[Frontiers in Bioscience 14, 2889-2903, January 1, 2009]

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

Charles R M Bangham[1], Kiran Meekings[1], Frederic Toulza[1], Mohamed Nejmeddine[1], Endre Majorovits[3], Becca Asquith[1], Graham P Taylor[2]

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

TABLE OF CONTENTS

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

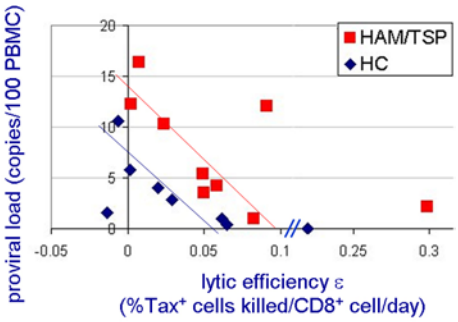

Figure 1. Proviral load is inversely correlated with CTL lysis rate. Proviral load was plotted against the lytic rate (or 'efficacy') of antiviral $CD8^+$ T cells, i.e. the rate at which $CD8^+$ cells suppress Tax protein-expressing cells. Data are shown on seven ACs and nine HAM/TSP patients. A negative 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).

predispose to a high risk of the subsequent onset of HAM/TSP.

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 1 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 inside the infected cell (41) to trigger reorientation of the infected cell's microtubule cytoskeleton towards the VS. HTLV-1 is then transferred directly to the target cell. However, until recently the precise form in which the virus is transferred between cells remained unclear. HTLV-1 requires Env protein for infectivity (42, 43), suggesting that enveloped virions are transmitted between cells across the VS. But lymphocytes naturally infected with HTLV-1 had not been shown to produce cell-free virions, and virions are usually undetectable in serum by electron microscopy or RT-PCR. Recently, this paradox has been resolved by Majorovits *et al* (44). Using electron tomography combined with immunostaining of viral protein, Majorovits *et al.* demonstrated the presence of enveloped HTLV-1 particles in confined intercellular clefts at the VS (Figure 2). The synaptic clefts are entirely enclosed by the tightly apposed plasma membranes of the two cells, preventing the virions from escaping into the extracellular space. These observations can reconcile the requirement for Env protein with the paucity (or absence) of detectable cell-free HTLV-1 virions in serum. The remarkable feature of the HTLV-1 VS is that the release of virions is directional, focal and triggered by a synergistic activation of T cell signalling pathways (M. Nejmeddine *et al* 2008, submitted) by HTLV-1 Tax protein (41) and engagement of ICAM-1 on the target cell (40).

Recent evidence (45) indicates that dendritic cells (DCs), unlike lymphocytes, can be efficiently infected by cell-free HTLV-1 particles *in vitro*. But it seems unlikely that cell-free spread plays a major role in maintaining the proviral load in T cells *in vivo* because of the paucity of cell-free virions and the observation by the same authors (45) that cell-free virions produced by HTLV-1-infected DCs cannot efficiently infect $CD4^+$ T cells, the chief host cell of HTLV-1.

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 $p30^{II}$ can also diminish HTLV-1 proviral transcription (48). Finally, the *HBZ* gene, on the anticoding strand of the provirus (49, 50), appears to act at both the RNA and protein levels: the RNA exerts a suppressive effect on proviral transcription (49, 51, 52). But it is not yet clear whether these regulatory elements of the virus are capable of complete termination of HTLV-1 proviral transcription.

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 heterogeneity 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

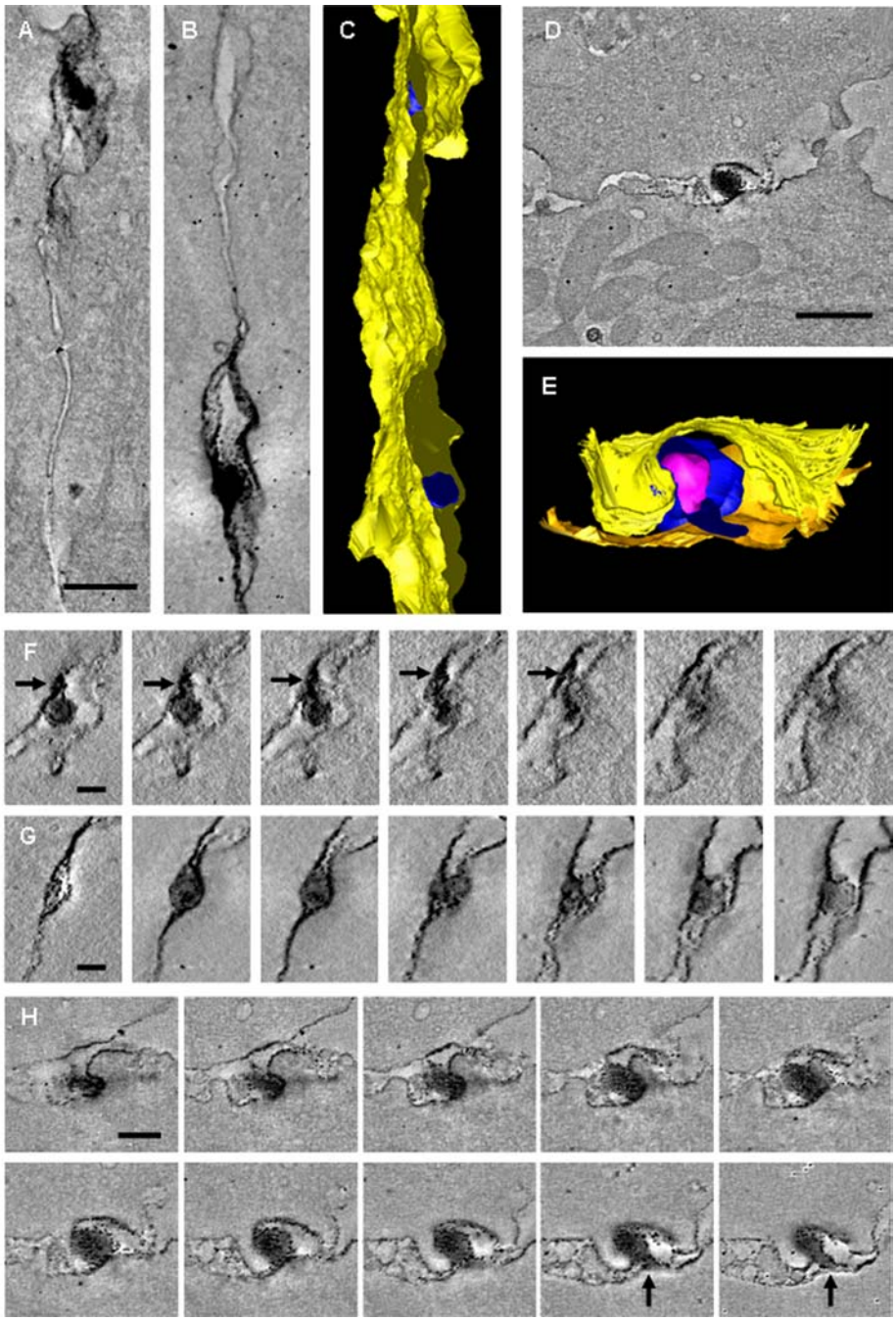

Figure 2. Three-dimensional ultrastructure of the VS shown by electron tomography. Cell-to-cell transmission of HTLV-1 as observed in tomograms of the VS formed between HTLV-1 infected $CD4^+$ T-cell (PBMC) and an autologous uninfected $CD4^+$ T-cell as a target cell. These cells were stained against 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).

