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T Cell Dynamics in HIV-1 Infection

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I. Introduction

One of the most prominent features of HIV-1 infection is $CD4^+$ T cell depletion. This statement is widely used in papers on HIV-1 research; however, while true, it is deceptively simplistic in that it fails to describe what is actually a complex change in the representation of T cell subsets during HIV-1 infection. Figure 1, adapted from Roederer (1995), shows this complex pattern of T cell subset composition with disease progression. Overall, $CD4^+$ T cells decline in number while the $CD8^+$ T cell population increases over time. The increase in the $CD8^+$ T cell pool is the result of massive peripheral expansion of memory cells. This subset only begins to decline shortly preceding AIDS diagnosis (Margolick *et al.*, 1995). The $CD4^+$ memory compartment also initially expands due to peripheral expansion, but memory cells are then progressively lost. Interestingly, in both $CD4^+$ and the $CD8^+$ subsets, the naive compartment begins to decline soon after infection (Rabin *et al.*, 1995; Roederer *et al.*, 1995). Thus, the T cell depletion observed in HIV-1 infection consists of naive cells of both $CD4^+$ and $CD8^+$ subsets, and memory cells of the $CD4^+$ subset. The majority of research has focused on the depletion of $CD4^+$ T cells and the bulk of the discussion that follows will concentrate on this subset.

Although the observation of T cell depletion in HIV-1 infection was made early, the mechanism for this decline is still not properly understood. Over the past 10 years of AIDS research, investigators have discussed several possible mechanisms for $CD4^+$ T cell depletion: virus-related killing, activation-induced apoptosis, and disturbed renewal mechanisms (Ho *et al.*, 1989; Ameisen and Capron, 1991; Meyaard *et al.*, 1992; Groux *et al.*, 1992; Gougeon and Montagnier, 1993; Bonyhadi *et al.*, 1993; Schnittman *et al.*, 1990; Kaneshima *et al.*, 1994; McLean and Michie, 1993). A major breakthrough in our understanding of HIV-1 infection came in 1994 with the introduction of protease inhibitors, a new generation of antiretroviral drugs that effectively blocks the replication of the virus in previously infected cells. Combination of these new drugs with other antiretroviral drugs made it possible to treat HIV-1-infected individuals more successfully

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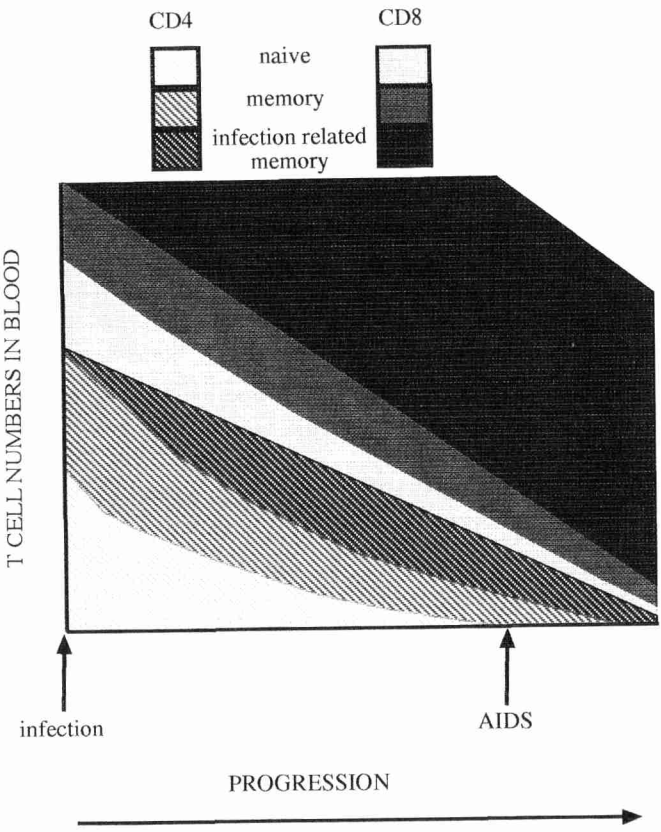

FIG. 1. Representation of T cell subset composition of blood during progression to AIDS. Adapted from Roederer (1995), by courtesy of the author and permission of *Nature Medicine*.

than before (Danner *et al.*, 1995; Markowitz *et al.*, 1995). In addition to the tremendous clinical benefit, new possibilities for research were created. For the first time, viral replication could be effectively blocked for a prolonged time, and the consequences for immune recovery could be studied. The advent of potent antiretroviral therapy gave renewed impetus to the debate over viral and T cell turnover. The application of mathematical models to viral load reduction and $CD4^+$ T cell increase after treatment, as was done by Ho *et al.* (1995), Perelson *et al.* (1996), and Wei *et al.* (1995) has changed our view on HIV pathogenesis dramatically. By calculating that 10^{10} virions were produced and destroyed per day (Wei *et al.*, 1995; Ho *et al.*, 1995; Perelson *et al.*, 1996), these investigators showed that HIV-1 infection is a highly dynamic process with much more virus "turn-

over" than was previously anticipated. From the same data, rapid turnover of $CD4^+$ T cells in HIV-1 was proposed as the mechanism leading to $CD4^+$ T cell depletion because the immune system would not be able to keep up with high rates of renewal infinitely. This view of rapid $CD4^+$ T cell turnover was challenged by studies on T cell telomere length, showing no evidence for increased $CD4^+$ T cell turnover or exhaustion (Wolthers *et al.*, 1996). These data provided the focal point for renewed debate over this issue (Mosier *et al.*, 1995; Grossman and Herberman, 1997; Hellerstein and McCune, 1997). Since then, several groups have tried to further elucidate the magnitude of T cell turnover in HIV-1 infection. The issue of turnover involves cell death, proliferation of existing cells, and development of new cells from progenitors. Here, we give an overview of what is known about normal T cell dynamics, T cell dynamics in HIV-1 infection, how the different studies relate to each other, and what insight they provide to explain T cell depletion in HIV-1 infection.

II. Normal T Cell Renewal from Progenitors

The process of T cell renewal in the maintenance of the T cell population can involve two separate mechanisms: proliferation of mature cells in the periphery and development of new T cells from a progenitor source. The relative contribution of each of these mechanisms to overall T cell renewal is dependent on the age of the individual and the profile of the remaining T cell pool. The common view is that early T cell development requires a thymus and involves a high degree of development from progenitor sources, but that as the organism ages the T cell population is maintained primarily through peripheral expansion of dividing mature cells (Mackall and Gress, 1997b; Rocha *et al.*, 1989; Tough and Sprent, 1994; Sprent and Tough, 1995). Here we summarize what is known about the mechanism of regeneration of T cells in a depleted individual, the effect of HIV-1 infection on T cell renewal, and the impact of therapy on regeneration of T cells.

A. T Cell Renewal in Mice

Our understanding of the importance of thymus-dependent and thymus-independent mechanisms for T cell regeneration comes initially from murine studies. Several studies compare the T cell regeneration from thymus-bearing and thymectomized animals, which were irradiated and repopulated with bone marrow. In the first studies, adult animals were given syngeneic T cell-depleted bone marrow as a source of progenitor cells and congenic lymph node cells as a source of mature T cells (Mackall and Gress, 1997b). In euthymic animals, T cell renewal occurred via

thymus-dependent development from progenitors with little expansion of the lymph node cells. In thymectomized animals, very little development from progenitors was detected while substantial expansion of mature lymph node cells occurred. Repopulation from T cell-depleted and undepleted bone marrow was compared (Dulude *et al.*, 1997). In the case where T cells were not present in the inoculum, regeneration occurred through extrathymic development of progenitor cells. However, when mature T cells were present, regeneration occurred primarily through expansion of these cells. It is important to note that the thymus-independent development was not able to restore normal T cell numbers (Mackall and Gress, 1997b; Dulude *et al.*, 1997). In addition, T cell renewal through peripheral expansion gave rise to cells bearing a memory phenotype based on expression of CD45RO and CD44 (Mackall and Gress, 1997b; Mackall *et al.*, 1993; Tanchot and Rocha, 1995).

