The immune control and cell-to-cell spread of human T-lymphotropic virus type 1

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Human T-lymphotropic virus type 1 (HTLV-1) varies little in sequence compared with human immunodeficiency virus type 1 (HIV) and it is difficult to detect HTLV-1 mRNA, proteins or virions in fresh blood. But the strong and chronically activated T cell response to the virus indicates that HTLV-1 proteins are expressed persistently. It now appears that the efficiency of an individual’s cytotoxic T cell (CTL) response to HTLV-1 is the chief single determinant of that person’s provirus load, which can differ between HTLV-1-infected people by more than 10 000-fold. Progress is now being made towards defining this CTL ‘efficiency’ in terms of host genetics, T cell function, T cell gene expression and mathematical dynamics. Lymphocytes that are naturally infected with HTLV-1 do not produce enveloped extracellular virions in short-term culture and this has reinforced the erroneous conclusion that the virus is latent. But recent evidence shows that HTLV-1 can spread directly between lymphocytes across a specialized, virus-induced cell–cell contact – a ‘viral synapse’. Instead of making extracellular virions, HTLV-1 uses the mobility of the host cell to spread within and between hosts. In this review the evidence is summarized on the persistent gene expression of HTLV-1 in vivo, the role of the immune system in protection and pathogenesis in HTLV-1 infection, and the mechanism of cell-to-cell spread of HTLV-1.

INTRODUCTION

Human T-lymphotropic virus type 1 (HTLV-1) is associated with two distinct types of disease: adult T cell leukaemia/lymphoma (ATL) and a range of chronic inflammatory diseases. The best-recognized chronic inflammatory disease is HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP), in which lesions in the central nervous system (CNS) cause progressive weakness, stiffness and paralysis of the legs. Much of the interest in HTLV-1 infection has been focused on two questions: why do certain individuals develop ATL or a chronic inflammatory disease such as HAM/TSP, and how does HTLV-1 persist in spite of a vigorous immune response? In this review I shall consider these two questions, with particular attention to the inflammatory diseases such as HAM/TSP. Whereas HTLV-1 was considered previously to be largely latent in vivo, the picture that is emerging is one of an intense, continuous battle between a highly adapted virus and the cellular immune response, in which the outcome is largely decided by the efficiency of the host’s response.

HTLV-1

HTLV-1 is classified as a complex retrovirus, in the genus Deltaretrovirus of the subfamily Orthoretrovirinae.

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The diploid plus-strand RNA genome is 9032 nucleotides long. In addition to the gag, pol and env genes found in a typical exogenous retrovirus, HTLV-1 encodes a number of small regulatory proteins, including Tax and Rex. The exact number and actions of these regulatory proteins are not yet agreed, but certain points are clear: Tax activates transcription of the HTLV-1 provirus, and Rex regulates the intracellular transport of unspliced and singly spliced HTLV-1 mRNAs. Tax protein also activates transcription of several host genes (Hollsberg, 1999; Yoshida, 2001): some – such as CD25 – by a direct effect on the host gene promoter, and others – such as IL-2 – as a secondary consequence of the powerful T cell activation induced by Tax. The reader is referred elsewhere for reviews of the molecular biology of HTLV-1 (Hollsberg, 1999; Green & Chen, 2001; Johnson et al., 2001; Yoshida, 2001; Albrecht & Lairmore, 2002), its epidemiology (Mueller & Blattner, 1997) and strain variation and phylogeny (Slattery et al., 1999). Reviews of the clinical features of HTLV-1-associated diseases may be found in Nakagawa et al. (1995) and Watanabe (1997).

The provirus load of HTLV-1 usually reaches a stable equilibrium ‘set point’ that fluctuates in most cases by no more than 2- to 4-fold over a period of years (Matsuizaki et al., 2001). This provirus load is frequently very high: in a Japanese population, the median provirus load was 5% PBMCs in patients with HAM/TSP and 0-3% in asymptomatic HTLV-1 carriers (Nagai et al., 1998; see below). In
contrast with HIV-1, the between-isolate and within-isolate sequence variation of HTLV-1 is very limited (Niewiesk et al., 1994; Slattery et al., 1999). However, there are minor variations in sequence between geographical regions (Slattery et al., 1999), and indeed certain HTLV-1 subgroups, defined by nucleotide sequence, are associated with different risks of HAM/TSP (Furukawa et al., 2000; see below).

The HTLV-1 provirus is found chiefly in CD4+ T cells in vivo, but up to a quarter of the provirus load may be carried by CD8+ T cells (Hanon et al., 2000a). The cellular tropism of HTLV-1 and the question of whether the virus is latent or persistent are considered below.

The immune response to HTLV-1
The immune response to HTLV-1 is typically strong. The serum antibody titre correlates with the provirus load of HTLV-1 (Nagai et al., 1998), and may exceed 1:256,000. However, it remains unclear whether this high antibody titre contributes significantly either to the protection from or pathogenesis of HTLV-1-associated disease, or to controlling the equilibrium provirus load. The fact that HTLV-1 appears to be able to spread directly from cell to cell, without the need to form enveloped extracellular virions (Igakura et al., 2003), suggests that HTLV-1 has a limited exposure to selection pressure exerted by antibody. However, Env protein is expressed on the surface of naturally infected lymphocytes (Igakura et al., 2003), albeit at a low level, and anti-Env antibodies could therefore reduce the efficiency of cell-to-cell transmission of HTLV-1.