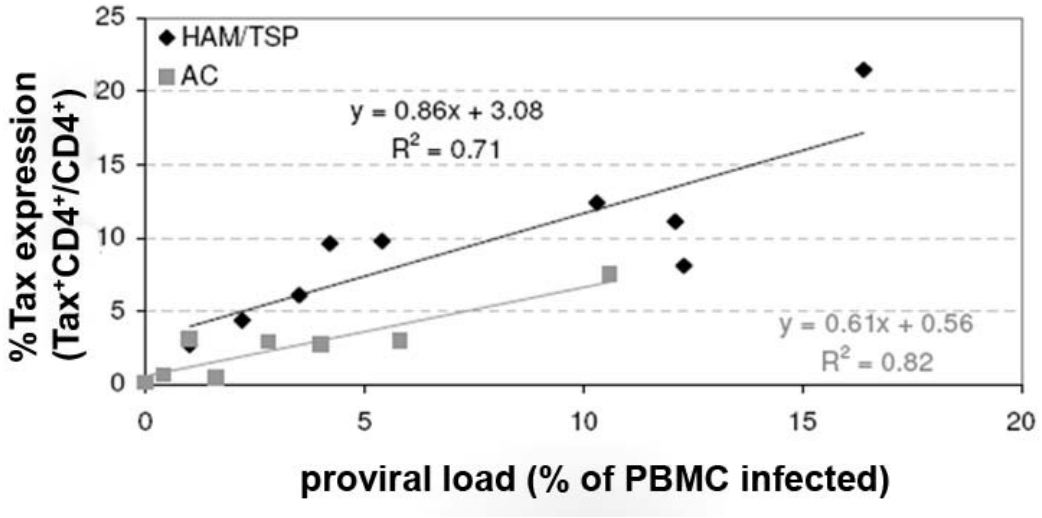

Figure 3. Proviral expression level is greater in HAM/TSP patients than in asymptomatic carriers at a given proviral load. Correlation between HTLV-1 Tax expression and proviral load in $CD4^+$ lymphocytes. The proportion of $CD4^+$ cells expressing the viral protein Tax after 18 h *ex vivo* incubation 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).

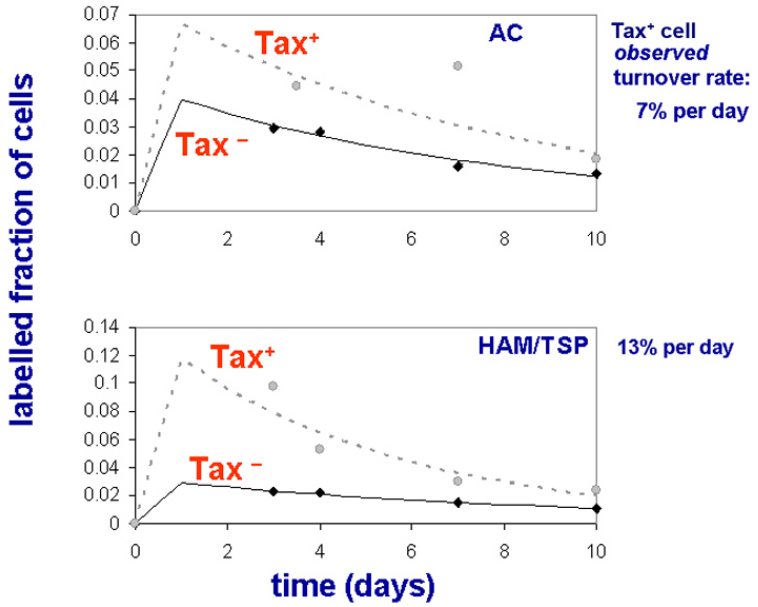

Figure 4. Tax^+ cells proliferate more rapidly than Tax^- cells in the same individual *in vivo*. Deuterium enrichment was measured in $Tax^+CD4^+CD45RO^+$ and $Tax^-CD4^+CD45RO^+$ cells in two individuals (L02-HAY and L07-TBI). Grey circles, enrichment in Tax^+ $CD4^+CD45RO^+$ cells; dashed line, theoretical 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).

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.

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

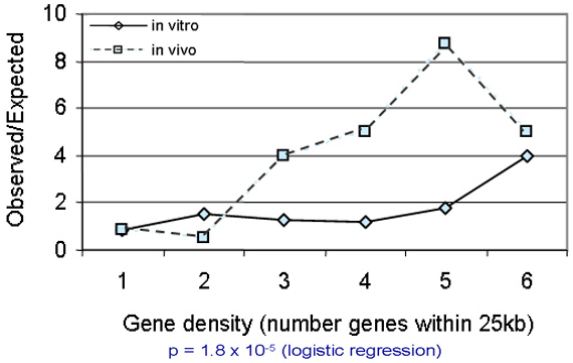

Figure 5. HTLV-1 shows an increased frequency of integration in gene-dense regions of the genome. Gene density in regions from 25kb to 1Mb around the integration site was analysed. In all region sizes, there was a greater association of HTLV-1 integration in gene-dense regions both *in vivo* and *in vitro*. However, there was a significantly stronger association between proviral integration frequency and gene density in persistent infection *in vivo* than was seen *in vitro*. Figure reproduced from (59).

