pool sizes of $CD4^+$ and $CD8^+$ T cells are independently regulated so as to maintain a constant number of T cells (Adleman & Wofsy 1993).

Our results also have a number of implications for AIDS pathogenesis. They provide direct evidence for an approximately threefold increase in both $CD4^+$ and $CD8^+$ turnover in SIV-infected animals as compared with normal controls. These results are significantly lower that the initial estimate of an almost 80-fold increase in $CD4^+$ T-cell turnover initially suggested (Ho *et al.* 1995). However, they are quite comparable with the estimates provided by several recent studies examining T-cell turnover in SIV-infected monkeys and HIV-infected humans. Using a similar approach of BrdU labelling coupled with mathematical modelling to estimate rates of cell death and proliferation, Mohri *et al.* (1998) calculated that the rate of $CD4^+$ T-cell death is increased by approximately threefold in SIV-infected monkeys. These authors also observed a comparable increase in the death rate of $CD8^+$ T lymphocytes. By analysing expression of the antigen Ki67, which is expressed in cells during the late G1–S–G2–M phases of the cell cycle, Sachsenberg *et al.* (1998) estimated that turnover of both $CD4^+$ and $CD8^+$ T cells was increased by two- to sixfold in HIV-infected people. A comparable increase in $CD4^+$ T-cell turnover has been calculated by Hellerstein *et al.* (1999) by labelling dividing T lymphocytes *in vivo* with glucose labelled with a non-radioactive isotope. Taken together, by documenting only a relatively modest increase in T-cell turnover, these studies suggest that $CD4^+$ T-cell depletion results from a modest increase in cell death coupled with an impaired production of $CD4^+$ T cells (Hellerstein *et al.* 1999; Hellerstein & McCune 1997).

6. CONCLUSIONS

AIDS virus infection results in significant alterations in immunological memory and T-cell turnover. While deficits in both cellular and humoral memory responses in HIV-infected people and SIV-infected macaques have been widely recognized, there has been only limited information on specific immunological mechanisms involved in the reactivation of opportunistic infections. Our ongoing studies examining the effects of SIV infection on CMV-specific humoral and cellular immune responses document that SIV induces multiple defects in both arms, even as soon as eight weeks after infection. Future studies should be able to use this model to more precisely define the mechanisms, both direct and indirect, by which SIV infection induces reactivation of opportunistic infections. Our studies of T-cell turnover using BrdU confirm recent publications from several other groups regarding a modest increase in $CD4^+$ and $CD8^+$ T-cell turnover in AIDS virus infection, but also reveal several new findings, including the coordination of $CD4^+$ and $CD8^+$ T-lymphocyte turnover rates and the usefulness of combined analysis of both CD45RA and CD62L for analysis of T-cell turnover and immunological memory. Future studies in non-human primates should help bridge the gap between studies on immunological memory carried out in murine models and our incomplete knowledge of AIDS pathogenesis in HIV-infected human subjects.

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