Microbial parasites versus developing T cells: An evolutionary ‘arms race’ with implications for the timing of thymic involution and HIV pathogenesis

PAUL W. TURKE
College of Human Medicine, Michigan State University, USA

Accepted 8 August 1995

Abstract. The thymus attempts to ensure that T cells which emerge from it are able to discriminate self from nonself. As such, it is a potential ‘backdoor’ through which microbial parasites can enter, manipulate the host into perceiving them as ‘self’, and thereby avoid immune surveillance. It is proposed that the host has evolved to overcome this parasitic strategy by rapidly producing large numbers of long-lived T cells very early in life (closing the backdoor), before the developing individual has significant contact with infectious organisms, and while still under the protection of its mother’s intact immune system. Hence the capacity of the thymus to function efficiently early in the lifespan would have been strongly favored by natural selection. It is well established in evolutionary biology that strong selection favoring enhanced early function easily accommodates, through pleiotropy, the accumulation of later occurring negative effects, and it is through this process that thymic involution and subsequent immune system senescence may have evolved. Once a large pool of competent T cells has been produced, even those microbes capable of contaminating the thymus usually can be eliminated, or at least contained. However, microbes that both destroy peripheral T cells (particularly peripheral T cells that are activated against them), and contaminate the thymus (leading to deletion of potential replacements of the destroyed peripheral cells), may be able to eventually overcome the immune system, thus producing disease after a long period of apparent latency. Human immunodeficiency virus, which is initially well controlled by the immune system, may become unleashed via this process.

Key words: Evolution, HIV pathogenesis, Immunosenescence, Thymic involution

Introduction

The solutions to two paradoxes in immunology are proposed to be related. The first of these concerns the timing of thymic involution, the second, the fact that the immune system initially controls human immunodeficiency virus (HIV) infection rather well but loses control, in most cases, over the course of a decade.

Evolution of thymic involution

The thymus has a key role in the generation and maintenance of immune function, serving as the site where immature T cells (thymocytes) are both
positively and negatively selected for their ability to distinguish self from nonself and react against only the latter. It is on these criteria that a proportion of thymocytes are induced to mature into functional T cells whereas the others are induced to undergo a programmed cell death termed apoptosis [1–3]. In this context it is perhaps surprising that in humans involution of thymic lymphoproliferative areas begins soon after the first year of life, and then progresses inexorably and rapidly [4]. Then again, perhaps it is not surprising: if – as several studies indicate – many of the T cells produced early in life have very long lifespans (either as individual cells or clones of the orginals), it might be redundant to maintain full thymic output beyond early childhood [5]. The fact that thymectomy during childhood, after the immune system is largely constituted, is generally without severe negative consequences testifies to the prodigious output by the young thymus of long-lived T cells, and suggests that the relatively low output later in life is not crucial to survival [6], under most circumstances (see below). Thus, thymic function seems to last about as long as it needs to last.

However, in a broader evolutionary sense – that is, if we recognize that lifespans evolve – maintaining a higher T cell output to older ages would seem to be beneficial, other things being equal. Several studies using mice indicate that 'normal' immune system senescence is due primarily to the inability of the involuted thymus to produce a continual supply of fresh (naive) T cells sufficient to replace those converted by encounters with antigen to memory T cells [7–10]. Miller and his colleagues have described the deficiencies that accrue to mouse memory T cells, and to immune systems replete with memory cells, at molecular, biochemical, physiological, and functional levels [7, 10, 11], but it may be added that memory cells, which by definition are cells which fit well with historical versions of antigen, stand a good chance of eventually becoming obsolete as a function of rapid evolution of the antigenic character of microbial parasites.

All of the above suggests that age-related deficits in human immune function, which can be detected by age 40 or 50, and which are obvious by age 70 [11], are due, at least in part, to a progressive increase in the ratio of memory to naive T cells [10]. If this understanding of immune system senescence is correct, or nearly so, an accelerated demise of the thymus should contribute to an accelerated demise of individual organisms, thus raising the issue of why involution occurs as early and rapidly as it does.