The helper T cell response
The CD4+ T cell response to HTLV-1 has been difficult to study because HTLV-1 infection of a CD4+ T cell – the main host cell of HTLV-1 – rapidly induces activation and proliferation of the cell and expression of many host genes, including IFN-γ. These events preclude the standard assays of antigen-specific CD4+ T cells, which depend on antigen-induced cellular proliferation or cytokine production. Using a short-term ELISPOT assay to circumvent this problem, Goon et al. (2002) found that the median frequency of HTLV-1-specific CD4+ T cells was 25 times greater in patients with HAM/TSP than in asymptomatic HTLV-1 carriers with a similar provirus load. Th1-type (IFN-γ-producing) cells predominated among the HTLV-1-specific helper T cells both in patients with HAM/TSP and in asymptomatic carriers. The high frequency of HTLV-1-specific CD4+ T cells is consistent with the hypothesis that such cells, activated by contact with HTLV-1 antigens in vivo or by infection of the cell itself by HTLV-1, cause the inflammatory lesions that result in tissue damage in the associated diseases such as HAM/TSP.

The dominant HTLV-1 antigen recognized by CD4+ T cells was Env protein, followed by Gag, Pol etc. (Goon et al., 2002). Interestingly, there was evidence of preferential HTLV-1 infection of these virus-specific CD4+ T cells: although most of the provirus was present in cells of other specificities, HTLV-1 was detected consistently at a higher frequency in HTLV-1-specific CD4+ T cells than in human cytomegalovirus-specific CD4+ T cells (Goon et al., 2002). The question arises whether such preferential infection impairs the immune response to HTLV-1. However, it is not possible to draw simple and robust conclusions on this point because of the complexity of the dynamics of interactions between helper T cells, virus-infected cells and other components of the immune response, notably cytotoxic T cells.

More precise analysis of the functions of HTLV-1-specific helper T cells will be possible when a convenient method is devised to isolate live antigen-specific CD4+ cells directly from fresh PBMCs.

CD8+ T cell response to HTLV-1
The CD8+ T cell response to HTLV-1 was first detected by Kannagi and her colleagues (Kannagi et al., 1983, 1984), who made the interesting observation that HTLV-1-infected cells become susceptible to CD8+ T cell-mediated lysis before the appearance of detectable Env protein on the cell surface. This observation presaged the discovery that cytotoxic T cells (CTL) recognize peptides derived from processed cytoplasmic proteins (Townsend et al., 1986), and suggests the important possibility of immunotherapy for ATL (see below).

The main features of this unusual CD8+ T cell response (Bangham, 2000, 2002) are the high frequency of HTLV-1-specific CD8+ T cells and their state of chronic activation. Tax protein dominates as the target antigen of HTLV-1-specific CTLs (Jacobson et al., 1990; Kannagi et al., 1991), but CTLs specific to Gag, Pol and Env have also been detected (Jacobson et al., 1990; Parker et al., 1992). Pique et al. (2000) also found CTLs specific to small putative regulatory proteins of HTLV-1, including ‘Tof’ and ‘Rof’, providing strong evidence that these proteins, whose existence and actions have been debated, are indeed produced in vivo. Interestingly, Rex protein does not appear to be a target for CTLs (Smith et al., 1997): the reason for this is not understood.

In most virus infections, CD8+ T cells play a critical role in limiting virus replication, by killing virus-infected cells and by secreting IFN-γ. It was therefore natural to propose (Bangham, 1996) that HTLV-1-specific CD8+ T cells played a major part in determining the provirus load at equilibrium, and that individual variation in provirus load was caused by individual variation in the efficiency of this response. This hypothesis was consistent with the observation (Niewiesk et al., 1994) that the tax gene was subject to stronger positive selection in asymptomatic carriers of HTLV-1, who in general have a lower provirus load, than in patients with HAM/TSP.
The hypothesis has been countered by a suggestion (Jacobson, 2002) that HTLV-1-specific CD8$^+$ T cells cause the tissue damage in HAM/TSP (see 'Pathogenesis of HAM/TSP' below). These two proposals are in fact not mutually exclusive, because there is always a trade-off between the beneficial and the harmful effects of CD8$^+$ T cells. For example, the CD8$^+$ response to lymphocytic choriomeningitis virus in the mouse is responsible both for clearing the infection and (under certain circumstances) for the fatal lymphocytic choriomeningitis (Buchmeier et al., 1980). But the question remains: is the net effect of CD8$^+$ T cells beneficial or harmful in HTLV-1 infection?