B. T Cell Renewal in Humans

Similar studies were conducted in humans that had been treated with T cell-depleting doses of chemotherapy. In these patients, there was an age-related regeneration of naive $CD4^+$ T cells (Mackall and Gress, 1997b; Mackall *et al.*, 1995; Weinberg *et al.*, 1995). Younger individuals showed regeneration of substantial numbers of $CD4^+$ $CD45RA^+$ T cells whereas older patients still showed $CD4^+$ depletion 6 months after therapy, though, after longer periods of time, the naive $CD4^+$ T cells did rise in these patients. The degree of regeneration of $CD45RA^+$ $CD4^+$ T cells was directly related to thymic function (Mackall *et al.*, 1995; Weinberg *et al.*, 1995; Heitger *et al.*, 1997), underscoring the importance of the thymus in the generation of naive $CD4^+$ T cells. In adult patients given doses of chemotherapy that only moderately depleted the T cell subset, the majority of regenerating T cells expressed a memory phenotype indicating they were derived by expansion of previously existing T cells (Hakim *et al.*, 1997). These results indicate that, in the case of $CD4^+$ T cells, regeneration from a progenitor source depends on thymic function and occurs at low rates in adults. The slow regeneration of naive $CD4^+$ cells can also be seen in adult patients undergoing monoclonal antibody (mAb) therapy for rheumatoid arthritis (Moreland *et al.*, 1994) or multiple sclerosis (Rep *et al.*, 1997) and in normal aged individuals (Mackall and Gress, 1997b).

Interestingly, requirements for the regeneration of $CD8^+$ T cells differ from those for $CD4^+$ T cells. In the same experiments described above, by 3 months after chemotherapy most individuals had recovered normal $CD8^+$ T cell numbers (Mackall, 1997). In addition, the age-related recovery rate seen for $CD4^+$ T cells was not observed for $CD8^+$ T cells. In one individual thymectomized before chemotherapy, the $CD45RA^+CD8^+$ T

cells were recovered though the $CD45RA^+CD4^+$ T cells were not (Heitger *et al.*, 1997). The results show that generation of naive $CD8^+$ T cells does not require a thymus and is faster than that of $CD4^+$ T cells (Mackall, 1997). One possible caveat to this conclusion is that the naive and memory $CD8^+$ subsets cannot be distinguished by the CD45 isoforms alone (Hamann *et al.*, 1997; Rabin *et al.*, 1995). The recovery of cells with a surface molecule expression pattern that does define these subsets in $CD8^+$ cells has not been measured. It is still possible that truly naive $CD8^+$ T cells do require a thymus for their development as well. The final conclusion of these studies is that maintenance of the T cell population in adults involves both mechanisms, but that the primary mechanism is peripheral expansion of previously existing T cells.

The age-related decline in thymic function and the slow rate of repopulation in depleted individuals has led to the proposal that T cell regeneration in adults normally occurs near maximum capacity to maintain normal T cell numbers and that this rate cannot be substantially increased (Zhang *et al.*, 1998). In mice depleted of $CD4^+$ T cells by mAb treatment and subsequently thymically injected with fluorescein to tag thymic immigrants, there was no change in thymic output compared to undepleted animals (Gabor *et al.*, 1997). In another study, mice were either oversupplied with thymic emigrants by grafting of additional neonatal thymi under the kidney capsule, or undersupplied by neonatal thymectomy. The thymic export rate was constant from both the intact and the graft thymi, regardless of whether the mouse was oversupplied or undersupplied, and the peripheral pool remained the same (Berzins *et al.*, 1998). These data suggest that the thymus-dependent regeneration rate is not altered by the size of the peripheral pool, or by the number of recent thymic emigrants in the periphery of adults. However, it remains to be determined whether the rate of peripheral mechanisms of regeneration can increase as a result of depletion.

Combining the data from these studies, maintenance of the overall T cell pool in the adult can be said to result from proliferation of existing cells. However, renewal of the naive pools may differ for the two subsets; $CD4^+$ cells are renewed through thymus-dependent mechanisms whereas $CD8^+$ cells can be renewed by both thymus-dependent and independent mechanisms.

III. T Cell Renewal from Progenitors in HIV-1 Infection

The impact of HIV-1 infection on T cell renewal from a progenitor source was originally thought to be minor based on the lack of infection of bone marrow progenitor cells (Stanley *et al.*, 1992; Davis *et al.*, 1991).

However, with the advent of potent antiretroviral therapies more interest has focused on parameters of immune reconstitution in infected individuals. For an adult infected with HIV-1 even the low thymic regeneration found in uninfected adults is likely to be lacking. Determination of the level of regeneration in humans is complicated by a lack of suitable markers for distinguishing between cells that developed from a progenitor and those arising by proliferation of existing cells. Because the naive compartment is thought to proliferate little, if at all, without alteration of the CD45 phenotype, increases in this pool are considered to result from development of new cells. By contrast, increases in the memory pool must be via proliferation either of existing memory cells or of activated naive cells, which will then change their CD45 phenotype. For newly developed cells, it is difficult to determine whether cells developed within the thymus or outside the thymus. Despite these difficulties, researchers have used a variety of means to try to assess the level of regeneration in HIV-1-infected individuals.

A. Regeneration Assessment by Bone Marrow Function

There are several pieces of evidence that the bone marrow of HIV-1-infected persons displays developmental abnormalities. This observation can be explained by changes in the progenitors and/or by alterations in the stroma that support development of the progenitors (Moses *et al.*, 1996). The first observations suggesting diminished development of bone marrow were of the multiple cytopenias experienced by many infected individuals (Scadden *et al.*, 1989). Enumeration of progenitor subsets by mAb staining of surface molecules showed the presence of all progenitor subsets in bone marrow from HIV-1-infected individuals (Weichold *et al.*, 1998), though some reports suggest that certain subsets have been reduced in HIV-1 infection (Marandin *et al.*, 1996; Bagnara *et al.*, 1990). No alteration in the number of very primitive progenitors in infected persons could be detected in assays that support the development of these cells (Weichold *et al.*, 1998). Therefore, there is no clear evidence that the number of progenitors has been adversely affected by HIV-1 infection.

Bone marrow stroma from HIV-1 infected persons is able to promote the development of progenitors from an uninfected individual (Sloand *et al.*, 1997). However, the progenitors from the infected individual were unable to develop on bone marrow stroma from the uninfected individual. Bone marrow from HIV-1-infected individuals was shown to have diminished capacity to develop cells of the granulocyte, erythrocyte, and megakaryocyte lineages in *in vitro* colony assays (Zauli *et al.*, 1992, 1996; Steinberg *et al.*, 1991). Because the T cell lineage is derived from the same common precursor as these lineages, it was reasonable to assume that development of this lineage would also be affected. These data add

to the accumulating evidence that the bone marrow progenitors of HIV-1-infected individuals are impaired in their ability to develop to mature hematopoietic cells of multiple lineages.

B. Progenitor Development Capacity Measurements

Progenitor development to the T cell lineage can be measured by *in vitro* T cell development systems, such as fetal thymus organ culture or thymic monolayer cultures, or by development in SCID-hu mice. HIV-1-infected SCID-hu mice show a thymic pathology, similar to that of infected children, with a lack of thymic subsets beyond the very primitive triple-negative stage and alterations in stromal architecture (Aldrovandi *et al.*, 1993). Interpretation of these results in the context of progenitor cell function is difficult because the thymic tissue is also of human origin and a potential target for infection. Additionally, the thymic and liver tissues used are of fetal origin, which may have a different pattern of response than adult thymic tissue and bone marrow.