In the context of the problem of distinguishing self from nonself, Roitt [12] notes that very early in life thymocytes are likely to be surrounded only by self, normally meeting nonself antigen such as viral and bacterial proteins with high frequency only later in the lifespan. Since antigen from infectious microbes can be expressed by thymic antigen presenting cells and thus
perceived as ‘self’, and since self-reactive immature T cells are induced to undergo apoptosis (see above), the thymus can become a ‘backdoor’ entrance through which parasites can avoid immune surveillance and destruction. The most significant increases in the thymus’s vulnerability to infiltration by foreign antigen (for mammals) would be expected to occur when the developing individual experiences diminished protection from its mother’s fully developed immune system, first at birth, later upon weaning, and later still upon first direct contact with large numbers of individuals other than mother. This implies that the value of the thymus to the host should decline with age.

How can the host fight back? A good strategy it seems would be to rapidly produce a large number of long-lived T cells early, before significant exposure to foreign antigen occurs. The idea is to quickly close the backdoor. However, doing so invites one enormous implication: strong selection for enhanced early function, of any biological system, can easily accommodate massive, later-occurring reductions in function due to pleiotropy [13–15]. It is this same process – selection for early advantage pleiotropically linked to later disadvantage – that is now widely believed to underlie the evolution of senescence, in general [16].

Natural selection is expected to strongly discount the negative pleiotropic effects that may be associated with enhanced early thymic output, for two reasons. First, as noted, older thymuses become intrinsically less valuable because the probability of contamination by foreign antigen is time-dependent. More generally, the power of natural selection declines over the lifespan as a function of declining reproductive opportunity; thus, selection fails to notice even very large detrimental effects, if they occur late enough [13–16]. Regarding human T cell function, negative effects – senescence – do not become significant until five or six decades after the beginning of thymic involution [9–11]. I am suggesting that this is a tolerable price to pay for enhanced, early output of long-lived competent T cells. One can imagine that, during youth, when reproductive potential is high, the benefit from even a small increase in survival accruing from foiling the attempt of a parasite to pass itself off as ‘self’ can easily outweigh the cost of even severe immune system senescence – again, provided that the cost occurs after the probability of future reproductive success has declined significantly anyway due to independent exogenous factors (saber-tooth tigers, starvation, warfare, and so on). Evidence suggesting that genetically heterogeneous mice with high memory-to-naïve T cell ratios at six months of age tend to have high ratios at about one year of age, and also are significantly more likely to die within the first eighteen months of life [17], is broadly consistent with the argument presented here, however, it remains to be determined whether these indicators of rapid immunosenescence correlate with enhanced early thymic function.
Parallel with the above general hypothesis, Kampinga et al. [18] demonstrate that thymic dendritic cells (antigen presenting cells of bone marrow origin) have a very rapid turnover (about 2 weeks, in rats), and they suggest that this minimizes the exposure of thymocytes to dendritic cells that may have been infiltrated by foreign antigen during transient infections. One might predict that the ability of dendritic cells to turnover rapidly in the thymus may be pleiotropically linked to effects that contribute to thymic involution and eventually to immune system senescence. It is important to note, in any case, that this strategy of rapid dendritic cell turnover is likely to be effective only for those pathogens which are quickly cleared by the immune system. Foreign organisms that produce smoldering or slowly progressive infection may be able to circumvent this host adaptation. Smoldering infection can theoretically result in progressive deletion of thymocytes which, as mature T cells, would have been capable of reacting to, and at least partially controlling, the invader. It is under such circumstances that a large reserve of competent long-lived T cells produced earlier, before thymic contamination, would be crucial. However, consider what might happen if the infecting pathogen is able to establish a smoldering infection that, in addition to deleting thymocytes which react to it, destroys mature peripheral T cells capable of reacting against it.