The hypothesis that a strong CD8$^+$ T cell response to HTLV-1 is beneficial faces two main problems. First, the frequency of HTLV-1-specific CD8$^+$ T cells is correlated positively with the provirus load (Kubota et al., 2000), especially in asymptomatic HTLV-1 carriers (Wodarz et al., 2001), and the frequency is slightly higher in patients with HAM/TSP than in asymptomatic HTLV-1 carriers. We have found that the mean (or median) frequency of such cells in the peripheral blood is 2- to 4-fold higher in patients with HAM/TSP than in asymptomatic carriers, whether the cells are assayed by limiting dilution analysis (Daenke et al., 1996), class I tetramer binding (Jeffery et al., 1999) or IFN-$

(1) Frequency of anti-HTLV-1 CD8$^+$ T cells

Ogg et al. (1998) observed an inverse correlation between the frequency of HIV-specific CD8$^+$ T cells and the plasma virus load in subjects in the quasi-equilibrium phase of HIV infection. The intuitive interpretation of this observation is that a strong immune response reached equilibrium with a low virus load. But the proliferation rate of virus-specific T cells is stimulated by the antigen (virus) load. Therefore, one can also argue that the frequency of CD8$^+$ T cells should be positively correlated with the virus load. In fact, both experiment (Ogg et al., 1998; Kubota et al., 2000; Betts et al., 2001; Wodarz et al., 2001; Addo et al., 2003) and theory (Wodarz & Bangham, 2000; Wodarz et al., 2001; Bangham, 2002) show that variations in experimental protocol or mathematical model can readily produce either a positive, negative or zero correlation between the specific CTL frequency and virus load. The conclusion is clear: when equilibrium is reached between a persistently replicating pathogen and the immune response, the frequency of specific CD8$^+$ T cells is an unreliable index of the efficiency or effectiveness of the T cell response.

(2) ‘Efficiency’ of the anti-HTLV-1 CTL response

Nowak & Bangham (1996) showed that individual variation in the efficiency of the CTL response to a persistent virus at equilibrium could lead to wide variation in the virus load between individuals whose frequency of specific CTLs was not significantly different. This model made two experimentally testable predictions. Firstly, polymorphisms in genes that influence the efficiency of the CTL response, notably the class I MHC genes, would be associated with individual variation in the provirus load and therefore in the risk of associated diseases such as HAM/TSP. Secondly, ‘CTL efficiency’, defined in precise and (in principle) experimentally testable terms, would be greater in subjects with a low provirus load than those with a high provirus load. We have now tested both of these predictions.

In a case control study of candidate gene polymorphisms in an endemically HTLV-1-infected population in Kagoshima, southern Japan, we found that possession of either of the class I MHC alleles HLA-A$^*$02 or HLA-Cw$^*$08 was associated with a significant reduction in both HTLV-1 provirus load and the risk of HAM/TSP (Jeffery et al., 1999, 2000; Vine et al., 2002; Table 1). The likely mechanism – indeed the only plausible mechanism suggested – is that

<table>
<thead>
<tr>
<th>Genotype</th>
<th>Reduction of provirus load in asymptomatic HTLV-1 carriers</th>
<th>Risk of HAM/TSP</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Provirus load* (N)</td>
<td>$P$†</td>
</tr>
<tr>
<td>HLA-A$^*$02$^+$</td>
<td>16-8 (100)</td>
<td>0.014</td>
</tr>
<tr>
<td>HLA-A$^*$02$^-$</td>
<td>50-1 (101)</td>
<td>0.046</td>
</tr>
<tr>
<td>HLA-Cw$^*$08$^+$</td>
<td>12.0 (43)</td>
<td></td>
</tr>
<tr>
<td>HLA-Cw$^*$08$^-$</td>
<td>45.7 (159)</td>
<td></td>
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*Median proviral copy number per 10$^4$ PBMCs.
†Mann–Whitney two-tailed test (uncorrected).
‡$\chi^2$ with Yates’ correction.

Table 1. Effect of class 1 HLA alleles

Class 1 HLA alleles HLA-A$^*$02 and HLA-Cw$^*$08 reduce both the risk of the inflammatory disease HAM/TSP and the provirus load of HTLV-1 in Kagoshima, Japan. Data taken from Jeffery et al. (1999, 2000).
HLA-A*02-restricted or HLA-Cw*08-restricted CTLs are particularly efficient at killing HTLV-1-infected cells. These observations therefore strongly supported the idea that variation in CTL efficiency accounts for variation in provirus load. In further support of this conclusion, individuals who were heterozygous at all three HLA class I loci had a significantly lower provirus load than those who were homozygous at one or more loci (Jeffery et al., 2000). This observation is consistent with the idea that CTL recognition of many epitopes contributes to the efficiency of antiviral surveillance (Weidt et al., 1995; Carrington et al., 1999).

There was compelling evidence that neither chance nor genetic stratification could explain the observed protective effects of HLA-A*02 and HLA-Cw*08 in southern Japan: each of these HLA alleles was associated with a significantly lower provirus load of HTLV-1 within the control subjects alone. This effect, which is consistent with the likely mechanism of protection against HAM/TSP conferred by HLA-A*02 and HLA-Cw*08, i.e. efficient CTL-mediated killing of HTLV-1-infected cells, was independent of the significant reduction in the risk of HAM/TSP, and of any possible systematic genetic difference between patients with HAM/TSP and asymptomatic HTLV-1 carriers.

