The immune control of HTLV-1 infection: selection forces and dynamics

Charles R M Bangham1, Kiran Meekings1, Frederic Toulza1, Mohamed Nejmeddine1, Endre Majorovits3, Becca Asquith1, Graham P Taylor2

1Department of Immunology, Imperial College, Norfolk Place, London W2 1PG, UK, 2Department of GU Medicine and Communicable Diseases, Imperial College, Norfolk Place, London W2 1PG, UK, 3Division of Structural Biology, Wellcome Trust Centre for Human Genetics, University of Oxford, Roosevelt Drive, Oxford OX3 7BN, UK

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1. ABSTRACT

Cytotoxic T lymphocytes (CTLs) play a central role in the protective immune response to human T-lymphotropic virus 1 (HTLV-1). Here we consider two questions. First, what determines the strength of an individual’s HTLV-1-specific CTL response? Second, what controls the rate of expression of HTLV-1 in vivo, which is greater in patients with HAM/TSP than in asymptomatic carriers with the same proviral load? Recent evidence shows that FoxP3+CD4+ T cells are abnormally frequent in HTLV-1 infection, and the frequency of these cells is inversely correlated with the rate of CTL lysis of HTLV-1-infected cells, suggesting that FoxP3+CD4+ cell frequency is an important determinant of the outcome of HTLV-1 infection. There is also new evidence that the rate of expression of HTLV-1 in vivo is associated with the transcriptional activity of the flanking host genome. We suggest that the frequencies of HTLV-1-infected T cell clones in vivo are determined by a dynamic balance between positive and negative selection forces that differ among the clones because of the distinct integration site of the HTLV-1 provirus in each clone.

2. RISK OF HAM/TSP DEPENDS ON HTLV-1 PROVIRAL LOAD

There is a strong and widely reproduced association between a high proviral load of HTLV-1 and a high risk of the inflammatory disease, HTLV-1-associated myelopathy/tropical spastic paraparesis (HAM/TSP) (1-3). The largest single study of this association was carried out in a population with endemic HTLV-1 infection in southern Japan by Nagai et al (4), who showed that the prevalence of HAM/TSP rose steeply as the proviral load exceeded 1% of peripheral blood mononuclear cells (PBMCs). The majority of such studies have been cross-sectional, i.e. they were designed to test the hypothesis that a high proviral load of HTLV-1 is associated with a high prevalence of established HAM/TSP, but did not test the important implication that an asymptomatic carrier (AC) of HTLV-1 carries a high risk of subsequent development of HAM/TSP. Because of the low annual incidence rate of HAM/TSP in the HTLV-1 seropositive population, this hypothesis is difficult to test with high statistical confidence. However, a small-scale study of Afro-Caribbean populations in the UK (5) suggested that a high proviral load of HTLV-1 does indeed
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![Figure 1. Provilal load is inversely correlated with CTL lysis rate. Provilal load was plotted against the lytic rate (or 'efficacy') of antiviral CD8+ T cells, i.e. the rate at which CD8+ cells suppress Tax protein-expressing cells. Data are shown on seven ACs and nine HAM/TSP patients. A negative correlation between proviral load and antiviral efficacy was observed in both ACs and HAM/TSP patients (P = 0.03 and 0.04, respectively; Spearman’s rank correlation two-tailed test). Furthermore, for a given rate of clearance the proviral load was lower in ACs than in HAM/TSP patients (P = 0.03; two-tailed permutation test). Figure reproduced (with modification), by permission from the publisher, from (17).](image)

There is a remarkable contrast between the stability of the proviral load of HTLV-1 within each infected person over time (6), and the variation of over 1000-fold in proviral load among infected hosts (4). As in other persistent viral infections, notably HIV infection, the load appears to be a characteristic attribute of each infected host, and returns to the “set point” of that host after perturbation by drug treatment (7, 8). Since HTLV-1 varies little in sequence either within or between hosts (9-12), it follows that the variation in proviral load among hosts is caused by differences in the host rather than in the virus. These host differences are likely to be genetic in origin: see the section below (section 8) on the role of the host genotype.