**HIV pathogenesis: The backdoor hypothesis**

A curious feature of HIV pathogenesis is that viremia associated with initial infection is almost always controlled within a matter of weeks. Furthermore, in spite of a reduction in numbers of peripheral CD4+ T lymphocytes (i.e. helper T cells, the primary targets of HIV) during acute infection, there is generally an initial recovery to normal or near normal levels [19]. Evidence now strongly suggests a major role for HIV-specific cytotoxic T lymphocytes (CTL's) in the early control of HIV infection [20–24]; and CTL response is of course contingent upon help from stimulated HIV specific CD4+ T cells. Moreover, although subtle laboratory signs of immune dysfunction appear not too long following acute infection, infection by HIV is clinically silent in a majority of individuals for at least 5–10 years during which time helper T cell levels decline slowly [19]. I propose that contamination of thymus antigen presenting cells by HIV is an important, perhaps primary, factor accounting for this slow reversal in host fortunes.

Fauci and Rosenberg [25] state that the available evidence ‘strongly’ suggests that in vivo infection of thymic epithelial cells occurs (1994: 63). They further report that in vitro infection of intrathymic T cell precursors (CD3− CD4− CD8−) occurs, and Ardavin et al. [26] report that thymic
dendritic cells and T cells develop simultaneously in the thymus from a common bone marrow-derived precursor population. In other words, it is likely that thymocytes frequently are infected by HIV and it is likely that thymic dendritic cells frequently are contaminated with HIV antigen.

Infection of thymocytes implies that a generalized depletion of helper T cells begins before they even reach the peripheral circulation [27, 28]; contamination of thymic dendritic cells with HIV antigen implies the deletion, by apoptosis, of T cells specifically reactive to HIV proteins. Together these processes may account for the reported low or absent response by helper T cells to HIV proteins in vivo [29]. Furthermore, of the two, unless the generalized infection-mediated depletion of thymocytes and T cells is massive, systematic apoptosis of thymocytes specifically reactive to HIV is a potentially more efficient means for losing control of HIV. Siegrist et al. [30] have made this point for infections occurring early in fetal development, and they suggest that contamination of the thymus accounts for the rapid progression of such individuals to the acquired immunodeficiency syndrome (AIDS). Note that by their hypothesis, infants infected during birth itself, or by contaminated breast milk, should progress more slowly than those infected earlier in development, other factors being equal (see below).

Siegrist et al. [30], in essence, have proposed a mechanism of pathogenesis for HIV in the fetus which overcomes the immune system by entering through the backdoor, represented by the thymus, before a large number of competent T cells is produced. (Note that in my hypothesis, closing the backdoor refers to rapidly producing T cells, not to thymic involution; thymic involution is proposed to be an undesirable, unavoidable consequence of strong selection for enhanced early thymic function.) Why are we not overwhelmed by other microbes entering the thymic backdoor before it closes? If my hypothesis is correct, others must have been successful in this in the past, thus creating the selective pressures that led to earlier and earlier closing of the backdoor. But, because the pregnant/lactating female’s mature immune system usually shields the fetus/infant from exposure to most infectious microbes, and in part for reasons discussed elsewhere involving vertical transmission and the evolution of benignness [31, Turk submitted], such early failures of host adaptation are expected to have been mostly overcome during the course of evolution.

AIDS obviously is not limited to individuals infected in utero. Unlike the very young, older individuals begin their infection with a large and effective armamentarium against HIV. But HIV is able to slowly eliminate this armamentarium, I suggest, by producing a persistent, smoldering infection – an infection that slowly destroys peripheral helper T cells, especially those directed against it (see below), and which simultaneously destroys their replace-
ments via negative selection in the thymus. Without this latter effect, AIDS may never develop, or at least it may develop more slowly.