The ‘efficiency’ of CTLs was embodied in two parameters in the models of Nowak & Bangham (1996): $c$, the rate of CTL proliferation in response to a given dose of antigen, and $p$, the rate at which CTLs kill virus-infected cells. We hypothesized that ‘efficient’ anti-HTLV-1 CTLs would overexpress genes concerned with cell division, or cell-mediated lysis, or both. To test this hypothesis, we used nucleotide microarrays to assay the mRNA expression of 12,000 genes in fresh ex vivo CD4+ cells and CD8+ cells from groups of asymptomatic HTLV-1 carriers with a low provirus load, carriers with a high provirus load, patients with HAM/TSP, and uninfected controls. Cluster analysis of the resulting gene expression profiles (A. M. Vine and others, unpublished data) showed a remarkably clear result in each of three independent experiments. A low provirus load of HTLV-1 was associated with overexpression of a ‘core group’ of about 12 genes in the CD8+ cells. Of these 12 genes, 10 encode proteins that are directly concerned with the lytic mechanism by which a CTL kills its target cell, including granzymes A, B, M, K, perforin, NKG2D and granulysin. In contrast, there was no distinct pattern of upregulation of cell cycle-related genes in these same cells. Thus, although the frequency of HTLV-1-specific CD8+ cells is lower in subjects with a low provirus load, such individuals express higher total levels of mRNAs of lysis-related genes in their circulating CD8+ cells than do individuals with a high provirus load.

We concluded that a vigorous CD8+ cell response to HTLV-1 reduces the equilibrium provirus load. But how important is this effect? More precisely, what proportion of the observed between-person variation in HTLV-1 provirus load is attributable to variation in the efficiency of their respective CTL response to the virus? In recent experiments we have measured the rate of CD8+ cell-mediated lysis of autologous ex vivo HTLV-1-infected cells (B. Asquith, and others, unpublished data). The results indicate that up to 50% of the variation in the provirus load observed between asymptomatic carriers is accounted for by variation in the rate of CTL-mediated lysis (see above). Differences in the rate of CTL-mediated lysis appear to account for a lower proportion (around 35%) of variation in provirus load between patients with HAM/TSP. The reasons for this difference are not yet known.

Pathogenesis of HAM/TSP: does the immune response contribute?

Even if the net effect of a strong immune (particularly CD8+) T cell response to HTLV-1 is to reduce both the provirus load and the risk of HAM/TSP, as argued above, it is possible that the immune response contributes to the tissue damage observed in the CNS. This hypothesis has been reviewed recently by Jacobson (2002). Activated CD4+ and CD8+ T cells have been found in white matter lesions in HAM/TSP, and activated lymphocytes and high titres of anti-HTLV-1 antibodies have been found in the cerebrospinal fluid (CSF) of patients with HAM/TSP. The frequency of HTLV-1-specific CD8+ T cells in CSF can exceed the frequency of such cells in the blood (Greten et al., 1998); the frequency of HTLV-1-specific CD4+ T cells in CSF has not been measured. The reason for this enrichment of HTLV-1-specific cells in the CSF is not known: a simple hypothesis is that circulating T cells, activated by the abundant HTLV-1 antigen, are more likely to leave the circulation and enter the CNS than are resting T cells (Wekerle et al., 1986).

The presence in the CNS of abundant antibody and T cells specific to HTLV-1 raised the questions whether and how they contribute to the tissue damage observed. Direct damage to HTLV-1-infected cells is unlikely to contribute, because few (Lehky et al., 1995) if any (Matsuoka et al., 1998) resident CNS cells become infected with HTLV-1. It is possible that HTLV-1-specific antibody or T cells also recognize a cell antigen expressed by CNS cells. Recently, Levin et al. (2002) have obtained intriguing evidence for such a mechanism in HAM/TSP. But although this mechanism might contribute to tissue damage in HAM/TSP, it cannot be the main or the only mechanism, because it is difficult to explain either the initiation or the distribution of inflammatory lesions by this mechanism alone.

Because HAM/TSP occurs only in the human CNS, formal tests of the mechanisms of pathogenesis are impossible and the evidence will therefore remain circumstantial.

Why do some individuals develop HAM/TSP?

The evidence reviewed above indicates that the CTL response to HTLV-1 plays a major role, perhaps the decisive
role, in determining the equilibrium provirus load of HTLV-1. But what are the factors that predispose an infected person to develop an inflammatory disease such as HAM/TSP? In a population immunogenetics study in Japan, in addition to class 1 HLA genotype, we examined the influence of *HLA-DRB1*/*0101* (‘DR1’), which had previously been reported to predispose to HAM/TSP, and 58 other single nucleotide polymorphisms in 39 other loci. Logistic and multivariate regression techniques were then used to identify the genes and other factors that had a statistically significant, independent effect in determining either the provirus load of HTLV-1 or the risk of HAM/TSP. The results (Vine et al., 2002; Table 2) showed that polymorphisms in the TNF-α promoter and the chemokine gene SDF-1 influenced the risk of HAM/TSP significantly. From these data we derived a logistic equation that allowed the calculation of the odds of HAM/TSP in an HTLV-1-infected person in Kagoshima of specified age, provirus load, and genotype at four loci: TNF-863, SDF-1 + 801, HLA-A and HLA-C. In addition, the subgroup (A or B) of HTLV-1 affected the risk of HAM/TSP (Furukawa et al., 2000; Vine et al., 2002).

These factors explained a remarkably high proportion (88%) of the risk of HAM/TSP in the study cohort. However, the provirus load is still present as a predictive factor in this logistic equation. A complete understanding of the factors that influence the control of HTLV-1 replication and the risk of HAM/TSP will not include the provirus load as a factor in the logistic equation, since the factors that determine provirus load will themselves be specified in the equation. It is likely that other host polymorphisms influence the provirus load and the risk of provirus load: however, the size of the cohort in Kagoshima limits the statistical power of population genetic surveys.