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

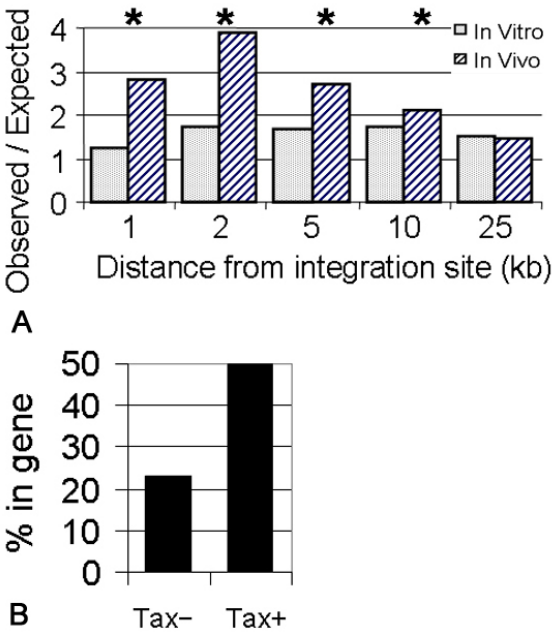

Figure 6. Differences between distributions of HTLV-1 proviral integration sites *in vivo* and *in vitro* suggest selection forces that operate *in vivo*. a) Integration of HTLV-1 in transcriptionally active genomic regions. HTLV-1 showed significantly greater than expected frequency of integration in the vicinity of CpG islands in cell culture *in vitro* and in persistent infection *in vivo*. Comparison of the frequency of observed to expected HTLV-1 integration sites between *in vivo* and *in vitro* infection revealed a significantly greater association with integration in the proximity of CpG islands (shown here) and transcriptional start sites (not illustrated). The asterisk indicates a significant difference between the frequency of HTLV-1 *in vivo* sites and *in vitro* sites, by logistic regression ($p < 0.005$). Figure reproduced from (59). b) There was a significant association between proviral (Tax protein) expression and integration of the provirus in a known (RefSeq) gene. $CD8^+$ cell-depleted PBMCs from an HTLV-1-infected individual were separated into Tax^+ and Tax^- fractions after 18hr incubation *in vitro*, and integration sites were cloned from each fraction. The integration sites from three independent experiments were combined and the distribution of sites between the Tax^+ and Tax^- fractions compared using logistic regression analysis. The Tax^+ fraction had significantly more integrations in RefSeq genes than did the Tax^- fraction ($p = 0.04$). Figure reproduced from (59).

forces that shape the distribution of HTLV-1 proviruses in the genome of a given host? Again, the immune response must play a critical role. It is presumed that initial integration of a retrovirus into accessible (transcriptionally active) regions of chromatin is largely random. Subsequent expression of the HTLV-1 provirus drives proliferation of the infected T cell clone and simultaneously exposes the T cell to immune destruction, particularly by CTLs (30, 31). The net result of these positive and negative selection 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 1 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 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, heterozygosity in the HLA class 1 loci was also significantly associated with a lower proviral load (20), suggesting that a broad class 1-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 1-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 (h^2) of proviral load from family studies or λ_s from twin studies.

The $p12^I$ 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 CTLs of defined antigenic specificities; sometimes both correlations are evident simultaneously in the same host (68). Recently, in an attempt to reconcile these conflicting data it has been suggested (69, 70) that CTLs whose frequency correlates negatively with viral load are effective or "driver" CTLs, while those that correlate positively with viral load are ineffective or "passenger" CTLs.

However, an "efficient" CTL response to a virus involves two important factors: rapid CTL-mediated killing of the infected cells and rapid proliferation of the CTLs to maintain the CTL population in the face of activation-induced cell death. Rapid CTL-mediated killing will reduce the antigenic load and so will reduce the antigenic stimulus to the CTLs to proliferate. These two forces act in opposition to each other, and there is no reliable method of predicting where the system will reach equilibrium (71, 72). Consequently, there is no simple way to predict whether CTL frequency and proviral load will be positively or negatively correlated, whether or not the CTL response is protective. The conclusion is that the frequency of virus-specific CTLs in a persistent infection at steady state is not a useful guide to their protective effect, still less to their efficacy (quantified as infected cells killed per CTL per day).

10. FOXP3$^+$ CD4$^+$ "T_{REG}" FREQUENCY IS NEGATIVELY CORRELATED WITH CTL LYSIS RATE

It is now recognized that certain $CD4^+$ T cell subsets, known as regulatory T cells or "T_{regs}" can suppress other T cell immune responses *in vivo* (73). These subsets are distinct from the illusory $CD8^+$ "suppressor T cells" or "T_S" reported in the 1980s. The definition of T_{regs} is complex, because more than one T cell subpopulation is capable of suppressing T cell responses, and because even within a single "T_{reg}" population, the minimal or optimal phenotypic markers of the population are not yet well defined. First identified as $CD4^+CD25^{HIGH}$, the main T_{reg} population was subsequently identified by the coexpression of the forkhead transcription factor FoxP3, and so became defined as $CD4^+FoxP3^+CD25^{HIGH}$.

T_{regs} have been studied most intensely in autoimmune disease (73), but there is growing evidence that T_{regs} can also influence the immune response to infections (74), such as HIV (75, 76), We therefore tested the hypothesis that T_{regs} 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 T_{regs}, 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 $CD25^{HIGH}$, 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 ("T_{regs}") 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

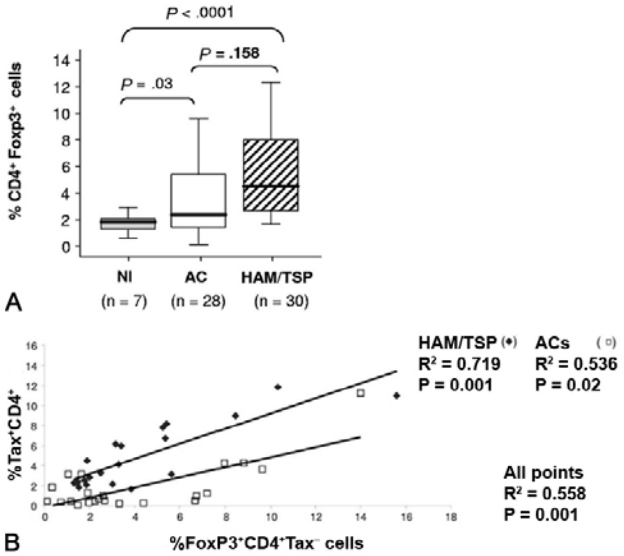

Figure 7. The frequency of FoxP3$^+$ cells is abnormally high in HTLV-1 infection and is correlated with HTLV-1 expression. a) The frequency of FoxP3 expression is abnormally high in CD4$^+$ T cells in HTLV-1–seropositive subjects. (A) FoxP3 expression in CD4$^+$ cells in 7 uninfected subjects, 28 ACs, 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).