The foremost requirement for producing smoldering infection is the ability to avoid complete elimination during acute infection. This ability by HIV probably is due to a number of characteristics. One may be that high rates of replication and mutation during acute infection may produce at least a few variants that are somewhat cryptic with respect to the initial assault mounted by the immune system [32]. Similarly, macrophage trophic HIV variants which seem to predominate early in infection may be relatively hidden from the immune system because of their relative inactivity within macrophages [33, 34]. In addition, HIV has the ability to exist in an unintegrated latent form within T cells [19]. This pattern of pathogenesis would yield minimal but persistent production of antigen following the initial, largely effective, immune response.

Smoldering infection should be able to overcome the strategy proposed by Kampinga et al. [18] (in which the rapid turnover of thymic dendritic cells eliminates contamination by foreign antigen), and therefore, as already suggested, should lead to persistent contamination of the thymus and deletion of HIV specific thymocytes (CD4+ and CD8+). This process of deletion would not apply to thymocytes that are not specific to HIV, thus return of T cell counts to near normal levels following acute infection is consistent with the hypothesis being proposed. Furthermore, those peripheral helper T cells which are reactive to HIV, but which manage to elude infection/destruction during acute infection, are more likely to later encounter residual HIV and thereby become activated (i.e. during the so-called latent phase of infection). Active cells are both more likely to be productively infected by HIV, and more likely to die from the production of virus than inactive helper T cells. The site of much of this activity is likely to be the lymph nodes, where the appropriate cells are concentrated [19]. The combination of intrathymic apoptosis of HIV reactive thymocytes and preferential deletion of HIV reactive mature T cells can be expected to lead to progressive diminution of control of HIV replication.

This proposed mechanism of pathogenesis is not assumed to account for all aspects of HIV pathogenesis (see below), and in particular probably cannot account for the generalized, but relatively subtle, immune dysfunction that soon follows acute infection. One plausible explanation for early disruption of cell-mediated immunity is that infection by HIV of macrophages may alter their ability to present antigen to T cells [34].
Discussion

Several studies describing HIV immunopathogenesis support the backdoor hypothesis by demonstrating that well circumscribed ‘holes’ accumulate in the T cell repertoire that was initially capable of controlling HIV. For instance, Cavacini et al. [35] demonstrate the loss of a serum antibody response to a specific epitope of HIV-1/gp120 in several asymptomatic infected patients they followed, and they found that this deficit is probably one cause of their disease progression. Antibody is produced by activated B cell lineages, but is of course highly dependent upon similarly activated helper T cells. It is also important to note that in the study cited the loss of control by antibody is apparently due to changes in host capabilities, not mutation of HIV gp120, since the response, or lack thereof, is to native HIV-1 gp120 from cells infected with HIV in vitro. Similarly, Voss and Hunsmann [36] report the ‘sudden loss of a vaccine-induced preexisting helper T cell activity’ in simian immunodeficiency virus infection in macaques. Strong, broad CTL responses in the early stages of HIV infection in people also routinely disappear completely by the time the acquired immunodeficiency syndrome appears [37]. In this instance, loss of response may be due to destruction of HIV specific CTL’s, or mutation-induced escape from T cell surveillance, or both. Experimental evidence presented by Phillips et al. [37] make a strong case for mutation-induced escape of HIV gag protein from CTL recognition, but does not exclude the possibility that deletion of specific CTL clones also contributes to the lack of response. This latter possibility remains cogent since in their study the CTL’s that failed to respond to the emergent gag proteins were from HIV infected individuals, not from individuals with intact immune systems. Moreover, it is significant that in the patients studied by Phillips et al., as infection progressed, all CTL responses that persisted became contingent upon in vitro stimulation by autologous helper T cells. That is, the patients’ own remaining helper T cells eventually were unable to stimulate their own HIV-specific CTL’s. Is this diminished response to HIV due to the evolution of escape mutants [37, 38] or to reductions in the HIV-specific T cell repertoire? Tests of the ability of T cells from intact immune systems to react to the HIV which emerges late in infection would help to discriminate the relative importance of each of these mechanisms.