Progenitor function has been measured by fetal thymus organ culture (FTOC). This technique uses a murine fetal thymus to support the development of human progenitors into mature T cells. This xenogeneic system eliminates the confounding factor of the thymic tissue as a target for infection. In the initial cross-sectional study, the ability of HIV-1-infected individuals to develop T cells in FTOC was shown to be significantly lower than that of uninfected individuals (Clark *et al.*, 1997). This impairment in T cell development capacity was seen in all individuals, even those with normal numbers of $CD4^+$ T cells. A longitudinal study comparing individuals who progressed to AIDS and long-term nonprogressors (LTNPs) showed that progressors lost T cell development capacity very early in infection but LTNPs still retained significant capacity after 8 years of infection (Clark *et al.*, 1998).

C. Thymic Function Assessment

Another way of measuring the effect of HIV-1 infection on T cell development is by assessing thymic function, particularly because the naive $CD4^+$ T cell compartment has been shown to require a functioning thymus for its maintenance. Thymic mass of HIV-1-infected adults has been measured by computerized tomography (CT) scan (McCune *et al.*, 1998). A number of individuals over 40 years of age were found to have larger thymic mass than the same aged uninfected individuals. The number of naive cells in the periphery could be correlated to the amount of thymic mass. These results can be interpreted two ways. The authors concluded that depletion in the periphery due to HIV-1 infection caused a response in the size of the thymus; the thymus got bigger to compensate for the loss of cells.

However, there is no evidence that this occurs in other cases of peripheral depletion and the size of the thymus in these individuals, prior to infection, is not known. Clearly, however, these results show that the key to maintaining the naive pool is development of naive cells, which requires a functional thymus.

Another technique has been used to measure thymic function. Excision circle polymerase chain reaction (PCR) detects T cells that have recently recombined their T cell receptor (TCR) genes. When a progenitor cell develops into a mature T cell, it must recombine gene segments to form a functional T cell receptor. During this process in $\alpha\beta$-TCR-bearing cells (the majority of T cells in the body), the entire δ locus is excised and forms a stable circle in the nucleus. These excision circles, detected by specific PCR, are only found in $CD45RA^+$ cells (Douek *et al.*, 1998). This technique has been described as a measure of thymic function. In fact, it is a measurement of development of T cells irrespective of thymic function, because any developing T cell must recombine TCR genes. In the case of $CD4^+$ cells, however, this technique would measure thymic function because development of this subset is thought to occur only in a thymus. Using this technique, Koup and co-workers (Douek *et al.*, 1998) have shown that the number of cells in the periphery expressing excision circles declines with HIV-1 infection. Taken together, results on thymic function support the contention that HIV-1 infection alters the T cell renewal capability of infected individuals.

These data show that HIV-1 infection has a direct inhibitory effect on the already low thymic-dependent development in adults. This translates to a reduced development of $CD4^+$ cells with a naive phenotype and places even more emphasis on the peripheral expansion of $CD4^+$ cells to maintain this population as it is being depleted by the virus. Immunodeficiency may be directly related to this thymus-independent T cell renewal. The cells that result from peripheral expansion have been shown to be less capable of responding to neoantigen (Mackall and Gress, 1997b), to have a skewed TCR repertoire (Mackall *et al.*, 1993), and to be prone to apoptosis (Hakim *et al.*, 1997). These data also suggest that the eventual depletion of $CD4^+$ cells is, at least in part, due to a block in the development of T cells from a progenitor source, which prevents the complete regeneration of the naive $CD4^+$ T cell compartment. In addition, these data explain the observation that naive $CD8^+$ T cells are also depleted in HIV-1 infection. Whether the rate of peripheral mechanisms of regeneration are incapable of increasing to make up for the loss of T cells, or whether HIV-1 infection directly interferes with these peripheral mechanisms as well, remains to be determined.

D. T Cell Renewal after Potent Antiretroviral Therapy

The effect of antiretroviral therapy on T cell renewal can provide additional information on the effect of HIV-1 infection on this parameter. Patients experience a rise in the number of cells in the periphery after initiation of therapy. Initially, the majority of this increase consists of cells with a memory phenotype; however there is a slow, consistent rise in the number of naive cells in most individuals (Kostense *et al.*, 1998; Pakker *et al.*, 1998; Gorochov *et al.*, 1998). Data from FTOC has shown that the T cell development capacity of progenitors increases after therapy (Clark *et al.*, 1998). This increase could be correlated with the number of naive cells in the periphery. In addition, the number of cells bearing TCR excision circles goes up after therapy (Douek *et al.*, 1998). In children infected with HIV-1, those with more thymic volume prior to therapy had the largest increase in number of $CD4^+$ T cells, in $CD4^+$ CD45RA/RO ratio, and in TCR repertoire (Vigano *et al.*, 1998). These data show that functional progenitors, in combination with functional thymic tissue, are required for reconstitution of treated individuals. They also support the contention that immune depletion in HIV-1 infection is, at least partially, the result of nonfunctional progenitors and/or nonfunctional thymic tissue.

IV. Getting Quantitative on $CD4^+$ T Cell Production

Ho *et al.* (1995) and Wei *et al.* (1995), based on the slopes of $CD4^+$ T cell increase in the first 30 days after strong antiretroviral therapy, concluded that there was high turnover of $CD4^+$ T cells in HIV-1-infected individuals. It was calculated that 2×10^9 $CD4^+$ T cells were destroyed and replaced per day. A high turnover of $CD4^+$ T cells in HIV-1 infection was compatible with the observed high viral replication, likely to lead to high numbers of infected cells with a short life span (Perelson *et al.*, 1996). At the time, few estimates of normal rates of $CD4^+$ T cell production were available. It was therefore assumed that normal production would be found in those infected patients with little increase in number of $CD4^+$ cells after start of therapy. Therefore, it was concluded that in other patients the $CD4^+$ T cell production was increased up to 70-fold as reflected by the enormous increase in $CD4^+$ T cell numbers in the blood after start of therapy. This conclusion was predicated on the basic assumption that the cells that appeared in the blood were newly produced and spared from killing by the virus due to the therapy. Apart from the debate on the origin of the $CD4^+$ cells that repopulate the blood, the issue of T cell turnover in HIV infection was hampered by the lack of proper data on normal T cell turnover in humans. To date, new studies have provided more insight

into the amount of ongoing T cell proliferation, both in HIV-1-infected persons and in healthy humans.

The dynamics of lymphocyte populations have largely been studied in mice, and for these small rodents it was typically concluded that the normal turnover is high (Freitas and Rocha, 1993; Freitas *et al.*, 1986; Sprent and Tough, 1994). The consensus is that murine naive T cells are relatively long-lived and are mostly produced by progenitor renewal in the thymus (Dutton *et al.*, 1998). In mice, thymectomy typically leads to loss of naive $CD4^+$ T cells on a time scale of weeks, whereas memory $CD4^+$ T cell numbers remain unaffected (Swain *et al.*, 1990). Indeed total body counts of naive and memory $CD8^+$ T lymphocytes seem to be regulated independently (Tanchot and Rocha, 1995). Memory T cells can be long or short-lived, are generated during immune reactions, and are partly maintained by low-level proliferation (Dutton *et al.*, 1998). There is strong evidence in favor of a homeostatic regulation of total body lymphocyte counts (Freitas *et al.*, 1996), and this homeostatic control involves lymphocyte specificity and repertoire diversity (Freitas *et al.*, 1996; Mclean *et al.*, 1997; DeBoer and Perelson, 1997). Finally, naive and memory $CD8^+$ T cells in transgenic mice have different survival and renewal requirements (Tanchot and Rocha, 1997); naive cells require the correct major histocompatability complex (MHC) restriction element for survival and the correct MHC with antigen for expansion. Memory $CD8^+$ T cells, on the other hand, require any MHC class I molecule for survival and the correct MHC restriction element for expansion (Tanchot and Rocha, 1997). It is not known how these rodent data translate to the human system. We will here focus on human $CD4^+$ T cell production and turnover and will suggest that the normal human lymphocyte turnover is low, about 1% per day, corresponding to a production of about 2.5×10^9 $CD4^+$ T cells per day.