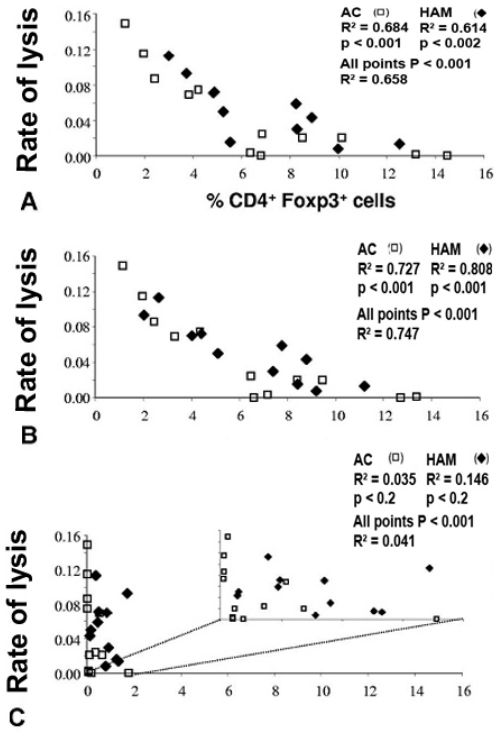

Figure 8. Correlation between frequency of FoxP3 expression and the rate of CD8$^+$ cell-mediated lysis. The percentage of FoxP3$^+$ cells in all CD4$^+$ cells (A), the percentage of CD4$^+$FoxP3$^+$ Tax$^-$ cells (B), and the percentage of CD4$^+$ FoxP3$^+$Tax$^+$ cells (C), were plotted against the 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).

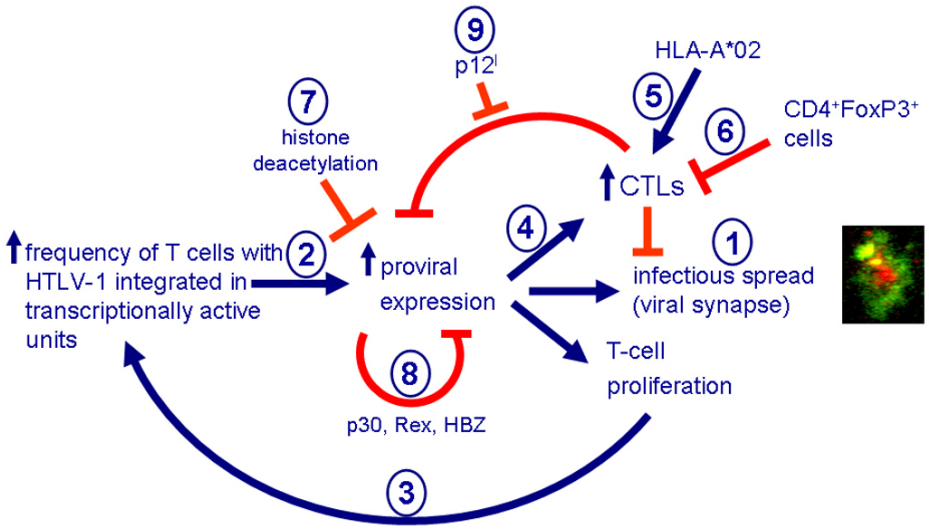

Figure 9. Determinants of HTLV-1 proviral load and the risk of HTLV-1-associated inflammatory disease. Recent evidence is consistent with the following sequence of events. 1) Early in infection, before the emergence of the adaptive immune response, HTLV-1 spreads by cell-cell contact through the virological 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 $P12^I$ may reduce the efficiency of CTL surveillance by targeting MHC Class 1 molecules for proteasomal degradation (63).

for this difficulty is that there is no currently known surface marker that is sufficiently specific to these T_{regs} to allow their isolation.

Our results appear to conflict with two recent studies of the role of T_{regs} 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 T_{regs} was reduced, especially in patients with HAM/TSP. However, in these studies T_{regs} were defined as $CD4^+FoxP3^+CD25^{HIGH}$. Our evidence (80) confirmed that the reported decrease in T_{reg} frequency associated with HTLV-1 infection could be attributed to the higher frequency of CD25 expression in these hosts, which reduced the fraction of $CD25^{HIGH}$ cells that expressed FoxP3 and therefore reduced the apparent frequency of T_{regs}.

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 T_{regs}, and that ATL cells, however they are generated, might themselves act as T_{regs}. It remains to be seen whether, as in non-malignant cases of HTLV-1 infection, CD25 expression merely confounds the measurement of T_{reg} frequencies. Also, physiologically relevant assays of T_{reg} 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 G_0, 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 naive T cells. In fact the opposite is true: individual memory T cells have a significantly shorter life expectancy than naive 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.

12. ACKNOWLEDGEMENTS

The authors thank the donors in the HTLV-1 clinic at the National Centre for Human Retrovirology, Imperial College Healthcare NHS Trust (St Mary's). We thank members of the Department of Immunology, Imperial College at St Mary's and Luc Willems and Stephen Fuller for invaluable discussions. This work was supported by the Wellcome Trust (UK) and by funding under the Sixth Research Framework Programme of the European Union, Project INCA (LSHC-CT-2005-018704).

13. REFERENCES

1. A. Gessain, F. Saal, O. Gout, M. T. Daniel, G. Flandrin, G. de The, J. Peries and F. Sigaux: High human T-cell lymphotropic virus type I proviral DNA load with polyclonal integration in peripheral blood mononuclear cells of French West Indian, Guianese, and African patients with tropical spastic paraparesis. *Blood*, 75 (2), 428-33 (1990)

2. J. Kira, Y. Koyanagi, T. Yamada, Y. Itoyama, I. Goto, N. Yamamoto, H. Sasaki and Y. Sakaki: Increased HTLV-I proviral DNA in HTLV-I-associated myelopathy: a quantitative polymerase chain reaction study. *Ann Neurol*, 29 (2), 194-201 (1991)

3. M. Yoshida, J. Inoue, J. Fujisawa and M. Seiki: Molecular mechanisms of regulation of HTLV-1 gene expression and its association with leukemogenesis. *Genome*, 31 (2), 662-7 (1989)

4. M. Nagai, K. Usuku, W. Matsumoto, D. Kodama, N. Takenouchi, T. Moritoyo, S. Hashiguchi, M. Ichinose, C. R. Bangham, S. Izumo and M. Osame: Analysis of HTLV-I proviral load in 202 HAM/TSP patients and 243 asymptomatic HTLV-I carriers: high proviral load strongly predisposes to HAM/TSP. *J Neurovirol*, 4 (6), 586-93 (1998)