In two recent studies Ho et al. and Wei et al. [39, 40] have presented evidence indicating that replication of HIV in vivo is continuous, highly productive, and causes a rapid turnover of helper T cells. A high rate of HIV replication is of course conducive to mutation and therefore bolsters the mutation escape hypothesis [37, 38]. It is important to recognize, however, that both studies were of patients with relatively advanced disease (mean of 80 and 120 CD4+ cells mm$^3$, respectively). Patients with less advanced
disease exhibit lower overall viral load and thus probably lower rates of T cell turnover [41]. The factors that underlie the progression to a higher rate of viral replication remain to be elucidated, and may include the progressive deletion of thymocytes and peripheral T cells specific for HIV peptides. The loss of such cells would tend to release extant HIV from immune system control, as argued above, and simultaneously should reduce the immune system’s ability to control emerging mutant versions of HIV. Thus, augmenting the supply of HIV-specific T cells may be the best way to prevent or reverse the situation described by Ho et al. and Wei et al. [39, 40]. In other words, in reference to the analogy drawn by Ho et al. [39] (which likens the dynamics of helper T cell depletion in advanced HIV disease to a sink in which the tap and drain are both wide open and the water level is declining), the best way to plug the drain may be to tinker with the tap, i.e. augment the supply of HIV-specific T cells (see below).

A number of factors not yet mentioned, but widely discussed in the literature, may also play important roles in HIV pathogenesis, such as syncytia formation [42], autoimmune phenomena [25], the production of superantigen [43,44], infectious cofactors [45], a switch from ‘type 1’ helper T cell cytokines (e.g. IL-2, IL-12) to ‘type 2’ cytokines (e.g. IL-4, IL-5), [46] and incomplete signal transduction leading to programmed cell death [47]. This latter idea involving programmed cell death has parallels to the hypothesis being proposed here. However, the authors are suggesting that cell death and the events that precipitate it occur in the periphery, not the thymus. More importantly, they suggest that inappropriate programmed cell death (which may be due to interaction of soluble HIV gp 120 with CD4) can occur in any helper T cell; they do not emphasize, as the present hypothesis does, destruction of thymocytes and T cells with receptors specific for HIV antigen. Neither hypothesis is mutually exclusive, but the mechanism of action proposed here might more efficiently unleash HIV from immune system control, in so far as cells specifically reactive to HIV are the same cells which initially, and effectively, control HIV infection.

Evidence of a role for superantigen in HIV pathogenesis is, in particular, increasingly compelling. Initially, it was suggested that HIV superantigen may promote destruction or anergy in broad classes (specific Vβ subsets) of mature T cells [44, 48]. One might propose a parallel argument for the deletion of broad classes of thymocytes by apoptosis induced by superantigen expressed in the thymus (as is known to occur with, for example, mouse mammary tumor virus superantigen) [44, 48]. However, more recent reports indicate that HIV superantigen does not preferentially delete specific Vβ subsets of T cells [49, 50]. Rather, these same studies indicate that HIV superantigen activates T cells carrying selected Vβ subsets, in turn promoting HIV infection
and replication. This issue, including the possibility of superantigen-induced apoptosis of thymocytes, deserves further study.

In short, a large number of factors probably contribute to HIV pathogenesis. However, the ability of the immune system to control the extensive viremia of early infection suggests that these factors become insurmountable only after the immune system’s ability to detect and eliminate HIV significantly deteriorates. Infiltration of the thymus and subsequent inability to replace killed peripheral HIV-specific helper and cytotoxic T cells may be a significant cause of this deterioration.