A. Total Body Numbers of $CD4^+$ and $CD8^+$ T Cells

Estimates for the total body numbers of $CD4^+$ T lymphocytes are calculated either by extrapolating from peripheral blood counts, or from small samples of lymphoid tissue. One should be careful, however, with extrapolations from blood measurements, because only a small fraction of the T lymphocytes resides in the peripheral blood. Such estimates are extremely sensitive to small changes in the distribution of T cells over the blood and lymphoid tissue. It is conventionally assumed that in a healthy human adult 2% of the lymphocytes resides in the blood (Westermann and Pabst, 1990). Considering that human adults have 5 liters of blood, with a typical $CD4^+$ T cell count of a 1000 cells/μl, one obtains a total body estimate of 2.5×10^{11} $CD4^+$ T cells (Ho *et al.*, 1995). Although the value 2% for the percentage of lymphocytes residing in the blood is used

throughout the literature, the different subsets of lymphocytes probably have somewhat different percentages. In the peripheral blood the CD4:CD8 ratio is approximately one, but in the lymphoid tissue $CD4^+$ T cells are overrepresented (Westermann and Pabst, 1990; Fleury *et al.*, 1998). Assuming that the conventionally used 2% of lymphocytes in the blood remains valid as an average over the different lymphocyte subsets, an average of 1.6% of the $CD4^+$ cells residing in the blood was calculated from measurements in lymph nodes and peripheral blood (Fleury *et al.*, 1998). This is reassuringly close to the conventional estimate of 2%. Similar studies (Zhang *et al.*, 1998) document the numbers of $CD4^+$ T cells in peripheral blood and lymphoid tissue (mostly tonsils). Assuming 700 g of lymphoid tissue in 70-kg individuals, the data from five HIV-negative subjects on average yield a total of 2.24×10^{11} $CD4^+$ T cells in the lymphoid tissue. The peripheral blood counts in the same group of subjects yield an average of 4.85×10^9 $CD4^+$ T cells in the peripheral blood. Thus, 2.1% of the $CD4^+$ T cells resides in the blood (Zhang *et al.*, 1998). Both this total body estimate and the distribution of $CD4^+$ T cells over the blood and lymphoid compartments are in close agreement with the earlier extrapolation from the peripheral blood. The $CD4^+$ T cells in a typical human adult are composed of naive $CD45RA^+$ and memory $CD45RO^+$ cells in an approximately 1:1 ratio (Cossarizza *et al.*, 1996; DePaoli *et al.*, 1988). This should correspond to an order of magnitude of 10^{11} cells in each subclass of $CD4^+$ T cells.

For $CD8^+$ T cells the situation is somewhat different, however, because they are underrepresented in the lymphoid tissue. Estimates for the CD4:CD8 ratio in lymphoid tissue vary between 2.5 (Westermann and Pabst, 1992), 3.7 (Tenner-Racz *et al.*, 1998), and 5 (Fleury *et al.*, 1998). Based on the assumption outlined above, an average of 5.6% of the $CD8^+$ cells residing in the blood was calculated in HIV-negative subjects (Fleury *et al.*, 1998). Thus, a $CD8^+$ T cell count of a 1000 cells/μl yields a body total of approximately 10^{11} cells, which is 2.5-fold less than that of the $CD4^+$ T cells.

In HIV-1-infected persons, the distribution of $CD4^+$ and $CD8^+$ lymphocytes differs in blood and lymphoid tissue (Fleury *et al.*, 1998; Zhang *et al.*, 1998). This distribution seems to normalize during highly active antiretroviral therapy (HAART) (Mosier *et al.*, 1995; Pakker *et al.*, 1998; Gorochov *et al.*, 1998; Zhang *et al.*, 1998; Hellerstein and McCune, 1997; Sprent and Tough, 1995). In a group of HIV-positive patients in early stage of disease (Fleury *et al.*, 1998), the total body numbers of $CD8^+$ T cells in the peripheral blood increase approximately 2-fold, and in the lymphoid tissue approximately 3-fold. As a consequence, the percentage of $CD8^+$ T cells residing in the blood decreases from 5.6% to approximately 3.2%. Thus, during HIV-1 infection there seems to be an increased trapping

of $CD8^+$ T cells in the lymphoid tissue. For the $CD4^+$ T cells in the same set of data (Fleury *et al.*, 1998), numbers in the peripheral blood and lymphoid tissue both decrease approximately 0.8-fold, such that the percentage of $CD4^+$ T cells residing in the blood is 1.8% (Westermann and Pabst, 1990). For a group of patients at later stages of disease, however, the distribution of $CD4^+$ T cells over the peripheral blood and the lymphoid tissue does suggest $CD4^+$ T cell trapping in the lymphoid tissue (Zhang *et al.*, 1998). Before the onset of HAART these patients have total body counts of 1.2×10^9 $CD4^+$ T cells in the blood, and 9.8×10^{10} $CD4^+$ T cells in the lymphoid tissue, which corresponds to 1.2% $CD4^+$ T cells in the blood. After 3 weeks of treatment this percentage has normalized to the conventional 2% in the blood (Zhang *et al.*, 1998). A 1% change in the distribution of $CD4^+$ T cells is more than sufficient to explain the marked increase in the $CD4^+$ T cell counts in the peripheral blood during HAART (Pakker *et al.*, 1998).

B. Measuring Division Rates by the Loss of Chromosome Damage

During radiotherapy, part of the lymphocytes become "marked" by chromosome damage. Radiation induces two types of microscopically detectable damage. Stable damage consists of breaks in the chromatid and is passed on to one daughter cell during cell division. Unstable damage consists of dicentric rings and leads to death of the cell in the next mitosis (Michie *et al.*, 1992). Because cells marked by the unstable chromosome damage die on cell division, the loss rate of such cells is a measure for their division rate (Michie *et al.*, 1992). Conversely, cells marked by stable chromosome damage should only disappear at their normal death rate (Mclean and Michie, 1995). The latter assumption is probably inaccurate, however, because cells with stable chromosome damage may continue to be produced, which probably accounts for the very long estimated average lifetime of both naive and memory T cells (Mclean and Michie, 1995). Thus, only data on unstable chromosome damage are discussed (Michie *et al.*, 1992). In a group of 19 patients treated with radiotherapy the lymphocyte count in the peripheral blood falls and slowly recovers. During the recovery period the number of $CD45RA^+$ cells marked by their unstable chromosome damage first increases and then declines. The marked $CD45RO^+$ T cells decline continuously (Michie *et al.*, 1992). To allow for an increase in marked $CD45RA^+$ T cells, the data are fitted to a mathematical model allowing for a reversion from the CD45RO to the CD45RA phenotype, and for different (division-associated) death rates for the $CD45RA^+$ and $CD45RO^+$ T cells. Assuming that most of the death is indeed associated with division, the results showed that naive T cells on

average divide every 1000 days, that memory T cells divide every 263 days, and that memory cells revert to the $CD45RA^+$ phenotype every 278 days (Michie *et al.*, 1992). This classic study established that memory T cells divide three times more frequently than naive T cells do, implying that long-lived memory is maintained by long-lived clones rather than by long-lived memory cells (Michie *et al.*, 1992). The fact that $CD45RO^+$ divide more frequently than naive T cells is now confirmed by a study using Ki67 as a marker for dividing cells (Sachsenberg *et al.*, 1998). One should keep in mind that these estimated division rates are the sum of the natural death and the true division rate, that this study lumps $CD4^+$ with $CD8^+$ T cells, and that the immune systems of these patients are recuperating from radiation therapy and are not at steady state. We expect that the $CD8^+$ T cell population is largely responsible for the estimated CD45RO to CD45RA reversion (Mackall and Gress, 1997a). Finally, one may employ these estimated division rates for calculating the total body T cell production. Assuming a total body count of 2.5×10^{11} $CD4^+$ T cells and 10^{11} $CD8^+$ T cells, a total of 3.5×10^{11} T cells, one obtains a production of about 3.5×10^8 naive T cells, and 10^9 memory T cells per day. Both figures are in good agreement with the results of studies employing Ki67 that are discussed below.