3. **HTLV-1 PROVIRAL LOAD IS CORRELATED WITH THE HTLV-1-SPECIFIC CTL ACTIVITY**

CD8+ cytotoxic T lymphocytes (CTLs) specific to HTLV-1 are typically abundant in the peripheral blood in HTLV-1-infected hosts, both patients with HAM/TSP (13-15) and ACs (14, 15), see (16) for review. CTLs are of central importance in the mammalian immune response to viruses, both acute and chronic; CTLs act by killing autologous cells that express viral antigens and by suppressing viral replication by secretion of IFNγ. CD8+ lymphocytes have indeed been shown to suppress HTLV-1 expression in autologous PBMCs ex vivo (17, 18) by a mechanism that depends on class I MHC, perforin, and CD8+ cell frequency. Furthermore, because of the very high frequencies of both infected cells and CTLs in the peripheral blood, it is possible to quantify the rate of this autologous CTL-mediated lysis in fresh peripheral venous blood by measuring the effect of varying the frequency of CD8+ cells on the rate of disappearance of HTLV-1-expressing (Tax+) cells (17). This “ex vivo lysis assay” produced an important finding: both in HAM/TSP patients and in asymptomatic carriers, there was a strong negative correlation between the proportion of HTLV-1 Tax+ cells eliminated per CD8+ cell per day and the proviral load in vivo (Figure 1). This negative correlation implies either that the low proviral load causes a high rate of CTL-mediated killing or that a high rate of CTL-mediated killing suppresses the proviral load in vivo. The second conclusion is consistent with the known physiological role of CD8+ T cells in other viral infections. It is also consistent with evidence from a range of experimental approaches for the importance of CTLs in controlling HTLV-1 infection, reviewed recently in (19). This evidence comes chiefly from host immunogenetics (20-23) and gene expression microarrays (24). In addition, two groups (25, 26) have obtained evidence that the tax gene of HTLV-1 is subject to positive selection in vivo. The tax gene encodes the dominant – though not the sole – antigen recognized by anti-HTLV-1 CTLs (13, 27, 28). We have therefore hypothesized that the observed positive selection of the virus is caused by the HTLV-1 specific CTL response, implying in turn that the CTLs limit the replication of HTLV-1 in vivo (29).

4. **HTLV-1 INFECTION ACCELERATES THE TURNOVER OF BOTH CD4+ AND CD8+ T CELLS IN VIVO**

If it is correct that HTLV-1 is suppressed in vivo by constant CTL-mediated killing of HTLV-1-infected cells then, because of the high observed frequencies of both infected cells and CTLs, HTLV-1 would be expected to exert a measurable impact on the rate of turnover of T lymphocytes in an infected person. Recently it has become possible to test this prediction directly, by labelling lymphocytes in vivo with deuterated glucose administered by intravenous infusion. The results (30) showed that there was indeed a strong increase in the turnover rate of both CD8+ CTLs and of HTLV-1-infected CD4+ T cells. The lifespan of an HTLV-1 Tax+ cell in the circulation was reduced from the normal 30 days to between 1 and 10 days. Further, from the measured mean turnover rate of CD8+ cells and the known range of frequency of HTLV-1-specific CD8+ T cells (27), it was estimated that the lifespan of an HTLV-1-specific CTL in the circulation was also reduced to approximately 1 day.

Since these data represent the true rates of turnover of cell populations in vivo, not in vitro, this study has provided the most direct evidence to date that HTLV-1 infection is associated with a persistently high rate of lymphocyte proliferation in vivo. The increasing evidence for the highly dynamic nature of HTLV-1 infection in vivo emphasizes the essential role of mathematics in reaching a comprehensive and coherent understanding of this persistent infection (31).
5. PERSISTENT EXPRESSION OF HTLV-1 IN VIVO: 
T-CELL PROLIFERATION AND DIRECTIONAL, 
TRIGGERED CELL-CELL SPREAD OF 
ENVELOPED HTLV-1 VIRIONS ACROSS THE 
VIROLOGICAL SYNAPSE