The effect of the TNF-α promoter polymorphism was particularly interesting because of a strong interaction with provirus load. That is, the −863A allele conferred a 10-fold increased risk of HAM/TSP among people whose HTLV-1 provirus load was greater than 2 copies per 100 PBMCs, but the TNF-α allele had no effect if the provirus load was below this apparent threshold. Asquith & Bangham (2000) suggested the following explanation for this effect. In a patient with HAM/TSP, abundantly expressed HTLV-1

### Table 2. Best-fit logistic regression equation for the risk of HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) in the Kagoshima HTLV-1 infected cohort (*n* = 402)

<table>
<thead>
<tr>
<th>Factor, condition</th>
<th>ln(odds of HAM/TSP)*</th>
<th>Odds ratio (P)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Constant</td>
<td>−1.716</td>
<td>−1†</td>
</tr>
<tr>
<td>Age</td>
<td>−(0.145 × age) + (0.003 × age²)</td>
<td>−†</td>
</tr>
<tr>
<td>Provirus load</td>
<td>+ (0.460 × load) + (0.487 × load²)</td>
<td>−†</td>
</tr>
<tr>
<td>TNF −863A⁻</td>
<td>3.057 −(4.616 × load) + (1.476 × load²)</td>
<td>−†</td>
</tr>
<tr>
<td>SDF-1 + 801AA</td>
<td>−0.808</td>
<td>0.45 (0.042)</td>
</tr>
<tr>
<td>SDF-1 + 801AA</td>
<td>−1.689</td>
<td>0.18 (0.003)</td>
</tr>
<tr>
<td>HLA-A*02⁺</td>
<td>−0.638</td>
<td>0.53 (0.043)‡</td>
</tr>
<tr>
<td>HLA-Cw*08⁺</td>
<td>−0.894</td>
<td>0.41 (0.046)‡</td>
</tr>
<tr>
<td>HTLV-1 subgroup B</td>
<td>−1.587</td>
<td>0.20 (0.017)</td>
</tr>
</tbody>
</table>

*The natural logarithm of an individual’s odds of HAM/TSP in the cohort is calculated as the sum of the components in the central column, contingent on the factors indicated in the left-hand column. Load denotes log₁₀ (proviral copy no.) per 10⁴ PBMCs; age is given in years. HTLV-1 ‘cosmopolitan’ type subgroups are either A or B. The odds ratio (OR) of developing HAM/TSP conferred by each respective genotype is shown in the right-hand column. This equation correctly classifies 88.0% of patients with HAM/TSP in this Japanese study cohort. The prevalence rate (P) of HAM/TSP in HTLV-1 infected individuals of a given genotype may be calculated as R = H × OR/(1 + OR), where H is the prevalence of HAM/TSP in the HTLV-1-infected population and OR is the OR of developing HAM/TSP associated with that genotype. For example, the prevalence of HAM/TSP in HLA-A*02⁺ individuals in Kagoshima = 0.01 (0.53/1.53) = 0.3%, taking H in Kagoshima = 1%.†ORs for the continuous variables (age and load) are omitted because their quadratic terms cause the ORs to vary over age and load. Similarly, an OR for TNF −863A is not given as its interaction term with provirus load causes the OR to vary over load.‡The HLA class 1 alleles A*02 and Cw*08 exert their strong effects on the outcome of HTLV-1 infection primarily through an effect on provirus load (Jeffery et al., 1999, 2000). The one-tailed *P* values given here relate to the additional effects of A*02 and Cw*08 after taking into account their effect on load. Table reproduced, with permission, from Vine et al. (2002), courtesy of the University of Chicago Press. Copyright 2002 by the Infectious Diseases Society of America. All rights reserved.
antigens – notably Tax – stimulate CD8+ T cells to produce inflammatory cytokines, including TNF-α and IFN-γ. In a healthy HTLV-1 carrier, by contrast, even one whose provirus load is as high as a typical patient with HAM/TSP, there is less HTLV-1 antigen expressed, perhaps because of more efficient CTL surveillance. The lower antigen abundance in these healthy carriers falls below the threshold required (Valitutti et al., 1996) for the CD8+ cells to produce TNF-α and IFN-γ, although there is still sufficient antigen to induce CTL-mediated lysis.

The mechanisms responsible for the association of HLA-DRB1*0101 and HLA-B*54 with an increased risk of HAM/TSP remain unexplained. Experiments are now under way to examine the gene and protein expression in TSP, but the associations seem highly improbable that the fundamental conclusion, that the CD8+ T cell response is a major factor determining the provirus load of HTLV-1 and the risk of HAM/TSP, will differ in other populations.

There are case reports of HAM/TSP developing within a few months of transfusion with HTLV-1-infected blood (Kaplan et al., 1991; Gasmi et al., 1997), but cases of ATL have not been reported so soon after transfusion. This observation raised the possibility that the route of infection determines the provirus load and the risk of different HTLV-1-associated diseases. It has also been suggested (Hasegawa et al., 2003) that infection by the oral route might lead to a degree of immunological tolerance of HTLV-1. However, in areas of endemic HTLV-1 infection the great majority of people have been infected by the mucosal route, by breast feeding or sexual contact, and cases of ATL and HAM/TSP both result. Further, the epidemiological evidence suggests that the provirus load (the 'set point' in each individual) is independent of the route of transmission (Nakagawa et al., 1995; Iga et al., 2002). A simpler explanation of the apparent association between ATL and HTLV-1 infection in childhood (or by the mucosal route) is that ATL requires the accumulation of several mutations, like other malignancies, and that many years are necessary, on average, for these unlikely events to occur. Since most people who have been infected for many years were infected as children or young adults, most will have been infected by breastfeeding or sexual contact, rather than by transfusion.