C. Recovery Rates Following $CD4^+$ T Cell Depletion

Adult human patients typically recover slowly from depletion of $CD4^+$ T cells by chemotherapy, radiotherapy, or CD4 monoclonal antibody treatment (Mackall *et al.*, 1995; Moreland *et al.*, 1995; Rep *et al.*, 1997). In most cases naive $CD4^+$ T cells were depleted more strongly than the memory $CD4^+$ T cells. $CD4^+$ T cell recovery correlated strongly with age and thymic function in a group of patients treated with chemotherapy, and this is especially true for the $CD4^+$ $CD45RA^+$ subset (Mackall *et al.*, 1995). In children the typical recovery rates are much better. Following treatment with CD4 mAb, the decrease in the number of CD4 T cells in the peripheral blood of two patients with juvenile chronic arthritis was only short-lasting, and numbers returned to normal values within 1 to 8 weeks (Horneff *et al.*, 1995). One can employ the recovery rates following lymphocyte depletion for estimating the total body production rate of $CD4^+$ T cells. Immune systems recuperating from $CD4^+$ T cell depletion by chemotherapy, radiotherapy, or CD4 mAb treatment are not at a natural steady state, however. Thus the estimated production rates could either be too high, when density-dependent effects increase $CD4^+$ T cell production at low total body counts, or too low, when only part of the TCR repertoire is recovering. A study of patients recovering from chemotherapy demonstrated the importance of thymic function (Mackall *et al.*, 1995). Here we estimate recovery rates for some of the adult patients in this

study. The three 24-year-old patients in this set recovered their $CD4^+$ T cell counts in the peripheral blood with linear recovery rates of 0.54, 0.26, and 0.63 cells/μl per day (Mackall *et al.*, 1995). For the total body such a recovery rate of about 0.5 cells/μl per day amounts to a production of a magnitude of 10^8 $CD4^+$ T cells/day. For a 23-year-old patient the study (Mackall *et al.*, 1995) provides more detailed data on the $CD4^+$ T cell recovery. Fitting the data by linear regression, both a linear growth and an exponential growth model fit reasonably well. This first yields an estimated growth rate of 1 cell/μl per day, the second a growth rate of 0.008 per day. By order of magnitude this amounts to a total production of about 10^8 $CD4^+$ T cells day. Note that these two estimates are about 10-fold lower than the estimates reviewed above.

Treatment of rheumatoid arthritis patients with CD4 mAb resulted in a severe $CD4^+$ T cell depletion to counts of about 300 $CD4^+$ T cells/μl (Moreland *et al.*, 1995). Figure 2 depicts these data and shows that following $CD4^+$ T cell depletion by this means, the recovery is slow. Six months after treatment, $CD45RO^+$ memory cells numbers had returned to normal, but the counts of $CD45RA^+$ naive $CD4^+$ T cells were only 50% of the normal count. By linear regression one finds an average daily increase of

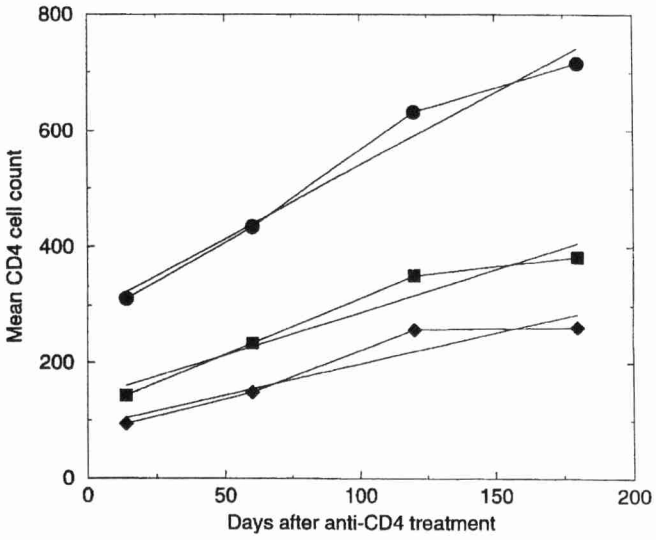

FIG. 2. Recovery of $CD4^+$ T cells in rheumatoid arthritis patients treated with CD4 mAb. Symbols indicate the mean CD4 count/μl peripheral blood: ● total $CD4^+$ T cells; ■, $CD4^+$ $CD45RO^+$ memory T cells; ◆, $CD4^+$ $CD45RA^+$ naive T cells. The three regression lines have slopes of 2.5, 1.5, and 1.1 cells/μl/day, respectively.

2.5, 1.5, and 1.1 $CD4^+$ T cells/μl, for the total, the memory, and the naive $CD4^+$ T cells, respectively. Because the production of memory cells is only marginally higher than that of the naive cells, the last two estimates amount to a total body production of approximately 10^8 naive $CD4^+$ T cells and 10^8 memory $CD4^+$ T cells per day. These production rates are very similar to those of the patients treated with chemotherapy (Mackall *et al.*, 1995). The production rate of 10^8 naive $CD4^+$ T cells per day corresponds closely to the similar estimate based on the unstable chromosome damage data (Michie *et al.*, 1992). For the memory $CD4^+$ T cells, however, the current estimate of 10^8 cells/day equals that of the chemotherapy patients (Mackall *et al.*, 1995), which was 10-fold lower than our other estimates. Similar data on $CD4^+$ T cell depletion come from a study of multiple sclerosis patients that had been administered CD4 mAb (Rep *et al.*, 1997). These patients received antibody until the $CD4^+$ T cell count in the peripheral blood dropped to 200 cells/μl. The subsequent recovery of the $CD4^+$ T cell count was exceedingly slow. Over the time course of 1 year, the average $CD45RA^+$ naive $CD4^+$ T cell count in the blood increased approximately linearly from 75 to 110 cells/μl. This increase in naive cells was paralleled by a similar increase in the $CD4^+$ memory cells, from 115 to 150 cells/μl (Rep *et al.*, 1997). Both correspond to a daily increase of only 0.1 cell/μl per day. For the total body production these figures amount to approximately 2.5×10^7 naive and only 2.5×10^7 memory $CD4^+$ T cells per day. Compared to the data reviewed below, both estimates are 10-fold and 100-fold lower.

D. BrdU Labeling of Proliferating Cells

The most direct method for estimating T cell production is counting the numbers of dividing cells. The DNA precursor bromodeoxyuridine (BrdU) (Tough and Sprent, 1994) was applied to study turnover of lymphocytes in mice. Labeling simian immunodeficiency virus (SIV)-infected and noninfected macaques with BrdU showed faster labeling with, and elimination of, BrdU in lymphocytes and natural killer (NK) cells in SIV-infected monkeys (Mohri *et al.*, 1998; Rosenzweig *et al.*, 1998). Labeling and elimination rates of both $CD4^+$ and $CD8^+$ T cells were reported to increase 2- to 3-fold in SIV-infected animals compared to healthy control animals. Conversion of the elimination rates into estimates of average life spans showed that naive T cells live about 16 weeks and memory cells about 7 weeks in normal animals, which is much shorter than previous estimates of T cell life span in humans (Mclean and Michie, 1995). By mathematical modeling, Mohri *et al.* (1998) calculated a proliferation rate of 3.6×10^7 $CD4^+$ T cells per day and a death rate of 3.6×10^8 $CD4^+$ T cells per day in normal animals, and in SIV-infected animals a proliferation rate of $4 \times$

10^8 T cells and a death rate of 7.5×10^8 $CD4^+$ T cells per day. Therefore, the production rates in infected monkeys were two to three times increased. The same increase in turnover rates was found in $CD8^+$ T lymphocytes, NK cells, and B cells from infected animals.