The vigorous, persistently-activated cellular 
immune response to HTLV-1 provides unequivocal 
evidence of persistent HTLV-1 antigen expression in vivo. 
This conclusion appeared to conflict with evidence that 
HTLV-1 is latent in vivo, and persists instead by 
proliferation of T cells that contain silent (untranscribed) 
proviruses (32-34). The relative lack of variation of HTLV- 
1 sequence within and between isolates (9-12) also 
appeared to favour the conclusion that HTLV-1 persists 
mainly by “mitotic” replication (i.e. infected T cell 
proliferation), rather than by “infectious” replication (35) 
which is mediated by the error-prone reverse transcriptase. 
a refined development of this view is that HTLV-1 might 
be strongly expressed in the early stages of infection, and 
that expression is subsequently shut down in the second 
stage of a supposedly “two-step” trajectory (36). However, 
a simpler explanation for these observations is that, after 
the early phase of infection, the immune response – 
particularly the cell-mediated response – reduces the rate 
of replication of the virus (by both infectious and mitotic 
routes) by killing HTLV-1 antigen-expressing cells.

Cell-free HTLV-1 virions are typically 
undetectable in vivo, and cell-free blood products from 
HTLV-1-infected people are not infectious (37, 38). 
Transmission of HTLV-1 between individuals requires 
transfer of infected cells either in breast milk, semen, or 
blood. The reason that efficient spread of HTLV-1 requires 
cell-cell contact was revealed in 2003, when Igakura 
et al (39) showed that HTLV-1-infected cells form an organized 
contact with target cells that was called a virological 
 synapse (VS). Engagement of ICAM-1 on the surface of 
the infected cell (40) acted in synergy with HTLV-1 Tax 
protein (41) and engagement of ICAM-1 on 
the target cell (40).

It is likely that HTLV-1 transcription and cell-
cell spread via the virological synapse (39) are more 
widespread in the early stages of infection, before the 
emergence of the HTLV-1-specific T cell response (46). 
This putative early widespread dissemination via the 
‘infectious’ cell-cell route may be inferred from the 
observation that HTLV-1-infected hosts typically possess 
many distinct HTLV-1-infected T cell clones, although it 
has not yet been possible to estimate the total number 
of infected clones in each individual.

It remains unknown how HTLV-1 expression is 
terminated in T cells that escape destruction by CTLs. 
The small regulatory proteins of HTLV-1, notably Rex, control 
the splicing and transport of viral RNAs and so switch the 
pattern of infection in the single cell from early mRNA 
transcription to the production of mature virions that 
contain unspliced genomic RNA (47). The regulatory 
protein p30

6. THE RATE OF HTLV-1 PROVIRAL EXPRESSION 
VARIES AMONG HOSTS AND AMONG T-CELL 
CLONES WITHIN ONE HOST

There is no known association in HTLV-1 
infection between the sequence of the provirus (which, as 
noted above, varies little between hosts) and either the 
proviral load (53) or the rate of proviral expression. 
However, it is now becoming clear that there is significant 
hetogeneity among HTLV-1-infected hosts in the rate 
of expression of HTLV-1. Specifically, both we (54) and 
others (55) have shown that the level of spontaneous 
proviral expression in PBMCs ex vivo, even in the absence 
of CTLs, is systematically greater in patients with 
HAM/TSP than in ACs at a given proviral load (Figure 3). 
Further, there are indications that the rate of HTLV-1 
proviral expression varies among individual T cell clones in 
a single host. Evidence for this conclusion came from the in 
vivo lymphocyte labelling with deuterated glucose (30): it 
was shown that cells that spontaneously expressed Tax 
within 18 hours of incubation ex vivo had a greater rate of
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Figure 2. Three-dimensional ultrastructure of the VS shown by electron tomography. Cell-to-cell transmission of HTLV-1 as observed in tomograms of the VS formed between HTLV-1 infected CD4⁺ T-cell (PBMC) and an autologous uninfected CD4⁺ T-cell as a target cell. These cells were stained against HTLV-1 Gag p19 matrix protein with a specific monoclonal antibody (GIN7). A, B, Projections along the z-axis of two subvolumes of the same tomogram showing viral transmission at two different locations. C, Surface representation of the VS shown in (A, B): Several virions (blue) are trapped between the closely apposed plasma membranes (yellow). D, Tomogram slice showing an HTLV-1 particle held between the cell membranes. E, Surface representation of the virus transmission shown in D (cell membranes: yellow and orange, virus envelope: blue, virus core: magenta). F, G, Tomogram slices through the two areas of virus transmission shown in (A) and (B), respectively, with a spacing of about 17 nm (F) and 25 nm (G) between subsequent slices. Black arrows indicate a protrusion linking the virus with the cell membrane. H, Subsequent slices through the area of virus transmission shown in (D) with a spacing of about 17 nm. Black arrows indicate a protrusion linking the virus with the cell membrane. Scale bars: A,B 300 nm, D 500 nm, F,G 100nm, H 200 nm. Figure reproduced from (44).
Figure 3. Proviral expression level is greater in HAM/TSP patients than in asymptomatic carriers at a given proviral load. Correlation between HTLV-1 Tax expression and proviral load in CD4⁺ lymphocytes. The proportion of CD4⁺ cells expressing the viral protein Tax after 18 h ex vivo incubation in the absence of CD8⁺ cells was measured by flow cytometry. The frequency of Tax expression was significantly higher in lymphocytes from HAM/TSP patients than from ACs of comparable proviral load (ANOVA, two tailed test, P = 0.014. Permutation test, two-tailed, P = 0.017). This result was robust to removal of outliers: the P value either remained unchanged or decreased on removal of outliers. Figure reproduced from (54).