5. G. P. Taylor, J. H. Tosswill, E. Matutes, S. Daenke, S. Hall, B. J. Bain, R. Davis, D. Thomas, M. Rossor, C. R. Bangham and J. N. Weber: Prospective study of HTLV-I infection in an initially asymptomatic cohort. *J Acquir Immune Defic Syndr*, 22 (1), 92-100 (1999)

6. T. Matsuzaki, M. Nakagawa, M. Nagai, K. Usuku, I. Higuchi, K. Arimura, H. Kubota, S. Izumo, S. Akiba and M. Osame: HTLV-I proviral load correlates with progression of motor disability in HAM/TSP: analysis of 239 HAM/TSP patients including 64 patients followed up for 10 years. *J Neurovirol*, 7 (3), 228-34 (2001)

7. G. P. Taylor, S. E. Hall, S. Navarrete, C. A. Michie, R. Davis, A. D. Witkover, M. Rossor, M. A. Nowak, P. Rudge, E. Matutes, C. R. Bangham and J. N. Weber: Effect of lamivudine on human T-cell leukemia virus type 1 (HTLV-1) DNA copy number, T-cell phenotype, and anti-tax cytotoxic T-cell frequency in patients with HTLV-1-associated myelopathy. *J Virol*, 73 (12), 10289-95. (1999)

8. A. Oxenius and B. Hirschel: Structured treatment interruptions in HIV infection: benefit or

disappointment? *Expert Rev Anti Infect Ther*, 1 (1), 129-39 (2003)

9. S. Daenke, S. Nightingale, J. K. Cruickshank and C. R. Bangham: Sequence variants of human T-cell lymphotropic virus type I from patients with tropical spastic paraparesis and adult T-cell leukemia do not distinguish neurological from leukemic isolates. *J Virol*, 64 (3), 1278-82 (1990)

10. T. Kinoshita, A. Tsujimoto and K. Shimotohno: Sequence variations in LTR and env regions of HTLV-I do not discriminate between the virus from patients with HTLV-I-associated myelopathy and adult T-cell leukemia. *Int J Cancer*, 47 (4), 491-5 (1991)

11. F. Komurian, F. Pelloquin and G. de The: *In vivo* genomic variability of human T-cell leukemia virus type I depends more upon geography than upon pathologies. *J Virol*, 65 (7), 3770-8 (1991)

12. J. P. Slattery, G. Franchini and A. Gessain: Genomic evolution, patterns of global dissemination, and interspecies transmission of human and simian T-cell leukemia/lymphotropic viruses. *Genome Res*, 9 (6), 525-40 (1999)

13. S. Jacobson, H. Shida, D. E. McFarlin, A. S. Fauci and S. Koenig: Circulating CD8+ cytotoxic T lymphocytes specific for HTLV-I pX in patients with HTLV-I associated neurological disease. *Nature*, 348 (6298), 245-8 (1990)

14. C. E. Parker, S. Daenke, S. Nightingale and C. R. Bangham: Activated, HTLV-1-specific cytotoxic T-lymphocytes are found in healthy seropositives as well as in patients with tropical spastic paraparesis. *Virology*, 188 (2), 628-36 (1992)

15. C. E. Parker, S. Nightingale, G. P. Taylor, J. Weber and C. R. Bangham: Circulating anti-Tax cytotoxic T lymphocytes from human T-cell leukemia virus type I-infected people, with and without tropical spastic paraparesis, recognize multiple epitopes simultaneously. *J Virol*, 68 (5), 2860-8 (1994)

16. C. R. Bangham: The immune control and cell-to-cell spread of human T-lymphotropic virus type 1. *J Gen Virol*, 84 (Pt 12), 3177-89 (2003)

17. B. Asquith, A. J. Mosley, A. Barfield, S. E. Marshall, A. Heaps, P. Goon, E. Hanon, Y. Tanaka, G. P. Taylor and C. R. Bangham: A functional CD8+ cell assay reveals individual variation in CD8+ cell antiviral efficacy and explains differences in human T-lymphotropic virus type 1 proviral load. *J Gen Virol*, 86 (Pt 5), 1515-23 (2005)

18. E. Hanon, S. Hall, G. P. Taylor, M. Saito, R. Davis, Y. Tanaka, K. Usuku, M. Osame, J. N. Weber and C. R. Bangham: Abundant tax protein expression in CD4+ T cells infected with human T-cell lymphotropic virus type I (HTLV-I) is prevented by cytotoxic T lymphocytes. *Blood*, 95 (4), 1386-92 (2000)

19. C. R. Bangham and M. Osame: Cellular immune response to HTLV-1. *Oncogene*, 24 (39), 6035-46 (2005)

20. K. J. Jeffery, A. A. Siddiqui, M. Bunce, A. L. Lloyd, A. M. Vine, A. D. Witkover, S. Izumo, K. Usuku, K. I. Welsh, M. Osame and C. R. Bangham: The influence of HLA class I alleles and heterozygosity on the outcome of human T cell lymphotropic virus type I infection. *J Immunol*, 165 (12), 7278-84 (2000)

21. K. J. Jeffery, K. Usuku, S. E. Hall, W. Matsumoto, G. P. Taylor, J. Procter, M. Bunce, G. S. Ogg, K. I. Welsh, J. N. Weber, A. L. Lloyd, M. A. Nowak, M. Nagai, D. Kodama, S. Izumo, M. Osame and C. R. Bangham: HLA alleles determine human T-lymphotropic virus-I (HTLV-I) proviral load and the risk of HTLV-I-associated myelopathy. *Proc Natl Acad Sci U S A*, 96 (7), 3848-53 (1999)

22. A. M. Vine, A. D. Witkover, A. L. Lloyd, K. J. Jeffery, A. Siddiqui, S. E. Marshall, M. Bunce, N. Eiraku, S. Izumo, K. Usuku, M. Osame and C. R. Bangham: Polygenic control of human T lymphotropic virus type I (HTLV-I) provirus load and the risk of HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Infect Dis*, 186 (7), 932-9 (2002)

23. C. R. M. Bangham: Human T-lymphotropic virus type 1 (HTLV-1)-associated diseases. In: *Genetic Susceptibility to Infectious Diseases*. Ed J. M. M. R. A. Kaslow, A. V. S. Hill. Oxford University Press, Oxford UK (2008)

24. A. M. Vine, A. G. Heaps, L. Kaftantzi, A. Mosley, B. Asquith, A. Witkover, G. Thompson, M. Saito, P. K. Goon, L. Carr, F. Martinez-Murillo, G. P. Taylor and C. R. Bangham: The role of CTLs in persistent viral infection: cytolytic gene expression in CD8+ lymphocytes distinguishes between individuals with a high or low proviral load of human T cell lymphotropic virus type 1. *J Immunol*, 173 (8), 5121-9 (2004)