BrdU labeling studies as described for mice and macaques cannot be performed in humans. Another approach to evaluate proliferation in peripheral blood lymphocytes (PBLs) is to incubate freshly isolated PBLs *ex vivo* with BrdU (Tissot *et al.*, 1998). In this study, increased DNA synthesis could be observed only in patients with less than 100 $CD4^+$ T cells/mm^3 who had opportunistic infections. Thus, labeling peripheral blood T cells *ex vivo* provided no evidence for extensive proliferation in these cells in asymptomatic HIV-1 infection.

V. Measuring Cell Division with the Ki67 mAb

The Ki67 antigen is expressed by human proliferating cells during the late G_1, S, G_2, or M phases of the cell cycle (Bruno and Darzynkiewicz, 1992; Schwarting *et al.*, 1986; Tsurusawa *et al.*, 1992), and the number of cells can be quantified with the Ki67 mAb by flow cytometry. Because the vast majority of lymphocyte cell divisions takes place in the lymphoid tissue, measurements within the lymphoid tissue seem most reliable (Zhang *et al.*, 1998; Fleury *et al.*, 1998; Tenner-Racz *et al.*, 1998). In HIV-infected patients there is the additional problem that dividing $CD4^+$ T cells are targets for HIV-1 infection. If $Ki67^+$ $CD4^+$ T cells die by HIV-1 infection, the total body numbers of such cells can only be a lower bound for the true $CD4^+$ T cell production.

A. Healthy Human Adults

Few groups have published data on Ki67 expression in the peripheral blood and lymphoid tissue of HIV-negative subjects. The percentages of $CD4^+$ cells expressing Ki67 and the calculations of cell production based on these results are presented in Table I. Two studies (Zhang *et al.*, 1998; Fleury *et al.*, 1998) report that about 0.5% of the $CD4^+$ T cells in lymphoid tissues express the Ki67 antigen. Having about 2.5×10^{11} $CD4^+$ T cells in lymphoid tissue, and assuming that the Ki67 antigen is expressed over most of the cell cycle of about 24 hr, these percentages correspond to a total body production of about 10^9 $CD4^+$ T cells per day. Another group distinguished between the T cell zone and the germinal center area in lymph nodes, and reports somewhat higher percentages (Tenner-Racz *et al.*, 1998). In the T cell zone 2.6% of the $CD4^+$ T cells expresses the Ki67 antigen, whereas in germinal centers 1.4% of the $CD4^+$ T cells is $Ki67^+$. Recalculating these percentages in terms of total body production is more

TABLE I
CD4$^+$ T CELL PRODUCTION RATES ESTIMATED BY THE Ki67 mAb

		Ki67$^+$		Estimated total		Production[d]	
Source[a]	Status[b]	%	Fold[c]	Number	Fold	Number	Fold
PBMC[e]	HIV−	0.60		3.9×10^9		2.2×10^7	
PBMC[e]	HIV+	0.80	1.3	3.4×10^9	0.89	2.7×10^7	1.2
PBMC[e]	4–8 wk H					1.0×10^7	0.45
PBMC[e]	30 wk H					6.9×10^7	3.14
PBMC[f]	HIV−	1.1		4.2×10^9		4.4×10^7	
PBMC[f]	HIV+	6.5	5.9	2.0×10^9	0.46	8.3×10^7	1.9
CD45RO$^+$[f]	HIV−	2.8		1.8×10^9		4.4×10^7	
CD45RO$^+$[f]	HIV+	6.7	2.4	9.1×10^8	0.52	7.0×10^7	1.6
CD45RA$^+$[f]	HIV−	0.8					
CD45RA$^+$[f]	HIV+	2.7	3.4				
LN[e]	HIV−	0.54		2.5×10^{11}		1.3×10^9	
LN[e]	HIV+	0.39	0.72	1.9×10^{11}	0.78	6.5×10^8	0.51
LN[e]	30 wk H					3.5×10^9	2.7
LN[g]	HIV−	0.4		2.2×10^{11}		8.0×10^8	
LN[g]	HIV+	1.2	3	9.5×10^{10}	0.47	1.1×10^9	1.4
LN[g]	2 day H	1.0	2.5	9.0×10^{10}	0.45	9.0×10^8	1.1
LN[g]	3 wk H	1.0	2.5	1.1×10^{11}	0.55	1.1×10^9	1.4
LN[g]	24 wk H	0.4	1.0	1.2×10^{11}	0.60	4.8×10^8	0.6
GC[h]	HIV−	1.4					
GC[h]	HIV+	18.7	13.4				
T cell zone[h]	HIV−	2.6					
T cell zone[h]	HIV+	3.7	1.4				

[a] PBMC, peripheral blood mononuclear cells; LN, lymph node; GC, germinal center.
[b] H, highly active antiretroviral therapy.
[c] Fold increase compared to the HIV-negative value.
[d] Estimated daily production in the compartment (assuming Ki67 is expressed for most of the 24-hr cycle).
[e] Fleury *et al.* (1998).
[f] Sachsenberg *et al.* (1998).
[g] Zhang *et al.* (1998).
[h] Tenner-Racz *et al.* (1998).

difficult because it is not known what fraction of CD4$^+$ T cell population resides in T cell zones, and in the germinal centers, respectively. In germinal centers CD4$^+$ T cell numbers tend to be low (McHeyzer-Williams and Davis, 1995).

Ki67 measurements in the peripheral blood also suggest low division rates of the CD4$^+$ T cells. Comparing peripheral blood with lymphoid tissue, in HIV-negative subjects the percentage of Ki67$^+$ CD4$^+$ T cells was similar in both compartments (Fleury *et al.*, 1998). Sachsenberg *et al.* (1998) reported a very similar percentage of Ki67$^+$ CD4$^+$ T cells in the

blood of healthy adults. This study also distinguished $CD4^+$ $CD45RA^+$ naive from $CD45RO^+$ memory T cells, and reports that in the blood, four times more $CD45RO^+$ $CD4^+$ T cells were $Ki67^+$ than naive $CD45RA^+$ $CD4^+$ T cells (Sachsenberg *et al.*, 1998). Although it remains difficult to extrapolate total body estimates for $CD4^+$ T cell production from data in peripheral blood, these confirm that, on average, memory T cells divide more frequently than naive T cells (Michie *et al.*, 1992; Mclean and Michie, 1995; DeBoer and Noest, 1998).

Data regarding Ki67 expression in $CD8^+$ T cells are summarized in Table II. In the normal situation the total production of $CD8^+$ T cells seems to be almost 10-fold lower than $CD4^+$ T cell production (Fleury *et al.*, 1998). In the lymphoid tissue one finds that only 0.2% of the $CD8^+$ T cells expresses the Ki67 antigen. This would correspond to a total body production of about 10^8 $CD8^+$ T cells per day. Measurements in the T cell zone of lymph nodes of healthy human adults, however, suggest a more than 10-fold higher percentage $Ki67^+$ $CD8^+$ T cells (Tenner-Racz *et al.*, 1998), which would correspond to a more than 10-fold higher produc-