Figure 4. Tax⁺ cells proliferate more rapidly than Tax⁻ cells in the same individual in vivo. Deuterium enrichment was measured in Tax⁺CD4⁺CD45RO⁺ and Tax CD4⁺CD45RO⁻ cells in two individuals (L02-HAY and L07-TBI). Grey circles, enrichment in Tax⁺CD4⁺CD45RO⁺ cells; dashed line, theoretical fit; black diamonds, enrichment in Tax⁻CD4⁺CD45RO⁺ cells; solid line, theoretical fit. (Note the different y axis scales in the two parts of the figure.) Figure reproduced (with modification), by permission from the publisher, from (30).

These observations are consistent with the hypothesis that each provirus-containing T cell clone has a characteristic rate – more precisely, a characteristic probability per unit time – of onset of spontaneous proviral turnover in vivo (Figure 4). However, these cells were not detectably expressing Tax immediately after venepuncture, suggesting that Tax expression in vivo is either intermittent or occurs at a level below the threshold of detection by flow cytometry.
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However, there is no evidence that epigenetic modifications transformed infected cells and in adult T cell leukaemia, in materialy alter HTLV-1 proviral expression, both in non-unpublished data) and DNA methylation (58) can evidence that both histone acetylation (56, 57); FT, CRMB, might affect the rate of proviral expression. There is conceivable that repeated or persistent infections, unrelated to proviral expression, and that this characteristic rate is maintained in the daughter cells of that clone.

7. THE GENOMIC INTEGRATION SITE OF HTLV-1 DETERMINES THE RATE OF HTLV-1 PROVIRAL EXPRESSION AND THE RISK OF HAM/TSP

From the evidence summarized above, it appears that the rate of expression of a single HTLV-1 provirus in vivo differs both among individual hosts, and among T cell clones in the same host, independently of the proviral sequence. The question arises: what determines the rate of expression of HTLV-1 in an individual T cell? There are at least three possible factors, which are not mutually exclusive: T-cell activation, epigenetic modifications, or the genomic integration site of the provirus. First, activation of the infected T cell by either antigen or cytokines (such as IL-2 or IL-15) might result in expression of the integrated provirus: in this case, proviral expression will depend on the antigen specificity of the T cell and on the presence of antigen or cytokines in the local environment of the T cell. T-cell activation is indeed likely to be an important cause of proviral expression, but it is an interesting and unanswered question whether systematic variation in T-cell excitability among hosts might cause the observed variation in the rate of proviral expression. It is conceivable that repeated or persistent infections, unrelated to HTLV-1, activate HTLV-1-carrying T cells and so complicate the dynamics of HTLV-1 infection.

Second, epigenetic changes in the infected T cell might affect the rate of proviral expression. There is evidence that both histone acetylation (56, 57); FT, CRMB, unpublished data) and DNA methylation (58) can materially alter HTLV-1 proviral expression, both in non-transformed infected cells and in adult T cell leukaemia, in which T cells are malignantly transformed by HTLV-1. However, there is no evidence that epigenetic modifications explain the systematic differences observed in the rate of HTLV-1 expression between individuals with a high proviral load and those with a low proviral load, or between patients with HAM/TSP and ACs (57).