25. R. Kubota, K. Hanada, Y. Furukawa, K. Arimura, M. Osame, T. Gojobori and S. Izumo: Genetic stability of human T lymphotropic virus type I despite antiviral pressures by CTLs. *J Immunol*, 178 (9), 5966-72 (2007)

26. S. Niewiesk, S. Daenke, C. E. Parker, G. Taylor, J. Weber, S. Nightingale and C. R. Bangham: The transactivator gene of human T-cell leukemia virus type I is more variable within and between healthy carriers than patients with tropical spastic paraparesis. *J Virol*, 68 (10), 6778-81 (1994)

27. P. K. Goon, A. Biancardi, N. Fast, T. Igakura, E. Hanon, A. J. Mosley, B. Asquith, K. G. Gould, S. Marshall, G. P. Taylor and C. R. Bangham: Human T cell lymphotropic virus (HTLV) type-1-specific CD8+ T cells: frequency and immunodominance hierarchy. *J Infect Dis*, 189 (12), 2294-8 (2004)

28. M. Kannagi, S. Harada, I. Maruyama, H. Inoko, H. Igarashi, G. Kuwashima, S. Sato, M. Morita, M. Kidokoro,

M. Sugimoto and *et al.*: Predominant recognition of human T cell leukemia virus type I (HTLV-I) pX gene products by human CD8+ cytotoxic T cells directed against HTLV-I-infected cells. *Int Immunol*, 3 (8), 761-7 (1991)

29. C. R. M. Bangham, Kermode, A. G., Hall, S. E., and Daenke, S.: The cytotoxic T-lymphocyte response to HTLV-I: the main determinant of disease? *Seminars in Virology*, 7, 41-48 (1996)

30. B. Asquith, Y. Zhang, A. J. Mosley, C. M. de Lara, D. L. Wallace, A. Worth, L. Kaftantzi, K. Meekings, G. E. Griffin, Y. Tanaka, D. F. Tough, P. C. Beverley, G. P. Taylor, D. C. Macallan and C. R. Bangham: *In vivo* T lymphocyte dynamics in humans and the impact of human T-lymphotropic virus 1 infection. *Proc Natl Acad Sci U S A*, 104 (19), 8035-40 (2007)

31. B. Asquith and C. R. Bangham: Quantifying HTLV-I dynamics. *Immunol Cell Biol*, 85, 280-286 (2007)

32. M. Cavrois, A. Gessain, S. Wain-Hobson and E. Wattel: Proliferation of HTLV-1 infected circulating cells *in vivo* in all asymptomatic carriers and patients with TSP/HAM. *Oncogene*, 12 (11), 2419-23 (1996)

33. E. Wattel, M. Cavrois, A. Gessain and S. Wain-Hobson: Clonal expansion of infected cells: a way of life for HTLV-I. *J Acquir Immune Defic Syndr Hum Retrovirol*, 13 Suppl 1, S92-9 (1996)

34. E. Wattel, J. P. Vartanian, C. Pannetier and S. Wain-Hobson: Clonal expansion of human T-cell leukemia virus type I-infected cells in asymptomatic and symptomatic carriers without malignancy. *J Virol*, 69 (5), 2863-8 (1995)

35. J. Overbaugh and C. R. Bangham: Selection forces and constraints on retroviral sequence variation. *Science*, 292 (5519), 1106-9 (2001)

36. F. Mortreux, M. Kazanji, A. S. Gabet, B. de Thoisy and E. Wattel: Two-step nature of human T-cell leukemia virus type 1 replication in experimentally infected squirrel monkeys (Saimiri sciureus). *J Virol*, 75 (2), 1083-9 (2001)

37. N. E. Mueller, W. A. Blattner: Retroviruses: HTLV. In: *Viral infections of humans: epidemiology and control*. Ed A. S. a. R. K. Evans. Plenum Medical Press, New York (1997)

38. K. Okochi and H. Sato: Transmission of ATLV (HTLV-I) through blood transfusion. *Princess Takamatsu Symp*, 15, 129-35 (1984)

39. T. Igakura, J. C. Stinchcombe, P. K. Goon, G. P. Taylor, J. N. Weber, G. M. Griffiths, Y. Tanaka, M. Osame and C. R. Bangham: Spread of HTLV-I between lymphocytes by virus-induced polarization of the cytoskeleton. *Science*, 299 (5613), 1713-6 (2003)

40. A. L. Barnard, T. Igakura, Y. Tanaka, G. P. Taylor and C. R. Bangham: Engagement of specific T-cell surface molecules regulates cytoskeletal polarization in HTLV-1-infected lymphocytes. *Blood*, 106 (3), 988-95 (2005)

41. M. Nejmeddine, A. L. Barnard, Y. Tanaka, G. P. Taylor and C. R. Bangham: Human T-lymphotropic virus, type 1, tax protein triggers microtubule reorientation in the virological synapse. *J Biol Chem*, 280 (33), 29653-60 (2005)

42. D. Derse, S. A. Hill, P. A. Lloyd, H. Chung and B. A. Morse: Examining human T-lymphotropic virus type 1 infection and replication by cell-free infection with recombinant virus vectors. *J Virol*, 75 (18), 8461-8. (2001)

43. L. Delamarre, C. Pique, A. R. Rosenberg, V. Blot, M. P. Grange, I. Le Blanc and M. C. Dokhelar: The Y-S-L-I tyrosine-based motif in the cytoplasmic domain of the human T-cell leukemia virus type 1 envelope is essential for cell-to-cell transmission. *J Virol*, 73 (11), 9659-63 (1999)

44. E. Majorovits, Nejmeddine, M., Tanaka, Y., Taylor, G.P., Fuller, S.D. & Bangham, C.R.M.: Human T-Lymphotropic Virus-1 Visualized at the Virological Synapse by Electron Tomography. *PLoS ONE*, 3 (5), e2251 (2008)

45. K. S. Jones, C. Petrow-Sadowski, Y. K. Huang, D. C. Bertolette and F. W. Ruscetti: Cell-free HTLV-1 infects dendritic cells leading to transmission and transformation of CD4 (+) T cells. *Nat Med*, 14 (4), 429-36 (2008)

46. A. Manns, W. J. Miley, R. J. Wilks, O. S. Morgan, B. Hanchard, G. Wharfe, B. Cranston, E. Maloney, S. L. Welles, W. A. Blattner and D. Waters: Quantitative proviral DNA and antibody levels in the natural history of HTLV-I infection. *J Infect Dis*, 180 (5), 1487-93 (1999)