Non-lytic protective effects of CD8+ cells

As shown in Table 1, possession of HLA-A*02 or HLA-Cw*08 was associated with a lower provirus load of HTLV-1 in southern Japan. However, the logistic regression analysis (Table 1) also showed that possession of either of these alleles was associated with a significant additional reduction in the risk of HAM/TSP, even after the effect on provirus load had been taken into account. This observation suggests that part of the protective effect of class I MHC-restricted T cells against HAM/TSP is exerted through a mechanism that is independent of provirus load. One possibility is that these T cells inhibit HTLV-1-induced inflammation. Finally, the possibility cannot be formally excluded that these genetic effects are the result of the action of non-HLA genes that are linked to MHC class 1.

Coevolution of HTLV-1 and the immune response: reciprocal selection between the virus and CD8+ T cells

The Tax protein is usually immunodominant in the CTL response to HTLV-1 (Jacobson et al., 1990; Kannagi et al., 1991; Parker et al., 1992), although in some individuals vigorous responses can also be detected with the other HTLV-1 proteins, especially Pol (Parker et al., 1992).

This abundant, chronically activated CD8+ T cell response would be expected to exert significant selection pressure on the virus and evidence of such selection was indeed obtained by Niewiesk et al. (1994). Naturally occurring sequence variants of Tax escape recognition by fresh autologous CTLs (Niewiesk et al., 1995), consistent with the idea that CTL selection favoured the emergence of these variant Tax sequences. However, recombinant Tax proteins that contained these putative CTL escape mutations were highly defective in their transactivating activity (Niewiesk et al., 1995). It is therefore likely that negative selection on the virus due to defective Tax function balances the positive selection exerted by anti-Tax CTLs. The inability of Tax to tolerate amino acid changes (Smith & Greene, 1990; Niewiesk et al., 1995) may explain the continued effectiveness of CTL-mediated control of HTLV-1 replication: Tax is essential to the infectious cycle of HTLV-1, and is the first HTLV-1 protein to be expressed.

Just as the strong CTL response appears to exert selection on the tax gene, so the persistently expressed Tax protein would be expected to exert selection on the T cells. Specifically, after months or years of continuous antigenic stimulation, one should observe selection of T cell antigenic receptors (TCRs) with a particularly high affinity for HTLV-1 Tax peptides. Such selection has been observed by Saito et al. (2001): in HTLV-1-infected subjects with the HLA-A*02 allele there was a strong predominance of
a four-aminio-acid motif (Gly-Leu-Ala-Gly) in the hyper-variable region (CDR3) of the TCR V\textsubscript{b}13.1 that makes contact with the A2/Tax\textsubscript{11–19} antigenic complex. By chance, the first complex of human TCR/MHC/peptide whose X-ray crystallographic structure was determined (Garboczi et al., 1996) also consisted of TCR V\textsubscript{b}13.1/HLA-A2/Tax\textsubscript{11–19}. Remarkably, the same motif (Gly-Leu-Ala-Gly) was present at the tip of the TCR CDR3 loop in this complex. The crystallographic structure (Fig. 1) showed that the Leu residue at position 98 in the CDR3 loop made particularly strong hydrophobic interactions with the A2/Tax\textsubscript{11–19} complex. Substitution of single amino acids in the Tax\textsubscript{11–19} peptide reduced the affinity of binding of the A2/Tax peptide complex by the TCR of two T cell clones (Hausmann et al., 1999), strengthening the conclusion that the sequence of Tax\textsubscript{11–19} appears particularly well suited to binding HLA-A2.

It is therefore possible that the interaction between HLA-A2 and the Tax\textsubscript{11–19} peptide, in particular the hydrophobic interaction made between the TCR V\textsubscript{b}13.1 CDR3 Leu-98 and two positions on the Tax peptide, accounts for a significant fraction of the population-level protection given by HLA-A*02 in HTLV-1 infection in southern Japan (Jeffery et al., 1999; see above).

**How much does reverse transcriptase contribute to the maintenance of HTLV-1 provirus load?**

The evidence of the CD8\textsuperscript{+} T cell response indicates that there is persistent widespread HTLV-1 antigen expression in vivo. But how frequently can HTLV-1 complete the replication cycle? That is: is the HTLV-1 provirus load maintained mainly by proliferation of infected CD4\textsuperscript{+} T cells – the ‘mitotic’ route of retrovirus replication (Wodarz et al., 1999; Overbaugh & Bangham, 2001) – or by full-cycle replication via reverse transcriptase – the ‘infectious’ route? The genetic evidence of positive selection on the HTLV-1 tax gene (Niewiesk et al., 1994; see above) indicated that reverse transcription had made a discernible contribution to the provirus load, because retroviruses are exposed to strong selection only when they replicate by the ‘infectious’ route, using reverse