The possibility remained that the transcriptional activity of the provirus is determined by the transcriptional activity of the host genome flanking the provirus. We therefore tested the hypothesis that the rate of proviral expression depends on specific attributes of the genomic integration site of the provirus. We mapped the genomic integration sites of over 300 HTLV-1 proviruses from naturally-infected cells isolated from 10 patients with HAM/TSP and 10 asymptomatic carriers, and compared them with integration sites derived from in vitro infection with HTLV-1 and with random control sites in the human genome. As surrogate markers of the transcriptional activity of the genomic region surrounding the proviral integration site, we used the gene coding density (number of genes per Mb), the CpG island density, and proximity to CpG islands or to transcriptional start sites.

The results (59) showed that HTLV-1 integrates preferentially in transcriptionally active genomic regions, both in vitro and in vivo. This preferential integration is usually ascribed to the increased accessibility of the unfolded chromatin around the expressed genes. In vitro studies had shown that other retroviruses, like HTLV-1, integrate in transcriptionally active regions of chromatin (60, 61). However, there were few data on the genomic integration site of any retrovirus in vivo. The comparison of integration sites of HTLV-1 in vitro and in vivo made it possible to ask three further questions of special significance and interest in HTLV-1 infection. First, does the distribution of integration sites in vivo differ systematically from the distribution in vitro, which might indicate the action of selection forces in vivo? Second, is the rate of proviral expression associated with a particular characteristic of the genomic integration site? Third, is the character of the integration site associated with the risk of inflammatory disease such as HAM/TSP?

The study by Meekings et al (59) gave answers to these three questions. The observed bias towards integration in transcriptionally active genomic regions – for example, regions of high gene density (Figure 5) and close to CpG islands (Figure 6a) – was consistently stronger in vivo than in vitro, suggesting that frequent or sustained HTLV-1 proviral expression in vivo confers a selective advantage on that T cell clone (31). This conclusion is consistent with the evidence for the increased proliferation rate of provirus-expressing cells in vivo (30). But the result of particular interest was that integration in a transcriptionally active region of the genome was significantly associated both with a higher rate of Tax expression (Figure 6b) and with the disease HAM/TSP (59).

These data show that HTLV-1 proviruses of the same sequence that differ only in genomic location can cause different patterns of viral gene expression and so result in different disease manifestations. But what are the
forces determines the relative frequencies of individual T cell clones in vivo and, therefore, generates the spectrum of proviral integration sites present in an individual. It is increasingly clear that there is a dynamic equilibrium between spontaneous HTLV-1 proviral expression and immune surveillance in vivo, in which the frequency distribution of provirus-carrying T cell clones both determines the strength of the T cell response, by establishing the rate of proviral expression, and reflects the strength of the anti-HTLV-1 T cell immune response.

8. HOST GENOTYPE DETERMINES THE EFFICIENCY OF THE IMMUNE CONTROL OF HTLV-1 AND THE OUTCOME OF HTLV-1 INFECTION

If the equilibrium “set point” of proviral load of HTLV-1 is indeed set by a balance between spontaneous proviral expression and CTL-mediated surveillance, what then accounts for the wide variation in this set point among hosts? The above evidence suggests that two main factors are involved: the rate of proliferation of T cells in response to HTLV-1 expression, driven mainly by the Tax protein, and the rate at which CTLs kill HTLV-1-expressing cells. It is possible that the average rate of HTLV-1-induced proliferation of one individual’s T cells differs substantially from that of another individual, but there is no direct evidence that bears on this point. However, we know that individuals differ sharply in their immune responsiveness to antigens. One important cause of this variation in immune responsiveness is clear: genotypic heterogeneity in the MHC antigens, which present antigenic peptides to the T cells.