47. B. Albrecht and M. D. Lairmore: Critical role of human T-lymphotropic virus type 1 accessory proteins in viral replication and pathogenesis. *Microbiol Mol Biol Rev*, 66 (3), 396-406, table of contents (2002)

48. C. Nicot, M. Dundr, J. M. Johnson, J. R. Fullen, N. Alonzo, R. Fukumoto, G. L. Princler, D. Derse, T. Misteli and G. Franchini: HTLV-1-encoded p30II is a post-transcriptional negative regulator of viral replication. *Nat Med*, 10 (2), 197-201 (2004)

49. G. Gaudray, F. Gachon, J. Basbous, M. Biard-Piechaczyk, C. Devaux and J. M. Mesnard: The complementary strand of the human T-cell leukemia virus type 1 RNA genome encodes a bZIP transcription factor that down-regulates viral transcription. *J Virol*, 76 (24), 12813-22 (2002)

50. M. H. Cavanagh, S. Landry, B. Audet, C. Arpin-Andre, P. Hivin, M. E. Pare, J. Thete, E. Wattel, S. J. Marriott, J. M. Mesnard and B. Barbeau: HTLV-I antisense transcripts initiating in the 3'LTR are alternatively spliced and polyadenylated. *Retrovirology*, 3, 15 (2006)

51. J. Matsumoto, T. Ohshima, O. Isono and K. Shimotohno: HTLV-1 HBZ suppresses AP-1 activity by impairing both the DNA-binding ability and the stability of c-Jun protein. *Oncogene*, 24 (6), 1001-10 (2005)

52. J. M. Mesnard and C. Devaux: Multiple control levels of cell proliferation by human T-cell leukemia virus type 1 Tax protein. *Virology*, 257 (2), 277-84 (1999)

53. Y. Furukawa, M. Yamashita, K. Usuku, S. Izumo, M. Nakagawa and M. Osame: Phylogenetic subgroups of human T cell lymphotropic virus (HTLV) type I in the tax gene and their association with different risks for HTLV-I-associated myelopathy/tropical spastic paraparesis. *J Infect Dis*, 182 (5), 1343-9 (2000)

54. B. Asquith, A. J. Mosley, A. Heaps, Y. Tanaka, G. P. Taylor, A. R. McLean and C. R. Bangham: Quantification of the virus-host interaction in human T lymphotropic virus I infection. *Retrovirology*, 2, 75 (2005)

55. Y. Yamano, M. Nagai, M. Brennan, C. A. Mora, S. S. Soldan, U. Tomaru, N. Takenouchi, S. Izumo, M. Osame and S. Jacobson: Correlation of human T-cell lymphotropic virus type 1 (HTLV-1) mRNA with proviral DNA load, virus-specific CD8 (+) T cells, and disease severity in HTLV-1-associated myelopathy (HAM/TSP). *Blood*, 99 (1), 88-94 (2002)

56. A. Lezin, N. Gillet, S. Olindo, A. Signate, N. Grandvaux, O. Verlaeten, G. Belrose, M. de Carvalho Bittencourt, J. Hiscott, B. Asquith, A. Burny, D. Smadja, R. Cesaire and L. Willems: Histone deacetylase mediated transcriptional activation reduces proviral loads in HTLV-1 associated myelopathy/tropical spastic paraparesis patients. *Blood*, 110 (10), 3722-8 (2007)

57. A. J. Mosley, K. N. Meekings, C. McCarthy, D. Shepherd, V. Cerundolo, R. Mazitschek, Y. Tanaka, G. P. Taylor and C. R. Bangham: Histone deacetylase inhibitors increase virus gene expression but decrease CD8+ cell anti-viral function in HTLV-I infection. *Blood* (2006)

58. Y. Taniguchi, K. Nosaka, J. Yasunaga, M. Maeda, N. Mueller, A. Okayama and M. Matsuoka: Silencing of human T-cell leukemia virus type I gene transcription by epigenetic mechanisms. *Retrovirology*, 2, 64 (2005)

59. K. N. Meekings, J. Leipzig, F. D. Bushman, G. P. Taylor and C. R. Bangham: HTLV-1 Integration into Transcriptionally Active Genomic Regions Is Associated with Proviral Expression and with HAM/TSP. *PLoS Pathog*, 4 (3), e1000027 (2008)

60. F. Bushman, M. Lewinski, A. Ciuffi, S. Barr, J. Leipzig, S. Hannenhalli and C. Hoffmann: Genome-wide analysis of retroviral DNA integration. *Nat Rev Microbiol*, 3 (11), 848-58 (2005)

61. R. S. Mitchell, B. F. Beitzel, A. R. Schroder, P. Shinn, H. Chen, C. C. Berry, J. R. Ecker and F. D. Bushman: Retroviral DNA integration: ASLV, HIV, and MLV show distinct target site preferences. *PLoS Biol*, 2 (8), E234 (2004)

62. N. D. Collins, G. C. Newbound, B. Albrecht, J. L. Beard, L. Ratner, and M. D. Lairmore. Selective ablation of human T-cell lymphotropic virus type 1 p12I reduces viral infectivity in vivo. Blood, 91 (12), 4701-7 (1998)

63. J. M. Johnson, C. Nicot, J. Fullen, V. Ciminale, L. Casareto, J. C. Mulloy, S. Jacobson and G. Franchini: Free major histocompatibility complex class I heavy chain is preferentially targeted for degradation by human T-cell leukemia/lymphotropic virus type 1 p12 (I) protein. *J Virol*, 75 (13), 6086-94 (2001)

64. D. Wodarz, S. E. Hall, K. Usuku, M. Osame, G. S. Ogg, A. J. McMichael, M. A. Nowak and C. R. Bangham: Cytotoxic T-cell abundance and virus load in human immunodeficiency virus type 1 and human T-cell leukaemia virus type 1. *Proc R Soc Lond B Biol Sci*, 268 (1473), 1215-21 (2001)

65. R. Kubota, T. Kawanishi, H. Matsubara, A. Manns and S. Jacobson: HTLV-I specific IFN-gamma+ CD8+ lymphocytes correlate with the proviral load in peripheral blood of infected individuals. *J Neuroimmunol*, 102 (2), 208-15 (2000)

66. R. Kubota, M. Nagai, T. Kawanishi, M. Osame and S. Jacobson: Increased HTLV type 1 tax specific CD8+ cells in HTLV type 1-asociated myelopathy/tropical spastic paraparesis: correlation with HTLV type 1 proviral load. *AIDS Res Hum Retroviruses*, 16 (16), 1705-9 (2000)