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**Fig. 1.** Crystal structure illustration of the A6 TCR HLA-A2-Tax11–19. One corner of the apex of the V\textsubscript{b} CDR3 loop (GLAG) inserts into a hydrophobic pocket formed by the \(\alpha\)-1 helix of HLA-A2 and the side-chain of the Tyr residue at position 8 in the Tax 11–19 peptide. The hydrophobic Leu residue at position 98 on the TCR V\textsubscript{b} loop makes several strong interactions with both the Tax peptide and HLA-A2. Leu is strongly favoured (34 of 38 clones) at this position in the V\textsubscript{b}13.1-containing clonally expanded CD8\textsuperscript{+} T cells that bind the HLA-A2-Tax11–19 tetramer. Reproduced, with permission, from Saito et al. (2001), courtesy of the American Society for Microbiology.
transcriptase. Furthermore, the ability of nucleoside analogues to reduce provirus load (Taylor et al., 1999), albeit temporarily, showed that reverse transcriptase makes an important contribution to the provirus load, at least in some individuals. But it remains difficult to quantify the ratio of mitotic : infectious replication of HTLV-1 in vivo and, therefore, to test the important possibility that this ratio varies between individuals.

Theory (Wodarz et al., 1999) indicates that, even if the ratio of the per-cell rates of infectious and mitotic spread of HTLV-1 remains constant throughout infection – the most economical hypothesis – the net contribution of reverse transcription (infectious spread) may be very small when the system reaches equilibrium. This explanation could reconcile the evidence for persistent replication of HTLV-1 by the infectious pathway with the observed relative sequence constancy of the HTLV-1 provirus.

**Why is HTLV-1 expressed at a low level in peripheral blood?**

If the above analysis is correct, i.e. that HTLV-1 is not latent in vivo but is transcribed and replicating persistently, then one would expect to detect expression of HTLV-1 proteins in freshly isolated PBMCs. HTLV-1 proteins can indeed be detected in fresh (uncultured) PBMCs in some infected individuals (Moritoyo et al., 1999; Hanon et al., 2000b), but the fraction of CD4+ T cells that express HTLV-1 is always considerably lower than the proportion of provirus-containing cells in the blood (Moritoyo et al., 1999; Hanon et al., 2000b). What is the cause of this discrepancy? It has been postulated that HTLV-1 transcription is repressed by a factor in serum or by a product of HTLV-1 itself, such as the Rex protein (Mortreux et al., 2001; Yoshida, 2001), leading to transient HTLV-1 expression by individual cells. But there is no evidence of such a factor in serum, and Rex is expressed from the same mRNA as Tax, so Rex-expressing cells must already have been exposed to lysis by Tax-specific CTLs.

A simpler explanation of the low HTLV-1 protein expression in peripheral blood is as follows. T cells spend the majority of their lives in the lymph and the solid lymphoid organs, not in the blood. The typical transit time of a T cell in the mammalian lymphoid system is of the order of several hours: ~5 h in the spleen and 12 to 24 h in peripheral lymph nodes (Ford, 1975; Westermann et al., 1988, 1993; Fabst et al., 1993). Furthermore, because Tax protein upregulates the expression of several adhesion molecules (Valentin et al., 1997; Yamamoto et al., 1997), an HTLV-1-infected T cell may progress abnormally slowly through the lymphoid system. However, the T cell transit time in the blood is only around 30 min (Schick et al., 1975). A T cell that starts to express Tax during its transit in the lymphoid system is therefore unlikely to re-emerge into the blood, because most will be killed by the abundant activated Tax-specific CTLs before they can do so. Thus the lymph nodes and the spleen may act as a filter that removes HTLV-1-expressing lymphocytes from the circulation. An HTLV-1 provirus-containing cell that emerges into the circulation has only approximately 30 min in the blood during which it can start to express Tax. Therefore, if we make the simplest assumption that the kinetics of Tax expression in vivo are the same as the kinetics in vitro (Hanon et al., 2000a, b), it follows that the fraction of provirus-positive cells that express detectable levels of Tax protein in the blood will be very low.

The above discussion relates only to non-malignant HTLV-1 infections. In ATL similarly, HTLV-1 provirus transcription rises spontaneously and rapidly during short-term in vitro incubation of lymphocytes in a proportion of cases. The Tax gene appears to be conserved selectively in ATL. However, HTLV-1 transcription in ATL may be subject to quite different constraints and selection forces (Kannagi et al., 1993), and is beyond the scope of this review: the reader is referred to Uchiyama (1997) and Yoshida (2001) for a useful discussion. The immune response to HTLV-1 has not been well-studied in ATL patients. However, it is worth noting that Kannagi et al. (1983, 1984) first detected the anti-HTLV-1 CTL response by assaying the lysis of ATL cells, and a CTL response was detected only in patients in remission from ATL. More work is needed on the immune response to HTLV-1 in ATL because of the important possibility of immunotherapy for this serious and refractory illness.

**How is HTLV-1 transmitted?**

Because the provirus load of HTLV-1 is often so high, one would expect to see evidence of HTLV-1 virions released from naturally infected lymphocytes, either spontaneously or after incubation in vitro. However, HTLV-1 virions are produced only by certain continuous in vitro T cell lines: fresh, naturally infected lymphocytes do not produce cell-free particles. Furthermore, of the cell-free HTLV-1 virions that are produced by transfected T cells or continuous producer T cell lines, only one in 105 to 106 is infectious (Fan et al., 1992). This particle : infectivity ratio is considerably lower even than that of other RNA viruses.

It had been known for many years that cell–cell contact is required for efficient transmission of HTLV-1 both in vivo (Okochi & Sato, 1984) and in vitro (Yamamoto et al., 1982; Popovic et al., 1983). But the mechanism of cell-to-cell transmission, and therefore the reason why it was so much more efficient than transmission by free virions, was unexplained.