The hypothesis that HLA genotype influences the outcome of HTLV-1 infection, i.e. the proviral load and the risk of HAM/TSP, was tested in a case-control candidate gene association study in a population with endemic HTLV-1 infection in Kyushu, southern Japan (20-22). The results showed that each of two class I HLA alleles was independently associated with both a lower proviral load and a lower risk of HAM/TSP. These associations remained statistically significant even after correction for multiple comparisons. That this association was real was strongly corroborated by two further observations. First, the fact that possession of HLA-A2 or HLA-Cw8 was strongly corroborated by two further observations. First, the fact that possession of HLA-A2 or HLA-Cw8 was associated with a low proviral load within the asymptomatic HTLV-1 carrier group alone demonstrated that both the quantitative phenotype (proviral load) and the qualitative phenotype (presence or absence of HAM/TSP) were independently associated with the protective effect of these HLA alleles and that genetic stratification could not explain the observed effects. Second, heterozygosities in the HLA class I loci was also significantly associated with a lower proviral load (20), suggesting that a broad class I-restricted immune response reduces the proviral load. The influence of host genetics on the control of HTLV-1 infection has recently been reviewed elsewhere (23).

The conclusions of these studies are that a strong class I-restricted CTL response provides significant protection against HTLV-1-induced disease, and that the HLA genotype is an important determinant of the
efficiency of this protective immune response. A number of other genetic polymorphisms have since been shown to contribute to the genetic determination of the outcome of HTLV-1 infection (reviewed in (23)). However, the genetic factors identified to date do not account for all of the observed variation in proviral load or in the risk of HAM/TSP (22, 23), and it is likely that other genetic polymorphisms play a part. No estimate has been made of the total contribution of host genetic factors to the outcome of HTLV-1 infection: an attractive approach would be to estimate the genetic heritability (h²) of proviral load from family studies or by twin studies.

The p12I regulatory protein of HTLV-1, which increases HTLV-1 infectivity in vivo (62) has been shown to reduce the expression of MHC class 1 molecules on the surface of HTLV-1-infected lymphocytes (63). This putative immune escape mechanism bears witness to the importance of CTLs in controlling HTLV-1 replication in vivo, but it is not known whether this mechanism contributes to the observed variation between individuals in the efficiency of CTL surveillance.

9. CTL FREQUENCY IS NOT A USEFUL GUIDE TO CTL EFFICACY

The evidence from the diverse techniques reviewed above indicates that the specific CTL response is effective in limiting HTLV-1 replication in vivo. But two groups (64-66) have observed a positive correlation between proviral load and the frequency of HTLV-1-specific CTLs, which might appear to suggest that the CTL response merely passively follows the proviral load, rather than controlling the load. An analogous problem is evident in other persistent infections such as HIV, in which the plasma viral load has been shown to be correlated either negatively (67) or positively (68) with the frequencies of virus-specific CTLs in a persistent infection at steady state. At present it remains impossible to distinguish this explanation and the alternative possibility that Tregs can also influence the “efficiency” of the cell-mediated immune response to HTLV-1. In this study, we avoided the use of CD25 as one of the phenotypic markers of Tregs because it is well established that HTLV-1 Tax protein strongly induces expression of CD25 on the infected cell (77-79). Instead, we quantified cells with the simpler phenotype CD4⁺FoxP3⁻. There were two surprising findings (80). First, in patients with HTLV-1 infection the mean frequency of CD4⁺FoxP3⁺ cells was abnormally high (Figure 7a) and was positively correlated with both proviral expression (Figure 7b) and the proviral load (80). Second, we examined the relationship between the frequency of CD4⁺FoxP3⁺ cells and the CD8⁺ cell-mediated immune response to HTLV-1. We used the ex vivo lysis assay (17) to quantify the effect of CD4⁺FoxP3⁺ cells on the CD8⁺ cell-mediated response to autologous naturally-infected T cells in fresh PBMCs. The results (Figure 8) (80) showed a remarkably strong negative correlation between the frequency of CD4⁺FoxP3⁺ cells and the per-cell rate of CD8⁺ cell-mediated suppression of Tax⁺ cells in vitro – the HTLV-1-specific CTL lysis rate. Furthermore, the frequency of FoxP3⁺CD4⁺ cells that themselves expressed Tax, and were therefore CD25HIGH, did not correlate with the CTL lysis rate: only the CD4⁺FoxP3⁺Tax⁻ cells were associated with a low lysis rate. More than 90% of the observed individual variation in CTL lysis rate can be accounted for by variation in the frequency of circulating CD4⁺FoxP3⁺Tax⁻ cells.