In addition to the immunological evidence discussed earlier, the epidemiological pattern of disease progression also is consistent with the backdoor hypothesis. One would expect that individuals infected early in utero, before a significant number of HIV reactive thymocytes can develop into mature T cells, would progress to AIDS more quickly than any other category of individual [30]. Individuals infected in childhood (after about age two), when the pool of HIV-specific T cells is probably near a maximum, should accordingly be the slowest to progress to AIDS. Infants infected perinatally should progress at an intermediate rate, albeit many additional factors may contribute significantly to progression, such as generalized immune suppression secondary to drug addiction during uterine development, poor maternal nutrition, perinatal coinfection, and so on. Following adolescence, the rate of progression should again begin to accelerate, slowly at first, because the pool of T cells potentially able to control HIV infection is expected to become less vigorous as the combination of thymic contamination and involution greatly reduce the output of new cells capable of controlling HIV infection.

The existing data accord well with each of these predictions: Anecdotal reports suggest that early in utero infection correlates with extremely rapid disease progression and almost complete inability to mount a response to HIV [30]. Furthermore, there is a well established positive association between age and rate of disease progression [51, 52], with one study of 111 patients (ages 2–77 years) demonstrating that (a) slowest progression is among 2–9 year olds, and (b) controlling for helper T cell levels, older patients have a greater risk of progressing to AIDS at any given time during the course of infection [51]. These findings are intriguing because one possible explanation for them is that – due to thymic involution and the progressive conversion of naive T cells to memory T cells – the elderly begin with a smaller pool of naive T cells, including those with the potential to control HIV, and they are less able to replenish killed HIV-specific T cells.

The backdoor hypothesis suggests several therapeutic strategies. First, it might be appropriate to try to use reverse transcriptase inhibitors or other antivirals in a manner that would minimize HIV contamination of the thymic compartment.
mus. Treatment during acute viremia should probably be avoided since this is a time when virus numbers are high and generation-time is short, which is a recipe for the rapid evolution of resistance [31]. However, antiretroviral therapy started soon following acute viremia might offer a few advantages over later treatment. The ability of HIV to produce escape mutants may be lowest immediately following acute infection (when virus numbers, replication, and thus variability on which selection can act are lowest), and therefore treatment at this time may be the most effective for reducing HIV's ability to continuously contaminate the thymus. This assumes, however, that the thymus can be effectively cleared of HIV antigen following acute infection (see above) [18].

Second, since host T cells are capable of controlling HIV early in infection, identifying such cells, expanding them, and reinfusing them at later dates (or transfusing them from suitably matched donors) should, in principle, be therapeutic [53]. That is, we should attempt to do what the contaminated thymus is no longer able to do: provide a fresh supply of HIV reactive T cells.

Third, therapeutic vaccines initiated early in infection, while significant numbers of peripheral T cells reactive to HIV still exist, should augment the peripheral armamentarium against HIV, making replacement T cells coming from the thymus less crucial. Simultaneously, an upregulated response to infection may help to clear the contaminated thymus of HIV.

Fourth, strategies for reconstituting the integrity of the thymus can be imagined. For example, Kampinga et al. [18] have developed methods for vascularized thymus transplants, however, their technique uses syngeneic rats, thus overcoming what otherwise might prove to be insurmountable difficulties with regard to donor matching. The immunosuppressive drugs normally necessary with less than perfectly matched transplants seem antithetical to the mission here. Moreover, the rapid turnover of transplanted thymic dendritic cells and their replacement by host cells is extremely problematic.

Additional methods for protecting the thymus from contamination should be sought. For example, it may be possible to alter the major histocompatibility complex of some thymic dendritic cells so that they would be unable to present particular HIV proteins, and hence be unable to delete HIV reactive thymocytes.

Acknowledgments

I thank Khalid Ahmed, Laura Betzig, Kevin Flurkey, Eyassu Habte-Gabr, Richard Lenski, George Williams, and one anonymous referee for a number of very helpful suggestions.
References

12. Ref. 6, p. 179.

Address for correspondence: Paul W. Turke, PhD, College of Human Medicine, Michigan State University, Hurley Medical Center, One Hurley Plaza, Flint, MI 48503-5993 USA
Phone: (810) 232-7000; Fax: (810) 232 7020