TABLE II
$CD8^+$ T Cell Production Rates Estimated by the Ki67 mAb

		$Ki67^+$		Estimated total		Production[d]	
Source[a]	Status[b]	%	Fold[c]	Number	Fold	Number	Fold
$PBMC^{+e}$	HIV−	0.41		3.2×10^9		1.2×10^7	
PBMC[e]	HIV+	1.2	3	5.7×10^9	1.8	7.1×10^7	5.8
PBMC[f]	HIV−	1		2.2×10^9		2.1×10^7	
PBMC[f]	HIV+	4.3	4.3	3.3×10^9	1.5	1.3×10^8	6.2
$CD45RO^{++f}$	HIV−	2		4.7×10^9		8.5×10^6	
$CD45RO^{++f}$	HIV+	6.8	3.4	1.0×10^9	2	4.8×10^7	6
$CD45RA^{++f}$	HIV−	0.9					
$CD45RA^{+f}$	HIV+	1.7	1.9				
LN[e]	HIV−	0.17		5.4×10^{10}		9.5×10^7	
LN[e]	HIV+	0.38	2.2	1.7×10^{11}	3.1	6.5×10^8	6.8
LN[e]	30 wk H					9.1×10^8	9.6
T cell zone[g]	HIV−	2.9					
T cell zone[g]	HIV+	5.5	1.9				

[a] PBMC, peripheral blood mononuclear cells; LN, lymph node.
[b] H, highly active retroviral therapy.
[c] Fold increase compared to the HIV-negative value.
[d] Estimated daily production in the compartment (assuming Ki67 is expressed for most of the 24-hr cycle).
[e] Fleury *et al.* (1998).
[f] Sachsenberg *et al.* (1998).
[g] Tenner-Racz *et al.* (1998).

tion of $CD8^+$ T cells per day. The reasons for this discrepancy remain unclear. For the $CD8^+$ T cells in the peripheral blood one finds smaller discrepancies. Fleury *et al.* (1998) report that 0.41% of the $CD8^+$ T cells in the blood expresses the Ki67 antigen, whereas Sachsenberg *et al.* (1998) reported that 1.0% of the $CD8^+$ cells is $Ki67^+$. Distinguishing CD45 subsets, the latter study also reports that in blood $CD8^+$ T cells 0.9% of the $CD45RA^+$ cells, and 2.0% of the $CD45RO^+$ cells, express the Ki67 antigen (Sachsenberg *et al.*, 1998). This again demonstrates that $CD45RO^+$ T cells divide more frequently than $CD45RA^+$ T cells.

B. HIV-Infected Patients

In HIV-positive patients the percentages of $Ki67^+$ T cells tend to increase in both $CD4^+$ and $CD8^+$ T cell compartments (Fleury *et al.*, 1998; Sachsenberg *et al.*, 1998; Tenner-Racz *et al.*, 1998). This is at least partly due to generalized immune activation that is associated with HIV-1 infection (Mohri *et al.*, 1998). A mere increase in the percentages of $Ki67^+$ T cells need not imply, however, that the total production in the CD4 and CD8 compartments is proportionally increased. In the lymphoid tissue of a group of early-stage HIV-patients, the percentage of $Ki67^+$ cells has changed 0.7-fold for $CD4^+$ cells, and 2.2-fold for $CD8^+$ cells, compared to healthy controls (Fleury *et al.*, 1998). However, because the total numbers of $CD4^+$ T cells have decreased in these patients, the total production of $CD4^+$ T cells seems somewhat less than normal (Fleury *et al.*, 1998). The total numbers of $CD8^+$ T cells have increased, such that the total production of $CD8^+$ T cells is also increased (Fleury *et al.*, 1998). Note that in the peripheral blood of these patients both the percentage of $Ki67^+$ $CD4^+$ T cells and the percentage of $Ki67^+$ $CD8^+$ T cells have increased (Fleury *et al.*, 1998). In HIV-positive patients, dividing ($Ki67^+$) T cells are apparently overrepresented in the blood, thus one cannot simply extrapolate from peripheral blood measurements to total body estimates.

In a group of HIV patients at a later stage of disease Zhang *et al.* (1998) report that an average of 1.2% of the $CD4^+$ T cells in the lymphoid tissue expresses the Ki67 antigen. This is 3-fold higher than their control value (Zhang *et al.*, 1998), and 3-fold higher than the percentages found in early-stage patients (Fleury *et al.*, 1998). The total body $CD4^+$ T cell counts are substantially depleted in these patients, however, on average 0.44-fold lower (Zhang *et al.*, 1998). The 3-fold higher percentage of dividing $CD4^+$ T cells could therefore be due to a more generalized hyper-activation at this stage of disease, and/or due to an increased $CD4^+$ T cell growth rate by density mechanisms. Although the percentage of dividing $CD4^+$ T cells has increased 3-fold because of the HIV-1 infection, the total body production of $CD4^+$ T cells has increased only 1.27-fold because of the

depletion of total $CD4^+$ T cell numbers. Over 24 weeks of HAART the percentage of $Ki67^+$ $CD4^+$ T cells in the lymphoid tissue normalizes, whereas the number of $CD4^+$ T cells in lymphoid tissue increases only slightly (Zhang *et al.*, 1998). Thus, $CD4^+$ production decreases with HAART. Similar results were reported by Fleury *et al.* (1998). This drop in Ki67 expression during HAART has two important implications. First, it demonstrates that HIV-1 infection is involved with a level of hyperactivation that falls when the viremia drops. Second, it suggests that there is little masking of cells expressing Ki67 by HIV-1 killing of dividing cells.

In a group of 12 HIV patients with $CD4^+$ T cell counts >500 cells/μl, much higher percentages of $Ki67^+$ T cells were found in the T cell zone and in germinal centers of lymph nodes (Tenner-Racz *et al.*, 1998). We have already mentioned that the healthy control data from this group are also higher than those of other studies (Zhang *et al.*, 1998; Fleury *et al.*, 1998). For the T cell zone the fold increases remain comparable to those of others (Zhang *et al.*, 1998; Fleury *et al.*, 1998). The reason for the high percentage in the germinal centers remains unclear, however.

Sachsenberg *et al.* (1998) documented Ki67 percentages in the peripheral blood and showed that for both $CD4^+$ and $CD8^+$ T cells the percentages increase with HIV-1 infection. The majority of the production was in the $CD45RO^+$ subset. Moreover, it was shown that the percentage of $Ki67^+$ T cells increased with decreasing $CD4^+$ T cell counts. Again, this could be due to density-dependent mechanisms, or to stronger immune activation due to higher viral loads that are associated with lower $CD4^+$ T cell counts (Sachsenberg *et al.*, 1998). Finally, note that if dividing cells are indeed over represented in the peripheral blood (Fleury *et al.*, 1998), all these percentages should be regarded as overestimates.

VI. What Is the Cause of $CD4^+$ T Cell Depletion in HIV-1 Infection?

With respect to $CD4^+$ T cell depletion it should be noted that this is, in general, measured in the blood. It is now thought that the steep increase in $CD4^+$ and $CD8^+$ T cell numbers in the blood immediately after initiation of antiviral therapy should be interpreted as redistribution of T cells that were retained in the lymphoid tissues and at other inflammatory sites (Pakker *et al.*, 1998; Autran *et al.*, 1997). Interestingly, the magnitude of this redistribution is inversely correlated with baseline $CD4^+$ T cell counts and is very limited in patients with baseline $CD4^+$ cell counts greater than 400 mm^3 (Fleury *et al.*, 1998). This indicates that, in patients with $CD4^+$ cell counts below 400 mm^3, but rarely in patients with higher $CD4^+$ T cell counts, $CD4^+$ T cell depletion is overestimated based on the $CD4^+$ T cell counts in the blood. In patients with very low $CD4^+$ T cell counts, in fact,

$CD4^+$ T cell counts sometimes do increase 6-fold within days of the start of therapy due to redistribution of cells that, apparently, were present in tissue before therapy (Pakker *et al.*, 1998; J. M. Prins, personal communication). In line with these observations, it has been reported that in lymphoid tissue the depletion of $CD4^+$ T cells is less severe compared to that in blood (Zhang *et al.*, 1998; Rosok *et al.*, 1996).