These results suggest that the largest single determinant of the CTL lysis rate of HTLV-1-infected cells is the frequency of CD4⁺FoxP3⁺Tax⁻ cells ("Tregs") in the circulation. At present it remains impossible to distinguish between this explanation and the alternative possibility that the HTLV-1-specific CTL activity determines the frequency of CD4⁺ FoxP3⁺Tax⁻ cells in the blood, although this second possibility seems unlikely. The reason...
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Figure 7. The frequency of FoxP3+ cells is abnormally high in HTLV-1 infection and is correlated with HTLV-1 expression. a) The frequency of FoxP3 expression is abnormally high in CD4+T cells in HTLV-1-seropositive subjects. (A) FoxP3 expression in CD4+ cells in 7 uninfected subjects, 28 ACs, and 30 HAM/TSP patients, gated on the CD4+ cells. The P value was calculated by an unpaired t test (2-tailed). Figure reproduced, by permission from the publisher, from (80). b) Correlation between FoxP3 expression and Tax expression. The percentage of FoxP3 expression in CD4+Tax- cells was correlated with the percentage of CD4+Tax+ cells both in ACs (N = 23) and in HAM/TSP patients (N = 22). The P values were calculated by a two-tailed Spearman test. Figure reproduced, by permission from the publisher, from (80).

Figure 8. Correlation between frequency of FoxP3 expression and the rate of CD8- cell-mediated lysis. The percentage of FoxP3+ cells in all CD4- cells (A), the percentage of CD4 FoxP3+Tax- cells (B), and the percentage of CD4 FoxP3+Tax+ cells (C), were plotted against the efficiency of lysis. The data represent the result obtained with samples from 15 ACs and 19 patients with HAM/TSP. P values were determined by a two-tailed Spearman test. For the percentage of CD4 FoxP3+Tax- cells we have also represented the correlation with the efficiency of lysis on a smaller scale, to clarify the lack of correlation here (C). Figure reproduced, by permission from the publisher, from (80).
Figure 9. Determinants of HTLV-1 proviral load and the risk of HTLV-1-associated inflammatory disease. Recent evidence is consistent with the following sequence of events. 1) Early in infection, before the emergence of the adaptive immune response, HTLV-1 spreads by cell-cell contact through the virological synapse (39). Integration of the HTLV-1 provirus into host DNA is biased towards transcriptionally active areas of the genome (59). 2) The rate of HTLV-1 proviral expression, i.e. the probability that a given T cell expresses HTLV-1 in a given time interval, is also positively correlated with the transcriptional activity of the host genome in the vicinity of the provirus (59). 3) Proviral expression drives proliferation of the infected cell and so favours expansion of that T cell clone (30, 32, 34). 4) The viral antigen expression also elicits a strong CTL response (13-15) which kills the HTLV-1-expressing cells (17, 18, 31). The main factor that varies among hosts and so accounts for the variation in the equilibrium set point of proviral load is the rate of CTL-mediated lysis (17). Important determinants of this CTL “efficiency” include: 5) the host genotype (22, 23) – notably the HLA Class 1 genotype (21) – and 6) the frequency of CD4+ FoxP3+ cells in the circulation (80). The rate of HTLV-1 proviral expression by a given T cell is also influenced by: 7) epigenetic modifications of host DNA, i.e. histone acetylation (57) and DNA methylation (58), and 8) regulatory proteins of HTLV-1 (47). 9) HTLV-1 P121 may reduce the efficiency of CTL surveillance by targeting MHC Class 1 molecules for proteasomal degradation (63).

Our results appear to conflict with two recent studies of the role of Tregs in HTLV-1 infection (81, 82). Yamano et al (81) reported a lower frequency of FoxP3 expression in CD4+CD25+ T cells in subjects with HTLV-1 infection than in uninfected individuals, and the authors concluded that the frequency of Tregs was reduced, especially in patients with HAM/TSP. However, in these studies Tregs were defined as CD4+FoxP3+CD25HIGH. Our evidence (80) confirmed that the reported decrease in Treg frequency associated with HTLV-1 infection could be attributed to the higher frequency of CD25 expression in these hosts, which reduced the fraction of CD25HIGH cells that expressed FoxP3 and therefore reduced the apparent frequency of Tregs.