67. G. S. Ogg, X. Jin, S. Bonhoeffer, P. R. Dunbar, M. A. Nowak, S. Monard, J. P. Segal, Y. Cao, S. L. Rowland-Jones, V. Cerundolo, A. Hurley, M. Markowitz, D. D. Ho, D. F. Nixon and A. J. McMichael: Quantitation of HIV-1-specific cytotoxic T lymphocytes and plasma load of viral RNA. *Science*, 279 (5359), 2103-6. (1998)

68. M. R. Betts, D. R. Ambrozak, D. C. Douek, S. Bonhoeffer, J. M. Brenchley, J. P. Casazza, R. A. Koup and L. J. Picker: Analysis of Total Human Immunodeficiency Virus (HIV)-Specific CD4 (+) and CD8 (+) T-Cell Responses: Relationship to Viral Load in Untreated HIV Infection. *J Virol*, 75 (24), 11983-91. (2001)

69. A. Zafiropoulos, E. Barnes, C. Piggott and P. Klenerman: Analysis of 'driver' and 'passenger' CD8+ T-cell responses against variable viruses. *Proc Biol Sci*, 271 Suppl 3, S53-6 (2004)

70. R. Zuniga, A. Lucchetti, P. Galvan, S. Sanchez, C. Sanchez, A. Hernandez, H. Sanchez, N. Frahm, C. H. Linde, H. S. Hewitt, W. Hildebrand, M. Altfeld, T. M. Allen, B. D. Walker, B. T. Korber, T. Leitner, J. Sanchez and C. Brander: Relative dominance of Gag p24-specific cytotoxic T lymphocytes is associated with human immunodeficiency virus control. *J Virol*, 80 (6), 3122-5 (2006)

71. B. Asquith and C. R. Bangham: An introduction to lymphocyte and viral dynamics: the power and limitations of mathematical analysis. *Proc R Soc Lond B Biol Sci*, 270 (1525), 1651-7 (2003)

72. B. Asquith and C. R. Bangham: How does HTLV-I persist despite a strong cell-mediated immune response? *Trends Immunol*, 29 (1), 4-11 (2008)

73. S. Sakaguchi and F. Powrie: Emerging challenges in regulatory T cell function and biology. *Science*, 317 (5838), 627-9 (2007)

74. Y. Belkaid: Regulatory T cells and infection: a dangerous necessity. *Nat Rev Immunol*, 7 (11), 875-88 (2007)

75. A. L. Kinter, R. Horak, M. Sion, L. Riggin, J. McNally, Y. Lin, R. Jackson, A. O'Shea, G. Roby, C. Kovacs, M. Connors, S. A. Migueles and A. S. Fauci: CD25+ regulatory T cells isolated from HIV-infected individuals suppress the cytolytic and nonlytic antiviral activity of HIV-specific CD8+ T cells *in vitro*. *AIDS Res Hum Retroviruses*, 23 (3), 438-50 (2007)

76. S. Tsunemi, T. Iwasaki, T. Imado, S. Higasa, E. Kakishita, T. Shirasaka and H. Sano: Relationship of CD4+CD25+ regulatory T cells to immune status in HIV-infected patients. *AIDS*, 19 (9), 879-86 (2005)

77. J. Inoue, M. Seiki, T. Taniguchi, S. Tsuru and M. Yoshida: Induction of interleukin 2 receptor gene expression by p40x encoded by human T-cell leukemia virus type 1. *Embo J*, 5 (11), 2883-8 (1986)

78. E. Hanon, P. Goon, G. P. Taylor, H. Hasegawa, Y. Tanaka, J. N. Weber and C. R. Bangham: High production of interferon gamma but not interleukin-2 by human T-lymphotropic virus type I-infected peripheral blood mononuclear cells. *Blood*, 98 (3), 721-6 (2001)

79. S. L. Cross, M. B. Feinberg, J. B. Wolf, N. J. Holbrook, F. Wong-Staal and W. J. Leonard: Regulation of the human interleukin-2 receptor alpha chain promoter: activation of a nonfunctional promoter by the transactivator gene of HTLV-I. *Cell*, 49 (1), 47-56 (1987)

80. F. Toulza, A. Heaps, Y. Tanaka, G. P. Taylor and C. R. M. Bangham: High frequency of CD4+FoxP3+ cells in HTLV-1 infection: inverse correlation with HTLV-1-specific CTL response. *Blood*, 111, 5047-5053 (2008)

81. Y. Yamano, N. Takenouchi, H. C. Li, U. Tomaru, K. Yao, C. W. Grant, D. A. Maric and S. Jacobson: Virus-induced dysfunction of CD4+CD25+ T cells in patients with HTLV-I-associated neuroimmunological disease. *J Clin Invest*, 115 (5), 1361-8 (2005)

82. U. Oh, C. Grant, C. Griffith, K. Fugo, N. Takenouchi and S. Jacobson: Reduced Foxp3 protein expression is associated with inflammatory disease during human t lymphotropic virus type 1 Infection. *J Infect Dis*, 193 (11), 1557-66 (2006)

83. T. Kohno, Y. Yamada, N. Akamatsu, S. Kamihira, Y. Imaizumi, M. Tomonaga and T. Matsuyama: Possible origin of adult T-cell leukemia/lymphoma cells from human T lymphotropic virus type-1-infected regulatory T cells. *Cancer Sci*, 96 (8), 527-33 (2005)

84. G. Roncador, J. F. Garcia, L. Maestre, E. Lucas, J. Menarguez, K. Ohshima, S. Nakamura, A. H. Banham and M. A. Piris: FOXP3, a selective marker for a subset of adult T-cell leukaemia/lymphoma. *Leukemia*, 19 (12), 2247-53 (2005)

85. S. Chen, N. Ishii, S. Ine, S. Ikeda, T. Fujimura, L. C. Ndhlovu, P. Soroosh, K. Tada, H. Harigae, J. Kameoka, N. Kasai, T. Sasaki and K. Sugamura: Regulatory T cell-like activity of Foxp3+ adult T cell leukemia cells. *Int Immunol*, 18 (2), 269-77 (2006)

86. D. C. Macallan, D. Wallace, Y. Zhang, C. De Lara, A. T. Worth, H. Ghattas, G. E. Griffin, P. C. Beverley and D. F. Tough: Rapid turnover of effector-memory CD4 (+) T cells in healthy humans. *J Exp Med*, 200 (2), 255-60 (2004)

87. C. A. Michie, A. McLean, C. Alcock and P. C. Beverley: Lifespan of human lymphocyte subsets defined by CD45 isoforms. *Nature*, 360 (6401), 264-5 (1992)

Key Words: HTLV-1, Persistent Viral Infection, Ctl, Dynamics, Retrovirus, Immune Response, Review

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

http://www.bioscience.org/current/vol14.htm