Experimental results on the number of cells productively infected with HIV have shown that these numbers are in fact very low, in particular given the enormous numbers of viral particles produced per day. Chun *et al.* (1997) reported a total of 5×10^7 CD4 cells with integrated proviral HIV and Haase and co-workers (Embretson *et al.*, 1993) reported a total of 5×10^8 cells that are positive for viral DNA. $CD4^+$ T cell death may also involve uninfected cells that die through activation-related apoptosis, although this is reportedly much higher for $CD8^+$ T cells (Finkel *et al.*, 1995; Meyaard *et al.*, 1992). Zhang *et al.* (1998) report a twofold increase in $CD4^+$ T cell apoptosis in lympoid tissue. Taken together, the number of productively infected cells and the number of cells involved in activation-induced apoptosis are indicative of a modestly increased destruction of $CD4^+$ cells in HIV-1-infected patients. Moreover, the fact that production of $CD4^+$ T cells is not immediately increased after start of HAART, but increases only later during HAART (Fleury *et al.*, 1998), indicates that in HIV-1 infection there is not a masking of high cell production by efficient killing of dividing cells. The question is whether this modestly increased destruction of $CD4^+$ T cells could still be the main cause of the gradual $CD4^+$ T cell depletion. This could be the case if $CD4^+$ T cell renewal is very limited and is incapable of meeting the higher demand, as has been argued by Haase and colleagues (Zhang *et al.*, 1998).

As pointed out previously, much of the controversy on the magnitude of T cell production and destruction in HIV-1 infection relates to what the investigators regard as high turnover or high $CD4^+$ T cell production. The calculated production of 2×10^9 cells/day, based on the initial rise of $CD4^+$ T cell numbers in the blood, was believed to be very high and was suggested to be at least an order of magnitude higher than normal (Ho *et al.*, 1995). It was argued that the system was highly stressed trying to keep up with the increased demand due to high levels of $CD4^+$ T cell death caused by HIV-1. To account for $CD4^+$ T cell depletion, this highly increased level of $CD4^+$ T cell production was believed to exhaust the renewal potential. As we have discussed, new experimental evidence on T cell production numbers in normal controls and HIV-infected patients suggests that $CD4^+$ T cell production is about two- to threefold increased with HIV-1 infection and is on the order of 10^9 cells per day (Table I). Studies that provided a mathematical interpretation of telomere length

dynamics in $CD45RA^+$ and $CD45RO^+$ $CD4^+$ cells demonstrated that telomere shortening rates comparable to uninfected controls are compatible with a small increase in production of $CD4^+$ T cells (Wolthers *et al.*, 1996, 1998). Therefore, the data suggest that there is a modest increase in the amount of proliferation of T cells in HIV-1-infected individuals. This low increase in production of $CD4^+$ T cells is not likely to result in exhaustion of the $CD4^+$ renewal potential, however, which is also indicated by the lack of significant telomere shortening.

There is now considerable data suggesting that the low level of thymic development that occurs in adults may be further reduced with HIV-1 infection. Studies of development of bone marrow from HIV-infected persons all show a lower capacity to develop cells of multiple hematopoietic lineages (Steinberg *et al.*, 1991; Zauli *et al.*, 1992, 1996). The data from FTOC show that changes in T cell developmental capacity occur rapidly in individuals who progress to AIDS but not in long-term nonprogressors (Clark *et al.*, 1998). In addition, studies in SCID-hu mice and measurement of thymic tissue have shown that the thymus is also affected by HIV-1 infection (Joshi and Oleske, 1985; Aldrovandi *et al.*, 1993; Mosier *et al.*, 1991; Vigano *et al.*, 1998). The export of new cells from the thymus, as measured by excision circle PCR, have shown that the number of new naive cells in the periphery is reduced after infection (Douek *et al.*, 1998). Many of these deficiencies improve with potent antiretroviral therapy (Douek *et al.*, 1998; Clark *et al.*, 1998; Vigano *et al.*, 1998). Therefore, there is now good evidence that in HIV-1 infection the capacity to develop new cells from progenitors is perturbed.

Finally, we can propose a coherent model for $CD4^+$ cell depletion based on the data we have summarized. During the course of asymptomatic infection an increasing number of naive cells will be activated and become memory cells (Roederer, 1995). If these cells cannot be replaced by development of new naive cells, as described above, the naive pool will be effectively depleted over time. Loss of naive cells could also be accelerated in those individuals harboring syncitium-inducing virus, because these viruses can infect naive cells (H. Blaak, personal communication). The memory pool is gradually depleted by activation-induced cell death and, in the case of $CD4^+$ T cells, by HIV-1-related death. Because development is inhibited, there are few naive cells to feed into the memory population, so it must be maintained by increased proliferation of already existing cells. As discussed, the increase in proliferation does not appear to be vast and perhaps, though not directly shown, is insufficient to keep up with cell loss. In any case, the cells that result from this proliferation would be more likely to die sooner (Mackall and Gress, 1997b). The result is the eventual loss of the memory $CD4^+$ cells, and concomitant with AIDS diagnosis, the

loss of the memory $CD8^+$ T cells. Thus $CD4^+$ T cell depletion is likely to be due to the combination of inhibition of development of new cells, and the increase in proliferation of already existing cells.

VII. Appendix: Summarizing in Terms of a Mathematical Model

Because most studies suggest that the production of naive $CD45RA^+$ cells requires a functional thymus (Mackall *et al.*, 1995; Mackall and Gress, 1997a; Hellerstein and McCune, 1997), we ignore cell division in the naive compartment, and write for the naive $CD4^+$ T cells N,

$$dN/dt = \sigma - \delta_N N - \alpha N - \varepsilon_N N^2, \tag{1}$$

where the σ term is the source of naive $CD4^+$ T cells from the thymus, the $\delta_N N$ term represents death, the αN term represents activation of naive cells due to priming by antigen, and the $\varepsilon_N N^2$ term reflects a possible additional density-dependent death rate by competition within the naive compartment (Tanchot and Rocha, 1995). The best current parameter estimate seems to be a production $\sigma = 10^8$ cells/day in human adults. The average life span and the priming rate remain uncertain, however. Additionally, there are no data allowing any direct estimate of the density-dependent additional death rate, ε_N. One can estimate the average life span of a naive $CD4^+$ T cell, however, from the estimated production σ and the steady-state total body count of 2.5×10^{11} cells. With a steady-state source of $\sigma = 10^8$ cells/day, the average life span in the naive compartment is $2.5 \times 10^{11}/10^8 = 2500$ days (or approximately 7 years).

For the memory $CD4^+$ T cells M we allow for a source from the naive compartment, for renewal, and death. Thus we write that

$$dM/dt = c\alpha N + \rho M/(1 + M/K) - \delta_M M - \varepsilon_M M^2, \tag{2}$$

where the δ and ε parameters again allow for a normal and for a density-dependent death rate. The $c\alpha N$ term represents the clonal expansion of the activated naive $CD4^+$ T cells (where c is the clonal expansion factor). The renewal term allows for an expansion/proliferation rate of ρ cell divisions per day when memory cell numbers are low (i.e., when $M \ll K$), and a total production of ρK cells/day when memory cell numbers are high (i.e., when $M \gg K$). This proliferation rate ρ should reflect that typically only a small fraction of the memory cells is dividing (ρ should be small). Most studies reviewed above suggest a production of 10^8 to 10^9 memory $CD4^+$ T cells per day, which seems fairly independent of the total body

lymphocyte counts. This suggests that the memory $CD4^+$ T cell density K at which the renewal levels off should be fairly low, or $K \ll 2.5 \times 10^{11}$. If memory $CD4^+$ T cell production is indeed 10-fold higher than that of their naive counterparts, their average life span at steady state should also be 10-fold lower, about 250 days. What fraction of the total memory production is due to the activation and clonal expansion of naive $CD4^+$ T cells, due to the $c\alpha N$ term, and what fraction is due to renewal, remains the major open question, however. Data establishing cell division rates of $CD4^+$ memory T cells (Sachsenberg *et al.*, 1998; Michie *et al.*, 1992; McLean and Michie, 1995) unfortunately fail to distinguish between the two possibilities.

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