It therefore becomes a matter of some importance to identify the mechanism by which HTLV-1 infection causes an increase in the frequency of CD4+FoxP3+ cells: at present this mechanism is quite unknown. It will also be of great interest to examine the role of CD4+FoxP3+ cells in adult T cell leukaemia. ATL cells are typically CD25+, and some also express FoxP3 (83-85). It has therefore been postulated that HTLV-1 might selectively infect or selectively transform Tregs, and that ATL cells, however they are generated, might themselves act as Tregs. It remains to be seen whether, as in non-malignant cases of HTLV-1 infection, CD25 expression merely confounds the measurement of Treg frequencies. Also, physiologically relevant assays of Treg function must be used to quantify their effect.

11. CONCLUSION: SELECTION AND PERSISTENCE OF HTLV-1-INFECTED T CELLS IN VIVO

With the increasing understanding of the positive and negative selection forces that act on HTLV-1-infected T cells in vivo, we can begin to piece together a qualitative and quantitative understanding of HTLV-1 persistence (Figure 9). HTLV-1-infected T cell clones differ from each other not only in their antigen specificity, but also in the genomic site of proviral integration. The character of the genomic site appears to influence the proviral expression, which in turn results in simultaneous strong positive and negative selection on the T cell. The net effect of these opposing forces on the T cell, which determines its chance of survival in the host and so its contribution to retroviral persistence, is not, however, a simple function of the total rate of proviral expression. In addition, the timing of proviral expression in relation to the cell cycle will be...
influenced by the transcriptional activity of the genome in the vicinity of the provirus (59). This timing of proviral expression will be a critical phenotype on which selection can act. Specifically, one can predict that constitutive proviral expression throughout the cell cycle will increase the probability of selective elimination of that infected T cell clone by the immune response, because the constant exposure to immune surveillance is not compensated by sufficiently rapid T cell division: the T cell has a limited rate of division. Conversely, a T cell that expresses a provirus more rarely will derive a smaller selective advantage from the virus-driven T cell replication. Therefore, there is likely to be an optimal frequency of expression of the provirus during the cell cycle: this frequency will differ between individual hosts, because the hosts vary in the strength of (mainly cell-mediated) immune selection that acts on the virus. Extending this argument, we conjecture that the virus will confer a strong selective advantage on the T cell if it is expressed selectively at particular points in the cell cycle, such as the cell cycle checkpoints. Transient proviral expression at cell cycle checkpoints would prevent the cell from entering G0, maintain it in cycle and so maintain the population of that infected T cell clone.

Direct evidence from in vivo lymphocyte labelling showed that HTLV-1-expressing cells turn over much faster than uninfected cells (Figure 4) (30). If the lifespan of an HTLV-1-infected cell is indeed reduced to between 1 and 10 days (30) then how is the infection maintained in the host? The answer is that HTLV-1 persistence in vivo depends not on the longevity of individual lymphocytes, but rather on the longevity of each respective clone of infected T cells. In this respect, there is a close analogy between HTLV-1 persistence and immunological memory. Before lymphocyte turnover rates were measured in vivo, it was believed that memory T cells – those that have previously made contact with their specific antigen – lived longer than naïve T cells. In fact the opposite is true: individual memory T cells have a significantly shorter life expectancy than naïve T cells (86, 87). But what persists is the clone of T cells: both immunological memory and HTLV-1 infection persist in the clones of T cells, not simply in long-lived individual T cells.

Factors that influence the outcome of HTLV-1 infection have been identified at the level of the human population, the host immune response, gene expression in host lymphocytes and the provirus itself, the genomic integration site and the sequence of the provirus. The challenge is to integrate the factors at these different levels, to produce a coherent qualitative and quantitative explanation of the persistence and pathogenesis of HTLV-1, and to account for the remarkably variable clinical manifestations of HTLV-1 infection: disabling chronic inflammatory diseases, malignant disease, or health.

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Send correspondence to: Charles R M Bangham, Department of Immunology, Wright-Fleming Institute, Imperial College, London W2 1PG, Tel: 44- (0)20-7594-3730, Fax: 44- (0)20-7402-0653, E-mail: c.bangham@imperial.ac.uk