We observed that HTLV-1-specific T cells are themselves infected more frequently with HTLV-1 than are T cells specific to other antigens. This preferential infection was evident in both CD8+ T cells (Hanon et al., 2000a) and in CD4+ T cells (Goon et al., 2002; P. Goon and C. R. M. Bangham, unpublished data). These observations, together with the requirement for cell-to-cell contact and the poor
infectivity of cell-free particles, raised the possibility that HTLV-1 transmission was assisted by the process of T cell antigen recognition. More precisely, HTLV-1 might spread across the ‘immunological synapse’ (Grakoui et al., 1999), the specialized area of contact that is formed between a lymphocyte and another cell in which distinct protein microdomains mediate adhesion, antigen recognition and secretion of cytokines or lytic granules.

Confocal microscopy (Fig. 2) of conjugates formed spontaneously between ex vivo CD4+ cells from an HTLV-1-infected person and autologous (or allogeneic) lymphocytes revealed a structure at the cell–cell junction which indeed resembles the immunological synapse (Igakura et al., 2003). Polarization of the adhesion molecule talin and the microtubule organizing centre (MTOC) to the cell–cell junction was accompanied by accumulation of the HTLV-1 core protein Gag and the HTLV-1 genome at the cell–cell junction (Fig. 2). After 2 h, both the Gag protein and the HTLV-1 genome were transferred from the infected to the uninfected cell (Igakura et al., 2003; Fig. 2).

The polarization and organization of talin at the cell–cell junction and the polarization of the MTOC are also characteristic of the immunological synapse (Grakoui et al., 1999): The MTOC is polarized to the cell–cell junction inside the responding T cell, i.e. the T cell that recognizes antigen presented on the surface of the other cell in the conjugate. However, there was an important difference in the conjugates formed with an HTLV-1-infected T cell: here, the MTOC was polarized inside the infected cell, not towards it (Table 3). This observation implies that the polarization events were not triggered by TCR-mediated recognition of HTLV-1 antigens, but rather by a combination of two signals: HTLV-1 infection and cell contact. Therefore, the structure observed at the cell–cell

![Fig. 2](http://vir.sgmjournals.org)
Table 3. HTLV-1-infected lymphocytes polarize their MTOCs to the cell–cell junction in CD4+ T cell conjugates

In Gag p19− cells the frequency of polarization of the MTOC to the cell–cell junction (24.4%) was similar to the frequency (18.7%) in lymphocytes from an uninfected control subject. However, the frequency of polarization in Gag p19+ cells (56.8%) was significantly greater ($\chi^2=99; P<0.001$). Data taken from Igakura et al. (2003) supplementary online material at http://www.sciencemag.org/cgi/content/full/1080115/DC1.

<table>
<thead>
<tr>
<th>MTOC orientation</th>
<th>Uninfected control subject</th>
<th>HTLV-1-infected subjects</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Gag p19−</td>
<td>Gag p19+</td>
</tr>
<tr>
<td>Polarized (%)</td>
<td>79 (18.7)</td>
<td>130 (24.4)</td>
</tr>
<tr>
<td>Not polarized/not seen (%)</td>
<td>343 (81.3)</td>
<td>403 (75.6)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>422 (100.0)</td>
<td>533 (100.0)</td>
</tr>
</tbody>
</table>

CONCLUSIONS

HTLV-1 is persistently transcribed in natural infection. However, full cycle replication makes a small net contribution to HTLV-1 replication at equilibrium. The actual ratio of mitotic replication (as provirus) to infectious replication (via reverse transcriptase) remains difficult to quantify.

The molecular mechanisms that trigger the formation of the ‘viral synapse’ are not yet identified. However, recently it was shown (Manel et al., 2003; Nath et al., 2003) that an early activation marker on T cells determines their susceptibility to HTLV-1 infection. Candidate molecules therefore include, among others, CD25, CD54 and CD69.

We concluded that HTLV-1 has lost the need to release cell-free virions in order to spread from cell to cell. Instead, HTLV-1 uses the mobility of the host cell to spread both within and between hosts. Since HIV-1 also spreads much more efficiently from cell to cell than by release of virus particles, it is possible that HIV-1 uses a mechanism similar to that used by HTLV-1.

The median frequency of HTLV-1-specific CD4+ cells is between 10- and 25-fold greater in HAM/TSP patients than in asymptomatic carriers with a similar provirus load. The median frequency of specific CD8+ cells is 2- to 4-fold greater in patients with HAM/TSP than in carriers with an equivalent provirus load. Since CD4+ T cells predominate in early, active lesions in HAM/TSP, the possibility must be considered that CD4+ T cells are primarily responsible for initiating the inflammatory lesions.

Finally, a higher risk of HAM/TSP in southern Japan is associated with the host genotype HLA-A2+, HLA-Cw8+, HLA-DR1+, TNFα-863A+, SDF-1+801A− and with infection with HTLV-1 subgroup A. Although the efficiency of the CTL response to HTLV-1 can account for most of the observed variation in provirus load among asymptomatic carriers and for a significant proportion of the variation in patients with HAM/TSP, it is insufficient to explain why some infected people progress to HAM/TSP. The factors responsible for this progression remain to be discovered.

REFERENCES


